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Assessment of the effects of intrauterine dextrose infusion in post-partum dairy cows diagnosed with clinical metritis

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ABSTRACT

The objective of this study was to assess the effects of intrauterine dextrose infusion on clinical metritis cure rate, daily milk yield and rumination time, metabolic stress, and systemic inflammation in dairy cows diagnosed with clinical metritis (CM). Cows (n=641) from a farm located in central Pennsylvania were screened at 7±3 DIM to assess vaginal discharge. Cows that presented a fetid red-brownish watery vaginal discharge (n=77) were classified as CM cows, blocked by parity and randomly assigned to one of two groups: 1) CONV (n=39): two injections of ceftiofur (per label;6.6 mg/Kg) 72 h apart; and 2) DEX (n=38): three intrauterine infusions of a 50% dextrose solution (1 L/cow) every 24 h. Cows that presented a normal vaginal discharge at 7±3 DIM (NOCM; n=45) were randomly selected and matched by parity to CONV and DEX cows. Cows were re-screened at 14±3 and 21±3 DIM to assess CM cure rate. Daily milk yield and rumination time for the first 150 DIM was collected from on-farm computer records. Body condition score was assessed, and blood samples were collected at enrollment, and at 9±3, 14±3 and 21±3 DIM. The data were analyzed using the MIXED and GLIMMIX procedures of SAS as a randomized complete block design. There was no difference in CM cure rate between treatment groups at 14±3 DIM (CONV=68.59±7.95%; DEX= 61.12±8.25%) and 21±3 DIM (CONV=88.26±5.586%; DEX=85.74±5.994%). CONV and DEX cows had higher concentration of haptoglobin at 7±3 DIM and 9±3 DIM, but there was no difference between study groups at 14 and 21±3 DIM. Overall, cows in the CONV and NOCM groups produced 3.67 kg/d and 3.68 kg/d more milk, respectively, compared with cows in the DEX group during the first 150 DIM. These results suggest that, although intrauterine infusion of dextrose may achieve similar clinical

cure rates as ceftiofur treatment, dextrose may not be as effective at restoring milk production in CM cows.

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Chapter 1

Literature Review

Clinical Metritis

Definition and incidence

The uterus of a dairy cow is sterile throughout the pregnancy; however, during and after parturition it can become contaminated with a variety of bacteria (Sheldon et al., 2009). The dilation of the reproductive tract anatomical barriers, such as vulva, vestibule, vagina, and cervix, is one of the main contributing factors for the aforementioned bacterial contamination (Sheldon 2004). The most commonly reported bacteria in clinical metritis cases include *E coli*, *T pyogenes*, *F necrophorum*, and *Prevotella* species (Sheldon 2004). It has been reported that *E. coli* in the intrauterine environment at 1-3 DIM has a strong association with metritis and is likely among the first bacteria to colonize in the uterus (Bicalho et al., 2012). *T. pyogenes* is a self-generated bacterium with serious virulence determinants including several methods of host cell adhesion, pyolysin, and the ability to survive within epithelial cells (Jost and Billington 2005). This bacterium causes the disintegration of endometrial cells, after the epithelium is lost following calving, allowing the bacteria to act as a pathogen (Carneiro et al., 2016). As a result of the use antimicrobials, a significant amount of *T pyogenes* isolates were found to be resistant to chlortetracycline, oxytetracycline, and tetracycline (Trinh et al., 2002). Most research related to bovine uterine health has mentioned the significance of *T pyogenes* and *E coli*, however, a recent study showed that cows with metritis had a higher number of *Fusobacterium* *necrophorum*, *Prevotella melaninogenica*, *Bacteroides pyogenes*, *Porphyromonas levii*, and *Helcococcus ovis* when compared to healthy cows; Meanwhile, bacteria such as *E coli* had no difference in concentration between healthy and metritic cows, challenging the impact this species has on the infection (Cunha et al., 2018).

While many cows can naturally fight off the bacteria, some cows go on to develop a persistent bacterial infection resulting in uterine disease. The incidence of metritis can greatly vary among dairy herds, ranging from 1% up to 40% (Sheldon, 2004), depending heavily on farm management. Severity of infection depends on the concentration and species of bacteria as well as each cow's individual immune response (Drillich 2006). The immune system of the animal will rely on neutrophils to kill the internalized bacteria contributing to the formation of pus in the uterus (Sheldon, 2004).

There are two main classifications of metritis in dairy cows: puerperal metritis and clinical metritis. Sheldon et al. (2006) defines puerperal metritis as an animal with an abnormally enlarged uterus and a fetid watery red-brown uterine discharge, associated with signs of systemic illness (decreased milk yield, dullness or other signs of toxemia) and fever >39.58 °C, within 21 days after parturition. Clinical metritis is also characterized by the presence of an enlarged uterus and fetid red-brown vaginal discharge, however, it may or may not be accompanied by systemic signs (Barragan et al., 2018, 2019).

Clinical metritis can be diagnosed using several different techniques. For instance, transrectal palpation of the uterus can be used to feel whether the uterus is enlarged and to detect any abnormal discharge, however, one downfall of this technique is that it is subjective (Leblanc et al., 2002). Vaginoscopy has been found to be a useful technique in diagnosing cows with uterine disease (Dohmen et al., 1995), however, this method has a high number of false negatives

(Kasimanickam et al., 2004). Ultrasound is a useful technique to determine the amount of fluid in the uterine lumen. A positive correlation has been found between the amount of fluid in the uterus and the presence of pathogenic bacteria (Mateus et al. 2002). Two more applied, economical and user-friendly diagnostic options for practicing veterinarians are the gloved hand technique and the use of the Metricheck device to assess the vaginal discharge's features (Simcro Tech Ltd., Hamilton, New Zealand; Pleticha et al., 2009). A five point scale adapted from Williams et al. (2005) is used to classify vaginal discharge samples. Discharge receiving a score of 1 should be interpreted as consisting of clear fluid, a score of 2 is less than 50% white purulent fluid, a score of 3 is greater than 50% white purulent fluid, discharge scoring a 4 consists of red-brownish fluid lacking odor, and a score of 5 is fetid red-brown watery discharge.

Losses and economics

It is estimated that the annual cost of uterine disease in the U.S. is around $650 million, this is contributed through a number of factors including decreased milk production, decreased cow fertility and increased culling rate (Sheldon et al., 2009). Several studies have shown decreased milk yield (Rajala and Gröhn, 1998), dry matter intake, and pregnancy per AI at first service in cows diagnosed with metritis compared to a healthy animal (Huzzey et al., 2007; Wittrock et al., 2011). Metritic cows are also more likely to be culled than healthy cows, one study found that multiparous metritic cows were 30% more likely to be culled than a healthy cow (Wittrock et al., 2011). After considering all of these economic costs, it is estimated that, on average, each case of metritis costs a farm $512 (Pérez-Báez et al., 2021). Furthermore, animal welfare may be compromised in cows with clinical metritis. Heart rate variability has been used to show increased sympathetic activity in metritic cows, supporting the idea that metritis causes pain to the animal (Stojkov et al., 2015). Additionally, when palpated, metritic cows were seen to have a more pronounced back arch than healthy cows indicating pain from the inflammation of the uterine wall (Stojkov et al., 2015). Another recent study showed that cows diagnosed with clinical metritis had decreased body condition scores and increased lying time as well as increased concentrations of inflammation, stress, and pain biomarkers (Barragan et al., 2018). These studies were able to quantitatively demonstrate the negative effects that metritis has on dairy cows, reflecting the need for the development of new treatment approaches to minimize the negative consequences of the bacterial infection.

Risk Factors

One of the most common health events that affects animal welfare and productivity in dairy farms around the globe is uterine diseases. Diseases, such as clinical metritis, are a major concern not only in respect to individual animal health but also the profitability of the entire herd. Around the time of calving, 30 to 50% of dairy cows are affected by a metabolic or infectious diseases (Leblanc 2010). These production diseases (e.g., hypocalcemia, ketosis, retained placenta, displacement of the abomasum, clinical metritis) are the result of the cow's inability to cope with the high metabolic demands of colostrum and milk production shortly after calving (Mulligan and Doherty 2008). The three weeks before calving to the three weeks after calving is the most challenging period for dairy cows during the lactation cycle and is known as the transition period (Grummer 1995). As the demand of output energy increases, feed intake is decreased 30 to 35% during the transition period; This coupled to the increase of nutrient demands, leads to a negative energy balance (Grummer 1995). The transition cow also experiences immunosuppression, dietary changes, and likely environmental stressors such as group changes during this period (Mulligan and Doherty 2008). All of these stressors coupled with an increase level of systemic stress and inflammation related to the birthing process makes the transition period the time of highest risk for production diseases (Mulligan and Doherty 2008), specifically clinical metritis.

Retained placenta

A standardized definition for retained placenta is generally agreed to be when the fetal membranes are present at the vulva, vagina, or uterus more than 24 hours after calving (Kelton et al., 1998). There is a distinct association between the cows diagnosed with retained placenta and the development of metritis. A study, that investigated the risk factors of uterine diseases in dairy cattle, found that among cows with retained placenta, 33.2% developed metritis (Dubuc et al., 2010). The odds ratio of developing metritis after being diagnosed with retained placenta is significantly higher (OR= 9.45) when compared to a cow without retained placenta (Hossein-Zadeh and Ardalan 2011). Retained placenta has a large impact on the development of metritis because it provides the ideal environment for bacteria to prosper with the large amount of necrotic tissue (Sheldon, 2004) as well as a direct pathway for bacteria in the environment to enter the uterus. The incidence of retained placenta is typically between 6% and 8% but can be increased to 40% in cows that experience twins and dystocia (Sheldon, 2004). Abortion also increases the risk of retained placenta. For instance, one study found that 61.6% of aborting cows retained their placenta (Joosten et al., 1987). Other retained placenta risk factors include stillbirth, gestation length, birth weight, and time of the year (Joosten et al., 1987). Overall, it was found that cows were most at risk of retained placenta if they had a low birth weight, shorter gestation length (less than 270 days), and gave birth during the summer months (June, July, or

August; Joosten et al., 1987). Additionally, it was reported that cows who had retained their placenta previously, were three times more likely to do so in a subsequent parturition, and up to six times as likely after two consecutive retentions (Joosten et al., 1987). Three processes must take place, as described by Sheldon (2004), for the normal expulsion of placenta: placental maturation during the time around parturition, collapse of the villi, and uterine contractions with distortion of the placentomes.

Dystocia

Dystocia is defined as a difficult calving due to a prolonged calving or a calving requiring severe assistance (Mee 2004). A 5-point scale is commonly used to describe the level of difficulty. A score of 1 indicates no problem, a score of 2 indicates some minor assistance occurred such as one individual pulling without mechanical assistance, a score of 3 indicates that assistance was needed requiring multiple individuals and/or chains, while a score of 4 indicates that considerable force was used (e.g., calf-pullers) and a score of 5 indicates extreme difficulty often needing a Caesarean section (Djemali et al., 1987; Dematawewa and Berger 1997; Lombard et al., 2007). Other scoring systems are available including a 0-3 point scale (Ettema and Santos 2004) and a 7-point scale (McClintock, 2005). It is estimated that on average each case of dystocia costs a farm $154 (Mahnani et al., 2017). Not only does dystocia cause economic losses, but it also has a negative impact on welfare of both the dam and the calf, as it has been found to increase morbidity and mortality (Mee 2004). Ghavi Hossein-Zadeh and Ardalan (2011) found that cows with dystocia were four times more likely to develop metritis than those with an easy calving. Additionally, it has been reported that dystocic calves have higher cortisol concentrations than calves with unassisted births (Barrier et al., 2013). According to Mee (2004), the majority of dystocia cases are caused by feto-pelvic incompatibility, abnormal fetal disposition, and incomplete dilation of the vulva and the cervix.

Twins

As a monotocous species, cows typically produce one calf per pregnancy; however, there is a chance that cows will have twins and possibly even triplets. The incidence of twinning in the United States is around 4.2% and is more likely to occur in multiparous cows (Cabrera and Fricke 2021). While twinning has the benefit of producing more calves, there are several negative consequences that make twinning undesirable in dairy farming. It has been shown that twin calving increases the risk of abortion, dystocia, retained placenta, the need for antibiotic therapy, calf mortality, the number of days open, and number of inseminations per conception (Nielen et al., 1989; Mee, 1991; Beerepoot et al., 1992; Echternkamp and Gregory, 1999; Andreu-Vázquez et al., 2012). Specifically looking at metritis, a study performed by Ghavi Hossein-Zadeh and Ardalan (2011) found that cows who gave birth to twins were six times more likely to experience metritis than cows delivering a single calf.

Risk factors that increase the chances of twins include parity (multiparous cows more prone to having twins; Neilen et al., 1989; Kinsel et al., 1998), calving during the summer season (Neilen et al 1989), previous twin pregnancies, and receiving antibiotics prior to conception (Kinsel et al., 1998). Perhaps the greatest correlation that has been found is that an increase in milk production leads to an increase in twinning (Kinsel et al., 1998; Neilen et al., 1989). Cows that delivered twins were found to have a greater peak milk production of almost 3 kg (Kinsel et al., 1998).

Collectively, the negative results of twinning lead to major economic consequences in a dairy herd. Twinning has been found to cost $97 to $225 per case to the American dairy industry, accounting for an annual loss of around $2,415 per farm (Mur-Novales et al., 2018). In an effort to reduce the occurrence of twin births, different approaches have been researched. A study performed by Mur-Novales et al. (2018) researched management strategies to alleviate the negative impact of twinning. The three management options focused upon were: no interference, induced abortion, and attempted manual embryo reduction (Mur-Novales et al., 2018). It was found that attempting the manual embryo reduction was the best management alternative from an economic point of view for any lactation, DIM, or type of twin pregnancy (Mur-Novales et al., 2018). While pregnancy loss was increased by almost 20% when performing embryo reduction when compared to doing nothing, the economic savings were shown to be related to the decrease of postpartum disease and increased life span of the cow (Mur-Novales et al., 2018).

Ketosis

A transition cow typically experiences a negative energy balance due to the increased energy demands at parturition and the decreased dry matter intake (Raboissen et al., 2014). In addition to the negative energy balance, nearly all peripartum cattle experience a period of insulin resistance, lipolysis, weight loss, hypocalcemia, reduced immune function, and bacterial contamination of the uterus (Leblanc, 2010). While clinical hyperketonemia (i.e., elevated blood ketones) will show signs such as decreased appetite, weight loss, and decreased milk production, a large percentage of cows suffer from subclinical ketosis, defined as an excess of circulating ketone bodies without the clinical signs of ketosis (i.e. a serum β-Hydroxybutyrate (BHB) ≥1,200 μmol/L; Andersson, 1988). The average incidence of subclinical ketosis is 43%

and ranges from 26 to 56% (McArt et al., 2012). The circulation of BHB indicates that the cow is in a negative energy balance (Leblanc 2010). Elevated blood ketones indicates that little to no cellular glucose uptake is occurring (Dhatariya 2016); therefore, the body mobilizes fat as an alternative energy source resulting in a higher ketone body serum concentration.

The threshold concentration of BHB to be considered subclinical ketosis varies between studies, the most commonly agreed upon threshold begins at a concentration of serum BHB ≥1,200 µmol/L (Leblanc 2010). It has been found that cows with a BHB concentration greater than 1,2000 µmol/L had a higher risk of developing displaced abomasum and metritis and decreased milk production (Duffield et al., 2009). More specifically, nonketotic cows produced 1.2 kg/day more milk than those with subclinical ketosis (McArt et al., 2012). It has been reported that cows diagnosed with subclinical ketosis were over 19 times more likely to develop displaced abomasum and 3 times more likely to die or be culled than nonketotic cows (McArt et al., 2012). Overall, cows that poorly adapt to the energetic challenges during the transition period are more likely to face metritis, milk loss, and be culled (Duffield et al., 2009).

Treatment Approaches

Systemic antibiotics

While many cows will naturally recover from metritis, a significant number of cows will require additional treatment. There are several treatment approaches that have been researched in dairy operations aimed on diminishing the negative effects associated with metritis. The ideal treatment would eliminate bacteria from the uterus and subendometrial layers without constraining the natural uterine defense mechanisms (Drillich 2006). Other considerations for potential treatments would include improving reproductive performance, increasing milk production, and overall minimizing the economic losses created by uterine disease.

As metritis is a bacterial infection in the uterus, it is reasonable to conclude that a systemic antimicrobial would be a good therapy option for the disease. Ceftiofur is a broad-spectrum antibiotic that has been shown to successfully treat cows diagnosed with metritis (Smith et al., 1998). One advantage of this antibiotic is the absence of a withholding period since the residue found in the milk is below the tolerance level for human consumption (Lima et al., 2014). However, only 77% of cows treated with this drug (2.2 mg/kg injections for five days) may recover from metritis (Chenault et al., 2004). Additionally, in the United States, ceftiofur is the only third-generation cephalosporin labeled to treat metritis leading to selection pressure, which has been suggested to be involved with emergence of cephalosporin microbial resistance (Tragesser et al. 2006).

Due to ceftiofur hydrochloride treatments requiring daily injections for extended periods of time, McLaughlin et al. (2012) predicted that a single dose of ceftiofur (EXCEDE; Pfizer Animal Health Inc., New York, NY) would be more convenient and just as effective as 5 daily injections of ceftiofur hydrochloride. It was shown that the ceftiofur crystalline free acid sterile suspension (CCFA-SS) when injected at the base of the ear increased the clinical cure rate 19% compared with the control animals (74.3 vs. 55.3% respectively) and was an effective therapy to cows with metritis (McLaughlin et al., 2012).

Other antibiotics that have been studied for the treatment of metritis include ampicillin, which was found to have a faster cure than Ceftiofur (Lima et al., 2014). Ampicillin has an amine group that is thought to enable the penetration into gram-negative bacteria increasing its range of action when compared to penicillin (Lima et al., 2014). While these antibiotics are effective treatments for metritis, the main concern when administering antimicrobials to a lactating dairy cow is the high dosage required to properly treat the animal increases the risk for antibiotic residue in the milk which is not in accordance to legal drug regulations and guidelines (Drillich et al., 2006).

Although the conventional antibiotic treatment approach is effective in treating this disease, there is a growing concern about misusing antibiotics in dairy farms leading to antimicrobial resistance. It has been shown that cows treated with systemic antibiotics (i.e., ceftiofur) have a rapid emergence of antimicrobial resistant Enterobacteriaceae when compared to an untreated cow (Sheedy et al., 2021; Taylor et al., 2019). It was also reported that these resistant phenotypes had cyclical re-emergence 15 days post treatment indicating that horizontal gene transfer of resistance genes had taken place within the animal's gut flora (Sheedy et al., 2021). There is a need for more research on alternative treatments for metritis in order to evaluate their treatment efficiency. This will allow producers that have current issues with this prevalent disease to be able to make informed treatment decisions.

Local: Intrauterine infusions

Intrauterine infusion (**IUI**) treatments are a good alternative to the systemic antibiotic. Two antibiotic-free treatments that have potential to cure metritis when infused into the uterus are iodine and dextrose. Maquivar et al. (2013) found that cows treated with intrauterine infusion of iodine had over a 15% greater cure rate than cows treated with dextrose infusion. While iodine may be effective at curing metritis, it has been shown that cows infused with a single treatment of povidone-iodine had reduced reproductive performance when compared to the control animals (Youngquist and Shore 1997). Additionally, the presence of organic materials inside the bovine uterus deactivates the iodine making it an ineffective treatment for metritis (Mido et al., 2016). As research continues to reveal drawbacks of an iodine intrauterine infusion, more research is needed to assess the dextrose treatment method.

Dextrose has the ability to kill the bacteria present in the uterus by creating a hypertonic environment and dehydrating the bacteria (Chirife et al., 1983). When studying the effects of sugar on human wounds, Chirife et al. (1983) concluded that in patients with lowered defense mechanisms, sugar can play a role in in the control of infection by lessening bacterial virulence. This suggests that an IUI of dextrose has the potential to kill bacteria in the uterine lumen and cure metritis without the use of antibiotics. Previous studies have applied an IUI dextrose treatment to a milder uterine disease, known as clinical endometritis; however, there have been inconsistent results (Brick et al., 2012; Maquivar et al., 2015; Machado et al., 2015). A study by Brick et al. (2012) compared intrauterine dextrose administration (50%; 200 mL) to subcutaneous ceftiofur (CCFA; 6.6 mg/kg; single-dose). Cows were enrolled in the study based on the appearance of vaginal discharge (i.e., 3-point scale; 0= normal uterine discharge, 1 = flakes pus, 2 = >50% pus, 3 = hemorrhagic uterine discharge mixed with pus) and allocated into three groups: dextrose, CCFA, or untreated (Brick et al., 2012). Uterine swab samples were taken from the cows diagnosed with clinical endometritis and found that the two predominant types of bacteria were *A. pyogenes* and *E. coli* (Brick et al., 2012). Clinical cure increased when treated with dextrose or CCFA, however, the rate of pregnancies per first artificial insemination (PAI) only increased in the cows treated with dextrose (Brick et al., 2012). Conversely, a recent study showed that the intrauterine administration of dextrose was detrimental to uterine health, decreased cure rate, and had no effect on first service conception rate (Machado et al., 2015). The data showed that the cure rate of cows treated with the dextrose decreased by ten percentage points when compared to the untreated cows (Machado et al., 2015). One explanation for the conflicting results to previous studies may be the concentration of dextrose was too low to create a strong hypertonic environment, consequently the dextrose may have nurtured the pathogens rather than inhibiting their growth (Machado et al., 2015). It is worth noting that while both studies evaluated vaginal discharge to diagnose the cows, Machado et al. (2015) is the only study that used a Metricheck device and a five-point rating scale rather than the gloved hand technique and a three-point rating scale. Collectively, these studies indicate that alternative therapies for metritis in dairy cows, other than antibiotics, have potential, but require additional research to refine the protocols and define the impacts. With the limited data available, more research is warranted to assess the effects of different treatment regimens using IU dextrose in cows diagnosed with clinical metritis.

Prevention

While complete elimination of metritis is unfeasible, management strategies such as maximizing cow comfort and dry matter intake, preventing ketosis and nutritional deficiencies (i.e., selenium and vitamin E (Harrison et al., 1984)), and keeping good facility hygiene can be taken to decrease incidence of this prevalent disease (Galvão, 2013). Additionally, attempting to prevent calving problems (i.e., dystocia, twins, retained placenta, etc.) to lower the risk of metritis can be done by having smaller sized calves, for example, breeding Holsteins to Jersey sires (Ribeiro et al., 2013). During the late lactation period, cows drop in their total amount of food intake and increase the risk of metritis (Gilbert, 2016). The removal of social stressors such as group changes and competition for food can help increase dry matter intake however, it is still not clear how to avoid complete reduction of feed consumption (Gilbert, 2016). Additionally, overcrowding of pens can contribute to feeding behavior, so it is important that dry cows have plenty of space, limited group changes, and have separation between heifers and multiparous cows (Gilbert, 2016).

Facility hygiene plays a critical role in preventing metritis, specifically in calving pens (Gilbert, 2016). Metritis is mainly caused by environmental bacteria; therefore, minimizing the number of bacteria to enter the uterine lumen during and after calving will decrease the risk of infection. The type of bedding used in stalls is an important factor in the number of bacteria in the environment, it has been shown that cows using sand stalls were cleaner than cows using straw stalls (Norring et al., 2008). Sand bedding has many advantages since it does not support bacterial growth and will not retain urine (Godden et al., 2007). Fresh cows housed in free-stalls had a 16% lower incidence of endometritis than cows housed in bedded pack (Cheong et al., 2011); however, other studies have found no difference in hygiene between free-stalls and bedded pack barns (Eckelkamp et al., 2016).

Study Rationale

Due to the severe implications that CM has in the profitability and animal welfare of dairy farms, it is critical to develop an effective yet affordable treatment strategy for this condition. While antibiotic treatments have proven to be an effective treatment for CM, there are increasing concerns regarding antibiotic misuse in dairy operations, which could lead to antibiotic resistance (Cuong et al., 2018). In a recent meta-analysis, dairy cattle operations were found to have the third greatest antimicrobial use among food animal operations, which is considered a key contributor to antimicrobial resistance (Cuong et al., 2018). It has been found that antimicrobial use in animals contributes to the selection and spread of resistance in bacteria populations in animals (McEwen and Cray 2002). With a large number of cows in closely confined facilities, the spread of resistant bacteria is certain to increase (McEwen and Cray 2012). Efforts to reduce the spread of antimicrobial resistant bacteria begin with restricting and monitoring of antibiotic use, specifically in food animals (McEwen and Cray 2002). Alternatives to antibiotics is crucial to human health as the resistance among animal pathogens reduces the effectiveness antibiotics used in human medicine (McEwen and Cray 2002). Studies have found that herds that reported ceftiofur use were more likely to have cows with reduced susceptibility *E coli* (Tragesser et al., 2006, Mann et al., 2011).

Administration of hypertonic solutions, such as dextrose, inside the uterus, is an antibiotic free and economical treatment approach that have been shown to be effective in treating a milder uterine disease known as endometritis (Brick et al., 2012; Maquivar et al., 2015). Not only would this treatment approach be beneficial to decreasing the antibiotic resistance trend, it is also would be a more affordable option for dairy operations to consider. The cost of administering 1 liter of dextrose to an animal for 3 days is estimated to be around $55 including the dextrose, labor and disposables. This is a major decrease from the cost of administering 6.6. mg of ceftiofur/kg which is estimated to be between $80 and $97 per treatment (Pérez-Báez et al., 2021). By determining the effectiveness of dextrose as a treatment to metritis, we will be able to verify if this cost-effective solution could be an alternative to the conventional antibiotics.

The specific objective of this study is to assess the effects of intrauterine dextrose (50%) infusion on metritis cure rate, daily milk yield, daily rumination, metabolic stress, and systemic inflammation in post-partum dairy cows diagnosed with metritis. We hypothesis that the proposed new treatment approach will be as effective as the conventional farm treatment on curing metritis.

Twice a week, throughout the summer months (i.e., May, June, July, August), the study team will administer treatments and collect samples (blood and vaginal discharge). Post-partum cows (7±3 days in milk; DIM) will be screened using a Metricheck device (Simcro Tech Ltd., Hamilton, New Zealand) to assess vaginal discharge. The discharge will be evaluated based on the proportion of pus, color, density and smell, using a 5-point scale (i.e., 1- clear fluid, 2- <50% white purulent fluid, 3- >50% white purulent fluid, 4- red-brownish fluid without fetid smell, and 5- fetid red-brownish watery fluid).

Cows with a vaginal discharge score of 4 or 5 will be categorized as metritis cows. Metritis cows will be blocked by parity, and randomly allocated to one of two treatments: 1) = two injectable (SC) administrations of ceftiofur antibiotic (6.6 mg/Kg) 72 hours apart and 2) intrauterine infusion with dextrose (50%; 1 L/cow) every 24 h for 3 consecutive days. Study cows will be re-screened with the Metricheck device (Simcro Tech Ltd., Hamilton, New Zealand) at 14±3 and 21±3 DIM to assess cure rate. Blood samples and vaginal discharge will be collected at each screening point and used to assess the concentrations of metabolic stress and inflammatory biomarkers. Furthermore, the study team will assess the body condition score (BCS) of study animals as well as daily milk yield, and daily rumination from the on-farm computer records.

Chapter 2

Introduction

Clinical metritis (CM) can be defined as the inflammation of the uterus that occurs within three weeks of parturition, characterized by an enlarged uterus with the presence of red-brownish, watery, and fetid vaginal discharge without the presence of systemic signs such as fever (Sheldon et al., 2009). The incidence of CM can greatly vary, ranging from 15% up to 40% (Gilbert, 2016), depending heavily on farm management. It is estimated that the annual cost of CM in the U.S. is around $650 million, this is contributed through a number of factors including decreased milk production, decreased fertility and increased culling rate (Sheldon et al., 2009). While many cows will naturally recover from CM, a significant number of cows will require additional treatment (Giuliodori et al., 2013). There are several treatment approaches that have been assessed to treat and diminish the negative effects associated with CM in dairy operations. Ideally, the perfect treatment should be capable to eliminate bacteria from all of the layers of the uterus without constraining the natural uterine defense mechanisms (Drillich 2006).

Antibiotics are an effective treatment, specifically, cephalosporins are recommended for the treatment of uterine bacterial infections in cattle (Sheldon 2004). While these antibiotics have the capability to kill the bacteria in the uterus, there is an increasing pressure to limit antimicrobial use in food producing animals. Emerging antibiotic resistance has led to the restrictions of use of cephalosporins in food animals in the United States, and as of now, only ceftiofur crystalline-free acid (Excede Sterile Suspension; Zoetis) and ceftiofur hydrochloride (Excenel RTU-EZ; Zoetis, Madison, NJ) are labeled for the treatment of bovine CM (Reppert, 2015). In a recent study, cows diagnosed with CM from three large U.S. dairy farms were treated with two doses of ceftiofur crystalline-free acid and it was determined that the treated animal groups had a higher concentration of resistant *E. coli* than untreated groups (Taylor et al., 2019). While ceftiofur has no withdrawal time for milk, there is a 13-day withdrawal period for meat, and the results from this study show a higher number of resistant colonies until 16 days after treatment, therefore, a recommendation to elongate the withdrawal time to ensure that resistant strains fall below baseline levels is proposed (Taylor et al., 2019). Therefore, an antimicrobial free treatment able to ensure the health and welfare of the cow, while keeping production and fertility high, would be extremely beneficial to dairy farms across the country. Additionally, a more affordable option for producers to choose from would aid in increasing farm profitability.

Intrauterine infusions (IUI) of dextrose have the potential to be an effective treatment for CM. Adding a high concentration of a sugar solution to the uterine environment may create a hypertonic environment, causing water to diffuse from the bacteria ultimately killing them (Chirife et al., 1983). Previous studies have utilized IUI of dextrose to treat a milder uterine disease known as endometritis; however, there have been inconsistent results (Brick et al., 2012; Maquivar et al., 2015; Machado et al., 2015). Clinical endometritis (CE) can be defined as the presence of purulent vaginal discharge 21 days or more after parturition (Sheldon et al., 2006). Studies have shown that a 200 mL administration of 50% dextrose intrauterine treatment increased clinical cure rate and rate of pregnancies per first artificial insemination in CE cows compared to cows in the control group (Brick et al., 2012; Maquivar et al., 2015). Conversely, a similar study showed opposite results and reported that the cure rate of CE cows treated with IUI dextrose decreased by ten percentage points when compared to the untreated cows (Machado et al., 2015). Although collectively these studies may indicate that IUI of dextrose may have potential as an effective CM therapy, there is a lack of research assessing the effects of this therapy on CM cows and additional studies are needed to refine treatment protocols and identify impacts in cow health and performance.

The objective of this study was to assess the effects of IUI of dextrose on clinical cure rate, daily milk yield and rumination time, fertility, metabolic stress and systemic inflammation in dairy cows diagnosed with CM. We hypothesize that cows treated with IUI of dextrose will have similar clinical cure rates, milk production, rumination time, fertility, metabolic stress, and systemic inflammation compared with cows treated with the conventional antibiotic treatment.

Chapter 3

Materials and Methods

Animals, facilities, and feeding

Dairy cows from a dairy farm located in central Pennsylvania were enrolled in this study. The facilities housed approximately 700 cows and had approximately 600 cows milking. The herd average yearly rolling milk production was 11,143 kg. Animals were housed in naturally ventilated free-stall pens with deep sand bedding, equipped with additional fans and sprinklers. Cows were milked three times a day, approximately 8 hours apart, and fed a TMR diet formulated to meet or exceed dietary nutritional requirement (NRC, 2001) twice daily.

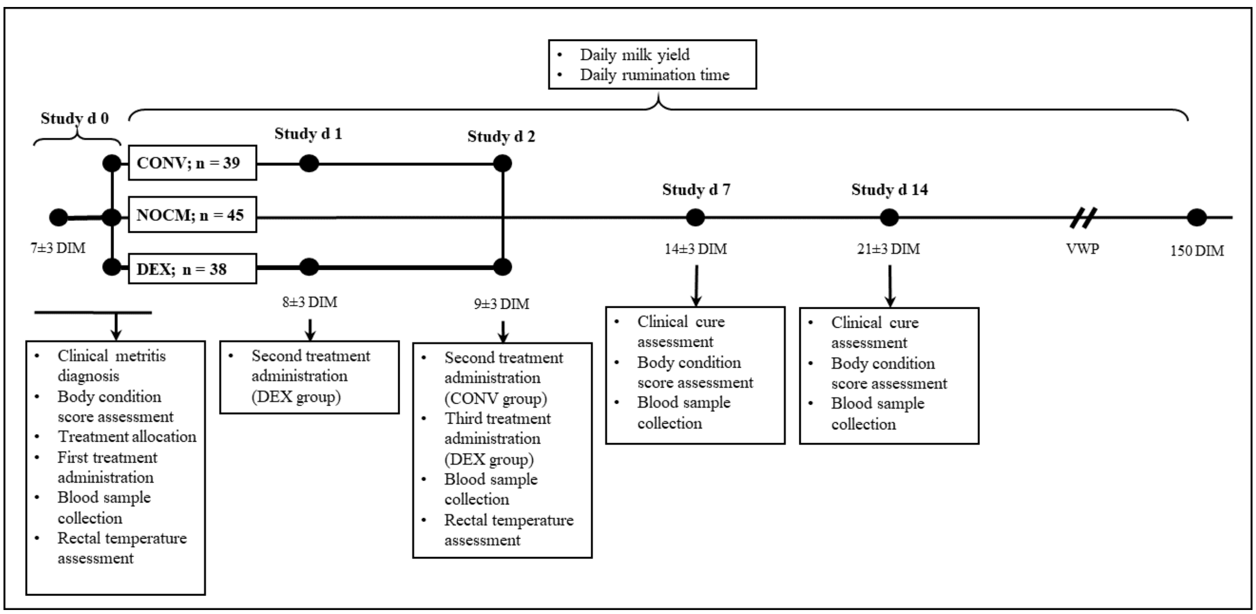
Animal Enrollment, Diagnosis of CM and Assessment of CM Cure Rate

Study animals were enrolled from May 2019 until August 2020. Briefly, a list of cows was obtained from on-farm computer records. Cows at 7±3 DIM and without any recorded previous health event from calving, such as lameness and displaced abomasum, were selected for screening. Eligible cows (n = 641) were screened for CM using the Metricheck device (Simcro Tech Ltd., Hamilton, New Zealand). Briefly, vaginal discharge was scored using a five-point scale (i.e., 1- clear fluid, 2- <50% white purulent fluid, 3- >50% white purulent fluid, 4- red-brownish fluid without fetid smell, and 5- fetid red-brownish watery fluid; Lima et al., 2014). Cows with a vaginal discharge score ≥ 4 were categorized as having CM.

Cows diagnosed with CM were blocked by parity and randomly allocated into one of two treatment groups (study d 0): 1) Dextrose (**DEX**): cows received IUI of 50% dextrose solution (1

L/cow; AgriLabs) every 24 hours for three days, or 2) Conventional (**CONV**): Two injectable (SC) administrations of ceftiofur (6.6 mg/Kg of BW; Excede, Zoetis Inc.) 72 h apart (Figure 1). A group of cows that had a vaginal discharge score of ≤2 (**NOCM** group) was randomly selected and matched by parity to DEX and CONV cows as a negative control group (Figure 1). For the DEX group, before each treatment, as much as possible abnormal fluid was siphoned out of the uterus using a stainless-steel half-inch infusion rod and transrectal uterine massage. Enrolled cows were re-screened to assess cure rate at Study d 7 and 14. A vaginal discharge score of ≤3 was considered as cure for CM.

Figure 1. Diagram of the study experimental design



Blood Sample Collection and BHB and HP Concentration Assessment

Blood samples were collected into a 10 ml vacutainer tubes on study days 0, 2, 7, and 14 (Figure 1). Samples were kept on ice until they were centrifuged (within ~2h) for 15 min at 1,400

× g, at room temperature (25°C) to collect serum. Serum was frozen at -20 °C until further analysis. Concentration of β-hydroxybutyrate was determined using a BHB handheld device (PortaCheck, Moorestown, NJ). Cows with a concentration above 1.2 mmol/L were diagnosed with subclinical ketosis (Iwersen et al., 2009). Haptoglobin concentration was assessed using a commercially available HP bovine ELISA kit (Life Diagnostics, West Chester, PA). The coefficient of variation for intraassay variability was 2.94% and the interassay variability was 3.99%. The analytical sensitivity of the assay was 3.91 ng/mL (lower limit of quantification) and 250 ng/mL (upper limit of quantification).

BCS, Rectal Temperature assessment, Daily Milk Yield and Daily Rumination Time

Body condition score (**BCS**) of enrolled animals was assessed on days 0, 7, and 14 (Figure 1) using a five-point scale (Ferguson et al., 1994). To maintain consistency, BCS were always assessed by the study team. Additionally, rectal temperature was recorded for DEX and CONV cows using a digital thermometer (AmerisourceBergen, Mandeville, LA) on study d 0 and 2.

Daily milk yields and daily rumination time were collected using parlor meters (SCR Dairy, Netanya, Israel) and electronic monitors (neck mounted electronic tags; HR tags; SCR Dairy, Netanya, Israel) from calving to 150 DIM.

Statistical Analysis

This randomized complete block design was analyzed using the SAS statistical software (version 9.4, SAS Institute Inc., Cary, NC). The UNIVARIATE procedure of SAS was used to assess the homogeneity and normality of variances (graphical method, such as histogram and Q-Q plot, and Barlett's tests; Shapiro-Wilk statistic) for the quantitative variables.

Continuous variables were analyzed with the MIXED procedure of SAS. For analysis of BCS, HP, BHB, daily milk yield and daily rumination, the REPEATED statement was included in the MIXED procedure. In addition, because the first sample (i.e., study d 0) for these variables was collected immediately before treatment administration, the study d 0 values were forced into these models as covariates to be used as baseline data between treatment groups (Montgomery et al., 2019). The covariate structures were selected using the best fit according to Schwarz's Bayesian information criteria. The variables that remained in the model were selected using the Wald statistic backward selection criterion ($P > 0.15$). The variable cow was included in the RANDOM statement of the MIXED procedure. The variable treatment, day, and day by treatment interaction (where the REPEATED statement was used) were forced in the models. The results are presented as least squares means (LSM) and standard error of the mean (SEM), calculated and adjusted with Tukey-Kramer method using the LSMEAN statement. When the interaction between treatment and day or other variable was significant ($P < 0.05$), the "slice" option in the "lsmeans" statement was used to determine differences among treatments on each level (single comparison) of the interacting variable. Due to lack of normality, the BHB (i.e., root square) and HP (LOG 10) variables were transformed. Results are presented as geometric means and backtransformed 95% confidence interval. The percentage of cows that cured (cure rate), subclinical ketosis and pyrexia was assessed using multivariable logistic regression models generated by the GLIMMIX procedure of SAS. The main variables of interest and their interactions were considered statistically significant if $P < 0.05$, and $0.05 < P < 0.10$ was considered a tendency.

Chapter 4

Results

Six hundred and forty-one Holstein dairy cows were screened for CM at 7±3 days after parturition. Seventy-seven cows were diagnosed with CM in the present study. The incidence of CM was found to be 21.08%. A total of 80 multiparous cows (DEX = 26; CONV = 26; NOCM = 28) and 42 primiparous cows (DEX = 12; CONV = 13; NOCM = 17) were enrolled in this cohort.

CM Cure Rate

There was no difference in CM cure rate at study d 7 (DEX = 61.12±8.25%; CONV = 68.59±7.95%;) or d 14 (DEX = 85.74±5.99%; CONV = 88.26±5.58%;) between DEX and CONV groups (Figure 2).

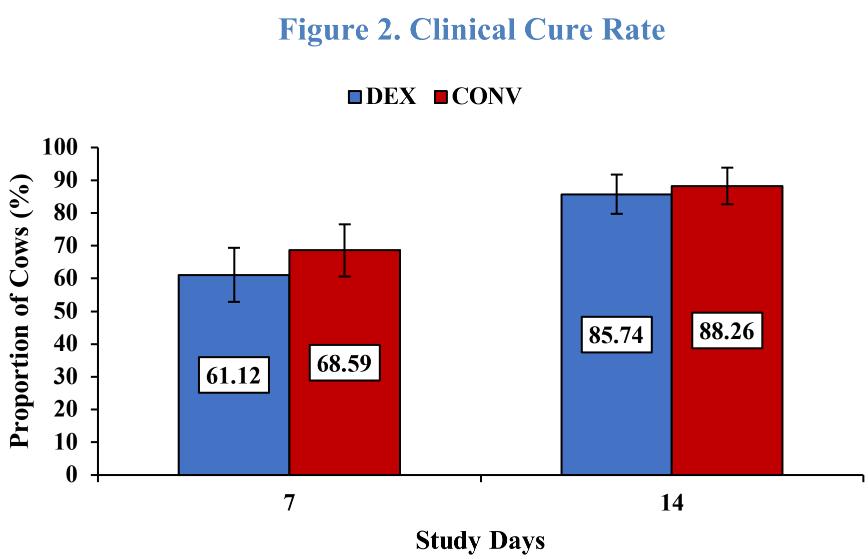


Figure 2. Clinical cure rate at study day 7 and 14 in dairy cows diagnosed with clinical metritis and were treated with either intrauterine infusion of dextrose (DEX) or injectable ceftiofur (CONV). Values are presented as LSM±SEM.

Pyrexia, BHB and HP Concentration Assessment

Although cows treated with DEX and CONV had overall higher HP concentrations compared with NOCM cows (*p* <.0001; DEX= 365.76 μg/mL; 95% CI: 283.85-471.19; CONV = 275.35 μg/mL, 95% CI: 208.16-364.16; NOCM = 95.60 μg/mL, 95% CI: 65.81-138.93; Figure 3), by study d 7 HP concentration of DEX and CONV did not differ from NOCM cows (Figure 3). NOCM cows had lower BHB concentrations at study d 0 compared with DEX and CONV cows (*p* = 0.02; DEX=1.68 mmol/L, 95% CI: 1.39-2.01; CONV = 1.63 mmol/L, 95% CI: 1.34-1.95; NOCM = 1.20 mmol/L; 95% CI: 0.97-1.45; Table 1), but there was no difference on BHB concentrations at study d 7 and 14 between study groups (Table 1). The incidence of subclinical ketosis and pyrexia of study groups is presented in Table 2. Cows in the DEX and CONV group had higher incidence of subclinical ketosis on study d 0 (*p* = 0.008; DEX = 66.68±7.95%; CONV = 77.16±7.17%; NOCM = 41.85±7.63%.; DEX=3.15±0.10 pts; Table 2). There was no difference in incidence of pyrexia between DEX and CONV cows (Table 2).

Figure 3. Serum Haptoglobin Concentration

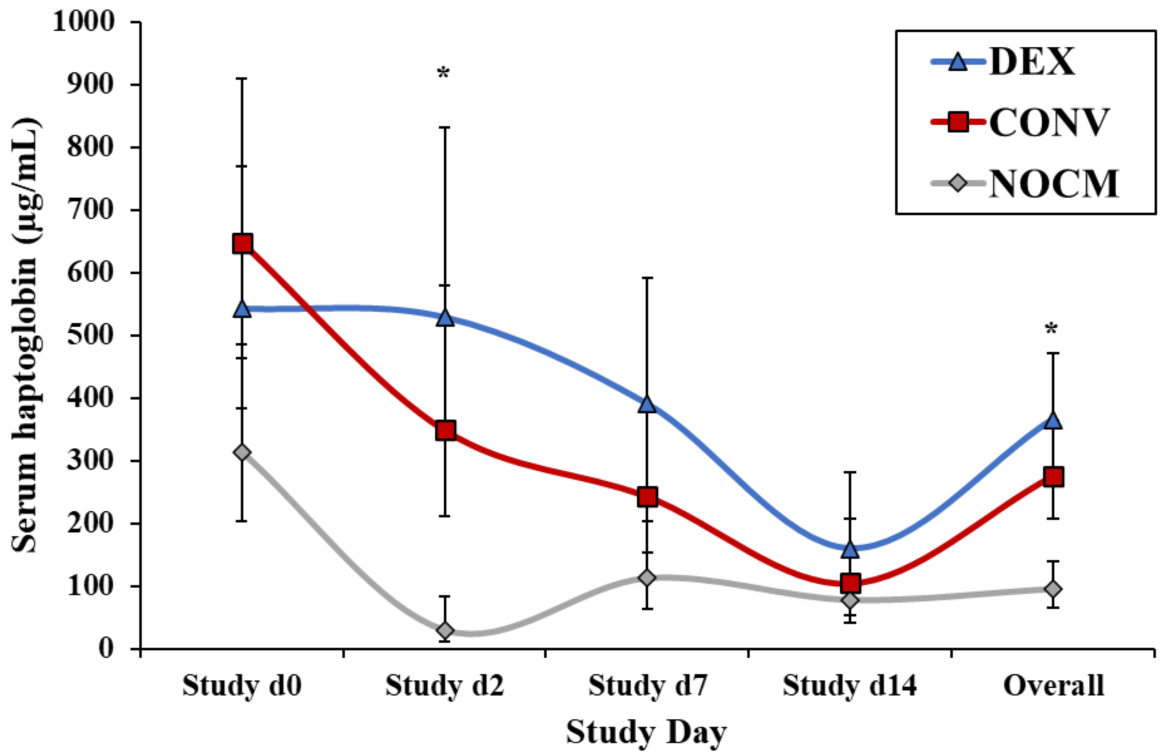


Figure 3. Serum haptoglobin concentration (µg/mL) at study d 0, 2, 7, 14 and overall, in cows diagnosed with clinical metritis and treated with either intrauterine infusion of dextrose (DEX) or injectable ceftiofur (CONV), and cows that did not have clinical metritis (NOCM) at 7±3 days after parturition. Values are presented as geometric mean ± 95% CI. * Indicates a *p*-value of <0.05

Table 1. Concentration of serum β-hydroxybutyrate (BHB) and body condition scores (BCS) in cows diagnosed with clinical metritis treated with an intrauterine 50% dextrose infusion (DEX), or with injectable ceftiofur (CONV), and non-clinical metritis cows (NOCM) at 7±3 days after calving.

		Treatment						
Variable	**Study day**	**DEX (n = 38)**	**95% CI**	**CONV (n = 39)**	**95% CI**	**NOCM (n = 45)**	**95% CI**	***P*-value**
Serum BHB[1]	Overall[i]	1.39	1.18-1.63	1.27	1.07-1.50	1.26	1.07-1.47	0.62
	Study d 0	1.68 [a]	1.39-2.01	1.63[a]	1.34-1.95	1.20 [b]	0.97-1.45	0.02
	Study d 7	1.27	1.01-1.55	1.14	0.90-1.41	1.26	1.01-1.54	0.74
	Study d 14	1.25	0.99-1.54	1.09	0.84-1.36	1.33	1.06-1.63	0.42
		DEX (n = 38)	.	**CONV (n = 39)**	.	**NOCM (n = 45)**	.	.
BCS	Overall[i]	3.32±0.09	.	3.41±0.09	.	3.48±0.08	.	0.25
	Study d 0	3.48±0.10	.	3.49±0.10		3.66±0.09	.	0.19
	Study d 7	3.33±0.10	.	3.47±0.09	.	3.40±0.09	.	0.34
	Study d 14	3.15±0.10	.	3.27±0.10	.	3.36±0.09	.	0.15

[a, b] different letters represent statistically significant at P-value <0.05.

[1] The values are presented in mmol/L as geometric means ± back-transformed 95% CI.

[i] Overall = concentration of BHB (Study d 0, d 7 and d 14) in study cows.

Table 2. Percentage (LSM ± SEM) of pyrexia and subclinical ketosis assessed by research team in cows diagnosed with clinical metritis treated with an intrauterine 50% dextrose infusion (DEX), or with injectable ceftiofur (CONV), and non-clinical metritis cows (NOCM) at 7±3 days after calving.

Variable		Treatment			
		DEX (n = 38)	CONV (n = 39)	NOCM (n = 45)	*P* -value
Pyrexia[1]	Study d 0	19.41±6.70	15.78±6.01	.	0.68
	Study d 2	19.93±8.19	15.99±7.50	.	0.72
Subclinical ketosis[2]	Study d 0	66.68±7.95[ab]	77.16±7.17[a]	41.85±7.63[b]	0.008
	Study d 7	22.83±8.39	15.66±6.60	50.98±11.02	0.05
	Study d 14	35.65±11.77	16.43±8.94	36.88±13.09	0.24

[a, b] different letters represent statistically significant at P-value <0.05.

[1] Percentage of cows with pyrexia, which was defined as a rectal temperature of >39.5°C, assessed by the research team.

[2] Percentage of cows with subclinical ketosis, which was defined as a cow that presented a BHB serum concentration of > 1.2 mmol/dL assessed by the research team.

Body Condition Score, Daily Milk Yield, and Daily Rumination Time

DEX cows tended to have lower BCS at study d 14 compared with NOCM cows (p = 0.06; NOCM =3.36±0.09 pts.; DEX=3.15±0.10 pts; Table 1).

Overall, cows in the CONV and NOCM groups produced 3.67 kg/d and 3.68 kg/d more milk, respectively, compared with cows in the DEX group during the first 150 DIM (Figure 4).

There was a day by treatment interaction (p <.0001) in daily rumination time between study groups. Cows in the DEX and CONV groups had lower daily rumination time on d 4, 5, 6, 7 and 8 after calving.

Figure 4. Daily Milk Yield

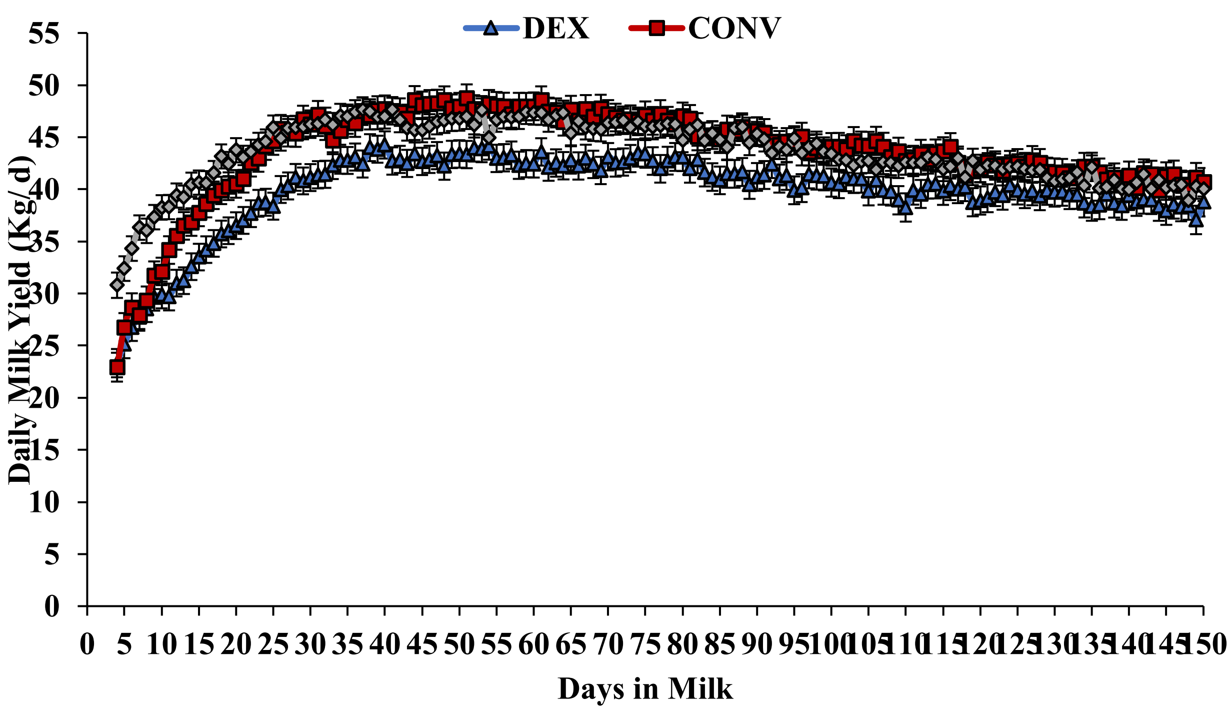


Figure 4. Daily milk yield (kg/day) during the first 150 days in milk in cows diagnosed with clinical metritis and were treated with either intrauterine infusion of dextrose (DEX) or injectable ceftiofur (CONV), and cows that did not have clinical metritis (NOCM) at 7±3 days after parturition. Values are presented as LSM±SEM.

Chapter 5

Discussion

The main results of this study were: 1) there was no difference in CM clinical cure rate between DEX and CONV cows at either 14±3 DIM or 21±3 DIM; 2) cows treated with DEX and CONV had overall higher HP concentrations compared with NOCM cows, but by study d 7 HP concentration of DEX and CONV did not differ from NOCM cows; and 3) Cows in the CONV and NOCM groups produced more milk compared with cows in the DEX group during the first 150 DIM.

Previous studies report conflicting results regarding the effectiveness of an IUI of dextrose on the clinical cure rate of postpartum dairy cows with uterine disease. A previous study on an organic dairy farm reported that a 1 L IUI dextrose treatment resulted in a 31.3% cure rate in cows diagnosed with CM (Maquivar et al., 2013). This suggests that IUI dextrose is not an effective treatment, especially when considering that self-cure rates for CM have been reported to be as high as 55.3% (McLaughlin et al., 2012). Other literature reports that a single 200 mL IUI of 50% dextrose solution increased the cure rate in dairy cows diagnosed with a milder uterine infection known as clinical endometritis (CE) (Brick et al., 2012; Maquivar et al., 2015). Cows diagnosed with CE and treated with dextrose had a similar clinical cure rate to those treated with a systemic antibiotic (Brick et al., 2012). Similarly, CE cows treated with dextrose had a higher cure rate than untreated cows (Brick et al., 2012; Maquivar et al., 2015). On the other hand, another study disagrees with these findings and reported that a single 200 mL IUI of 50% dextrose solution was detrimental to the health of CE cows (Machado et al., 2015). In the latter study, it was found that CE cows treated with dextrose had a clinical cure rate of 48.4% whereas untreated control CE cows had a cure rate of 63.2% (Machado et al., 2015). The inconsistencies in the reported clinical cure rates of cows treated with an IUI of dextrose may be the result of discrepancies in enrollment criteria and the differences in solute concentrations. The first explanation for a difference in findings is recognized by Machado et al. (2015) who discusses the inconsistency in the definition of CE and the definition of clinical cure between studies. While Brick et al. (2012) enrolled cows with mucopurulent or worse discharge, Machado et al. (2015) enrolled all cows that had anything more than flecks of pus in the discharge. Clinical cure in Brick et al. (2012) was defined as a cow with completely clear mucus whereas Machado et al. (2015) included cows with flecks of pus in the discharge as clinically cured. Another explanation for the inconsistent clinical cure rates relates to the concentration and dosage of the dextrose solution. Dextrose antimicrobial effects lies in the creation of a hyperosmotic environment, which dehydrates the microorganisms (Chirife et al., 1983). In this study, CM cows were treated with 1 L of IUI 50% dextrose for 3 consecutive days and abnormal fluid was siphoned out of the uterus right before each treatment, while in Maquivar et al. study (2013) cows were treated only once.

Our results show that DEX and CONV cows had higher concentrations of serum haptoglobin (HP) at study d 0 and study d 2, but not at study d 7 and d 14. It has been reported that CM cows have higher concentration of HP compared to NOCM cows (Barragan et al., 2018). By study d 0 and d 2, DEX and CONV cows were being treated for CM, while NOCM cows were healthy. By study d 7, around 50% of the DEX and CONV cows were cured, which may explain, at least in part, the lack of difference on HP concentration between treatment groups and control NOCM cows.

Our present study found no difference in milk production between CONV cows and NOCM; while DEX cows produced less milk in the first 150 DIM. Conversely, Piccardi et al.

(2016) reported that the antibiotic treatment did not reverse the negative impact of metritis on milk production when treating CM cows with 2.2 mg/kg of ceftiofur hydrochloride. A possible explanation for the difference in these results is the treatment frequency and dose used. In the present study, cows were treated with two injectable administrations of 6.6 mg/kg of BW, while Piccardi et al. (2016) did a single treatment with a dose of only 2.2 mg/kg of BW. It is interesting that the DEX cows did not produce as much milk as the other two treatment groups. One explanation for decreased milk production could be increased inflammation. A previous study assessed the effects of inflammation on dairy cow performance and found that when inflammation was most evident, particularly in the first month of lactation, there was a significant relationship between the severity of inflammatory phenomena and milk loss (Bertoni et al., 2008). Similarly, another study reported that inflammation during the transition period was directly associated with a reduced milk yield (Huzzey et al., 2015). However, this is not consistent with our study since CONV cows had the same HP concentrations as DEX cows yet produced as much milk as NOCM cows. More research is needed to fully understand the inability of an IUI of dextrose to restore milk production.

Chapter 6

Conclusion

These results suggest that the IUI of dextrose treatment used in this study is as effective as the conventional antibiotic treatment in curing postpartum dairy cows diagnosed with CM. However, the dextrose treatment was not as effective at restoring milk production. Future research should focus on refining the treatment protocols, such as treatment dose and frequency, of IUI dextrose as well as proposing additional measures to aid in restoring milk yield.

Chapter 7

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ACADEMIC VITA

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Education Highlights:

- Pennsylvania State University (Fall 2018- present)
  - College of Agricultural Sciences – Veterinary and Biomedical Sciences
  - Schreyer Honors College Class of 2022
- Seneca High School- Erie, PA
- Regional Choice Initiative program (RCI) (2016-2018)
- Cornell Summer Program 2017
  - Conservation Medicine from a Veterinarian's Perspective

Professional Experiences:

- Part Time Research Assistant with Penn State Veterinary Extension Team (2018-present)
  - On-farm sample collection, lab analysis, data entry, extension events, etc.
- Part Time Dairy Barn Worker- Penn State Dairy Barns (2021-present)
- Part Time Teaching Assistant (2020-present)
  - Lecture and grade homework/quizzes for Penn State ANSC 201 course
- Undergraduate Thesis- Assessment of the effects of intrauterine dextrose infusion on clinical cure rate, daily milk yield, daily rumination, metabolic stress and inflammation in post-partum dairy cows diagnosed with clinical metritis
  - 2020 ADSA Student Affiliate Division- 2nd place undergraduate student poster for original research/independent study
  - Presented at 2021 Penn State Extension Annual Conference
  - Presented at Penn State 2021 and 2020 Undergraduate Research Exhibition (virtual)
  - Presented at 2021 Gamma Sigma Delta Research Expo (virtual), abstract accepted for 2020 expo (expo cancelled)
- Dog walking (2019-present)
- 2020 Hospitality Chair for Penn State Pre-Vet Stayover Program- a three-day recruitment program for senior high school students
- Union City Pet Hospital (2016-2018)
- Secretary of Emmanuel Presbyterian Church Board of Deacons (2018)

Other Activities:

- Gamma Sigma Delta- honor society for agriculture and agricultural sciences (2020-present)
- Phi Eta Sigma Honor Society (2019-present)
- Penn State Pre-Vet Club Member (2018-present)
- Erie Animal Rabbit Society and Rescue Volunteer (2018)
- Emmanuel Presbyterian Church
  - Board of Deacons (2015 – 2018) – Shut-in / Hospital caller, worship service assistance
  - Pianist – Special music
- Second Harvest Food Bank Volunteer (2016)