Investigating the effect of dexamethasone on productivity, immune function, and behavior in dystocic periparturient dairy cattle

Dana Marie Bryant

Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Master of Science

In

Dairy Science

Christina S. Petersson-Wolfe, Committee Chair

Cynthia M. Wood

Robin R. White

December 13th, 2021

Blacksburg, VA

Keywords: dystocia, dexamethasone, postpartum, animal well-being

Investigating the effect of dexamethasone on productivity, immune function, and behavior in dystocic periparturient dairy cattle

Dana Marie Bryant

ACADEMIC ABSTRACT

Dairy cows are increasingly predisposed to diseases in the periparturient time period due in part to immunosuppression. Dystocia amplifies the disease risk due to the increased tissue trauma and stress the animal endures during the lengthened parturition. To decrease the increased inflammatory response seen in dystocic animals and improve their well-being in the postpartum period, we administered either a potent steroid, dexamethasone (DEX), or a saline control (CON) to cows within 12 hours after a dystocic parturition. The inflammatory marker haptoglobin was measured as well as behavioral and production measures. We observed that primiparous DEX cows exhibited a greater haptoglobin concentration on d 3 and d 7 postpartum compared to primiparous CON cows. Behavior was seen to be altered between the treatments, with DEX cows having reduced locomotion and increased lying times in the week following parturition. These measures could indicate pain reduction, suggesting improved comfort. Milk yield was affected, with a reduction of 7.3 kg/d in multiparous DEX cows in comparison to CON cows for almost the entirety of the first month following dexamethasone treatment. No treatment effects were seen for milk production of primiparous cows. Additional research is needed for further investigation of the immunological and production effects of steroids on postpartum dairy cows, especially between parities.

Investigating the effect of dexamethasone on productivity, immune function, and behavior in dystocic periparturient dairy cattle

Dana Marie Bryant

GENERAL ABSTRACT

Dairy cows are most likely to develop metabolic diseases in the weeks leading up and following calving. This risk can be heightened in cows that experience a difficult calving process, most likely due to an increased inflammatory response. A potent steroid, dexamethasone, was assessed after a difficult calving to see if postpartum animal recovery is accelerated through dampening of the inflammatory response. Dairy cows were given either dexamethasone (DEX) or saline control (CON) within 12 hours after a difficult birth to counter the inflammatory response. Our study found that primiparous cows that received dexamethasone, exhibited a greater concentration of the inflammatory marker, haptoglobin, on d 3 and d 7 postpartum than in CON cows. This may be attributed to increased tissue trauma in first time calvings since there was no treatment difference in multiparous cows. Cows that received dexamethasone also had a reduction in locomotion and an increased amount of total lying time in the days following calving. Additionally, multiparous cows that received dexamethasone exhibited a reduction in milk production by 7.3 kg/d for almost the entirety of the first month following treatment. Future research needs to examine the long-term effects of dexamethasone on the mammary gland and the inflammatory response in different parity cows postpartum.

Acknowledgements

Thank you to my advisor Dr. Petersson-Wolfe for taking me on as a master's student and working tirelessly with me to understand statistics and to motivate me on my thesis! I also appreciate all your help on keeping the project running at the farm through Covid-19. I cannot thank you enough!

Thank you to Turner Swartz for your statistical expertise and editing abilities. I really don't know what I would have done without your statistical genius and constant ability to respond to any questions I had. Thank you is not enough!

Thank you to my trusty lab manager Jane Duncan for being a constant person to rely on, keeping me entertained during our many hours in the lab, and working on the farm with me—no matter the weather, holiday, or birthday—during this crazy Covid-19 pandemic. I appreciate your guidance and help working out the ELISAs. I honestly don't know what I would have done without you!

Thank you to the undergraduates and fellow graduate student that helped me with project—Ahmerah Thompson, Sarah Thomas, Gianna Novak, and Kayla Alward—I appreciate all the time each of you spent helping me in the lab and/or enrolling and sampling cows with me.

Thank you to the Virginia Tech Dairy Farm staff, especially Clayborne and Shane. I appreciate your help through my many questions about the cows, texts informing me about calvings, and especially working through the AfiMilk data! Thank you so much!!

Thank you to my parents and siblings—Mom, Dad, Donna, David, and Daniel—for keeping me sane through either campus visits or time at home, and especially the two weddings during 2021 that I was able to forget about school for a while and celebrate you!

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: LITERATURE REVIEW

1.1. INTRODUCTION

The peripartum period (the period three weeks before and after calving) is highlighted by immunocompromised animals and an increased susceptibility to metabolic diseases. Parturition is considered a stressful event exhibited by an increased output of stress hormones: vasopressin and cortisol (Preisler et al., 2000, Vannucchi et al., 2015) and can be scored (1-5) to denote difficulty. The scores range from 1 (easy birth without assistance) to 5 (extremely difficult with assistance). Some researchers have utilized the length of active labor as an objective measure of calving difficulty where ≥70 minutes is defined as dystocia and <70 mins is associated with eutocia (Schuenemann et al., 2011, Funnell and Hilton, 2016). Dams that experience dystocia are presented with heightened risk factors progressing throughout the lactation. Dystocia exacerbates susceptibility and increases metabolic disease predisposition (Correa et al., 1993, Duffield et al., 2009). The incidence of dystocia varies from farm to farm and can be affected by management factors and breeding decisions.

Animal well-being has become a point of focus in animal production, but pain mitigation during the parturition process has not been well evaluated. Animal well-being and productivity may be improved through introduction of the use of analgesics—known anti-inflammatory agents—after a difficult calving. This review focuses on detailing dystocia in dairy cows and the impact of a difficult birth postpartum. The inflammatory response triggered by trauma and the role of steroid application for disease prevention as well as production improvement will be discussed.

1.2.Dystocia

Calving is an important part of a dairy cow's life, with the duration and outcome impacting her productive life. Parturition is comprised of two stages: initiation of parturition by the fetus causing cervical dilation and the process of subsequent expulsion of the fetus (Putnam, 1982). The second stage is the most important for farm management, as cows can experience difficulty with calf expulsion which leads to dystocia. Dystocia is defined as a difficult birth which results in a prolonged calving process and even assisted extraction of the calf, which is recommended 70 minutes after the amniotic sac appears (Schuenemann et al., 2011, Funnell and Hilton, 2016). Calving ease can be defined with the use of scores (1-5), where scores 3 and above are considered difficult, hence dystocic (Dematawena and Berger, 1997). Dystocia occurrences on farms tend to vary, with the USDA (2018) reporting 4.7% of cows experience dystocia, while other reports show dystocia occurrences up to 28.7% in primiparous cows and 10.7% in multiparous cows (Meyer et al., 2001). The range of occurrences could be attributed to varying calving intervention management styles, calving observation, and farm record keeping. Since dystocia occurrences seem to vary greatly from herd to herd, it is important to take precautionary steps towards prevention.

1.2.1. Factors Contributing to Dystocia

The predisposition of dystocia can begin at insemination, but many other causes also exist. Factors include fetal malpresentation, fetal to dam size, and dam related causes (Schuenemann et al., 2011). Although dystocia can happen to any cow, varying predisposing factors are related to parities. Primiparous cows are more prone to dystocia through feto-pelvic disproportion, abnormal fetal position, and vulva stenosis (Mee, 2008). However, for multiparous cows the predisposing factors differ a bit: abnormal fetal position, feto-pelvic disproportion, multiple fetuses, and uterine inertia/torsion (Mee, 2008). Feto-pelvic

disproportion, the most common cause of dystocia, is defined by the inverse relationship between calf birthweight and maternal pelvic size, which both gestational length and sex of calf can negatively impact (Mee, 2008).

It has been documented that primiparous cows exhibit a higher rate of dystocia compared to multiparous counterparts (Dematawena and Berger, 1997, Atashi et al., 2012, Mainau et al., 2014). In contrast, a study by Juozaitiene et al. (2017), found that primiparous and parities 6-8 had the majority of the extremely difficult births. The discrepancy may be attributed to the unequal quantity of parities used in different studies. Through an observational study, amniotic sac appearance is used as the baseline from which assistance should be required 70 minutes after (Schuenemann et al., 2011). Although assistance is needed in some cases, assisting prematurely will increase the number of cows that experience a difficult calving, and assisting too late can affect calf vitality and the cows' reproductive health (Mee, 2004).

Dystocia not only negatively affects dams, but also increases the multitude of health hurdles for the newborn calf such as prolonged hypoxia, internal trauma, and even stillbirths (Meyer et al., 2001, Lombard et al., 2007). Surviving a difficult birth can adversely impact a calf's future productive life. The first lactation milk yield, lifetime milk production, and protein production are negatively correlated with the subsequent increase of calving score (Heinrichs and Heinrichs, 2011). Primiparous cows have been seen to have a greater percentage of stillbirths than multiparous counterparts, with most of the stillbirths being attributed to an extremely difficult birth (Meyer et al., 2001, Juozaitiene et al., 2017).

1.2.2. Dystocia and Metabolic Diseases Risk

The peripartum period is a time in which the dairy cow is under a significant amount of metabolic stress preparing for parturition, colostrogenesis, and lactogenesis, consequently more disease prone. Decrease in dry matter intake (DMI) leading up to parturition exacerbates negative energy balance, impairing bodily defenses. With all the metabolic changes happening during the peripartum period, it is not surprising that disease incidence increases, with the most occurrences in the postpartum period, especially when risk factors like twins or dystocia are present (Correa et al., 1993).

Neutrophils and macrophages have decreased phagocytic ability during the last week of gestation (Sordillo, 2005). As part of the inflammatory process, neutrophil and macrophage phagocytosis produce reactive oxygen species (ROS), which are free radicals that ultimately help defend the host from pathogens (Sordillo and Raphael, 2013). After parturition, buildup of ROS called oxidative stress, may also contribute to the periparturient immunocompromised state. Tissue damage from oxidative stress is attributed to the degradation of protein and DNA (Sordillo and Aitken, 2009, Sordillo and Raphael, 2013). Not only does the innate immune response leave the animal susceptible to pathogens, but the adaptive immune response does as well. It has been observed that during the postpartum period, there is an increase in Th2 CD4+ cells. These cells produce anti-inflammatory cytokines (IL-4, IL-10) which can increase disease susceptibility through decreasing the host attack ability on foreign pathogens (Shafer-Weaver et al., 1999).

A traumatic birth has a greater impact on both the dam and calf. Dystocia has long term negative affects on milk yield, fat and protein losses, reproductive viability, and mortality of calves and/or cows, while also increasing veterinary costs (Dematawena and Berger, 1997). Lactational milk, protein, and fat yield was seen to decrease by 135, 3.16, and 6.52 kg,

respectively, due to dystocia (Atashi et al., 2012). Dystocia also reduces the health of cows by predisposing cows to postpartum diseases such as retained placenta (RP), metritis, and left displaced abomasum (LDA); and additionally is seen as a risk factor for ketosis (Correa et al., 1993, Duffield et al., 2009). Somatic cell count (SCC) and mastitis incidences are positively correlated with dystocia, with increasing prevalence of *Staphylococcus aureus* and *Streptococcus agalactiae* in milk associated with difficult birth (Juozaitiene et al., 2017). Using a database containing farm record keeping of calving ease scores (1-5), Dematawena and Berger (1997) were able to analyze a variety of production factors in association with the calving ease score. They found cost of dystocia differs between parities when examining losses based on milk production, fertility, and animal loss, with the average primiparous cow associated with a greater loss ($29) compared to average multiparous cows (~$10) (Dematawena and Berger, 1997). However, looking at the cost associated with calving scores—going from 1 to 5—$0, $50.45, $96.48, $159.82, $379.61 respectively, an observed difference is seen due to dystocia. Dematawena and Berger (1997) concluded that based on probabilities of occurrences and cost per parity, the cost of a difficult birth compared to an easy calving was $380, regardless of parity.

1.2.3. Pain during Parturition

When a human is giving birth, multiple pain mitigation options are offered during the process ((UK), 2007). However, when a cow is in the process of parturition, pain medication is not usually given. Looking at this from an animal well-being point of view, cows may be in a considerable amount of pain during and after a difficult birthing process (Singh, 2010). There are five freedoms outlined to improve upon animal well-being, including: freedom from thirst, hunger, and malnutrition, freedom from discomfort and exposure, freedom from pain, injury, and disease, freedom from fear and distress, and freedom to express normal behavior (Mellor, 2016).

When looking at the topic of parturition the question is, how do we know if pain is associated after parturition?

Cows are prey animals, which means they tend not to outwardly indicate pain or illness. Thus, pain is hard to evaluate. Through studies assessing pain, criteria have been formulated that allow us to decide if an animal is in pain. These criteria are: possession of noxious stimuli sensitive receptors, possession of brain structures analogous to human cerebral cortex, possession of nervous pathways connecting nociceptive receptors to higher brain structures, possession of opioid substance receptors, analgesics modify response to noxious stimuli, avoiding noxious stimuli, and a persistence response to noxious stimuli with association (Bateson, 1991, Sneddon et al., 2014). Sympathetic nervous system and HPA axis activation can be an indicator of an animal perceiving a potentially painful stimulus (Sneddon et al., 2014). This is measured through catecholamine and glucocorticoid production. Observing for abnormal behavioral responses is another way to assess for pain (Sneddon et al., 2014).

Parturition in general is a stressful event for cattle with cortisol levels increasing seven-fold during calving (Preisler et al., 2000). A prolonged or an assisted calving contributes even more stress to the event. This is seen through an elevated serum cortisol concentration in dystocic cows immediately after calving and up to an hour postpartum (Vannucchi et al., 2015). Hyperglycemia was exhibited in all study animals' postpartum, with the dystocic treatment group maintaining this status from pre-partum (Vannucchi et al., 2015). In addition to the elevated cortisol levels, heifers that had assistance with calving expressed heightened concentrations of vasopressin, a hormone secreted in response to pain and/or stress (Hydbring et al., 1999). Cortisol is also known for anti-inflammatory properties, which with an increased stress response, could lead to immunosuppression in dystocic cows (Lord et al., 2014).

By reducing inflammation and pain cows experience during and after birth, milk production could be subject to change. Previous research has shown that the administration of meloxicam, a non-steroidal anti-inflammatory drug (NSAID), to eustocic cows increased milk yield, but did not affect the milk yield for dystocic cows (Swartz et al., 2018). Through administration of pain-relieving medication, animal well-being could be improved in addition to animal productivity.

1.3.Behavioral and Physiological Indicators

Since animals rarely express pain, producers are left to monitor for signs of discomfort and changes in behavior. There are multiple factors that may indicate discomfort including rumination time, lying time, activity, and resting bouts. Literature suggests cows display a consistent time budget each day. Therefore, cows that are not feeling well will decrease feed intake which reduces rumination times; a decrease in their daily activity will also be noted (Proudfoot et al., 2014). Conversely, healthy cows display longer time spent lying down during each session (Cattaneo et al., 2020).

1.3.1. Behavioral Aspects

When not in the milking parlor, cows spend time lying, eating, and drinking. By altering any of these, there could be consequences on health and production. Limitations on lying behavior are related to excessive head lock time for herd checks, limited bunk space, and hoof trimming, amongst others. Standing behavior can be an indicator of animal discomfort. Lying time is necessary to remove constant pressure from the hooves/legs, increase blood flow through udder, and improve rumination effectiveness (Grant, 2007). Cows should be spending 12-14 hours daily lying down and resting, which is associated with the highest producing cows (Grant,

2007). Research has demonstrated cows two weeks postpartum exhibit reduced lying time in comparison to later lactation cows (Mattachini et al., 2020). Other studies have reported lying time increased with parity and stage of lactation (Chaplin and Munksgaard, 2001, Westin et al., 2016). Additionally, lying bouts were seen to be more frequent in the morning (Mattachini et al., 2020).

Activity levels of cows not only tend to fluctuate due to illness, but also with season. Healthy cows have increased activity levels in the winter and spring in comparison with fall and summer months (Edwards and Tozer, 2004). In contrast, Steensels et al. (2012) saw the inverse, where lying times were greater in the winter. These differences may be attributed to study location (Florida vs Israel) and different cooling practices. Looking at parities, first parity cows had greater activity levels than multiparous cows (Edwards and Tozer, 2004). Activity levels fluctuate with different illnesses in the first 30 days in milk (DIM), as seen with ketosis and LDA; these cows exhibited greater activity levels compared to healthy cows 8-9 days before diagnosis then subsequently declining (Edwards and Tozer, 2004). Research has shown no significant difference in activity levels between healthy and metritis affected cows (Cattaneo et al., 2020). However, RP was associated with significantly greater lying times than in healthy controls. Lying bouts showed no significant difference between the three groups (Cattaneo et al., 2020).

Altered animal behavior has been shown following calving. Looking at the first four days after unassisted calving, overall lying time was reduced, while the number of lying bouts and activity was higher on the first day in comparison to the later three days (Jensen, 2012). Reduction in lying time and increased activity in the first couple days postpartum was also reported by Steensels et al. (2012). In contrast, Swartz et al. (2018) observed decreased

locomotion following either pre or post calving meloxicam treatment in comparison to a negative control dystocia group.

1.3.2. Physiological Aspects

Illness can be detected not only through behavioral changes, but also biologically. Beta-hydroxybutyrate (BHBA) concentration can be determined from blood as an indicator of ketosis in postpartum cows. Each case of ketosis, or hyperketonemia, is estimated to cost approximately $289, but that cost increases in primiparous animals (McArt et al., 2015). Body temperature fluctuations from normal range indicate when the animal is ill. During the inflammatory process, cytokines are produced which cause the telltale sign of fever. Body temperature is essential to gauge if an animal is ill.

Haptoglobin, a positive acute phase protein (APP), is a metabolic measure of the inflammatory cascade. This APP is a measure of inflammation. One study examined postpartum haptoglobin in healthy cows compared to animals diagnosed with RP or metritis (-14, -3, 1, 3, 7, and 28 days relative to calving) (Cattaneo et al., 2020). At d 7 postpartum, haptoglobin was significantly greater in the sick cows than the healthy cows, as the healthy cow's haptoglobin concentration decreased after d 3 (Cattaneo et al., 2020).

1.3.3. Production Aspects

Dairy cattle are easily assessed through production values such as milk yield, fat, and protein percentages. Illness can be ascertained through production drops or inversions. Following normal milk yield curves after parturition, production increases 8% and 10% each day for the first 18 and 14 days in primiparous and multiparous animals respectively (Grant, 2007). It has been observed that growth hormone is significantly increased in high producing cows compared to

lower yielding counterparts (Hart et al., 1978). Growth hormone works in allocating nutrients to the mammary gland from other tissues. Behavioral aspects are also linked to production; this is seen through lying time and milk production. A study examining if behavior affects production looked at the effects of lying time on growth hormone concentrations. Milk yield did not differ between the groups even though lower concentrations of growth hormone were found in the lying deprived group (Munksgaard and Løvendahl, 1993). These results may seem in contrast with the previous study. However, Munksgaard and Løvendahl (1993) utilized late lactation cows which are not as severely affected by growth hormone as early lactation cows (Hart et al., 1978). Lying time does have an effect on milk production as seen by Bach et al. (2008) who reported a positive correlation between lying time and milk production. Consequently, milk yields may not recover for up to at least 10 days post disease diagnosis (LDA and ketosis specifically) (Edwards and Tozer, 2004).

1.4. Inflammation

Redness, swelling, pain, heat, and loss of function are the cardinal signs of inflammation (Lees et al., 2004, Bradford et al., 2015). Initiation occurs during tissue trauma or pathogen invasion and is necessary to eliminate the threat, return to homeostasis and reduce tissue damage. Inflammation occurs due to an influx of pro-inflammatory mediators such as cytokines, enzymes, and chemokines amongst others, which are facilitated through pro-inflammatory gene transcription (Barnes and Adcock, 2003). The acute phase response (APR) is initiated due to inflammation, causing a decline in protein (-APP) secretion by the liver and an increase of positive acute phase proteins (+APP) (Bradford et al., 2015). Although inflammation is triggered to resolve the initiating event and bring the body back to homeostasis, the body can be overwhelmed, and death can occur.

1.4.1. Immune Factors

The immune system is composed of two arms: innate and adaptive, which work in concert to identify and eliminate pathogens. The innate immune system is non-specific and consists of leukocytes, neutrophils, and natural killer cells (Sordillo, 2005), while the highly specific adaptive immune system is composed of B and T cells (lymphocytes) that produce antibodies (Mallard et al., 1998). While these two arms are separated by functionality, they still work together. Cells, like macrophage presenting cells (MPC), bring antigens over to lymphocytes, that can then produce antigen specific antibodies.

Each cell in the immune system has its own function and responsibility in times of defense, beginning with the innate immune system, as it is the first to react to non-host material. There is a wide variety of cells that aid in initiating inflammation, each regulated by cytokine release. Neutrophils are the first cells to react and defend against foreign material, using primarily phagocytosis and ROS against pathogens (Paape et al., 2003). Macrophages are also an important part of the immediate immune response, utilizing phagocytosis and inducing increased production of pro-inflammatory mediators (Sordillo, 2005). Natural killer cells seem to be a shared cellular player for both innate and adaptive immune functions, but has an overall bactericidal activity (Sordillo, 2005). The innate immune response initiates the inflammatory response and works quickly to resolve the triggering event.

The adaptive immune system is the slower reacting part of the immune system but holds memory of specific antigens to help the body respond quicker to repeat threats. It is composed primarily of lymphocytes: B and T cells. B cells are known for production of antibodies while T cells are divided on function based on cell receptors (Mallard et al., 1998). Naive helper T cells, identified through expression of CD4+, are known for secreting cytokines (Berger, 2000).

Cytokine secretion leads to further differentiation; Th1 secretes pro-inflammatory cytokines (IL-2, TNF-α), while Th2 secretes anti-inflammatory cytokines (IL-4, IL-10) (Berger, 2000, Franchimont et al., 2000, Sordillo, 2005). The CD8+ helper T cells are known as cytotoxic T cells and work with MHC cells to destroy antigens. This cell type also acts to suppress other cellular activation (Sordillo, 2005). Specific cell profiles vary across body tissues, for example CD8+ is the main T lymphocyte in the mammary gland while CD4+ is predominant in the blood (Sordillo, 2005).

When a tissue injury initially occurs, the innate immune system is first to respond. The receptors on the surface of innate immune cells recognize danger associated molecular patterns (DAMPs) or pathogen associate molecular patterns (PAMPs) following trauma or tissue injury (Liu et al., 2017). These pattern recognition receptors (PRRs) initiate the degradation of IκBα through phosphorylation causing the release of NF-κB complexes into the cell nucleus (Hayden et al., 2006, Liu et al., 2017). Activation of NF-κB upregulates gene transcription of various pro-inflammatory cytokines production by monocytes both distal and proximal to the primary trauma location (Baumann and Gauldie, 1994, Hayden et al., 2006). Commencement of the inflammatory cascade is due to the surge of cytokine production initiated by Interleukin (IL) and Tumor-necrosis factor (TNF) families (Baumann and Gauldie, 1994). Along with an increase in cytokine production, there is an activation of monocytes and vasodilation causing leakage which allows passage of leukocytes to the site of trauma/infection (Chinenov and Rogatsky, 2007). The acute phase response is how the innate immune system responds rapidly to a threat based on trauma, inflammation, and infection. Once the initiating event is resolved, the body works to restore homeostasis.

1.4.2. Factors contributing to Inflammation

Pro-inflammatory cytokines, TNF-α, Interleukin-1, and Interleukin-6 (IL-1, IL-6), are secreted from lymphocytes and monocytes. Recognition by pattern-recognition receptors (PRRs), like toll-like receptor (TLR), initiates cytokine release (Medzhitov and Janeway, 2000, Ceciliani et al., 2012). Cytokines target many cell types to initiate the inflammatory response. This cytokine cascade causes a heightened inflammatory response, initiating events leading to elevated glucocorticoids, catecholamines, and glucagon (Grimble, 1990). Once glucocorticoid synthesis becomes involved, the type of cytokines produced fluctuates. These peptides are classified based on function: pro and anti-inflammatory. Pro-inflammatory cytokines work by activating immune cells such as neutrophils, causing the differentiation of naive adaptive immune cells, and promotion of pathogen clearance. Pro-inflammatory cytokines induce the symptoms that are typical of sickness behavior, such as fever, weakness, and behavioral changes (Kelley et al., 2003).

Chronic inflammation occurs when the body does not resolve the initial triggering event, sustaining inflammatory conditions and leading to further tissue damage (Flower, 1988). A factor that contributes to chronic inflammation is ROS through its activation of NF-κB, which induces the production of pro-inflammatory cytokines, therefore increasing the severity of oxidative stress and the inflammation (Sordillo and Raphael, 2013).

1.4.3. Inflammation and Pain

Inflammation is needed to expel the invading organism(s) and to promote healing. However, it is dangerous to the host in excess, as more tissue damage can occur, and can potentially cause death. Where there is a large inflammatory response, there may be an association with pain due to the activation of the acute phase response (Mainau et al., 2014). This may be the case in parturition, dystocia particularly. The use of anti-inflammatory drugs can attenuate inflammatory

responses and allow normality to resume. Both non-steroidal anti-inflammatory drugs (NSAIDs) and steroids are commonly used.

It is known that NSAIDs are COX (cyclooxygenase) inhibitors, but how does that fit in with reducing the inflammatory response? Tissue damage initiates phospholipase A2 action and the formation of arachidonic acid through the breakdown of cell membrane phospholipids. Arachidonic acid is converted to eicosanoids as a result of several enzyme reactions (Lees et al., 2004). Eicosanoids, such as prostaglandins, are known to be pro-inflammatory mediators since they increase vascular permeability and susceptibility to pain. Each of the enzyme actions on arachidonic acid form separate eicosanoids with prostaglandins originating from the COX enzyme mediated response. As NSAIDS inhibit COX action, this hinders the pro-inflammatory actions prostaglandins exhibit. However, there are two COX enzymes, COX-1 and COX-2. They have different effects, with COX-2 being pro-inflammatory and COX-1 having more normal functions like platelet aggregation (Lees et al., 2004). By only inhibiting COX-2, fewer side effects and more anti-inflammatory action will be seen.

Multiple studies have evaluated NSAIDS before and after parturition to determine if the analgesic effects impact production. Treating postpartum cows with meloxicam within the first 6 hours after calving, Mainau et al. (2014) measured milk production during the first month of lactation and saw no significant difference due to treatment. Swartz et al. (2018) gave meloxicam either pre (between 6-48 hours) or post (within 12 hours) calving with a placebo given pre/post depending on group. This study utilized all calvings and saw a significant daily yield increase in the eutocic meloxicam pre group in comparison to the control (full placebo) group. In addition, a significant increase of milk fat was seen in both meloxicam treatments in comparison to the control. However, no milk yield differences were seen in the dystocic cows (Swartz et al., 2018).

Mainau et al. (2014) looked at only eustocic and easily pulled parturitions. Focusing in on assisted calvings, dystocia, and the use of analgesics, we might be able to see more of a heightened response and possible increased well-being for the cows. Looking at meloxicam given after assisted births, there was no significant difference in DMI, milk production, or haptoglobin concentrations, which may be attributed to the treatment being given 24 hours postpartum (Newby et al., 2013). However, this was done to prevent potential interference with prostaglandin on fetal membrane expulsion. Although they saw no significant differences, they did observe the meloxicam treated animals spent more time in the 24 hours post treatment at the feed manger, potentially indicating some pain elimination (Newby et al., 2013).

1.4.4. Acute Phase Proteins and Haptoglobin

A key indicator of the acute phase response is an increase in the APP which are a part of the innate immune response (Grönlund et al., 2005, Bradford et al., 2015). The acute phase response is activated after inflammation and is an arm of the innate immunity. In diagnostic work APP are useful when deciding if the animal is undergoing a localized inflammatory response potentially due to an injury or well disguised illness (Van Leeuwen and Van Rijswijk, 1994, Gomez-Laguna et al., 2011). Hepatocytes in the liver produce APP and are activated by cytokines, typically TNFα, IL-1, and IL-6, and glucocorticoids (Gomez-Laguna et al., 2011). These proteins are defined as positive or negative due to action and concentration during inflammation. Positive APP are increased during inflammatory response while negative APP decrease. These proteins are pertinent during the inflammatory process to scavenge toxic molecules, opsonize pathogens, and other inflammatory regulatory processes (Ceciliani et al., 2012). There are multiple different APP, but depending on the species the most predominate can

vary during inflammatory responses. For bovine, the two most prominent +APP are haptoglobin and serum amyloid A (SAA) (Grönlund et al., 2005).

Specifically, haptoglobin is one of the highly reactive APP that is activated during tissue injury, increasing as much as 10-fold in the first two days after parturition and reaching the highest concentration 3 days postpartum (Petersen et al., 2004, Chan et al., 2010). This is due to the increased inflammation and parturition length with dystocia, with cows experiencing an increased stress response (Singh, 2010, Vannucchi et al., 2015). Dystocia also allows more outside exposure to the vagina, increasing the risk for metritis or retained placenta (Correa et al., 1993). However, haptoglobin is considered to be bacteriostatic binding to free hemoglobin, thus preventing bacterial utilization of iron, and preventing kidney mutilation from free heme (Natelson and Natelson, 1980, Ceciliani et al., 2012).

Haptoglobin, in its hapto-heme complex, exerts anti-inflammatory actions through binding to CD163 on macrophages/monocytes and triggering release of IL-10 and carbon monoxide (CO) (Philippidis et al., 2004). Haptoglobin can exhibit anti-inflammatory effects on neutrophils through inhibiting respiratory burst (Oh et al., 1990). Suppression of cytokines when challenged with lipopolysaccharide (LPS), potentially to prevent endotoxic shock due to an overproduction of pro-inflammatory cytokines is another immunoregulatory effect of haptoglobin (Arredouani et al., 2005).

Haptoglobin has been used as an easy measure of the inflammatory response due to the rapid increase after trauma or injury. Treating cows postpartum with meloxicam within the first 6 hours, Mainau et al. (2014) measured haptoglobin on four separate days during the first two weeks of lactation and did not observe any significant difference due to treatment. Still,

haptoglobin concentrations were seen to be greater in postpartum primiparous cows than in multiparous cows (Mainau et al., 2014).

The ability of milk haptoglobin to be used as an indicator of subclinical mastitis was also evaluated. Milk samples were collected over a two-month period to investigate milk sample assessment of APP (SAA and haptoglobin) (Grönlund et al., 2005). The control animals were below detection levels, which affirmed no abnormalities. Although APP were detected in some abnormal milk samples, it only correlated with SCC in 52% of the chronic subclinical mastitis cases. However, if only one of the APP was detected, it was typically haptoglobin which may indicate how different APP are more significant during certain times of inflammation (Grönlund et al., 2005).

1.5. Use of Steroids in Dairy Cattle

The use of steroids is important in the veterinary field due to the vast effects from suppression of the HPA axis, including anti-inflammatory and anti-pyretic effects. There are a variety of different ways steroids can be used on the farm. Unintentional abortion or induced calving can result from steroid application, as it is thought to replicate fetal cortisol production that signals parturition initiation (Black, 1974). Glucocorticoids have been used for the treatment of ketosis, as they induce hyperglycemia not through cellular gluconeogenesis, but total circulatory dissemination (Black, 1974, Herdt and Emery, 1992). Furthermore, mastitis recovery may be assisted with glucocorticoid treatment, due to the anti-inflammatory properties, reduction of swelling, and perhaps enhancement of the antimicrobial treatment effects (Black, 1974). Another udder specific area of interest would be treatment of udder edema, as glucocorticoid action on fluid and electrolyte balance allows proper fluid loss from the udder (Black, 1974, Gupta and Bhatia, 2008). In addition, glucocorticoids are used for various

inflammatory diseases or conditions. Nevertheless, prior to application the potential side effects need to be evaluated, due to the possibility of potentiating the disease and preventing recovery (Black, 1974).

Effect of steroid administration varies between species and is dependent on lymphocyte reaction. Steroid sensitive species have a pronounced lymphopenia effect after steroidal treatment, while in steroid resistant species it is not as easily reproduced (Claman, 1972). Bovine are considered steroid-resistant which prevents lymphocyte decline when glucocorticoids are administered (Pruett et al., 1987).

1.5.1. Immunosuppression

Glucocorticoids are known to exhibit an immunosuppressive action through up regulation of anti-inflammatory mediators and down regulation of pro-inflammatory mediators. Glucocorticoids also interact with the adaptive immune system, suppressing helper T cells, specifically Th1 cellular immunity, and promoting apoptosis of eosinophils (Franchimont et al., 2000, Elenkov and Chrousos, 2002). It has been seen through glucocorticoid injection that the animals become more susceptible to infection due to its effects on immune cells and mediators (Griffin, 1989).

Glucocorticoids can influence the immune system in several ways. In a study administering three dexamethasone intramuscular injections per week for two weeks, greater total leukocyte counts were observed on d 9 and 11 in the treatment group in comparison to the control group (Pruett et al., 1987). This study also noted after the dexamethasone injection, leukocytes tended to increase and peak at 48 hours post injection. In accordance with this, neutrophilia was expressed on half of the sampling days. However, no significant T-lymphocyte

decline was noted. In fact, the dexamethasone treated cows saw an increase throughout the study. Although a decline in the T-lymphocyte population was not seen, suppression due to the dexamethasone injection(s) was observed, but differed depending on the concentration of the mitogen exposure given (Pruett et al., 1987). This study elucidated glucocorticoid mode of action on the immune system in accordance with a variety of factors such as dosage.

Stress is an established precursor of poor immune function, increasing the risk for infectious diseases; a prominent example with transport stress being shipping fever. The stress response begins with the activation of the hypothalamic-pituitary-adrenal system (HPA) through the secretion of corticotropin releasing hormone (CRH), consequently influencing adrenocorticotropin hormone (ACTH) release, stimulating glucocorticoid secretion from the adrenal cortex (Butcher and Lord, 2004, Salak-Johnson and McGlone, 2007, Xavier et al., 2016). With the glucocorticoid secretion, cortisol blood levels increase, thus indicating stress levels.

As previously stated, blood cortisol levels increase seven-fold during parturition, which influence the immune cells (Preisler et al., 2000). Parturition was confirmed to increase the number of leukocytes, but not influence lymphocyte concentration. Cortisol was negatively correlated with lymphocyte and monocyte glucocorticoid receptor (GR) expression. Yet, GR expression is twice that in monocytes compared to lymphocytes. Lymphocyte GR expression was altered due to parturition, with a 42% reduction in comparison with the control and remained significantly lower than the control during the 14-day postpartum study period. Similarly, GR expression on monocytes resulted in a 57% reduction in comparison with the control which persisted for the remainder of the 14-day postpartum study. Multiparous cows showed a more enduring, but gradual GR reduction in leukocytes (Preisler et al., 2000). This shows with heightened glucocorticoid exposure, immunity can be altered or compromised.

1.5.2. Steroids and Inflammation

Glucocorticoids, naturally elicited in times of stress, injury, and infection, are produced during the process of parturition, starting as early as several days (4 days) prior to the day of parturition, and then declining in the two days postpartum (Smith et al., 1973, Flower, 1988). Endogenous glucocorticoids are known to inhibit factors like cytokines, which contribute to the inflammatory response (Sapolsky et al., 2000). Glucocorticoids do play a role in increasing the expression of APP, although they primarily work synergistically with cytokines in quest to stimulate APP (Baumann and Gauldie, 1994). It is theorized that exogenous glucocorticoids have more of a response on the production of APP than endogenous glucocorticoids (McGrotty et al., 2003). Due to the antagonistic nature of glucocorticoids towards cytokines, a negative feedback loop is exhibited allowing the acute phase reaction to end and a return to homeostasis (Baumann and Gauldie, 1994).

During the inflammation process, pro-inflammatory cytokines/stimuli activate pro-inflammatory transcription factors, like NF-κB, which then go on to bind to the promotor region of these inflammatory genes, initiating a sequence of events leading to pro-inflammation genes being switched on through gene transcription (Barnes and Adcock, 2003). Endogenous or synthetic glucocorticoids bind to the GR in the cytoplasm after diffusing through the cell membrane (Barnes and Adcock, 2003). Once bound, GR initiates protein dissociation, allowing translocation into the nucleus and binding to the glucocorticoid responsive elements (GRE) on the promotor region of the glucocorticoid responsive gene, causing gene transcription (Barnes and Adcock, 2003). This gene transcription triggers multiple genes with anti-inflammatory properties to switch on, increasing protein synthesis of proteins such as interleukin-10 and IκB-α (Barnes and Adcock, 2003). However, the primary function of glucocorticoids is to suppress the

expression of inflammatory protein genes, so pro-inflammatory protein synthesis does not occur (Barnes and Adcock, 2003, Barnes, 2006).

One of the first cell types to respond to inflammation are neutrophils, the most numerous leukocyte. Neutrophils are a prominent innate feature, proficient in pathogen elimination and as a mediator of inflammation. However, neutrophils are sensitive to glucocorticoids due to the large number of glucocorticoid receptors located on these cells and glucocorticoids cause an increase of neutrophils in the blood (Paape et al., 2003, Burton et al., 2005). For these cells to reach the site of inflammation, mobilization out of the blood stream into the target tissue is required, which is where adherence molecules come into play. Present on the inactivated surface, L-selectin allows the neutrophils to openly interact with the endothelium of an inflamed area, but once activation occurs, L-selectin is shed and Mac-1 is upregulated, allowing stable binding to the endothelium (Butcher, 1991). After stable binding, the neutrophil can extravasate into the inflamed tissue. Anti-inflammatory effects through treatment with glucocorticoids like dexamethasone exhibit down regulation of these surface molecules (L-selectin and Mac-1) that allow neutrophils to respond to inflammatory sites (Burton et al., 1995). Thus, glucocorticoids down regulate the neutrophil response to inflammation.

1.5.3. Steroid effects

Hyperglycemia due to glucocorticoid treatment is beneficial in treating ketosis in dairy cattle, though there may be some shortcomings associated with production. In clinically ketotic cows, glucocorticoid injection in addition to the propylene glycol treatment, resulted in reduced plasma BHBA concentration, which is an indicator of decreased lipid mobilization (van der Drift et al., 2015). An increased risk of subclinical ketosis has been associated with low protein and high fat in milk (Duffield et al., 2009). Elevation of serum BHBA has been linked to decreased

milk yield, milk protein percentage, and increased milk fat percentage (Duffield et al., 2009). Glucocorticoids can impact milk production due to its influence on glucose. Milk production has been seen to decrease with glucocorticoid application, even in healthy cows not treated for ketosis (Herdt and Emery, 1992). It has been proposed that the decline in milk production could be from the rerouting of glucose from the mammary gland. Research examining mammary flow and uptake of glucose after dexamethasone treatment noted glucose availability with the diminished milk yield, however, there was a reduced uptake and utilization (Hartmann and Kronfeld, 1973). Still, there must be other factors contributing to the reduced milk yield due to blood glucose recovering several days prior to the milk depression recovery (Shamay et al., 2000).

Not only is glucose metabolism affected, but protein as well. It has been noted that protein catabolism is increased with glucocorticoid treatment (Roth and Kaeberle, 1982). Antibody suppression after dexamethasone injection has also been observed (Fleshner et al., 2001). This is one of the reasons why it is recommended to vaccinate animals under low stress conditions since endogenous glucocorticoid production can prevent production of a sufficient concentration of antibodies in response to vaccines.

There are many different glucocorticoid treatments available for medical/therapeutic use, but the decision on which to choose depends on target area, action time (short, intermediate, and long acting), and potential side effects from treatment. Each glucocorticoid differs in their effect whether it be more anti-inflammatory, or more mineralocorticoid activity, each case is different with animal treatment.

1.5.4. Dexamethasone

Dexamethasone is a synthetic glucocorticoid with one of the most potent anti-inflammatory properties, thirty times more potent than cortisol (Dluhy et al., 1973). It is classified as a long action glucocorticoid since it has a biological half-life of 36-72 hours (Gupta and Bhatia, 2008). Dexamethasone interferes with the pro-inflammatory action of the NF-κB activation pathway by binding to the glucocorticoid receptors on monocytes, which becomes internalized into the cell nucleus (Brummer et al., 2005). Subsequently, IκBα is produced, causing an inhibitory effect on NF-κB, thus limiting the amount of pro-inflammatory cytokines produced (Brummer et al., 2005). Dexamethasone has also been found to inhibit pro-inflammatory cytokines, such as TNF-α (Smoak and Cidlowski, 2006). This is done through cytokine mRNA destabilization from glucocorticoid stimulation of the synthesis of zinc finger protein tristetraprolin (TTP). Hence, degradation of mRNA and decreasing protein synthesis commences (Smoak and Cidlowski, 2006).

Bovine mammary epithelial cells have a plethora of intracellular glucocorticoid binding sites that are divided into three different receptor types—two types with an elevated affinity for dexamethasone over cortisol (Van Der Kolk, 1990). This contrasts with dexamethasone binding in plasma, which has a low affinity for dexamethasone (Van Der Kolk, 1990). The lactation period may increase the affinity for glucocorticoid binding, therefore increasing effects according to research on glucocorticoid accumulation in mammary tissue (Gorewit and Tucker, 1976). Consequently, milk yield may be suppressed following dexamethasone injection, depending on dosage and mode of injection, since it was found to cause the most severe drop in milk production out of tested glucocorticoids (Van Der Kolk, 1990). The dosage dictates how many receptors are bound which influences the drug's effectiveness (Buttgereit et al., 2002). Prolactin appears to be inhibited by dexamethasone injections and may be a mechanism in which

glucocorticoids affect milk yield. Ponchon et al. (2017) through two experiments observed the repressive action of dexamethasone injections on prolactin secretion. The dexamethasone treated cows had lower prolactin concentrations, lower production, and saw higher concentrations of fat and protein percentages (Ponchon et al., 2017). Mid-late lactation animals treated with dexamethasone produced less milk than control animals for the first three milkings (Shamay et al., 2000). This slump in milk production took five days to regain to previous values. Milk components were also influenced. Total protein secretion (kg/day) decreased by 35% in the first 24 hours post treatment and returned to baseline by the next day. However, fat secretion was unaffected by the dexamethasone treatment. Nevertheless, concentration (%) of fat and total protein increased by 45%, taking three days to return to baseline values (Shamay et al., 2000). This difference (concentration vs secretion) is due to the lower milk yield in the treated animals (Varner and Johnson, 1983).

1.6. Conclusion

Dystocia is an unavoidable part of production life, but managing for as many contributing factors as possible can lower incidences. Management focused on prevention of dystocia is necessary to improve the productive life of not only the dam, but the calf. Pain associated from the event endured can affect normal feeding and activity habits which can increase the risk of metabolic disease. Evaluation of pain mitigation is warranted to ideally improve animal well-being and productive life within the herd.

Inflammation is a necessary response to trauma or pathogen invasion, but it is not always positive for the host. Excessive inflammation can cause death or reduce productive life. Pain from inflammation contribute to productive losses from decreased feeding times to susceptibility to more diseases from abnormal behavior. Alleviation of inflammation and/or pain, allows

animals to resume normal activities. This inflammatory reduction can be accelerated through NSAIDS or steroid application. By modulating the immune functions of a postpartum dairy cow, it may be possible to prevent metabolic diseases from occurring, increase productive life, as well as milk production. Using dexamethasone to decrease the inflammatory response in dystocic dairy cows may allow the body to recover without having a heightened inflammatory state.

As animal well-being continues to be brought to the forefront of animal production, it is important the animal industry continues to push forward and ensure that the five freedoms associated with animal well-being are met.

1.7. Research Objectives

The research objective of this study was to evaluate the inflammatory response, production, and behavior after a difficult calving (dystocia) with the administration of the steroid, dexamethasone. We hypothesize that with administration of a steroid, inflammatory responses would be dampened, allowing quicker postpartum recovery and an increase in milk yield, components, and daily activity.

CHAPTER TWO: THE EFFECTS OF DEXAMETHASONE ADMINISTRATION ON PHYSIOLOGICAL PARAMETERS IN THE PERIPARTUM DAIRY COW

2. INTRODUCTION

A dairy cattle's productive life is centered around parturition. Not only does calving play a significant role in the calf's life, but it determines productivity for the dams' upcoming lactation (Meyer et al., 2001, Lombard et al., 2007). Determination of calving difficulty is done using calving ease scores (1-5) which are given at parturition (Dematawena and Berger, 1997). The lower scores define eutocia with the process from appearance of amniotic sac to calf expulsion lasting < 70 mins without calving assistance. Dystocia is defined by the higher scores, taking ≥70 mins for calf expulsion and possibly entailing assistance. This extended parturition is associated with increased inflammation which can cause a predisposition to metabolic diseases (Singh, 2010). Additionally, dystocia negatively affects milk yield, fat and protein yields, and increases veterinary costs and mortality of cows/calves (Dematawena and Berger, 1997). Hence treatment to reduce the inflammatory response, potentially bypassing disease incidence is an avenue that should be increasingly explored.

Inflammation is effective in ridding the host of foreign invaders, but can cause damage in excess (Barnes and Adcock, 2003). The acute phase response (APR) is triggered due to the inflammation and stimulates the secretion of positive acute phase proteins (APP) from hepatocytes in the liver (Bradford et al., 2015). Haptoglobin is an APP and a known inflammatory marker due to its rapid increase (up to 10-fold) in response to inflammation, thus becoming a viable diagnostic tool in veterinary work (Van Leeuwen and Van Rijswijk, 1994, Petersen et al., 2004, Chan et al., 2010, Gomez-Laguna et al., 2011). Haptoglobin concentration has been measured for correlation with certain diseases. Plasma haptoglobin was found to be

greater in d3-10 postpartum cows with one or more diseases or that died within the first month postpartum (Huzzey et al., 2011). This study also indicated that the prepartum haptoglobin concentration was not correlated to postpartum disease incidence (Huzzey et al., 2011). Although haptoglobin is used as an indicator of inflammation, it tends to have anti-inflammatory properties. Research has shown that haptoglobin is an inhibitor of COX and LOX enzymes, both involved in inflammatory processes (Saeed et al., 2007). These anti-inflammatory properties work to reinstate homeostasis and to minimize tissue damage. However, research has shown that cows that experience dystocia exhibit greater haptoglobin concentration (>100 μg/ml) one week after calving, decreased milk yields for up to two months postpartum, and decreased conception rates (Shin et al., 2018).

Reducing inflammation through NSAID or steroid treatments may be associated with pain mitigation. The anti-inflammatory properties of NSAIDs are attributed to inhibition of COX enzymes (Lees et al., 2004). A study looking at minimizing systemic inflammation treated postpartum dairy cows with a NSAID (meloxicam) for four days (d10-13) postpartum (Pascottini et al., 2020). The meloxicam group had lower beta-hydroxybutyrate (BHBA) serum concentrations in the four days following the treatments (d11-14) in comparison to the control group. This decrease was also seen in haptoglobin. The meloxicam group had a lower serum haptoglobin concentration for 3 consecutive days after treatment (d11-13) in comparison to the control group (Pascottini et al., 2020). This study could indicate that NSAID treatment promotes efficient energy metabolization and the dampening of the inflammatory response.

Glucocorticoids are a more potent anti-inflammatory agent than NSAIDs (Becker, 2013). This is attributed to NSAIDs primarily inhibiting COX enzymes, while glucocorticoids inhibit the synthesis and release of a multitude of pro-inflammatory mediators on the transcriptional

level (Becker, 2013). This glucocorticoid inhibition of inflammatory mediators includes COX-2, IL-6, and IL-8 amongst others (Sapolsky et al., 2000, Becker, 2013). Although glucocorticoids have a large number of glucocorticoid receptor sites throughout the body, its effect on the immune system is one of the most investigated areas. Glucocorticoids do play a role in increasing the expression of APP, although they primarily work synergistically with cytokines for APP expression (Baumann and Gauldie, 1994). However, exogenous glucocorticoids have been theorized to have more of a response on the production of APP than endogenous glucocorticoids (McGrotty et al., 2003). Using NSAIDs in postpartum dairy cows within the first 6 hours after parturition, Mainau et al. (2014) measured haptoglobin on four separate days during the first two weeks of lactation. No significant difference in haptoglobin concentration was observed due to treatment. However, haptoglobin concentrations were seen to be greater in postpartum primiparous cows than in multiparous cows (Mainau et al., 2014). Since there was no observable difference in haptoglobin using a lower anti-inflammatory agent like meloxicam, a more potent anti-inflammatory like dexamethasone could cause a significant difference in haptoglobin concentrations between treatment groups.

The objective of this study was to determine if a more potent anti-inflammatory agent dampens the inflammatory response for a quicker recovery through physiological indicators of inflammation (haptoglobin concentration), energy metabolism (BHBA), and body temperature. We hypothesize that the use of dexamethasone on postpartum dystocic animals will initially increase the haptoglobin response in comparison to the control group, but then decline while the control has a later heightened inflammatory response. We also hypothesize that the BHBA concentration will be lower in dexamethasone treated animals due to the higher availability of

glucose from the induced hyperglycemia. Lastly, body temperature is hypothesized to be lower in the dexamethasone treated animals attributable to the induced decreased inflammatory state.

2.1 MATERIALS AND METHODS

2.1.1 ANIMALS, HOUSING, AND MANAGEMENT

All experimental procedures were approved by the Virginia Tech Institutional Animal Care and Use Committee (#19-100). A total of 264 dairy cows at the VT Dairy Center (Blacksburg, VA) were evaluated between November 2019 and March 2021 for the study beginning 10 days prior to expected calving. Starting approximately 21 days prior to expected calving and lasting until 21 days following partition, dry cows and pregnant heifers were housed in a bedded pack that was aerated daily following standard herd management protocols. Group size varied, but space was maintained at 100 ft^2 per cow. After calving, cows were moved into a separate pen that was also a compost bedded pack. At approximately 21 DIM, lactating cows were moved to a free stall barn which was bedded with sand. Cows were fed a total mixed ration (TMR) daily at 0830 h. Cows were milked twice a day in a double 12 parallel at 0100 and 1300 h.

DETERMINATION OF DYSTOCIA

Close up dry cattle were monitored 24 hr/d using three video cameras (Axis P1353-E, Axis Communications AB, Lund, Sweden). The videos were saved in three-hour intervals on the computer connected through an Ethernet cord (Media Recorder, Noldus Information Technology Inc., Leesburg, VA, USA). Videos were examined for each calving to determine the occurrence of dystocia by examining the amount of time that elapsed between amniotic sac appearance at vulva and calf expulsion. Dystocia was defined as a difficult birth resulting in a prolonged calving (≥ 70 minutes after the amniotic sac appears) (Schuenemann et al., 2011, Funnell and

Hilton, 2016), assisted calving, and/or twin births. Calving assistance was performed only by farm staff who were blinded to the treatments. Throughout the study period a total of 264 dairy cows were monitored starting 10 days prior to expected calving. Of those, 146 cows experienced eutocic calving. Due to technological issues including camera/computer crashes and out of frame angles, 31 cows were unable to be included in the study. This resulted in a final sample size of 87 animals that experienced dystocia and were enrolled onto the study.

2.1.2 DESIGN AND TREATMENTS

Ten days prior to the calving date, animals began study evaluation and mammary secretion samples as well as coccygeal vein samples were aseptically collected. Within 12 hours of calving, dystocia was determined using the aforementioned definition. Dystocic cows were randomly assigned to one of two treatments using an excel random number generated sheet. The two treatments were: (1) dexamethasone administration within 12 hours after a dystocic calving **(DEX,** n = 41), and (2) saline administration within 12 hours after a dystocic calving **(CON**, n = 42). Dexamethasone dose was determined based on pre dry off body weight and equal saline volume was used for controls. Body weights were obtained from an automated scale (AfiWeigh, Afimilk LTD., Kibbutz Afikim, Israel) at dry off. These weights were then used to determine the approximate dexamethasone dosage (0.1mg/kg of BW, administered intramuscularly; Vet One, Sparhawk Laboratories, Lenexa, KS, USA). Administration was done in headlock restraint.

2.1.3 HEALTH AND INFLAMMATORY MARKERS

Body condition scores (BCS; Wildman et al. (1982)) ranging from (1-5) were recorded on the day of calving. Rectal temperatures were obtained for the first seven days after calving. This was done at 0800 h using an automated thermometer (Dual Scale Digital Thermometer, Vet One,

Meridian, ID, USA). A single blood sample was taken on d -10, 0, 1, 3, and 7, relative to calving through coccygeal venipuncture with a 10 ml tube coated with silicon (Monoject Blood Collection Tube, Covidien, Mansfield, MA, USA). This sample was used for both BHBA (d 0, 3, and 7) and haptoglobin (d 0, 1, 3, and 7) analyses. A BHBA meter (Precision Extra, Abbott Diabetes Care Inc., Alameda, CA, USA) was used with a test strip (Blood β-Ketone Test Strip, Abbott Diabetes Care Inc., Witney, Oxon, UK) for each blood sample. Results (mmol/L) were given a few seconds after the blood was put on the strip.

Serum was collected after centrifugation (2000 x rpm for 15 min at 4 ºC) and stored at -20 ºC. Haptoglobin was analyzed using an enzyme-linked immunoassay (ELISA) (Catalog Number: HAPT-11, Life Diagnostics, Inc, West Chester, PA, USA). Serum samples were thawed and diluted in series of 1:10, 1:100, 1:1000, 1:2000, and up to 1:8000 based on how high the concentration was in each sample. The ELISA kit came with a plate that was pre-coated with cow haptoglobin antibody. The diluted samples were pipetted into the wells and incubated in a shaker for 45 minutes (25ºC, 150 rpm). After that, the plate was washed five times with the wash buffer provided with the kit. The horseradish peroxidase (HRP) conjugate was then pipetted into the plate wells and incubated in the shaker for another 45 minutes. The plate was washed five times and then TMB was pipetted into the plate wells. After 20 minutes of shaker incubation with TMB, the reaction was halted using the stop solution. The resulting color reaction was read immediately at 450 nm absorbance using a plate reader (μQuant Universal Microplate Spectrophotometer, Bio-Tek Instruments, Inc, Winooski, Vermont, USA).

2.2 STATISTICAL ANALYSIS

All data were compiled in Excel (Microsoft Office Excel 2013 for Windows, Microsoft Corporation, Redmond, WA, USA) and imported into SAS (SAS 9.4, SAS Institute Inc., Cary,

NC, USA). Data were analyzed using mixed models (PROC GLIMMIX) with the fixed effects of treatment (DEX or CON), parity (primiparous or multiparous), and time (day relative to calving), as well as all biologically plausible two- and three-way interactions. Time was used as a repeated measure with the random effect of cow. The first-order autoregressive error structure was used unless sampling time points were uneven, in which case spatial power error structure was used instead. Data collected just prior to treatment application were tested as a covariate in their respective models. For haptoglobin and BHBA, BCS recorded on the day of calving was tested a covariate as well. Stepwise backward elimination, from highest to lowest significance, was used to remove nonsignificant terms from the model, however treatment and time were forced into the model. Residuals were assessed for normality and outliers (PROC UNIVARIATE). If an observation had a Studentized residual greater than the absolute value of 4, the data point was removed. Significance was declared at $P \leq 0.05$.

2.3 RESULTS

General descriptive statistics are shown in Table 2.1. For haptoglobin, there was a significant three-way interaction between treatment, time, and parity ($P = 0.02$). While there was no significant difference in haptoglobin concentration between treatment groups for primiparous cows on d 1 ($P = 0.40$), haptoglobin concentration was significantly greater in DEX treated primiparous cows on d 3 (DEX vs. CON: 710.2 ± 55.4 vs. 203 ± 51 μg/ml, $P < 0.0001$) and on d 7 (DEX vs. CON: 395.1 ± 55.4 vs. 214.9 ± 51 μg/ml, $P = 0.02$; Figure 2.1.) as compared to CON primiparous cows. No difference in haptoglobin concentration was seen on any of the days for multiparous cows (Figure 2.1.).

BHBA had to be transformed for normality using natural log. Although there was a significant treatment by time interaction for blood BHBA ($P = 0.03$), there was no difference

between treatment groups on either d 3 (DEX vs. CON: 0.61 ± 0.07 vs. 0.75 ± 0.07 ln(BHBA,mmol/L) +1; $P = 0.13$) or d 7 (DEX vs. CON: 0.78 ± 0.07 vs. 0.68 ± 0.07 ln(BHBA,mmol/L) +1; $P = 0.29$) as seen in (Figure 2.2). Concentrations of BHBA were affected by parity ($P = 0.0001$). Body temperature was not different between treatment groups (DEX vs CON: 38.5 ± 0.04 vs. 38.5 ± 0.04 ºC; $P = 0.64$). Body temperature was affected by parity (primiparous vs. multiparous: 38.6 ± 0.04 vs. 38.4 ± 0.04 ºC, $P = 0.05$).

2.4 DISCUSSION

This study was designed to evaluate the effect of dexamethasone, a potent glucocorticoid, on the inflammatory process following dystocia. The APR is activated after initiation of inflammation and is an important part of innate immunity. A key indicator of the APR is an increase in the APP which constitute a part of the innate immune response (Grönlund et al., 2005, Bradford et al., 2015). In bovine specifically, haptoglobin is one of the highly reactive APP that is activated during tissue injury, increasing as much as 10-fold in the first two days and reaching the highest concentration 3 days postpartum (Petersen et al., 2004, Chan et al., 2010). Haptoglobin concentration in healthy cattle is below 20 mg/L, but within days of infection can increase to greater than 2 g/L (Eckersall and Bell, 2010). Although haptoglobin is induced during cases of inflammation, it is an anti-inflammatory mediator secreted to return the body back to homeostasis (Wang et al., 2001).

The process of parturition is an inflammatory process with IL-6 and IL-1β driving it (Leimert et al., 2021). Previous research using NSAIDs to decrease inflammation during the peripartum period have seen increases in milk production (Swartz et al., 2018) and a decrease in haptoglobin (Pascottini et al., 2020) in eutocic cows. However, these same effects have not been shown for dystocia. We hypothesized that through administration of a more potent anti-inflammatory agent

like dexamethasone the inflammatory response would be reduced following dystocia, contributing to physiological, behavioral, and productional improvements.

Physiological aspects like BHBA and body temperature are commonly measured during the postpartum period to monitor onset of disease. Haptoglobin is emerging in veterinary diagnostics as a non-specific indicator of inflammation. Thus, the reduction in serum concentration may demonstrate the ability to curtail the uncontrolled inflammatory response.

In the current study, DEX significantly increased the serum haptoglobin concentration in primiparous cows on d 3 and d 7. Our study confirmed what was seen by Mainau et al. (2014) where primiparous animals had greater haptoglobin concentrations than multiparous cows. This is suggested to be attributed to more severe tissue damage occurring for these first time calvings (Humblet et al., 2006). Additionally, it may also be attributed to a heightened stress response from primiparous cows. However, our results for primiparous cows contradict the results of observations made by Pascottini et al. (2020), who found a decrease in haptoglobin due to NSAID treatment. This difference might be due in part to the difference in treatment, NSAIDS vs steroids. As steroids are a more potent anti-inflammatory agent, promotion of anti-inflammatory factors like haptoglobin could be heightened in comparison to NSAID treatment. Additionally, dystocic parturitions were used in the current study, but Pascottini et al. (2020) only used cows that experienced an unassisted parturition. Pascottini et al. (2020) also administered the NSAID treatment on d 10-13 postpartum which would not impact the immediate inflammatory response following parturition.

It has been noted that cytokine IL-6 is a leading inducer for hepatocyte APP production (Gauldie et al., 1992). Studies have shown that IL-6, IL-1, and glucocorticoids are needed for the greatest expression of haptoglobin in rats (Marinković and Baumann, 1990). Though, in humans

only IL-6 and glucocorticoids are needed for greatest haptoglobin expression (Castell et al., 1988, Marinković and Baumann, 1990). Dexamethasone has been seen to increase IL-6 cytokine effects which could explain the haptoglobin expression seen in our study (Baumann et al., 1989, Wang et al., 2001). In addition, increased haptoglobin expression may be attributed to the influx of endogenous glucocorticoid binding due to the heightened stress response from a dystocic parturition (Uchida et al., 1993).

Stressors activate the HPA axis, stimulating the release of cortisol. This is especially seen at parturition with cortisol almost tripling in concentration from the baseline concentration (Uchida et al., 1993). Synthetic exogenous glucocorticoids mirror the endogenous increase in cortisol. Although synthetic glucocorticoids are similar to cortisol, different potencies are involved (Dluhy et al., 1973). Dexamethasone is 30 times more potent than cortisol (Dluhy et al., 1973), although it is important to note that exogenous glucocorticoids affect endogenously produced glucocorticoids at time of injection. Using rats injected with dexamethasone, Geisterfer et al. (1993) reported endogenous glucocorticoid levels significantly decreased post injection, but recovered 12 hours post injection. In addition, mRNA for hepatic IL-6 receptors increased 5.7-fold within 12 hours after the dexamethasone injection (Geisterfer et al., 1993). While glucocorticoids are known for their anti-inflammatory effects, especially inhibiting pro-inflammatory cytokines, increasing receptor mRNA for a pro-inflammatory cytokines like IL-6 is puzzling. This contrasting mechanism exhibited by glucocorticoids has been suggested to quicken the inflammatory response in order to accelerate the onset and offset (Wiegers and Reul, 1998). This could be what is exhibited in this study where dexamethasone treated primiparous cows had a significantly increased concentration of haptoglobin on d 3 and d 7 after treatment.

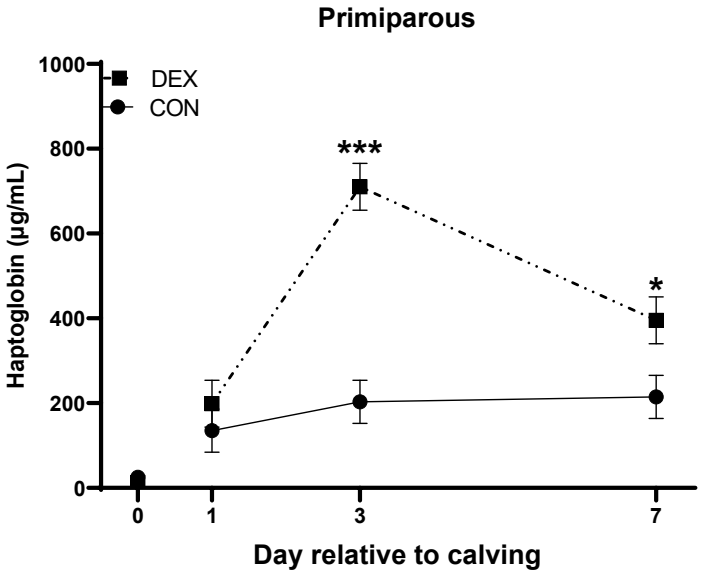
2. CONCLUSION

In conclusion, a single administration of dexamethasone to alleviate the inflammatory response in dystocic cows resulted in an increased haptoglobin concentration on d 3 and d 7 in primiparous cows only. There were no significant effects on BHBA or body temperature due to treatment effects. Further research is needed to look at other inflammatory mediators after dystocic or eutocic calvings to determine other factors involved. Once other inflammatory factors are determined, the results from this study can be used to expand more on why only primiparous cows—treated with a potent anti-inflammatory agent—had such a greater haptoglobin response in contrast to multiparous cows.

Table 2.1. Descriptive statistics with mean (± SD) shown. Treatments were dexamethasone (0.1mg/kg of BW intramuscularly) after calving (**DEX**) and a saline control (**CON**).

Treatment	Enrolled	Finished study	Parity	BCS at calving	Body weight (kgs)	Assistance given	Calving length (mins)
Dexamethasone	43	41	1.7 ± 1.2	3.4 ± 0.2	687 ± 110	30%	92 ± 46
Primiparous	23	22		3.3 ± 0.3	685 ± 110	34.8%	91 ± 48
Multiparous	20	19	1.8 ± 1.3	3.4 ± 0.2	687 ± 110	25%	85 ± 37
Saline	44	42	1.8 ± 1.3	3.4 ± 0.2	685 ± 109	45.4%	84 ± 41
Primiparous	28	28	---	3.4 ± 0.2	685 ± 109	46.4%	90 ± 48
Multiparous	16	14	1.8 ± 1.3	3.4 ± 0.2	694 ± 103	43.8%	83 ± 37

Figure 2.1. Least square means (±SE) of the treatment by parity by day interaction (multiparous; primiparous), Comparison of the d 1, 3, and 7 haptoglobin concentrations (µg/mL). Day 0 was day of calving and used as a covariate (prior to treatment application). Treatments were dexamethasone (0.1mg/kg of BW intramuscularly) after calving (**DEX**; primiparous, n = 19; multiparous, n = 23) and a saline control (**CON**; primiparous, n = 27, multiparous, n = 15). Differences between treatments: *$P \leqslant 0.05$, ***$P < 0.001$.



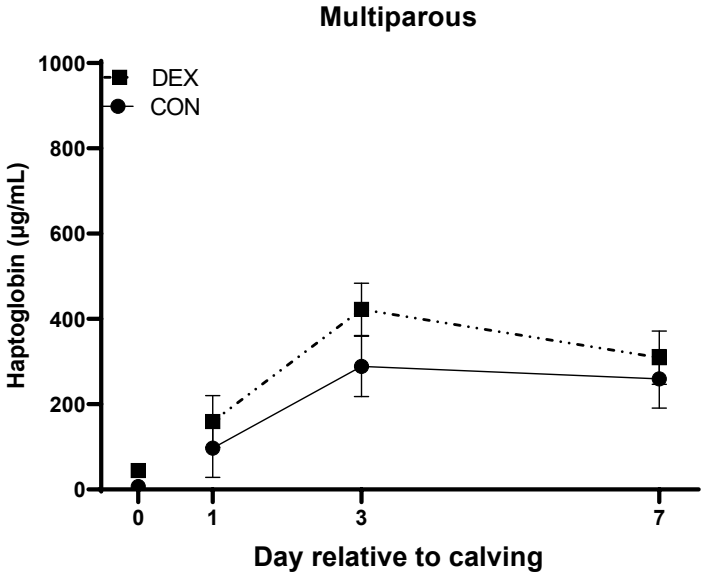
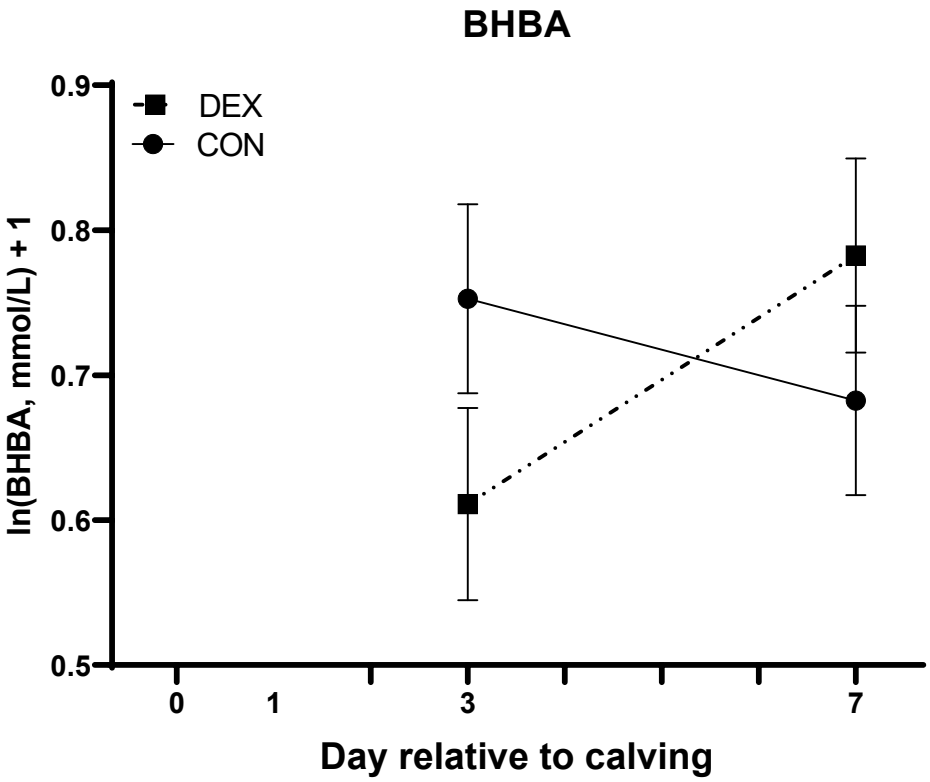


Figure 2.2. Least square means (±SE) of the treatment by day interaction for BHBA. Comparison of the d 3 and 7 haptoglobin concentrations (ln(BHBA,mmol/L)+1). Day 0 was day of calving and used as a covariate (prior to treatment application). Treatments were dexamethasone (0.1mg/kg of BW intramuscularly) after calving (**DEX**, n = 41) and a saline control (**CON**, n = 42).



CHAPTER 3: THE EFFECTS OF DEXAMETHASONE ADMINISTRATION ON BEHAVIORAL PARAMETERS AND PRODUCTION IN THE PERIPARTUM DAIRY COW

3. INTRODUCTION

Parturition begins the productive life of dairy cattle. However, deviations in the process can cause both parties involved, calf and dam, to incur potential lifelong issues. These deviations in the birth process can also be known as dystocia. Dystocia occurs when the time from the appearance of the amniotic sac to expulsion of the calf takes ≥ 70 minutes and can involve human intervention (Schuenemann et al., 2011, Funnell and Hilton, 2016). Eutocia defines the calving process taking <70 minutes without involving assistance. Dystocia negatively impacts milk yield, fat and protein yields, reproductive viability, and mortality of the calves and/or cows, while also increasing veterinary costs (Dematawena and Berger, 1997). Dystocia also predisposes cows to postpartum diseases such as retained placenta, metritis, and left displaced abomasum, and additionally is seen as a risk factor for ketosis (Correa et al., 1993, Duffield et al., 2009). These impacts from dystocia may be due in part to the increased inflammatory state, and thus potentially pain, that the cow is experiencing following parturition (Singh, 2010). Reduction of the amplified inflammatory response following a difficult birth could lead to improved animal well-being and production.

Previous studies have shown that anti-inflammatory drugs like NSAIDs can improve milk production in postpartum cows that experienced eutocia, but not dystocia (Newby et al., 2013, Swartz et al., 2018). Perhaps a more potent anti-inflammatory agent could enhance the effects seen in Swartz et al. (2018) and Newby et al. (2013), improving production in dystocic cows. Steroids are commonly used in the veterinary field due to their vigorous anti-inflammatory

action and can be used to treat a wide variety of ailments such as ketosis, udder edema, and calving induction (Black, 1974). Steroids are considered more potent than NSAIDs due to widespread inflammatory mediator inhibition (Becker, 2013). Dexamethasone is a synthetic glucocorticoid with one of the most potent anti-inflammatory properties, thirty times more potent than cortisol (Dluhy et al., 1973). Conversely, hyperglycemia has been seen due to glucocorticoid administration, as well as a short-term decrease in milk production (Van Der Kolk, 1990, Herdt and Emery, 1992). The hyperglycemia state glucocorticoids induce in the body is attributed to the increased muscle breakdown, reduced glucose and amino acid availability, and reduction of proteolysis (van der Drift et al., 2015). Treatment of glucocorticoids in ketotic cows was seen to decrease BHBA concentrations presumably due to increasing gluconeogenesis, allowing the body to use an energy source other than ketone bodies (van der Drift et al., 2015). Low glucose availability to the mammary gland may be to blame for the milk drop, but other postpartum mechanisms may also contribute.

Monitoring for signs of discomfort and changes in behavior is crucial since animals are unable to verbally express pain. In a 24 hr period, it is essential for cows to maintain certain behaviors. Due to the necessity of lying time to remove pressure from hooves/legs, increase blood flow through the udder, and improve rumination effectiveness, cows should be spending 12-14 hours lying down (Grant, 2007). Standing can be associated with animal discomfort, especially if any udder ailments are involved. However, not all cows exhibit the same behavioral patterns. As parity and stage of lactation increase, so does lying time (Chaplin and Munksgaard, 2001, Westin et al., 2016). An increase in lying time per session (lying bout) has been seen in healthy cows (Cattaneo et al., 2020). Additionally, behavior can also have effects on production. Bach et al. (2008) saw a positive correlation between lying time and milk production.

After calving, cows may exhibit discomfort through heightened activity and lying bouts, with a reduction in lying time (Jensen, 2012, Steensels et al., 2012). Recovery can be accelerated with anti-inflammatory agent administration. In response to either of the pre-or post-calving meloxicam treatment given to dystocic cows, Swartz et al. (2018) observed decreased locomotion in comparison to the dystocic control. Through inflammation reduction, pain may also be mitigated allowing cows to rest easier. Without anti-inflammatory administration, Steensels et al. (2012) had contrasting results with Swartz et al. (2018), as postpartum lying time was reduced and activity was increased the first couple days postpartum. These results from Steensels et al. (2012) could be indicative of the behavior exhibited when cows are in discomfort, without anti-inflammatory help.

The objective of this study is to determine if the potent anti-inflammatory agent dexamethasone dampens the heightened inflammatory response in dystocic cows for a swifter recovery measured through milk production and behavioral measures. We hypothesize that the use of dexamethasone on postpartum dystocic animals will overall increase the milk yield and decrease the postpartum restlessness behavior exhibited by dystocic cows.

3.1 MATERIALS AND METHODS

3.1.1 ANIMALS, HOUSING, AND MANAGEMENT

All experimental procedures were approved by the Virginia Tech Institutional Animal Care and Use Committee (#19-100). A total of 264 dairy cows at the VT Dairy Center (Blacksburg, VA) were evaluated between November 2019 and March 2021 for the study beginning 10 days prior to expected calving. Starting approximately 21 days prior to expected calving and lasting 21 following parturition, dry cows and pregnant heifers were housed in a bedded pack that was

aerated daily following standard herd management protocols. Group size varied, but space was maintained at 100 ft^2 per cow. After calving, cows were moved into a separate pen that was also a compost bedded pack. At approximately 21 DIM, lactating cows were moved to a free stall barn which was bedded with sand. Cows were fed a total mixed ration (TMR) daily at 0830 h. Cows were milked twice a day in a double 12 parallel at 0100 and 1300 h.

DETERMINATION OF DYSTOCIA

Close up dry cattle were monitored 24 hr/d using three video cameras (Axis P1353-E, Axis Communications AB, Lund, Sweden). The videos were saved in three-hour intervals on the computer connected through an Ethernet cord (Media Recorder, Noldus Information Technology Inc., Leesburg, VA, USA). Videos were examined for each calving to determine the occurrence of dystocia by examining the amount of time that elapsed between amniotic sac appearance at vulva and calf expulsion. Dystocia was defined as a difficult birth resulting in a prolonged calving (≥ 70 minutes after the amniotic sac appears) (Schuenemann et al., 2011, Funnell and Hilton, 2016), assisted calving, and/or twin births. Calving assistance was performed only by farm staff who were blinded to the treatments. Throughout the study period a total of 264 dairy cows were monitored starting 10 days prior to expected calving. Of those, 146 cows experienced eutocic calving. Due to technological issues including camera/computer crashes and out of frame angles, 31 cows were unable to be included in the study. This resulted in a final sample size of 87 animals that experienced dystocia and were enrolled onto the study.

3.1.2 DESIGN AND TREATMENTS

Ten days prior to the calving date, animals began study evaluation and mammary secretion samples as well as coccygeal vein samples were aseptically collected. Within 12 hours of

calving, dystocia was determined using the aforementioned definition. Dystocic cows were randomly assigned to one of two treatments using an excel random number generated sheet. The two treatments were as follows: (1) dexamethasone administration within 12 hours after a dystocic calving **(DEX,** n = 41**)**, and (2) saline administration within 12 hours after a dystocic calving **(CON**, n = 42**)**. Dexamethasone dose was determined based on pre dry off body weight and equal saline volume was used for controls. Body weights were obtained from an automated scale (AfiWeigh, Afimilk LTD., Kibbutz Afikim, Israel) at dry off. These weights were then used to determine the approximate dexamethasone dosage (0.1mg/kg of BW, administered intramuscularly; Vet One, Sparhawk Laboratories, Lenexa, KS, USA). Administration was done in headlock restraint.

3.1.3 BEHAVIOR

Body condition scores (BCS; Wildman et al. (1982)) ranging from 1-5 were recorded on the day of calving. When cows were brought to the close-up dry pen, farm staff fitted them with a leg accelerometer (AfiTag II, AfiMilk LTD., Kibbutz Afikim, Israel) allowing activity (average daily steps/h), lying time (min/d), and rests per bout (average number of minutes for duration of a lying bout) to be measured constantly. Restlessness ratio was calculated through these measurements and is a calculation by Afimilk that considers lying time, activity, and the number of lying bouts to determine restlessness of a cow. The researchers were not privy to the equation used by the farm management software to calculate restlessness ratio. These movements were transmitted to a computer (AfiAct II, Afimilk LTD., Kibbutz Afikim, Israel) which kept the records for each cow. Behavior measurements were recorded for the first 7 d (days 0-7) after parturition. Activity (steps/hr) was multiplied by 24 for steps/d.

3.1.4 SAMPLING

Milk samples were collected aseptically according to the sample collection guidelines set by NMC (NMC, 2004). Milk was collected on d -10, 1, 7, 14, 21, 35, 49, 63, 77, 91, 105, and 119, relative to calving in sterile 15 ml tubes (VWR® Centrifuge Tube, VWR, Radnor, PA, USA). Somatic cell count (SCC) was determined on each milk sample with a DeLaval cell counter (DeLaval cell counter, DCC; DeLaval, Tumba, Sweden). Somatic cell count was transformed into somatic cell score (SCS) using the formula from Ali and Shook (1980):

$$\mathbf{SCS = \log_2 (SCC/100{,}000) + 3}$$

Daily milk weights and components were obtained at each milking in the parlor starting 3 d after calving until 120 days postpartum. Milk weights were analyzed two ways. From d 3 until 30 days postpartum, daily milk weights were analyzed by day. Additionally, daily milk weights were averaged by month where month 1 was the average milk weight from d 3 through 30, month 2 averaged d 31 through 60, month 3 averaged d 61 through 90, and month 4 averaged d 91 through 120. Components were analyzed at each milking and averaged for daily percentage. Energy corrected milk (ECM) was calculated according to formula described by (Orth, 1992). The formula used to calculate ECM was:

$$\mathbf{ECM = (0.327 \times milk\ kg) + (12.95 \times fat\ kg) + (7.65 \times protein\ kg)}$$

3.2 STATISTICAL ANALYSIS

All data were compiled in Excel (Microsoft Office Excel 2013 for Windows, Microsoft Corporation, Redmond, WA, USA) and imported into SAS (SAS 9.4, SAS Institute Inc., Cary, NC, USA). Data were analyzed using mixed models (PROC GLIMMIX) with the fixed effects of treatment (DEX or CON), parity (primiparous or multiparous), and time (days relative to calving or month), as well as all two- and three-way interactions. Time was used as a repeated measure

with the random effect of cow. The first-order autoregressive error structure was used with the Kenward-Roger degrees of freedom approximation. For milk yield, milk component yields, and ECM, covariates included BCS at calving, genetic traits (PTAM, PTAF, and PTAP in their respective models), and the season (winter, spring, summer, fall) in which the cow calved. Stepwise backward elimination, from highest to lowest, was used to remove nonsignificant terms from the model, however, treatment and time were forced into the model. Residuals were assessed for normality and outliers (PROC UNIVARIATE). Rest per bout was not normally distributed and consequently a logarithmic transformation was used. If an observation had a Studentized residual greater than the absolute value of 4, the data point was removed. Significance was declared at $P \leq 0.05$.

3.3 RESULTS

3.3.1 BEHAVIORAL RESULTS

General descriptive statistics are shown in Table 3.1. The main effects and interactions for all behavioral data (activity, lying time, rest per bout, and restlessness ratio) are provided in Table 3.2. Restlessness ratio and rest per bout were transformed using natural log for normal distribution.

Cows treated with DEX were less active (DEX vs. CON: 3,847 ± 146 vs. 4,315 ± 145 steps/d; $P = 0.02$) than the CON cows in the week following parturition (Figure 3.1).

Lying times were greater in DEX cows (DEX vs. CON: 591 ± 15 vs. 524 ± 15 min/d; $P < 0.01$) than in CON cows. For lying time, the three-way interaction of treatment, time, and parity was significant ($P = 0.05$). Primiparous cows treated with DEX had a greater lying time than primiparous CON on d 1 (DEX vs. CON: 698 ± 33 vs. 477 ± 29 min/d; $P < 0.0001$) and d 2

(DEX vs. CON: 658 ± 33 vs. 428 ± 28 min/d; $P < 0.0001$), and no significant difference was found on d 3 (DEX vs. CON: 520 ± 32 vs. 450 ± 27 min/d; $P = 0.10$). Multiparous cows that received DEX spent more time lying than multiparous CON on d 2 (DEX vs. CON: 740 ± 35 vs. 573 ± 41 min/d; $P < 0.01$).

There was a significant three-way interaction seen in rest per bout between treatment, time, and parity ($P = 0.02$). Primiparous DEX cows had reduced rest per bout on d 0 (DEX vs. CON: 3.2 ± 0.10 vs. 3.5 ± 0.10 ln(rest per bout); $P < 0.01$), d 2 (DEX vs. CON: 3.7 ± 0.10 vs. 3.9 ± 0.10 ln(rest per bout); $P = 0.02$), and d 3 (DEX vs. CON: 3.6 ± 0.10 vs. 3.9 ± 0.10 ln(rest per bout); $P < 0.01$) in comparison to primiparous CON cows. However, multiparous DEX cows had increased rest per bout on d 0 (DEX vs. CON: 3.4 ± 0.10 vs. 3.0 ± 0.10 ln(rest time/bout); $P < 0.01$) in comparison to multiparous CON cows.

For restlessness ratio, the three-way interaction between parity, treatment, and time was significant ($P = 0.03$). In primiparous cows, the restlessness ratio was seen to be greater in CON cows on d 1 (DEX vs. CON: 1.0 ± 0.10 vs. 1.5 ± 0.10 ln(restlessness ratio); $P < 0.001$), d 2 (DEX vs. CON: 1.0 ± 0.10 vs. 1.4 ± 0.10 ln(restlessness ratio); $P < 0.001$), and no significant difference was found on d 3 (DEX vs. CON: 1.2 ± 0.10 vs. 1.4 ± 0.10 ln(restlessness ratio); $P = 0.10$) than in DEX cows. In multiparous cows, the restlessness ratio was greater in CON cows on d 0 (DEX vs. CON: 1.1 ± 0.10 vs. 1.6 ± 0.20 ln(restlessness ratio); $P = 0.02$) and d 2 (DEX vs. CON: 0.6 ± 0.1 vs. 1.0 ± 0.10 ln(restlessness ratio); $P = 0.03$) than in DEX cows.

3.3.2 MILK PRODUCTION AND COMPONENTS

The main effects and interactions for all milk data (milk yield for first 30 DIM, monthly milk, monthly fat, monthly protein, ECM, and SCS) are provided in Table 3.3. For milk yield

averaged by month, treatment had a significant interaction with time ($P = 0.05$; Figure 3.5.). DEX cows produced 2.7 kg/d less milk than CON cows in the first month after parturition ($P = 0.05$); however, no difference was found in months 2,3, or 4.

Because of this, daily milk yield was then examined for the first 30 days in milk. A significant three-way interaction between treatment, parity, and time ($P < 0.01$; Table 3.3.) was observed. For days 4 through 20 and days 22 and 23, multiparous DEX cows produced approximately 7.3 kg/d less milk than CON cows (Figure 3.6.). There were no significant effects seen for primiparous cows for the first month of milk production.

No treatment effects were found for either protein yield ($P = 0.34$), fat yield ($P = 0.21$), ECM ($P = 0.25$) or SCS ($P = 0.40$).

3.4 DISCUSSION

Previous research has investigated the effects of NSAIDs on postpartum behavior and milk production. Swartz et al. (2018) observed an increase in milk yield and components with meloxicam treatment in cows that experienced eutocia. However, administration of meloxicam after a difficult parturition yielded no difference in milk production (Newby et al., 2013, Swartz et al., 2018). Due to multiple studies exhibiting similar results for NSAIDs, it was hypothesized that a stronger anti-inflammatory agent could improve the milk yields in dystocic cows by promoting faster recovery. In the current study, a reduction in milk yield for nearly the first month was found only in the multiparous dexamethasone treated cows. It has been observed in several studies that dexamethasone treatment induces a reduction in milk yield (Shamay et al., 2000, Ponchon et al., 2017). Yet, the slump in production took only five days to regain previous values in the study by Shamay et al. (2000) and two days for Ponchon et al. (2017)—although it

must be taken into consideration that both these studies used animals in mid-lactation. The stage of lactation and parity of the cow must play a role in the effect of glucocorticoids from binding in the mammary gland.

Receptors are needed wherever glucocorticoids act. However, not all receptor sites are equal. Bovine mammary epithelial cells have a plethora of intracellular glucocorticoid binding sites that are divided into three different receptor types—two types with an elevated affinity for dexamethasone over cortisol (Van Der Kolk, 1990). In addition, the lactation period may increase the affinity for glucocorticoid binding, therefore increasing effects according to research on glucocorticoid accumulation in mammary tissue (Gorewit and Tucker, 1976). Gorewit and Tucker (1976) examined the cortisol binding capacities of each mammary cell and found that lactating cows are able to bind 1,300 molecules, virgin heifers ~400 molecules, and dry cows ~300 molecules. This binding capacity may help explain why multiparous cows in the current study had a significant reduction in milk in comparison to control cows, while primiparous cows did not see any significant change in milk yield. However, there could be other contributing factors to the reduction in milk yield. Questioning if glucocorticoids play a role in prolactin inhibition, two experiments using dexamethasone observed lower prolactin concentrations and lower production in the treated cows (Ponchon et al., 2017). Subsequently Ponchon et al. (2017) deduced that prolactin inhibition may be a mechanism in which glucocorticoids affect milk yield. Whereas another glucocorticoid mechanism potentially detrimental to production may be the effect of induced hyperglycemia potentially rerouting glucose from the mammary gland (Herdt and Emery, 1992). However, Hartmann and Kronfeld (1973) examined mammary flow with uptake of glucose after dexamethasone treatment and noted a reduction of uptake in utilization, not glucose availability.

Inflammation from parturition activates the acute phase response (APR) (Mainau et al., 2014). The APR is associated with an influx of positive acute phase proteins (+APP) which work as part of the innate immune system to reinstate homeostasis (Grönlund et al., 2005, Bradford et al., 2015). In bovine specifically, haptoglobin is the prominent +APP that is becoming increasingly used for diagnostic veterinary work due to its rapid increase following injury or infection (Grönlund et al., 2005). Endogenous and exogenous glucocorticoids can increase expression of haptoglobin, although since dexamethasone is more potent than cortisol, haptoglobin expression may be increasingly heightened due to treatment. Shin et al. (2018) has shown that cows exhibiting greater haptoglobin serum concentration postpartum are associated with a significant reduction in milk for the first two months in comparison to cows with low haptoglobin serum concentration. Haptoglobin concentration could play a part in the milk reduction seen in the current study. Many other mechanisms could be potentially involved with this extended reduction in milk from glucocorticoid treatment. However, this is the first study to the author's knowledge that has seen a reduction in milk production for nearly a month after a single dose of dexamethasone.

Reducing inflammation can influence postpartum cow behavior, as seen in several studies (Newby et al., 2013, Mainau et al., 2014, Swartz et al., 2018). With no treatment after unassisted parturitions, lying time was reduced and activity was heightened in the first day postpartum (Jensen, 2012). Subsequently after assisted parturitions, lying time was seen to be greater than in non-assisted parturitions—at least in the first 48 hours postpartum (Gladden et al., 2021). Following meloxicam treatment, Newby et al. (2013) reported more time spent at the feed manger in comparison to the control cows. Treatment influencing pain reduction could be to explain. In the current study behavior was altered between treatments. Dexamethasone treated

cows had reduced activity (total 7 d postpartum period) and greater lying time (d 1, 2, and 3) in comparison to the control cows. This is similar to the results seen in Swartz et al. (2018) where meloxicam treated cows saw a reduction in activity. However, the greater lying time seen in the current study potentially contrasts with the reduced lying time (lateral recumbency) seen in Gladden et al. (2021) which administered ketoprofen 3 hours after parturition. In the current study, distinctions between lying positions were not made like in Gladden et al. (2021), thus the contrast may not necessarily be accurate.

In the current study, control cows had a greater rest per bout and restlessness ratio in the first days following calving. Although the increased rest per bout seen in the control cows may seem contradictory when dexamethasone cows had greater lying time, rest per bout is looking at the average amount of time per each lying period. The dexamethasone treated cows are lying down more, but not for long periods. This potentially contrasts with Cattaneo et al. (2020) which saw healthy cows spending longer time lying per session. However, restlessness, alternation between lying and standing, is used in classifying cow discomfort or pain (Matamala et al., 2021). As seen in the current study, the restlessness ratio for the control cows was increased in the first 2 days postpartum. Per Matamala et al. (2021) definition of restlessness, this could be indicative of the control cows experiencing some discomfort or pain following dystocia.

3.5 CONCLUSION

This study demonstrated that the administration of a potent steroid, dexamethasone, after a dystocic birth has various effects on behavioral traits and milk production. Milk production in multiparous cows was seen to be reduced for almost the entirety of the month following parturition. There were no treatment effects seen on components, ECM, or SCS. Activity in the first 7 days after parturition was reduced in cows treated with dexamethasone. The inverse effect

was seen with lying behavior, where dexamethasone treated cows spent a greater amount of time lying down in the first days following parturition. These relationships are exhibited through the restlessness ratio which was seen to be greater in the control cows in the first two days. Dexamethasone may decrease the discomfort seen in postpartum dairy cows, but due to the negative effects seen in milk yield from multiparous cows experiencing dystocia, may not be advised for widespread farm application. Additional studies should examine more in depth on why dexamethasone affects multiparous mammary gland milk production for such a prolonged period.

Table 3.1. Descriptive statistics with mean (± SD) shown. Treatments were dexamethasone (0.1mg/kg of BW intramuscularly) after calving (**DEX**) and a saline control (**CON**).

Treatment	Enrolled	Finished study	Parity	BCS at calving	Body weight (kgs)	Assistance given	Calving length (mins)
Dexamethasone	43	41	1.7 ± 1.2	3.4 ± 0.2	687 ± 110	30%	92 ± 46
Primiparous	23	22		3.3 ± 0.3	685 ± 110	34.8%	91 ± 48
Multiparous	20	19	1.8 ± 1.3	3.4 ± 0.2	687 ± 110	25%	85 ± 37
Saline	44	42	1.8 ± 1.3	3.4 ± 0.2	685 ± 109	45.4%	84 ± 41
Primiparous	28	28	---	3.4 ± 0.2	685 ± 109	46.4%	90 ± 48
Multiparous	16	14	1.8 ± 1.3	3.4 ± 0.2	694 ± 103	43.8%	83 ± 37

Table 3.2. Main effects and interaction terms for activity (steps/d), lying time (min/d), rest per bout (min/bout), and restlessness ratio measured from d 0 after calving until d 7 after calving with cows receiving dexamethasone after dystocia (**DEX**, n = 41) or a saline control after dystocia (**CON**, n = 42).

Effect	Activity	Lying time	Rest per bout	Restlessness ratio
Treatment	0.022	0.0018	0.4068	0.0008
Parity	<0.0001	<0.0001	<0.0001	<0.0001
Time	<0.0001	0.0039	<0.0001	<0.0001
Treatment x Parity	---	0.7807	0.0463	0.877
Treatment x Time	---	0.0001	0.1925	0.0728
Parity x Time	---	0.0025	0.0014	0.1477
Treatment x Parity x Time	---	0.0468	0.0208	0.0337

Table 3.3. Main effects and interaction terms for milk yield in first 30 DIM, milk yield by month, monthly protein, monthly fat, ECM, and SCS measured from 3d after calving until 120d after calving with cows receiving dexamethasone after dystocia (**DEX**, n=41) or a saline control after dystocia (**CON**, n=42).

Effect	Milk yield 30 DIM	Monthly Milk	Monthly Protein	Monthly Fat	ECM	SCS
Treatment	<0.0001	0.4465	0.339	0.2094	0.245	0.4025
Parity	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	---
Time	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Treatment x Parity	0.0107	---	---	---	---	---
Treatment x Time	0.0036	0.0473	---	---	---	---
Parity x Time	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	---
Treatment x Parity x Time	0.0077	---	---	---	---	---

Figure 3.1. Least square means (±SE) of the treatment interaction for activity (steps/d) for the first 7 days after calving. Day 0 was day of calving. Treatments were dexamethasone (0.1mg/kg of BW intramuscularly) after calving (**DEX**, n = 41) and a saline control (**CON**, n = 42). Differences between treatments: *P ≤ 0.05.

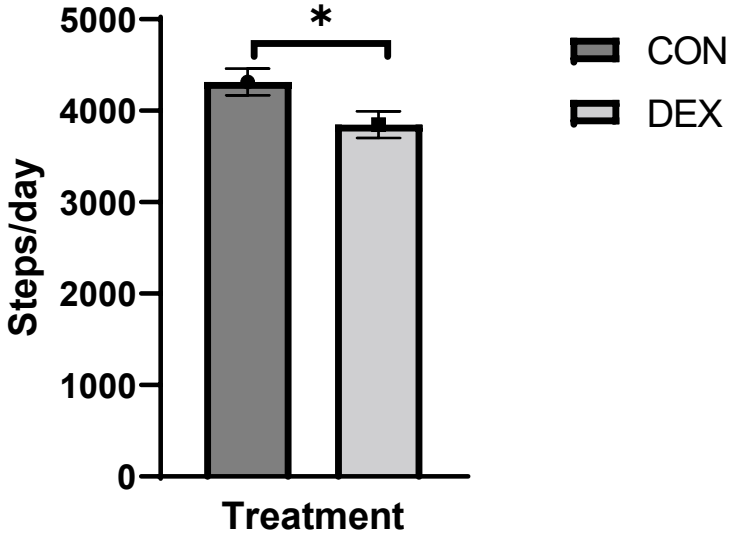


Figure 3.2. Least square means (±SE) of the treatment by parity by day interaction (multiparous; primiparous), Comparison of the first 7 days lying time (mins/day). Day 0 was day of calving. Treatments were dexamethasone (0.1mg/kg of BW intramuscularly) after calving (**DEX**; primiparous, n=22; multiparous, n=19) and a saline control (**CON**; primiparous, n = 28, multiparous, n = 14). Differences between treatments: *$P \leq 0.05$, **$P \leq 0.01$, ***$P < 0.001$, #$P < 0.10$.

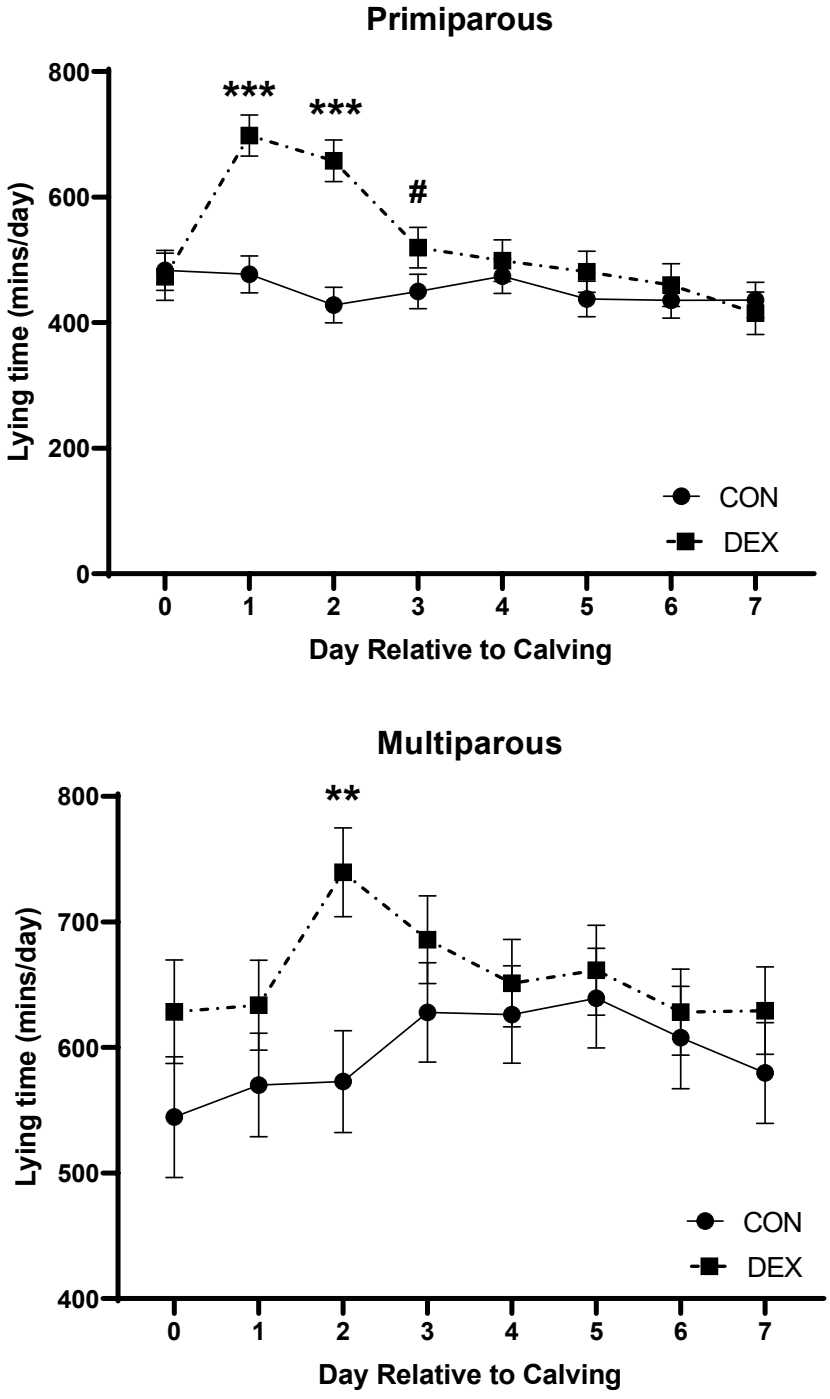


Figure 3.3. Least square means (±SE) of the treatment by parity by day interaction (multiparous; primiparous), Comparison of the first 7 days ln(rest per bout). Day 0 was day of calving. Treatments were dexamethasone (0.1mg/kg of BW intramuscularly) after calving (**DEX**; primiparous, n = 22; multiparous, n = 19) and a saline control (**CON**; primiparous, n = 28, multiparous, n = 14). Differences between treatments: *$P \leq 0.05$, **$P \leq 0.01$.

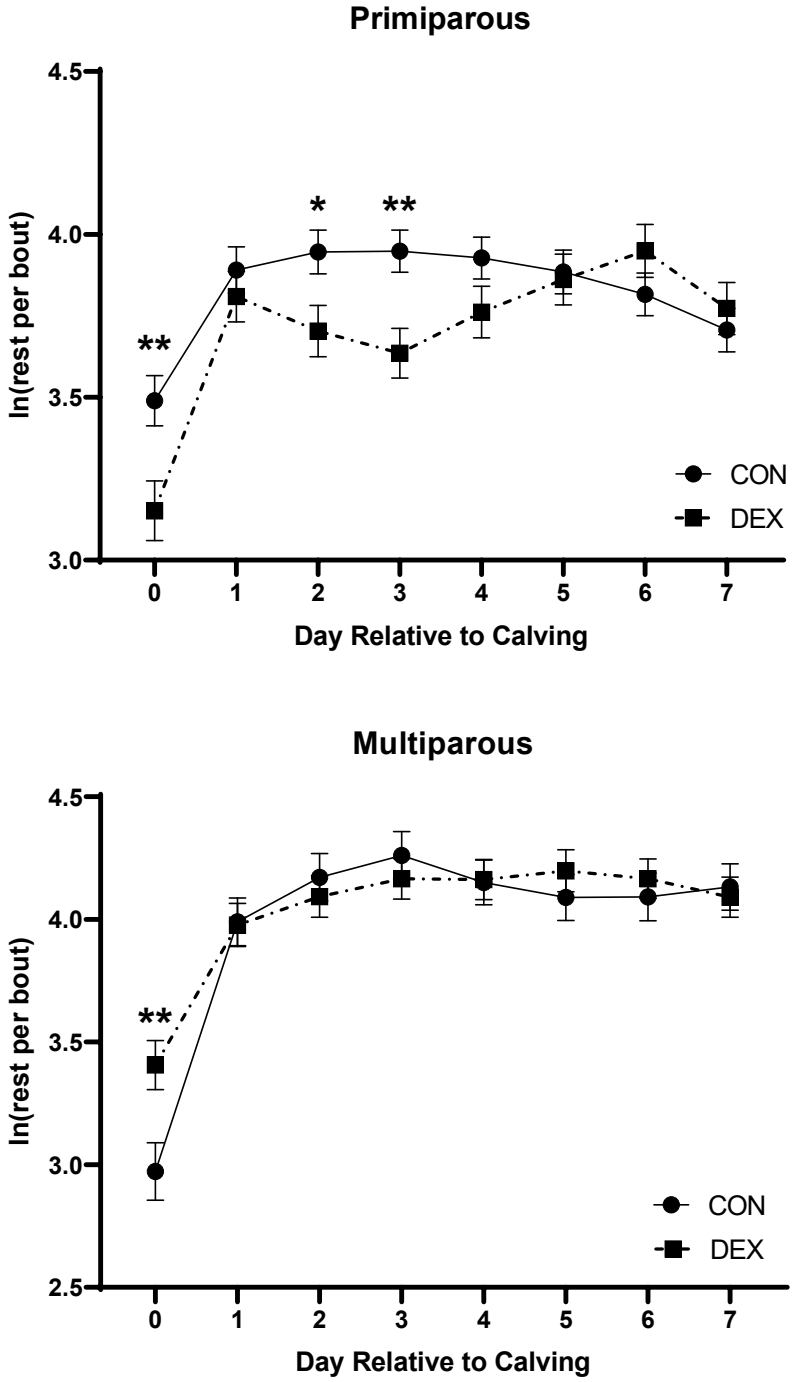


Figure 3.4. Least square means (±SE) of the treatment by parity by day interaction (multiparous; primiparous), Comparison of the first 7 days ln(restlessness ratio). Day 0 was day of calving. Treatments were dexamethasone (0.1mg/kg of BW intramuscularly) after calving (**DEX**; primiparous, n=22; multiparous, n=19) and a saline control (**CON**; primiparous, n=28, multiparous, n=14). Differences between treatments: *$P \leq 0.05$, **$P \leq 0.01$, ***$P < 0.0001$.

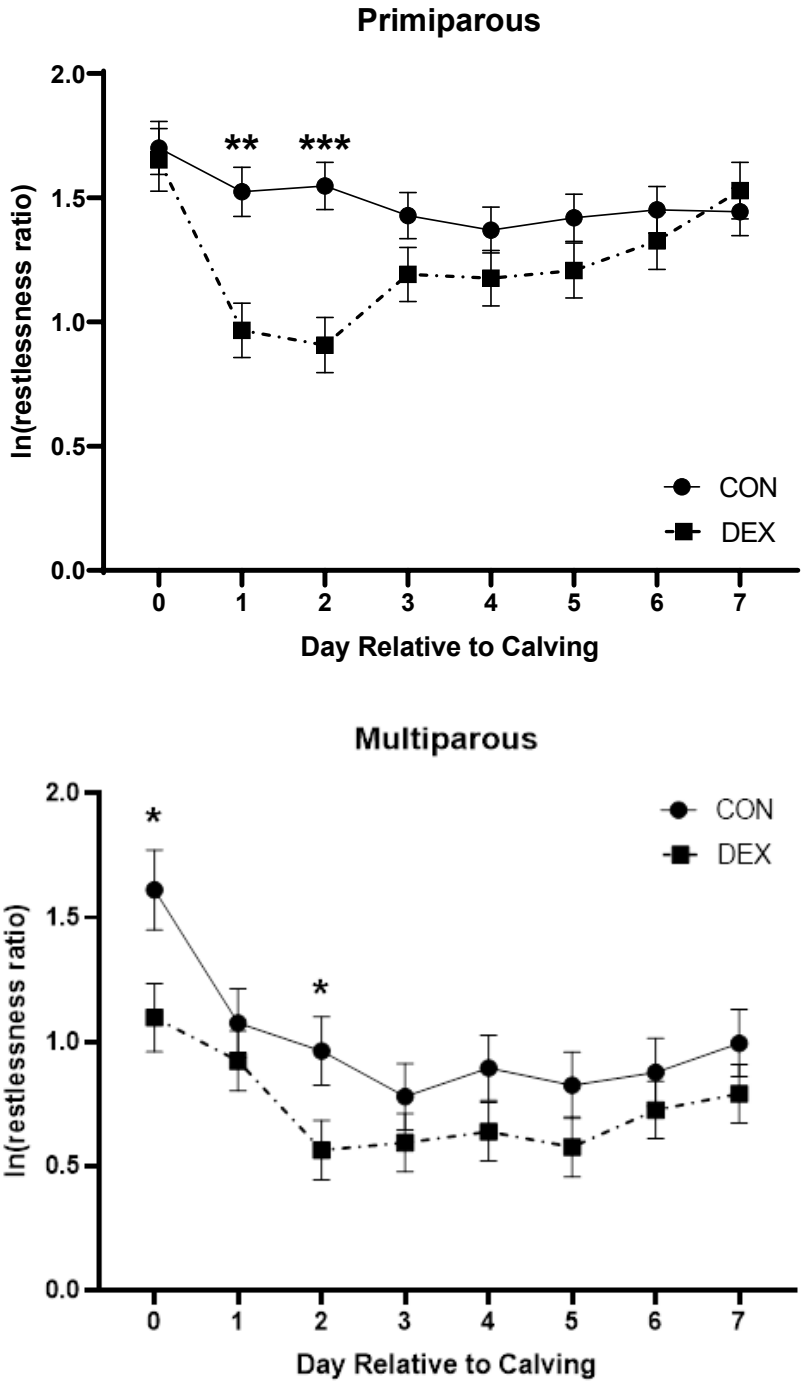


Figure 3.5. Least square means (±SE) of the treatment by month interaction. Comparison of the monthly milk yield (kg/day). Month 1 was month of calving. Treatments were dexamethasone (0.1mg/kg of BW intramuscularly) after calving (**DEX**, n = 41) and a saline control (**CON**, n = 42). Differences between treatments: *$P \leq 0.05$.

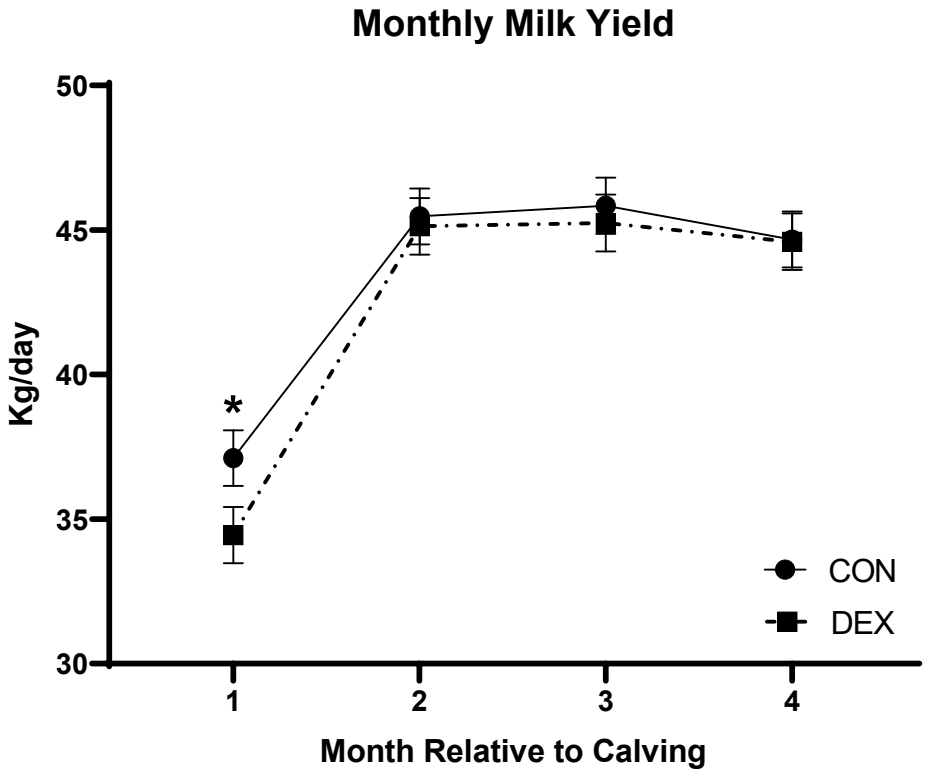
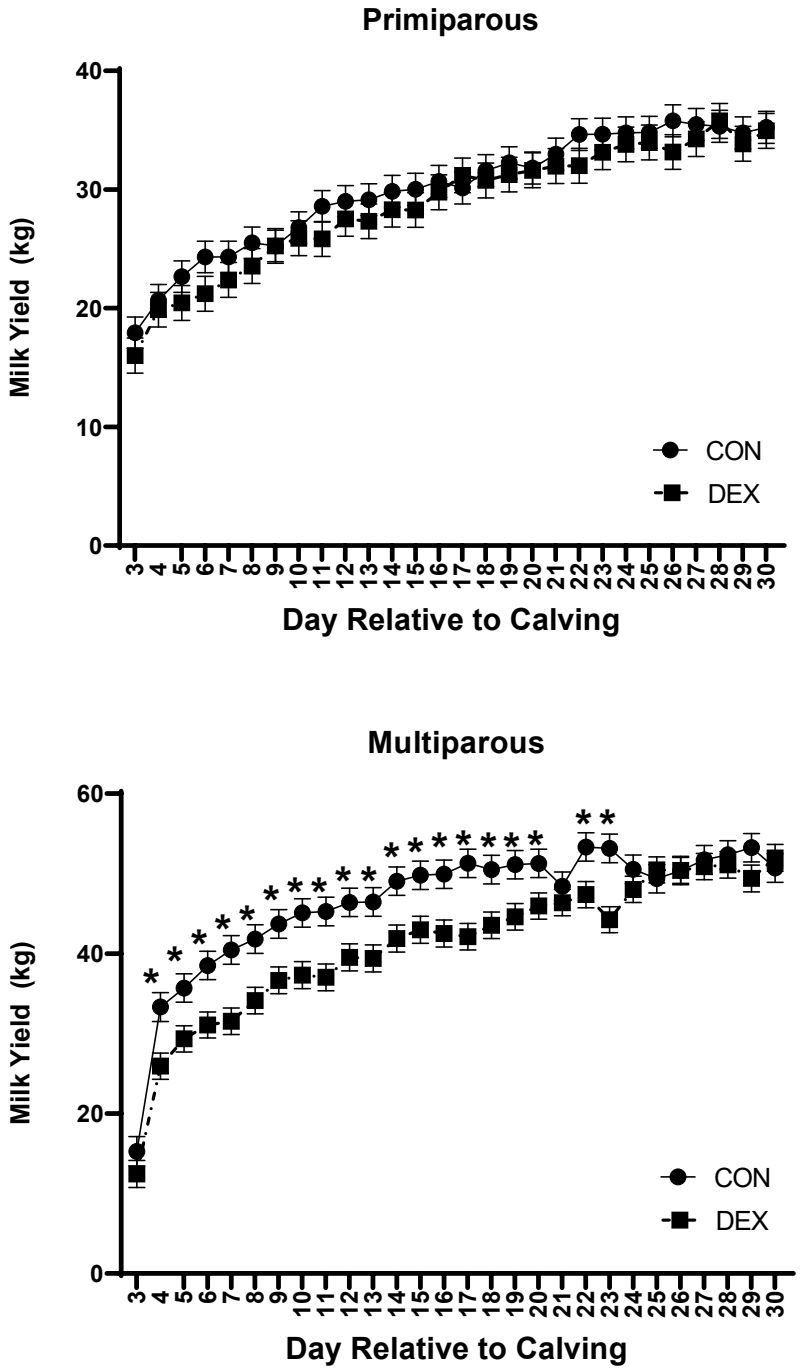


Figure 3.6. Least square means (±SE) of the treatment by parity by day interaction (multiparous; primiparous), Comparison of the first month milk yield (kg/day). Day 0 was day of calving. Treatments were dexamethasone (0.1mg/kg of BW intramuscularly) after calving (**DEX**; primiparous, n = 22; multiparous, n = 19) and a saline control (**CON**; primiparous, n = 28, multiparous, n = 14). Differences between treatments: *$P \leq 0.05$.



CHAPTER 4: GENERAL SUMMARY AND CONCLUSION

4.1 CONCLUSIONS

This study explored the effects of a potent steroid, dexamethasone, on the dystocic recovering dairy cow through various measured physiological, behavioral, and production parameters. With a one-time dose of dexamethasone within 12 hours after dystocia, the inflammatory marker measured, haptoglobin, was seen to be greater on d 3 and d 7 in DEX primiparous cows. Glucocorticoids have been shown to work in tandem with the inflammatory cytokine IL-6 to enhance the production of haptoglobin in rats and humans (Castell et al., 1988, Marinković and Baumann, 1990). Additionally, mRNA for hepatic IL-6 receptors increases with dexamethasone treatment (Geisterfer et al., 1993). Glucocorticoids are thought to quicken the inflammatory response through increasing the mRNA for pro-inflammatory receptors. This could be what is seen in the current study as the primiparous DEX cows exhibited heightened haptoglobin concentration on d 3 and d 7 in comparison to the CON cows. However, the question raised could be why only primiparous animals? This may be answered through the increased inflammatory response attributed to calving for the first time. Inflammatory mediators like cytokines may be greater in primiparous cows from the increased tissue damage (Humblet et al., 2006). In addition, not only from the tissue damage, but the stress response from a difficult calving may also contribute to an increased haptoglobin concentration in these DEX primiparous cows. Other physiological parameters measured like BHBA and body temperature however were not affected by treatment.

Treatment did influence the behavioral parameters measured. DEX cows had reduced locomotion during the 7 d postpartum period measured. Contributing to that, DEX cows had greater lying times on d 1, 2, and 3 in primiparous cows and on d 2 for multiparous cows.

Interestingly, DEX cows had reduced rest per bout on d 0, 2, and 3 in primiparous cows and a greater rest per bout only d 0 in multiparous cows. This is the average amount of time spent lying down per bout period which seems to contradict with the greater lying times DEX cows had. However, this could indicate that DEX cows were getting up to get food more often. This is indicated through the restlessness ratio calculated showing CON cows had greater values on d 1 and 2 in primiparous cows and d 0 and 2 in multiparous cows. Restlessness ratio is calculated considering lying time, activity, and the number of lying bouts. From this, CON cows are more restless than DEX on those days stated previously which could indicate pain preventing cow comfort.

Lastly, the first month of milk production was seen to be negatively affected by the DEX treatment, with a reduction of 2.7 kg/d in those cows. Dissecting this first month further into parities, it was seen that this difference was due to the multiparous DEX cows. For d 4 through 20 and d 22 and 23, multiparous DEX cows produced an average of 7.3 kg/d less milk than CON cows. It is unclear why there was no treatment effect seen on d 21. There were no treatment effects on primiparous cow milk production in the first month.

Although previous research has shown that heightened haptoglobin concentration contributes to a decreased milk yield, the current study showed primiparous DEX cows have greater haptoglobin concentrations, but multiparous DEX cows showed the milk reduction (Shin et al., 2018). This could be due to a variety of reasons. Even though primiparous DEX cows had a treatment effect for haptoglobin concentrations, multiparous DEX cows still saw a rise in haptoglobin concentrations above the normal undetectable amounts (Shin et al., 2018). Furthermore, a potential higher dose of dexamethasone given since multiparous cows typically

weigh more could play a factor, in addition to an increasingly developed mammary gland with more glucocorticoid receptor locations.

Although definition of dystocia may vary from farm to farm, it obviously has a profound impact on both the offspring and dam. By finding a treatment to reduce the immediate inflammation seen in the dams, and potentially the calves, production and other contributing factors may be improved. This is a necessary avenue to investigate since the dystocia occurrences vary so widely on farms, countries, and worldwide.

4.2 PROJECT LIMITATIONS

The current study may have some limitations. The pro-inflammatory cytokine TNFα was originally going to be measured in the serum samples in addition to haptoglobin, but due to the ELISA protocols being only for live cell culture samples, we were unable to do so without compromising the results. We wanted to ensure that our results were from a verified serum TNFα ELISA kit. Additionally, we did not use the calving ease scores on the animals we classified as dystocia. Separating dystocic cows in our study by calving scores they received, there could have been potentially more specific treatment effects for certain scores only.

There was an error with collecting the handwritten milk weights that were discarded for the first two days after calving. This was not realized until the study had completed. There might have been a potential first day(s) interaction, but with the error it is unknown.

4.3 FUTURE RESEARCH

This study has highlighted future research that should be done to improve animal well-being and production aspects in the postpartum dairy cow. Since the current study saw a large reduction in milk yield for almost the entirety of the first month, more research could be done in

vitro and/or in vivo on steroid effects on milk production in the mammary gland and other biological effects. Additionally, similar studies could be conducted using different steroids since they each have different properties. Dexamethasone could be too potent as a glucocorticoid, so potentially using a glucocorticoid with a lower potency the milk reduction seen in this study could be lessened, while still dampening the inflammatory response. In addition, since Swartz et al. (2018) observed milk yield and component improvement in eutocic cows with one dose of meloxicam, potentially giving two doses of meloxicam could show these same improvements for the dystocic cow. Also, timing of treatments could play a role in the results seen in this study as there was not a designated time per day that dexamethasone was administered. Naturally, endogenous glucocorticoids are secreted in the morning where they reach highest concentrations (Gross et al., 2015). By splitting cows up by when the glucocorticoid was administered, could the endogenous glucocorticoid secreted in the morning play an extra role in the results seen?

Shin et al. (2018) showed that a high haptoglobin concentration impacts reproductive performance through decreased conception rates. By thoroughly examining reproductive performance records after the dexamethasone treatment, a decline in reproductive performance may be seen in the primiparous cows or we could see potentially see different results. In addition to reproductive parameters, further immunological assays should be run to determine what is driving haptoglobin secretion. As mentioned previously, measurements of IL-6, since they are easily detected after trauma or infection, could show a difference between the different cytokine concentrations and APP concentration (Gauldie et al., 1992). Gauldie et al. (1992) saw a reduction of IL-6 and APP 24-36 hours after the traumatic event that initiated the uptick. In the current study the haptoglobin concentration was greater in DEX cows on d 3 and d 7 after dystocia which contrasts that statement by Gauldie et al. (1992). By measuring other

inflammatory mediators, we might be able to determine more about the inflammatory response in addition to glucocorticoid action.

For the behavioral effects seen, since CON cows had greater rest per bout on d 0, 2, and 3, measurement of feed intake may help paint the picture better. This is suggested due to DEX cows lying down for less time per lying period during those days which could imply they could be getting up to eat more. By measuring feed intake or time spent by the bunk, we might be able to gauge cows that are feeling less pain and are comfortable enough to eat more. This indicator could exhibit cows moving out of negative energy balance sooner, especially if it was DEX cows. This study has highlighted many new avenues that could be studied in tandem to the current study or as novel studies.

4.4 IMPLICATIONS

Due to the results found in this study, farmers may be more wary in treating multiparous cows with dexamethasone in the postpartum period due to the severe milk loss seen. The current study shows dexamethasone inhibition on the mammary gland or processes associated with milk production in multiparous cows. In addition, the current study shows a greater inflammatory marker response in the week after dexamethasone treatment in primiparous cows. This could potentially indicate an accelerated response to resolving inflammation with these cows which may suggest faster recovery. Lastly, dexamethasone was shown to decrease activity and increase lying time which could demonstrate reduction of pain allowing more rest to occur. This study is important since it will provide greater detail on postpartum inflammatory reduction work with a high potency steroid like dexamethasone.

REFERENCES

Ali, A. K. A. and G. E. Shook. 1980. An Optimum Transformation for Somatic Cell Concentration in Milk1. Journal of Dairy Science 63(3):487-490.

Arredouani, M. S., A. Kasran, J. A. Vanoirbeek, F. G. Berger, H. Baumann, and J. L. Ceuppens. 2005. Haptoglobin dampens endotoxin-induced inflammatory effects both in vitro and in vivo. Immunology 114(2):263-271.

Atashi, H., A. Abdolmohammadi, M. Dadpasand, and A. Asaadi. 2012. Prevalence, Risk Factors and Consequent Effect of Dystocia in Holstein Dairy Cows in Iran. Asian-Australasian Journal of Animal Sciences 25(4):447-451.

Bach, A., N. Valls, A. Solans, and T. Torrent. 2008. Associations Between Nondietary Factors and Dairy Herd Performance. Journal of Dairy Science 91(8):3259-3267.

Barnes, P. J. 2006. How corticosteroids control inflammation: Quintiles Prize Lecture 2005. British Journal of Pharmacology 148(3):245-254.

Barnes, P. J. and I. M. Adcock. 2003. How do corticosteroids work in asthma? Ann Intern Med 139(5 Pt 1):359-370.

Bateson, P. 1991. Assessment of pain in animals. Animal Behaviour 42(5):827-839.

Baumann, H. and J. Gauldie. 1994. The acute phase response. Immunology Today 15(2):74-80.

Baumann, H., K. R. Prowse, S. MarinkoviĆ, K. A. Won, and G. P. Jahreis. 1989. Stimulation of Hepatic Acute Phase Response by Cytokines and Glucocorticoidsa. Annals of the New York Academy of Sciences 557(1):280-296.

Becker, D. E. 2013. Basic and Clinical Pharmacology of Glucocorticosteroids. Anesthesia Progress 60(1):25-32.

Berger, A. 2000. Science commentary: Th1 and Th2 responses: what are they? BMJ 321(7258):424-424.

Black, W. 1974. Therapeutics of corticosteroids in the bovine animal and problems surrounding their use. Pages 63-66 in Proc. American Association of Bovine Practitioners Proceedings of the Annual Conference.

Bradford, B. J., K. Yuan, J. K. Farney, L. K. Mamedova, and A. J. Carpenter. 2015. Invited review: Inflammation during the transition to lactation: New adventures with an old flame. Journal of Dairy Science 98(10):6631-6650.

Brummer, E., J. H. Choi, E. Brummer, J. H. Choi, and D. A. Stevens. 2005. Interaction between conidia, lung macrophages, immunosuppressants, proinflammatory cytokines and transcriptional regulation. Medical Mycology 43(s1):177-179.

Burton, J. L., M. E. Kehrli Jr., S. Kapil, and R. L. Horst. 1995. Regulation of L-selectin and CD18 on bovine neutrophils by glucocorticoids: effects of cortisol and dexamethasone. Journal of Leukocyte Biology 57(2):317-325.

Burton, J. L., S. A. Madsen, L.-C. Chang, P. S. D. Weber, K. R. Buckham, R. van Dorp, M.-C. Hickey, and B. Earley. 2005. Gene expression signatures in neutrophils exposed to glucocorticoids: A new paradigm to help explain “neutrophil dysfunction” in parturient dairy cows. Veterinary Immunology and Immunopathology 105(3):197-219.

Butcher, E. C. 1991. Leukocyte-endothelial cell recognition: Three (or more) steps to specificity and diversity. Cell 67(6):1033-1036.

Butcher, S. K. and J. M. Lord. 2004. Stress responses and innate immunity: aging as a contributory factor. Aging Cell 3(4):151-160.

Buttgereit, F., J. A. P. Da Silva, M. Boers, G. R. Burmester, M. Cutolo, J. Jacobs, J. Kirwan, L. Köhler, P. van Riel, T. Vischer, and J. W. J. Bijlsma. 2002. Standardised nomenclature for glucocorticoid dosages and glucocorticoid treatment regimens: current questions and tentative answers in rheumatology. Annals of the Rheumatic Diseases 61(8):718.

Castell, J. V., M. J. Gómez-Lechón, M. David, T. Hirano, T. Kishimoto, and P. C. Heinrich. 1988. Recombinant human interleukin-6 (IL-6/BSF-2/HSF) regulates the synthesis of acute phase proteins in human hepatocytes. FEBS Lett 232(2):347-350.

Cattaneo, L., V. Lopreiato, E. Trevisi, and A. Minuti. 2020. Association of postpartum uterine diseases with lying time and metabolic profiles of multiparous Holstein dairy cows in the transition period. The Veterinary Journal 263:105533.

Ceciliani, F., J. J. Ceron, P. D. Eckersall, and H. Sauerwein. 2012. Acute phase proteins in ruminants. Journal of Proteomics 75(14):4207-4231.

Chan, J. P.-W., C.-C. Chang, W.-L. Hsu, W.-B. Liu, and T.-H. Chen. 2010. Association of increased serum acute-phase protein concentrations with reproductive performance in dairy cows with postpartum metritis. Veterinary Clinical Pathology 39(1):72-78.

Chaplin, S. and L. Munksgaard. 2001. Evaluation of a simple method for assessment of rising behaviour in tethered dairy cows. Animal Science 72(1):191-197.

Chinenov, Y. and I. Rogatsky. 2007. Glucocorticoids and the innate immune system: Crosstalk with the Toll-like receptor signaling network. Molecular and Cellular Endocrinology 275(1):30-42.

Claman, H. N. 1972. Corticosteroids and lymphoid cells. N Engl J Med 287(8):388-397.

Correa, M. T., H. Erb, and J. Scarlett. 1993. Path Analysis for Seven Postpartum Disorders of Holstein Cows. Journal of Dairy Science 76(5):1305-1312.

Dematawena, C. M. B. and P. J. Berger. 1997. Effect of Dystocia on Yield, Fertility, and Cow Losses and an Economic Evaluation of Dystocia Scores for Holsteins1. Journal of Dairy Science 80(4):754-761.

Dluhy, R. G., D. P. Lauler, and G. W. Thorn. 1973. Pharmacology and chemistry of adrenal glucocorticoids. Med Clin North Am 57(5):1155-1165.

Duffield, T. F., K. D. Lissemore, B. W. McBride, and K. E. Leslie. 2009. Impact of hyperketonemia in early lactation dairy cows on health and production. Journal of Dairy Science 92(2):571-580.

Eckersall, P. D. and R. Bell. 2010. Acute phase proteins: Biomarkers of infection and inflammation in veterinary medicine. The Veterinary Journal 185(1):23-27.

Edwards, J. L. and P. R. Tozer. 2004. Using Activity and Milk Yield as Predictors of Fresh Cow Disorders. Journal of Dairy Science 87(2):524-531.

Elenkov, I. J. and G. P. Chrousos. 2002. Stress hormones, proinflammatory and antiinflammatory cytokines, and autoimmunity. Ann N Y Acad Sci 966:290-303.

Fleshner, M., T. Deak, K. T. Nguyen, L. R. Watkins, and S. F. Maier. 2001. Endogenous Glucocorticoids Play a Positive Regulatory Role in the Anti-Keyhole Limpet Hemocyanin In Vivo Antibody Response. The Journal of Immunology 166(6):3813-3819.

Flower, R. J. 1988. Lipocortin and the mechanism of action of the glucocorticoids. British Journal of Pharmacology 94(4):987-1015.

Franchimont, D., J. Galon, M. Gadina, R. Visconti, Y. J. Zhou, M. Aringer, D. M. Frucht, G. P. Chrousos, and J. J. O'Shea. 2000. Inhibition of Th1 Immune Response by

Glucocorticoids: Dexamethasone Selectively Inhibits IL-12-Induced Stat4 Phosphorylation in T Lymphocytes. The Journal of Immunology 164(4):1768-1774.

Funnell, B. J. and W. M. Hilton. 2016. Management and Prevention of Dystocia. Veterinary Clinics of North America: Food Animal Practice 32(2):511-522.

Gauldie, J., C. Richards, and H. Baumann. 1992. IL6 and the acute phase reaction. Research in Immunology 143(7):755-759.

Geisterfer, M., C. Richards, M. Baumann, G. Fey, D. Gywnne, and J. Gauldie. 1993. Regulation of IL-6 and the hepatic IL-6 receptor in acute inflammation in vivo. Cytokine 5(1):1-7.

Gladden, N., K. Ellis, J. Martin, and D. McKeegan. 2021. Administration of ketoprofen affects post-partum lying behaviours of Holstein dairy cows regardless of whether parturition is assisted. Veterinary Record 189(6):e300.

Gomez-Laguna, J., F. J, F. J, I. M, I. Barranco, and L. Carrasco. 2011. Acute Phase Proteins as Biomarkers in Animal Health and Welfare. InTech.

Gorewit, R. C. and H. A. Tucker. 1976. Glucocorticoid Binding in Mammary Tissue Slices of Cattle in Various Reproductive States¹. Journal of Dairy Science 59(11):1890-1896.

Grant, R. 2007. Taking Advantage of Natural Behavior Improves Dairy Cow Performance Pages 225-236 in Proc. Western Dairy Management Conference, Reno, NV.

Griffin, J. F. 1989. Stress and immunity: a unifying concept. Vet Immunol Immunopathol 20(3):263-312.

Grimble, R. F. 1990. Nutrition and Cytokine Action. Nutrition Research Reviews 3(1):193-210.

Grönlund, U., C. Hallén Sandgren, and K. Persson Waller. 2005. Haptoglobin and serum amyloid A in milk from dairy cows with chronic sub-clinical mastitis. Vet Res 36(2):191-198.

Gross, J. J., O. Wellnitz, and R. M. Bruckmaier. 2015. Cortisol secretion in response to metabolic and inflammatory challenges in dairy cows1. Journal of Animal Science 93(7):3395-3401.

Gupta, P. and V. Bhatia. 2008. Corticosteroid physiology and principles of therapy. The Indian Journal of Pediatrics 75(10):1039-1044.

Hart, I. C., J. A. Bines, S. V. Morant, and J. L. Ridley. 1978. Endocrine control of energy metabolism in the cow: comparison of the levels of hormones (prolactin, growth hormone, insulin and thyroxine) and metabolites in the plasma of high- and low-yielding cattle at various stages of lactation. J Endocrinol 77(3):333-345.

Hartmann, P. E. and D. S. Kronfeld. 1973. Mammary Blood Flow and Glucose Uptake in Lactating Cows Given Dexamethasone. Journal of Dairy Science 56(7):896-902.

Hayden, M. S., A. P. West, and S. Ghosh. 2006. NF-κB and the immune response. Oncogene 25(51):6758-6780.

Heinrichs, A. J. and B. S. Heinrichs. 2011. A prospective study of calf factors affecting first-lactation and lifetime milk production and age of cows when removed from the herd. Journal of Dairy Science 94(1):336-341.

Herdt, T. H. and R. S. Emery. 1992. Therapy of Diseases of Ruminant Intermediary Metabolism. Veterinary Clinics of North America: Food Animal Practice 8(1):91-106.

Humblet, M.-F., H. Guyot, B. Boudry, F. Mbayahi, C. Hanzen, F. Rollin, and J.-M. Godeau. 2006. Relationship between haptoglobin, serum amyloid A, and clinical status in a survey of dairy herds during a 6-month period. Veterinary Clinical Pathology 35(2):188-193.

Huzzey, J. M., D. V. Nydam, R. J. Grant, and T. R. Overton. 2011. Associations of prepartum plasma cortisol, haptoglobin, fecal cortisol metabolites, and nonesterified fatty acids with postpartum health status in Holstein dairy cows. Journal of Dairy Science 94(12):5878-5889.

Hydbring, E., A. Madej, E. Macdonald, G. Drugge-Boholm, B. Berglund, and K. Olsson. 1999. Hormonal changes during parturition in heifers and goats are related to the phases and severity of labour. Journal of Endocrinology 160(1):75-85.

Jensen, M. B. 2012. Behaviour around the time of calving in dairy cows. Applied Animal Behaviour Science 139(3):195-202.

Juozaitiene, V., A. Juozaitis, A. Kardisauskas, J. Zymantiene, V. Zilaitis, R. Antanaitis, and M. Ruzauskas. 2017. Relationship between dystocia and the lactation number, stillbirth and mastitis prevalence in dairy cows. Acta Veterinaria Brno 86(4):345-352.

Kelley, K. W., R. M. Bluthé, R. Dantzer, J. H. Zhou, W. H. Shen, R. W. Johnson, and S. R. Broussard. 2003. Cytokine-induced sickness behavior. Brain Behav Immun 17 Suppl 1:S112-118.

Lees, P., M. F. Landoni, J. Giraudel, and P. L. Toutain. 2004. Pharmacodynamics and pharmacokinetics of nonsteroidal anti-inflammatory drugs in species of veterinary interest. Journal of Veterinary Pharmacology and Therapeutics 27(6):479-490.

Leimert, K. B., W. Xu, M. M. Princ, S. Chemtob, and D. M. Olson. 2021. Inflammatory Amplification: A Central Tenet of Uterine Transition for Labor. Frontiers in Cellular and Infection Microbiology 11:663.

Liu, T., L. Zhang, D. Joo, and S.-C. Sun. 2017. NF-κB signaling in inflammation. Signal Transduction and Targeted Therapy 2(1):17023.

Lombard, J. E., F. B. Garry, S. M. Tomlinson, and L. P. Garber. 2007. Impacts of Dystocia on Health and Survival of Dairy Calves. Journal of Dairy Science 90(4):1751-1760.

Lord, J. M., M. J. Midwinter, Y.-F. Chen, A. Belli, K. Brohi, E. J. Kovacs, L. Koenderman, P. Kubes, and R. J. Lilford. 2014. The systemic immune response to trauma: an overview of pathophysiology and treatment. The Lancet 384(9952):1455-1465.

Mainau, E., A. Cuevas, J. L. Ruiz-de-la-Torre, E. Abbeloos, and X. Manteca. 2014. Effect of meloxicam administration after calving on milk production, acute phase proteins, and behavior in dairy cows. Journal of Veterinary Behavior 9(6):357-363.

Mallard, B. A., J. C. Dekkers, M. J. Ireland, K. E. Leslie, S. Sharif, C. Lacey Vankampen, L. Wagter, and B. N. Wilkie. 1998. Alteration in Immune Responsiveness During the Peripartum Period and Its Ramification on Dairy Cow and Calf Health. Journal of Dairy Science 81(2):585-595.

Marinković, S. and H. Baumann. 1990. Structure, hormonal regulation, and identification of the interleukin-6- and dexamethasone-responsive element of the rat haptoglobin gene. Molecular and Cellular Biology 10(4):1573-1583.

Matamala, F., A. Strappini, and P. Sepúlveda-Varas. 2021. Dairy cow behaviour around calving: Its relationship with management practices and environmental conditions. Austral journal of veterinary sciences 53(1):9-22.

Mattachini, G., A. Tamburini, M. Zucali, L. Bava, E. Riva, G. Provolo, and A. Sandrucci. 2020. Relationships among lying and standing behaviour, body condition score and milk production in primiparous cows. Italian Journal of Animal Science 19(1):772-782.

McArt, J. A. A., D. V. Nydam, and M. W. Overton. 2015. Hyperketonemia in early lactation dairy cattle: A deterministic estimate of component and total cost per case. Journal of Dairy Science 98(3):2043-2054.

McGrotty, Y. L., C. M. Knottenbelt, I. K. Ramsey, S. W. Reid, and P. D. Eckersall. 2003. Haptoglobin concentrations in a canine hospital population. Vet Rec 152(18):562-564.

Medzhitov, R. and J. C. Janeway. 2000. The Toll receptor family and microbial recognition. Trends in Microbiology 8(10):452-456.

Mee, J. F. 2004. Managing the dairy cow at calving time. Veterinary Clinics of North America: Food Animal Practice 20(3):521-546.

Mee, J. F. 2008. Prevalence and risk factors for dystocia in dairy cattle: A review. The Veterinary Journal 176(1):93-101.

Mellor, D. 2016. Updating Animal Welfare Thinking: Moving beyond the “Five Freedoms” towards “A Life Worth Living”. Animals 6(3):21.

Meyer, C. L., P. J. Berger, K. J. Koehler, J. R. Thompson, and C. G. Sattler. 2001. Phenotypic Trends in Incidence of Stillbirth for Holsteins in the United States1. Journal of Dairy Science 84(2):515-523.

Munksgaard, L. and P. Løvendahl. 1993. Effects of social and physical stressors on growth hormone levels in dairy cows. Canadian Journal of Animal Science 73:847-853.

Natelson, S. and E. A. Natelson. 1980. Haptoglobins: Hemoglobin Binding. Pages 369-407. Springer New York.

Newby, N. C., D. L. Pearl, S. J. Leblanc, K. E. Leslie, M. A. G. Von Keyserlingk, and T. F. Duffield. 2013. Effects of meloxicam on milk production, behavior, and feed intake in dairy cows following assisted calving. Journal of Dairy Science 96(6):3682-3688.

NMC. 2004. Procedures for Collecting Milk Samples.

Oh, S. K., N. Pavlotsky, and A. I. Tauber. 1990. Specific binding of haptoglobin to human neutrophils and its functional consequences. J Leukoc Biol 47(2):142-148.

Orth, R. 1992. Sample day and lactation report, DHIA 200. Fact Sheet A-2. Mid-States Dairy Records Processing Center (DRPC), Ames, IA.

Paape, M. J., D. D. Bannerman, X. Zhao, and J. W. Lee. 2003. The bovine neutrophil: Structure and function in blood and milk. Vet Res 34(5):597-627.

Pascottini, O. B., S. J. Van Schyndel, J. F. W. Spricigo, M. R. Carvalho, B. Mion, E. S. Ribeiro, and S. J. LeBlanc. 2020. Effect of anti-inflammatory treatment on systemic inflammation, immune function, and endometrial health in postpartum dairy cows. Scientific Reports 10(1):5236.

Petersen, H. H., J. P. Nielsen, and P. M. H. Heegaard. 2004. Application of acute phase protein measurements in veterinary clinical chemistry. Veterinary Research 35(2):163-187.

Philippidis, P., J. C. Mason, B. J. Evans, I. Nadra, K. M. Taylor, D. O. Haskard, and R. C. Landis. 2004. Hemoglobin Scavenger Receptor CD163 Mediates Interleukin-10 Release and Heme Oxygenase-1 Synthesis. Circulation Research 94(1):119-126.

Ponchon, B., X. Zhao, S. Ollier, and P. Lacasse. 2017. Relationship between glucocorticoids and prolactin during mammary gland stimulation in dairy cows. Journal of Dairy Science 100(2):1521-1534.

Preisler, M. T., P. S. D. Weber, R. J. Tempelman, R. J. Erskine, H. Hunt, and J. L. Burton. 2000. Glucocorticoid Receptor Expression Profiles in Mononuclear Leukocytes of Periparturient Holstein Cows. Journal of Dairy Science 83(1):38-47.

Proudfoot, K. L., M. B. Jensen, D. M. Weary, and M. A. G. von Keyserlingk. 2014. Dairy cows seek isolation at calving and when ill. Journal of Dairy Science 97(5):2731-2739.

Pruett, J. H., W. F. Fisher, and J. R. DeLoach. 1987. Effects of dexamethasone on selected parameters of the bovine immune system. Vet Res Commun 11(4):305-323.

Putnam, M. R. 1982. Parturition: A Mechanism Review Induction, Intervention, and Calf Viability. in Proc. American Association of Bovine Practitioners Nashville, TN.

Roth, J. A. and M. L. Kaeberle. 1982. Effect of glucocorticoids on the bovine immune system. J Am Vet Med Assoc 180(8):894-901.

Saeed, S. A., N. Ahmad, and S. Ahmed. 2007. Dual inhibition of cyclooxygenase and lipoxygenase by human haptoglobin: Its polymorphism and relation to hemoglobin binding. Biochemical and Biophysical Research Communications 353(4):915-920.

Salak-Johnson, J. L. and J. J. McGlone. 2007. Making sense of apparently conflicting data: Stress and immunity in swine and cattle. Journal of Animal Science 85(suppl_13):E81-E88.

Sapolsky, R. M., L. M. Romero, and A. U. Munck. 2000. How Do Glucocorticoids Influence Stress Responses? Integrating Permissive, Suppressive, Stimulatory, and Preparative Actions*. Endocrine Reviews 21(1):55-89.

Schuenemann, G. M., I. Nieto, S. Bas, K. N. Galvão, and J. Workman. 2011. Assessment of calving progress and reference times for obstetric intervention during dystocia in Holstein dairy cows. Journal of Dairy Science 94(11):5494-5501.

Shafer-Weaver, K. A., C. M. Corl, and L. M. Sordillo. 1999. Shifts in bovine CD4+ subpopulations increase T-helper-2 compared with T-helper-1 effector cells during the postpartum period. J Dairy Sci 82(8):1696-1706.

Shamay, A., F. Shapiro, H. Barash, I. Bruckental, and N. Silanikove. 2000. Effect of dexamethasone on milk yield and composition in dairy cows. Annales de Zootechnie 49(4):343-352.

Shin, D. H., J. K. Jeong, I. S. Choi, S. H. Moon, S. C. Lee, H. G. Kang, S. B. Park, and I. H. Kim. 2018. Associations between serum haptoglobin concentration and peri- and postpartum disorders, milk yield, and reproductive performance in dairy cows. Livestock Science 213:14-18.

Singh, A. K. 2010. Histopathological observations in cervix of dystocia affected vis-à-vis normally calved buffaloes. Indian Journal of Animal Sciences 80(9):842-846.

Smith, V. G., L. A. Edgerton, H. D. Hafs, and E. M. Convey. 1973. Bovine Serum Estrogens, Progestins and Glucocorticoids during Late Pregnancy, Parturition and Early Lactation. Journal of Animal Science 36(2):391-396.

Smoak, K. and J. A. Cidlowski. 2006. Glucocorticoids Regulate Tristetraprolin Synthesis and Posttranscriptionally Regulate Tumor Necrosis Factor Alpha Inflammatory Signaling. Molecular and Cellular Biology 26(23):9126-9135.

Sneddon, L. U., R. W. Elwood, S. A. Adamo, and M. C. Leach. 2014. Defining and assessing animal pain. Animal Behaviour 97:201-212.

Sordillo, L. M. 2005. Factors affecting mammary gland immunity and mastitis susceptibility. Livestock Production Science 98(1):89-99.

Sordillo, L. M. and S. L. Aitken. 2009. Impact of oxidative stress on the health and immune function of dairy cattle. Veterinary Immunology and Immunopathology 128(1):104-109.

Sordillo, L. M. and W. Raphael. 2013. Significance of Metabolic Stress, Lipid Mobilization, and Inflammation on Transition Cow Disorders. Veterinary Clinics of North America: Food Animal Practice 29(2):267-278.

Steensels, M., C. Bahr, D. Berckmans, I. Halachmi, A. Antler, and E. Maltz. 2012. Lying patterns of high producing healthy dairy cows after calving in commercial herds as affected by age, environmental conditions and production. Applied Animal Behaviour Science 136(2):88-95.

Swartz, T. H., H. H. Schramm, J. M. Bewley, C. M. Wood, K. E. Leslie, and C. S. Petersson-Wolfe. 2018. Meloxicam administration either prior to or after parturition: Effects on behavior, health, and production in dairy cows. Journal of Dairy Science 101(11):10151-10167.

Uchida, E., N. Katoh, and K. Takahashi. 1993. Appearance of Haptoglobin in Serum from Cows at Parturition. Journal of Veterinary Medical Science 55(5):893-894.

(UK), N. C. C. f. W. s. a. C. s. H. 2007. National Institute for Health and Clinical Excellence: Guidance. in Intrapartum Care: Care of Healthy Women and Their Babies During Childbirth. RCOG Press Copyright © 2007, National Collaborating Centre for Women's and Children's Health., London.

USDA. 2018. Health and Management Practices on U.S. Dairy Operations, 2014. USDA–APHIS–VS–CEAH–NAHMS, ed, Fort Collins, CO

van der Drift, S. G. A., M. Houweling, M. Bouman, A. P. Koets, A. G. M. Tielens, M. Nielen, and R. Jorritsma. 2015. Effects of a single glucocorticoid injection on propylene glycol-treated cows with clinical ketosis. The Veterinary Journal 204(2):144-149.

Van Der Kolk, J. H. 1990. The bovine pituitary-adrenocortical axis and milk yield. Veterinary Quarterly 12(2):114-120.

Van Leeuwen, M. A. and M. H. Van Rijswijk. 1994. Acute phase proteins in the monitoring of inflammatory disorders. Baillière's Clinical Rheumatology 8(3):531-552.

Vannucchi, C. I., J. A. Rodrigues, L. C. Silva, C. F. Lúcio, G. A. Veiga, P. V. Furtado, C. A. Oliveira, and M. Nichi. 2015. Association between birth conditions and glucose and cortisol profiles of periparturient dairy cows and neonatal calves. Vet Rec 176(14):358.

Varner, M. A. and B. H. Johnson. 1983. Influence of adrenocorticotropin upon milk production, milk constituents, and endocrine measures of dairy cows. J Dairy Sci 66(3):458-465.

Wang, Y., E. Kinzie, F. G. Berger, S. K. Lim, and H. Baumann. 2001. Haptoglobin, an inflammation-inducible plasma protein. Redox Report 6(6):379-385.

Westin, R., A. Vaughan, A. M. De Passillé, T. J. Devries, E. A. Pajor, D. Pellerin, J. M. Siegford, E. Vasseur, and J. Rushen. 2016. Lying times of lactating cows on dairy farms with automatic milking systems and the relation to lameness, leg lesions, and body condition score. Journal of Dairy Science 99(1):551-561.

Wiegers, G. J. and J. M. H. M. Reul. 1998. Induction of cytokine receptors by glucocorticoids: functional and pathological significance. Trends in Pharmacological Sciences 19(8):317-321.

Wildman, E. E., G. M. Jones, P. E. Wagner, R. L. Boman, H. F. Troutt, and T. N. Lesch. 1982. A Dairy Cow Body Condition Scoring System and Its Relationship to Selected Production Characteristics. Journal of Dairy Science 65(3):495-501.

Xavier, A. M., A. K. O. Anunciato, T. R. Rosenstock, and I. Glezer. 2016. Gene Expression Control by Glucocorticoid Receptors during Innate Immune Responses. Frontiers in Endocrinology 7(31).