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Assessment of the Effects of Intrauterine Dextrose Infusion After Calving on Uterine Health, Metabolic Stress, Systemic Inflammation and Daily Milk Yield in Post-partum Dairy Cows

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ABSTRACT

The objective of this study was to assess the effect of intrauterine dextrose infusion at 4±1 days in milk (DIM) on uterine health (i.e., incidence of clinical metritis), metabolic stress (i.e., body condition score [BCS], β-hydroxybutyrate [BHB] concentration), systemic inflammation (i.e., haptoglobin [HP] concentration), daily milk yield and reproductive performance in post-partum dairy cows. Cows (n = 245) from a dairy farm located in southwest Pennsylvania were screened at 4±1 DIM using a Metricheck® device to assess vaginal discharge. In addition, overall cow health (e.g., lameness) and rectal temperature was assessed at this time. Cows that presented a clear dense vaginal discharge, were in good health status, and had a normal rectal temperature (i.e., <39.7 °C; n = 134) were blocked by parity (primiparous [PRIM] = 64; multiparous [MUL] = 70) and randomly assigned to one of three groups: 1) SAL (n=45): one intrauterine infusion of saline solution (1 L/cow); 2) DEX (n=44): one intrauterine infusion of a 50% dextrose solution (1 L/cow), and 3) CON (n=45): cows remained untreated. The goal of the experiment was to determine the effectiveness of the dextrose treatment compared to control groups in preventing the development of clinical metritis in otherwise healthy animals. Cows were re-screened at 7 days after enrollment to assess uterine health. Body condition score was assessed and blood samples were collected at enrollment and at study day 7, 14 and 21. Furthermore, daily milk yield and clinical disease events (for the first 60 DIM) and reproductive performance data were collected from on-farm computer records. The data were analyzed using the MIXED and GLIMMIX procedures of SAS as a randomized complete block design. Although not significant (RCMI p = 0.13; FCMI p =0.11), the Research Clinical Metritis Incidence (RCMI) and Farm Clinical Metritis Incidence (FCMI) in DEX and SAL cows was on average 14.52 and 18.16 percentage points higher, respectively, compared to CON cows. SAL cows had higher BHB and HP concentration and higher incidence of subclinical ketosis compared to CON cows on study d 7. In regards to daily milk yield, there was a day by treatment interaction, where CON cows produced more milk in certain days compared to DEX and SAL cows. Based on these findings, authors conclude that intrauterine dextrose infusion in postpartum cows with normal vaginal discharge may be prejudicial for cow health and performance.

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Chapter 1

Literature Review

The transition period can be referred to as the 3 weeks before to 3 weeks after parturition (Drackley, 1999). It is a period marked by many physiological changes including those in lipid and energy metabolism, endocrine signaling and immune function (Drackley, 1999; LeBlanc, 2010; Sundrum, 2015; Trimboli et al., 2020). During this period, the cow's energy requirements change due to initiation of lactation and final development of the calf and placenta. Many of the physiological challenges can be attributed to energy deficiency caused by the drop in dry matter intake (DMI) and increase nutrient demands. As a simplistic view of this interconnected and complex set of events, the drop in DMI and increased nutrient demands causes negative energy balance (energy expenditures exceed energy intake), which causes generation of alternative body energy sources such as ketone bodies (Trimboli et al., 2020). Nutrients must be re-partitioned by a process referred to as homeorhesis (LeBlanc, 2010). Due to this repartitioning, many cows become nutrient deficient and enter a state of negative energy balance, especially since feed intake is reduced during this period. Furthermore, lactation not only increases energy requirement, which causes hypoglycemia, but also the calcium requirements which predispose cows to develop hypocalcemia (Trimboli et al., 2020). Changes in circulating metabolites, hormones and neuroendocrine factors drive physiological tissue remodeling and metabolic adaptation (Trimboli et al., 2020). Additional physiological challenges such as, insulin resistance, lipolysis, immune depression, bacterial contamination and weight loss may develop during the transition period (LeBlanc, 2010).

These physiological challenges can lead to an increased incidence of disease during this period. As the mammary gland and uterus prepare and overpass calving and lactation during the transitional period, microorganisms can gain access to these susceptible organs and cause infection. It is believed at least one third of transition period dairy cows experience a metabolic or infectious disease during this early period of lactation (LeBlanc, 2010). If not managed properly, these conditions can lead to negative outcomes for cow and farm productivity and economics due to a longer recovery before becoming able to become pregnant again (LeBlanc, 2010).

Common Health Events During the Transition Period

During the transition period, cows are more susceptible to experiencing health events, especially during the first three weeks following parturition (Drackley, 1999; LeBlanc, 2010; Sundrum, 2015; Trimboli et al., 2020). Health events such as dystocia, RFM and hypocalcemia occur shortly after calving while others may take several days or weeks to occur (Figure 1).

Dystocia can be defined as a difficult calving where a cow requires assistance in expelling the calf. Like metritis, it is often diagnosed on a 5-point scale of ease of calving where a 4-5 represent high difficulty and the need for calf repositioning or caesarian section (Machado et al., 2014). This condition increases the risk of later developing metritis (Bartolome et al., 2014) and other health events such as stillborn calves and retained fetal membranes (RFM).

Retained fetal membranes is a condition in which a cow does not expel the placenta within 24 hours after parturition (Deng et al., 2015). Although the cause of RFM is not fully elucidated yet, it is believed that an impaired immune function from reduction in neutrophil function and interleukin (IL)-8 levels around two weeks before calving can be an important contributing factor (LeBlanc, 2010). Retained fetal membrane cows tend to have significantly higher serum cortisol and non-esterified-fatty-acid (NEFA) levels, which could increase the likelihood of developing further infections such as metritis or endometritis (LeBlanc, 2010).

Milk fever or post-partum hypocalcemia is characterized as a calcium deficiency that results from failure of a cow to adapt to increased calcium demands during initiation of lactation (Domino et al., 2017). Milk fever, also known as clinical hypocalcemia (cows display clinical signs such as lethargy and inability to stand up) affects approximately 5% of periparturient dairy cows; while the subclinical form of hypocalcemia (no clinical signs) affects 50% of postpartum multiparous cows (Domino et al., 2017). Cows with hypocalcemia suffer from increased rates of metabolic diseases and conditions such as hyperketonemia (i.e., ketosis), displaced abomasum (DA), RFM, metritis and mastitis, decreased reproductive performance and decreased milk production (Domino et al., 2017).

Hyperketonemia is defined as an increase serum BHB concentration (BHB > 1200 micromoles/liter; LeBlanc, 2010). It could be classified as clinical ketosis (presence of clinical signs such as drop in DMI and milk yield) or subclinical ketosis (lack of clinical signs; LeBlanc, 2010). The clinical form occurs in cows at a rate of 5 to 10 % and subclinical ketosis occurs at a reported incidence of 40% within the first 3 weeks of lactation (LeBlanc, 2010). Ketosis is commonly diagnosed by assessing blood serum BHB concentration in cows during the first 14 DIM (LeBlanc, 2010). This condition is associated with reduced feed intake, lower milk production, higher risk of uterine disease and long-term reproductive health effects such as decreased pregnancy rates (LeBlanc, 2010).

Clinical mastitis can be defined as an intramammary bacterial infection with altered milk appearance and potentially a swollen or inflamed quarter (Domino et al., 2017; Sordillo, 2015). It is one of the most common diseases in dairy production, affecting 21% of the animals during the post-partum period (Gröhn et al., 2003). Mastitis majorly impacts producers economically due to treatment costs, reduced milk production for the rest of lactation, culling and milk contamination (Gröhn et al., 2003).

Displaced abomasum (DA) can be defined as the displacement of the fourth forestomach of the cow, the abomasum (Caixeta et al., 2018). This condition can affect around 3.5% of the cows in the herd. The most common presentation is the left DA, where the abomasum displaced to the left side of the abdominal cavity in between the rumen and the abdominal wall (Caixeta et al., 2018). This disease is often diagnosed by the presence of a high-pitched ping in response to physical stimulation on the right or left side of the cow (Risco and Hernandez, 2003). Elevated serum NEFA concentrations have been associated with an increased risk of displaced abomasum development (LeBlanc, 2010). This condition can lead to a cow becoming lame, an increasing risk of fatty liver and associated conditions and reduce the amount of milk produced by the animal (Drackley, 1999).

Lameness can be defined as the inability to move normally (Leno et al., 2018). Lame animals may suffer reduced locomotion due to an illness or injury and may display sign such as a reduced response to stimuli, abnormal gait, hunched-back walking, limping, favoring of a limb while walking or similar signs (Leno et al., 2018). The incidence of lameness varies widely but tend to be lower in primiparous animals and up to 36% in multiparous animals (Leno et al., 2018).

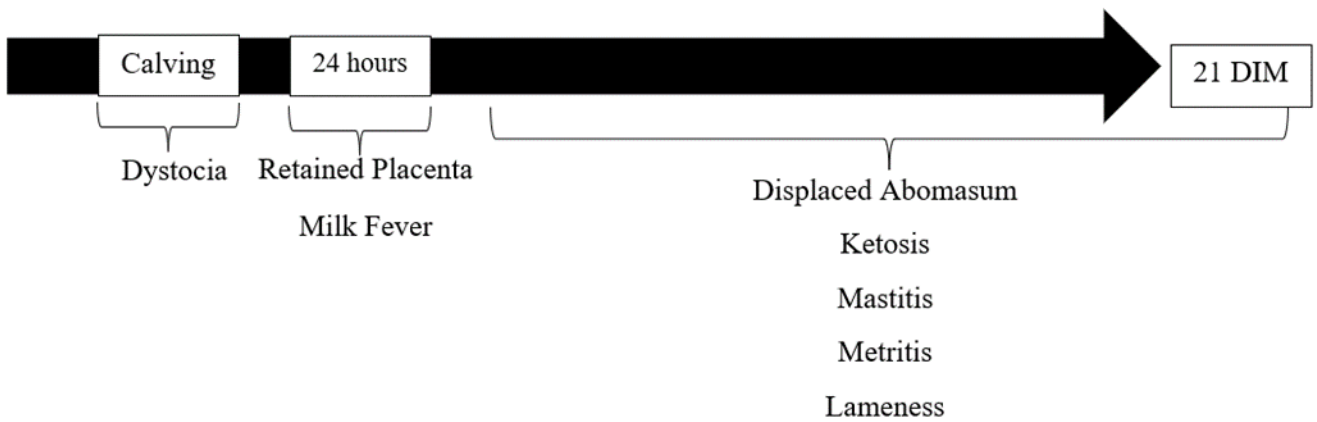

Figure 1: Timeline of post-partum health events in dairy cattle.

Clinical Metritis in Dairy Cattle

Definition

Metritis can be defined as the inflammation of the uterus due to bacterial infection that typically occurs during the first 21 days after calving (Genis et al., 2018). Metritis can affect up to 40% of dairy cows (Genis et al., 2018). Risk factors for this prevalent condition include difficult births and poor nutritional status (Bartolome et al., 2014).

Puerperal metritis can be defined as where a cow would have an abnormally enlarged uterus and a fetid watery red-brown discharge of the uterus and presents with signs of systemic illness and fever, while clinical metritis is where animals have abnormally enlarged uterus with purulent uterine discharge within 21 days after parturition but do not seem ill (Sheldon et al., 2006). The most common signs of puerperal metritis include systemic signs of sickness such as a fever over 39.5◦C, red-brown watery foul-smelling vaginal discharge, dullness, inappetence, elevated heart rate and low milk production (Machado et al., 2014; Sheldon et al., 2006). Clinical endometritis occurs when purulent or mucopurulent discharge from the uterus is found in the vagina after 21 days since partition (Sheldon et al., 2006). Metritis causes important economic losses to the dairy industry resulting from increased culling rates, treatment costs and delayed or altered ability to become pregnant again (Machado et al., 2014). The focus of this thesis will be on clinical metritis.

Animals are at a high risk for the development of any form of metritis during the transition period or after calving. During this time the immune system is weakened and the uterus is open for bacteria to enter since it is still in the process of healing. Cows with prior calving related disorders such as dystocia, RFM, acute hypocalcemia, twins, abortion, or stillbirths, are at an increased risk of developing metritis (Bartolome et al., 2014). Additional risk factors include reduced feed intake before calving, reduction in body condition score, parity and season (Bartolome et al., 2014).

Importance of Preventing Metritis

Preventing all forms of metritis is important for many reasons including the welfare of the animals and profitability of the farm. It has been reported that cows that had metritis in the first week after calving had an increased concentration of pain biomarkers (Barragan et al., 2018). Uterine infections such as metritis predispose dairy cows to impaired reproductive performance and often increase the likelihood of cows of being culled (Deng et al., 2015). In addition to increased losses due to culling, economic loss for producers is incurred from reduced fertility, treatment cost and lower milk production (Bartolome et al., 2014). On average, treating metritis on a farm can cost $329–386 in the United States, due to antibiotic treatment and the negative effects of metritis on reproductive performance, milk production and survivability (Machado et al., 2014). These economic losses could be devastating to producers if a large proportion of their animal become sick during the transition period (Machado et al., 2014). Additionally, since metritis in general is one of the most prevalent diseases in dairy cows, preventing this disease could significantly decrease the use of antibiotics in dairy farms contributing to decreasing antimicrobial resistance (Machado et al., 2014).

Causal Agents

The most common bacteria contributing to the development of uterine diseases including metritis are *Escherichia coli*, *Trueperella pyogenes* and *Fusobacterium necrophorum* (Machado et al., 2014). Damage of the uterine lining tissue from parturition allow for bacterial invasion and inflammation that are characteristic of metritis (Genis et al., 2018). Initially, during the post-partum period, *E. coli* is the predominant bacteria to infect the uterus which then allows for infection from *F. necrophorum* and *T. pyogenes* to follow (Machado et al., 2014). Overall, the inflammation in the uterus is caused by the need for substantial tissue repair while fighting off bad bacteria resulting in part recruiting of immune cells (Genis et al., 2018).

Diagnostic Methods

Metritis of all types primarily is diagnosed based on the presence of a reddish-brown, watery and fetid odor vaginal discharge, with or without fever (Barragan et al., 2018). Assessment of the vaginal discharge is more commonly performed using a Metricheck device (a soft rubber hemisphere connected to a stainless-steel rod; Genis et al., 2018). These samples may be scored 1-5 based on severity with 4 and 5 indicating a more severe case of metritis (Barragan et al., 2018, 2019). Cytology and uterine biopsy can also be used as a diagnostic method; however, it is in many cases impractical due to cost and potential reproductive concerns (Barlund et al., 2008). More common diagnostic techniques include gloved hand rectal palpation to obtain and evaluate vaginal mucus (LeBlanc, 2010).

Treatment and Alternatives

Traditionally metritis has been treated by using antibiotics, prostaglandins and nonsteroidal anti-inflammatory drugs (Bartolome et al., 2014), being systemic antibiotic treatment, the most common treatment implemented in conventional dairy farms. However, with the rising antibiotic resistance concern in the veterinary medicine field, alternative methods of metritis prevention are in high demand. Typical antimicrobial treatments for this condition have a 67–77% reduction in fever after 5–10 days of treatment without the guarantee of removing of the fetid odor vaginal discharge associated with metritis (Genis et al., 2018). Additionally, these treatments may not have positive effects on the impaired reproductive performance of metritic cows (Genis et al., 2018). Several other alternative methods have been investigated including the use of probiotic strains, calcium supplementation and vaccination. These will be discussed later.

Methods for Preventing/Treating Metritis

Several intervention methods for either preventing or treating metritis have been investigated. The most popular include intrauterine/vaginal infusions, injectable medications and calcium supplementations.

Local Treatments

Intrauterine/Intravaginal Infusions

The most effective prevention investigated thus far in the literature has been intrauterine and intravaginal infusions, though they have also been used therapeutically. One such study explored using an intravaginal administration of lactic acid bacteria (LAB), a common probiotic. This study compared two intravaginal administration doses of LAB per week during the 3 weeks prior to calving with an intra-uterine dose of LAB 1 day after calving with the goal of preventing metritis. Overall, this study found cows receiving pre-partum vaginal treatment had a lower incidence of metritis development compared to the control, but the intra-uterine group had no effect on metritis incidence (Genis et al., 2018). Another study, by the University of Alberta, aimed at determining if intravaginal infusion of a LAB could reduce uterine disease prevalence during the postpartum period and benefit the immune system. This study evaluated 3 treatment regimens: 1) administered 2 consecutive LAB doses on a weekly basis beginning 2 weeks after parturition and 1 carrier dose the week after; 2) administered 3 consecutive LAB doses; and 3) administered 3 consecutive carrier doses (control group). Significant positive findings from this study include results showing that LAB treatment decreased incidence of clinical metritis and other uterine diseases such as clinical endometritis, also it could lower the concentrations of systemic lipopolysaccharide-binding protein (LBP) and serum amyloid A (SAA) (Deng et al., 2015). Additionally, from the therapeutic side of this type of treatment, Maquivar et al. (2015) treated cows diagnosed with clinical endometritis at 26±3 DIM with an 200mL Intrauterine Infusion (IUI)of 50% dextrose solution and compared treated cows to a control group finding that treated cows had higher clinical cure rates, improved reproductive performance and faster return to a normal estrous cycle.

Systemic Treatments

Vaccines and injectable antibiotics

Vaccinations and injectable medications have been investigated and proved to be somewhat effective at preventing the development of metritis. Both antibiotics and bacterial protein vaccine injections have been cited to reduce the incidence of metritis (Dubuc et al., 2011; Machado et al., 2014). It has been shown that multivalent vaccines are some of the most effective preventive strategies (Machado et al., 2014).

Injectable systemic antibiotic administrations have been investigated as a preventive strategy for clinical metritis. One study conducted in Argentina investigated the effect of 1.1 mg/kg of ceftiofur hydrochloride on three consecutive days on the development of metritis versus a control (Bartolome et al., 2014). The study found a reduction in metritis only in high risk (prior calving-disorders) cows who had high body condition scores. Another study, that focused on assessing prostaglandin and antibiotic treatments as clinical metritis preventive strategies, administered ceftiofur crystalline free acid 24 hours after parturition at a dose of 6.6

mg/kg via a subcutaneous injection in the ear to their experimental groups. They also investigated the effect of 25 mg dinoprost administered intramuscularly both on days 35 and 49 days after calving. The only significant results from this study were that ceftiofur crystalline free acid treatment reduced incidence of metritis in high-risk cows without RFM (Dubuc et al., 2011).

Great advances have been done in the development of metritis vaccines. Cornell University investigated the effects of subcutaneously immunizing cows with inactivated, purified *Escherichia coli, Trurperella pyogenes* and *Fusobacterium necrophorum* bacterial proteins. In that study, the five vaccines were created. The first two were whole inactivated bacterial cells of *E. coli, T. pyogenes and F. necrophorum* and the proteins FimH, PLO and LKT in either the intravaginal or subcutaneous versions. The second two were made of the proteins FimH, PLO and LKT both an intravaginal and subcutaneous version. The final consisted of whole inactivated bacterial cells of *E. coli, T. pyogenes and F. necrophorum* in a subcutaneous form. Each vaccine was given twice at days 230 and 260 of pregnancy. The study concluded subcutaneous, but not intravaginal, vaccinations including inactivated bacterial components and/or protein subunits of *E. coli, F. necrophorum and T. pyogenes* did reduce the prevalence of metritis during the first lactation of dairy cows (Machado et al., 2014).

Overall, systemic treatments seem as though they are a promising prevention method for preventing metritis; however, they may be limited to reduce its incidence in certain populations of animals and therefore a more effective preventive method would be beneficial for the dairy industry.

Oral Calcium Supplementation

A third method that has been investigated for preventing metritis is via oral calcium bolus supplementation. This method is used to target the hypocalcemia challenge many cows face in the transition period that plays a role in weakened immune functions. Studies have shown that cows with hypocalcemia have more extreme negative energy balance and are more susceptible to develop metabolic conditions and metritis after parturition (Leno et al., 2018). This is also linked to a greater risk of being culled early on lactation and having a poor reproductive performance (Leno et al., 2018).

Several studies have been conducted to determine the effect of oral calcium supplementation on preventing health conditions including clinical metritis. In one study, cows were given 54 and 64 grams of calcium boluses postpartum. This study found that the calcium supplementation proposed had no effects on metritis prevention (Leno et al., 2018). Another study that investigated a similar calcium treatment supplementation found no effect on the incidence of metritis or other transition period health conditions (Domino et al., 2017). In that study, oral administration of 2 calcium boluses containing 43 grams of calcium, one after calving and one 12 hours later, was performed (Domino et al., 2017). Overall, the literature does not support that oral calcium supplementation shortly after calving decreases clinical metritis incidence during the post-partum period.

Problem Statement and Study Rationale

Clinical metritis is one of the most prevalent diseases in dairy cows, and greatly impairs animal welfare and profitability in dairy farms. For producers, there are documented reductions in milk yield, reproductive performance plus the cost of treatment. This disease costs the industry $650 million each year (Sheldon et al., 2009). For the animals, they face an increased risk of culling and discomfort that affects their quality of life (Deng et al., 2015). Preventing these diseases could increase the efficiency of the dairy industry, maximize profits by minimizing losses, all while increasing the welfare of production animals. However, current preventive practices are ineffective, focus on treatment rather than prevention, or rely on injectable/intrauterine antibiotics that contribute to the antimicrobial resistance (Espadamala et al., 2018).

The goal of this study is to investigate the effects of intrauterine 50% dextrose solution on prevention of clinical metritis in postpartum dairy cows, as well as on milk yield, body condition score and systemic inflammation. Intrauterine dextrose has been shown to effectively cure clinical metritis and clinical endometritis (Maquivar et al., 2015; Hamilton et al., 2020); however, its effects on metritis prevention have not been assessed yet. This strategy may offer a cost-effective and easy-to-implement treatment protocol to prevent clinical metritis. We hypothesized that cows treated with intrauterine infusion of dextrose would have lower incidence of clinical metritis, lower BHB and HP concentrations and higher daily milk yield and BCS compared to cows treated with intrauterine infusion of saline solution or that remained untreated.

Chapter 2

Introduction

The uterus of a dairy cow is free of pathogenic microbes throughout the pregnancy; however, during and after parturition the reproductive tract can become contaminated with a wide variety of bacteria (Sheldon et al., 2009). While many cows can naturally overcome this bacterial contamination, around 13.5%, and up to 40%, of cows will develop a persistent bacterial infection, known as clinical metritis (CM; Sheldon et al., 2006; Sheldon et al., 2008). CM is characterized by the presence of a watery, red-brownish, fetid-smelling vaginal discharge within 21 days of parturition, without systemic signs of illness (e.g., fever, anorexia; Sheldon et al., 2006).

Clinical metritis causes important economic losses to the dairy industry such as decreased milk yield, decreased reproductive performance and increased culling rate (Fourichon et al., 2000; Gröhn et al., 2003; Sheldon et al., 2006). It is estimated that the annual cost of uterine disease in the U.S. is around $650 million (Sheldon et al., 2009) as each case of CM has been estimated to be approximately $329–386 when accounting for antibiotic treatment, decreased reproductive performance, milk production and survivability of the animal (Machado et al., 2014). Additionally, it has been reported that CM has negative effects on cow welfare. For instance, Barragan et al. (2018), reported that cows with CM had higher concentrations of substance P (biomarker of nociception) and haptoglobin (HP), and that primiparous (PRIM) cows with CM spent more time lying down compared to PRIM cows without CM. Due to the important economical and animal welfare impacts of this disease for the dairy industry, it is imperative to identify effective preventative strategies.

The effects of intrauterine infusion (IUI) of solutions, such as solutions containing lactic acid bacteria (LAB), after calving has been investigated for their ability to enhance immune response and lower incidence of uterine disease. In one study, where cows were treated with two intravaginal administrations of LAB per week during the 3 weeks prior to calving or received an intra-uterine dose of LAB 1 day after calving, cows that received pre-partum vaginal treatment had a lower incidence of metritis compared to the control (Genis et al., 2018). Another study evaluated 3 intravaginal treatment regimens: 1) administered one LAB dose at week -2 and -1 (related to expected calving date) and 1 dose of the carrier solution the week after calving; 2) administered one LAB dose at week -2 and -1 and 1 week after calving; and 3) administered one dose of carrier at week -2 and -1 and 1 week after calving (control group). The authors reported that cows treated with regimens 1 and 2 had a 29 and 31 percentage points decrease, respectively, on the incidence of uterine infections (e.g., clinical metritis, clinical endometritis, pyometra), respectively, compared to cows treated with regimen 3 (control group). Although pre-partum intravaginal administration of LAB may have potential to prevent uterine diseases; the variability of predictive calving dates for which this strategy greatly relies on may challenge the use of this approach in conventional dairy farms, and more applicable strategies should be assessed.

Great advances have been done in the development of CM vaccines; however, the few published studies available showed inconsistent results. For instance, Machado et al. (2014) reported that cows treated with three formulations of metritis vaccines administrated subcutaneously 60 days and 30 days before expected calving date decreased metritis incidence and improve reproductive performance of treated cows compared to control animals. More recently, Freick et al. (2017) vaccinated 142 Holstein heifers subcutaneously at 6 and 3 weeks prior to expected calving date with a multivalent herd-specific vaccine produced with the most common bacteria isolated from uterus of cows at this farm and reported no difference in metritis incidence between treatment groups (CM incidence in vaccinated group = 46% vs CM incidence in unvaccinated = 48.9%).

Intrauterine infusion of a 50% dextrose solution has proven to be effective in treating uterine diseases. It has been reported that the use of hypertonic solutions such as sucrose or dextrose inhibited the growth or adhesion of common CM causing bacteria such as E. coli (Chirife et al., 1983; King et al., 2000). Maquivar et al. (2015) treated cows diagnosed with clinical endometritis with an IUI of 50% dextrose solution and found that treated cows had higher clinical cure rates, improved reproductive performance and faster return to a normal estrous cycle when compared to the untreated cows. Similarly, a more recent study (Hamilton et al., 2020) reported that CM cows treated with 3 consecutive daily treatments of 1 L of 50% dextrose IUI had similar clinical cure rates than cows treated with two systemic antibiotic treatments (i.e., ceftiofur). Nevertheless, the effects of dextrose IUI on uterine health and cow performance early after calving has not yet been assessed.

The objective of this study was to assess the effect of intrauterine dextrose infusion at 4±1 days in milk on incidence of clinical metritis, metabolic stress (i.e., body condition score [BCS], β-hydroxybutyrate [BHB] concentration), systemic inflammation (i.e., HP concentration) and daily milk yield in post-partum dairy cows. We hypothesized that cows treated with IUI of a 50% dextrose solution would have lower incidence of CM, lower BHB and HP concentrations, and higher daily milk yield compared to cows treated with IUI of saline solution or untreated control cows.

Chapter 3

Materials and Methods

Animals, Facilities and Feeding

Post-partum (4±1 DIM) Holstein dairy cows (n = 245) from a dairy farm located in Central Pennsylvania were enrolled in the present study. Pregnant heifers and cows were moved to a far-off pen at 60 days prior to their expected calving date and housed separately. At 28±3 days prior to expected calving date, pregnant heifers and cows were moved to a close-up pen and commingled together until calving. At the onset of calving (i.e., imminent signs of parturition observed by farm personnel), heifers and cows were move to individual straw bedded calving pens and remained there until calving was finalized and colostrum was milked. Then, PRIM and multiparous (MULT) cows were moved, and commingled together, into a post-partum pen until 21 days. Dry and post-partum cow were housed in 6-row freestall barns with water mattresses and bedded with sawdust, and a TMR diet was delivered twice daily. The TMR diet was formulated to meet or exceed dietary nutritional requirements for high-producing lactating dairy cows (NRC, 2001). Postpartum cows were milk three times daily. All the procedures described below were approved by the Institutional Animal Care and Use Committee at The Pennsylvania State University (Protocol number 202001502).

Animal Enrollment and Treatment

Two-hundred and forty-five dairy cows were screened for CM at 4±1 days after parturition using a Metricheck® device. Only cows that presented a clear dense vaginal discharge, were in good health status (did not present with any visual signs of disease) and had a normal rectal temperature (i.e., <39.7 °C) were included in the study (n=134; PRIM = 64; MULT = 70). Study cows were blocked by parity and randomly assigned (study d 0) to 1 of 3 treatment groups: CON (n=45): No treatment was administered, DEX (n=44): One IUI of a 50% dextrose solution (1 L/cow; VetOne®, Boise, Idaho, 83705) and SAL (n=46): One IUI of a saline solution (1 L/cow; VetOne®, Boise, Idaho, 83705).

Assessment of β-hydroxybutyrate, haptoglobin and body condition score

Blood samples were collected at study d 0, d 7, d 14 and d 21 to assess serum circulating concentration of HP and β-hydroxybutyrate (BHB). Samples were collected from coccygeal blood vessels into 8.5-mL evacuated sterile serum tubes, placed on ice immediately after collection and centrifuged (15 min at 1,400 × g, at room temperature [25°C]) within 2 h of collection to harvest serum, which was stored at −20°C until further analysis. An electronic hand-held device (PortaCheck, Moorestown, NJ) was used to assess serum concentrations of BHB. The serum concentration of HP was determined in a randomly selected subset of animals (DEX = 10; SAL = 11; CON = 11) at study d 0, d 7, d 14 and d 21 using a commercially available bovine haptoglobin ELISA kit (Life Diagnostics, West Chester, PA) following the manufacturer's instructions. All samples and standards were analyzed in duplicate wells. The intra-assay (within plate) coefficient of variation ranged from 2.87% to 5.29% and the interassay (between plates) coefficient of variation ranged from 1.73% to 4.02%. The analytical sensitivity of the assay was 3.91 ng/mL (lower limit of quantification) and 250 ng/mL (upper limit of quantification). Body condition score was assessed at study d 0, d 7, d 14 and d 21 using a 5-point scale (Ferguson et al., 1994).

Assessment of pyrexia, incidence of diseases, milk yield and reproductive performance

Rectal temperature was measured using an electronic thermometer (Cotran Corporation, Portsmouth, RI) at study d 7. Pyrexia was defined as a rectal temperature of >39.5°C (Jeon et al.,2016). Enrolled cows were screened for CM at study d 7 using the Metricheck® device. Briefly, vaginal discharge was scored using a 5-point scale (Barragan et al., 2019). Cows that were screened by the research team with the Metricheck® device and presented a watery, red-brownish discharge were categorized as having CM (RCMI; Barragan et al., 2018). On-farm computer records (i.e., Dairycomp 305) were also assessed for collecting CM incidence (FCMI), as well as other clinical disease events, such as mastitis and clinical ketosis, recorded by farm personnel in the first 60 DIM. Cows were classified based on the number of clinical disease events (CDEVT), as having no clinical disease events recorded or having one or more clinical disease events recorded. Serum BHB values were used to assessed subclinical ketosis incidence at study d 7. Subclinical ketosis was defined as a cow that presented a BHB serum concentration of > 1.2 mmol/L (Iwersen et al., 2009). Daily milk yield was collected for enrolled cows for the first 60 DIM from on-farm computer records (GEA Farm Technologies, Naperville, IL). On-farm computer records were used to collect reproductive performance data (i.e., DIM to conception [DIMC], number of services required to conceive [SPC], pregnancy per AI at first service [PAI]).

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 and daily milk yield, 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 variables originally offered to the models as fixed effects were CDEVT, subclinical ketosis at 7 ± 3, 14 ± 3 and 21 ± 3 DIM, BCS at 7 ± 3, 14 ± 3, and 21 ± 3 DIM, sold and died. 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 variable was transformed (i.e., root square). Results are presented as geometric means and back transformed 95% confidence interval.

The percentage of cows that had RCM, FCM, pyrexia at study d 7, subclinical ketosis at study d 7 and CDEVT were 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

There was a total of 134 cows enrolled in this study (DEX PRIM = 21, DEX MULT = 23; SAL PRIM = 21, SAL MULT = 24; CON PRIM = 22, CON MULT = 23). Enrolled cows were classified based on the number of clinical diseases farm personnel recorded during the first 60 DIM: CDEVT, NO-EVT (DEX PRIM = 11, DEX MULT = 17; SAL PRIM = 13, SAL MULT = 17; CON PRIM = 16, CON MULT = 17), EVT (DEX PRIM = 10, DEX MULT = 6; SAL PRIM = 8, SAL MULT = 7; CON PRIM = 6, CON MULT = 6). There were 8 cows that left the herd before 60 DIM (DEX Sold = 5; SAL Sold = 1; CON Sold = 0; DEX Died = 0; SAL Died = 1; CON Died = 1). Therefore, a total of 39 cows (PRIM = 21; MULT = 18), 43 cows (PRIM = 20; MULT = 23) and 44 cows (PRIM = 22; MULT = 22) remained in the DEX, SAL and CON groups, respectively.

Assessment of β-hydroxybutyrate, haptoglobin and body condition score

For BHB, the variables that remained in the model were day, treatment, day by treatment interaction, parity, BHB concentration at study d 0 and FCMI. For HP, the variables that remained in the model were day, treatment, day by treatment interaction, HP concentration at study d 0 and CDEVT. For analysis of BCS, day, treatment, day by treatment interaction, parity and BCS score at study d 0 remained in the model.

Overall, cows in the CON group had lower BHB concentrations compared to DEX and SAL cows (DEX = 1.11 mmol/L; 1.02-1.20 95% CI; SAL = 1.13 mmol/L; 1.05-1.23 95% CI; CON = 0.96 mmol/L; 0.88-1.05 95% CI; $p = 0.006$; Table 1). There was no difference in HP concentration between treatment groups (DEX = 119.72±21.89 μg/mL; SAL = 140.62±21.61 μg/mL; CON = 122.36±18.27 μg/mL; p = 0.65); however, there was a tendency for a day by treatment interaction (p = 0.07). Cows treated with SAL had higher concentration of HP on study d 7 compared to CON cows (DEX = 137.35±31.00 μg/mL; SAL = 181.23±30.09 μg/mL; CON = 86.26±27.78 μg/mL; p = 0.05; Table 1). There was no difference in BCS between treatment groups (DEX = 3.59± 0.03 pts.; SAL = 3.57±0.03 pts.; CON = 3.57±0.03 pts.; p = 0.53; Table 1).

Table 1 Concentration of serum β-hydroxybutyrate (BHB; Overall, study d 0 and 7) and haptoglobin (HP; study d 0, 7, 14 and 21) in cows treated with an intrauterine 50% dextrose infusion (DEX), or with an intrauterine saline infusion (SAL) or that remained untreated (CON) at 4±1 days after calving.

				Treatment				
Variable	**Study day**	**DEX (n = 44)**	**95% CI**	**SAL (n = 45)**	**95% CI**	**CON (n = 45)**	**95% CI**	***P*-value**
Serum BHB[1]	Overall[2]	1.11[a]	1.02-1.20	1.13[a]	1.05-1.23	0.96[b]	0.88-1.05	0.006
	Study d 0	1.04	0.93-1.16	0.97	0.87-1.08	0.97	0.86-1.09	0.63
	Study d 7	1.18[ab]	1.04-1.34	1.31[a]	1.17-1.47	0.96[b]	0.84-1.09	0.0008
		DEX (n = 10)	.	**SAL (n = 11)**	.	**CON (n = 11)**	.	.
Serum HP[3]	Overall	119.72±21.89	.	140.62±21.61	.	122.36±18.27	.	0.65
	Study d 0	181.23±31.00	.	142.26±30.08		179.88±27.78	.	0.52
	Study d 7	137.35±31.00[ab]	.	181.23±30.09[a]	.	86.26±27.78[b]	.	0.05
	Study d 14	77.62± 31.00	.	107.60±30.09	.	105.43±27.78	.	0.68
	Study d 21	82.69±31.00	.	131.37±30.09	.	117.85±27.78	.	0.43

[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.

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

[3] The values are presented in μg/mL as LSM± SEM.

Assessment of pyrexia, incidence of diseases, milk yield and reproductive performance

For RCMI, FCMI and subclinical ketosis the variables that remained in the model was treatment and parity, while for pyrexia and CDEVT only treatment remained in the model. The percentage of cows with RCMI, pyrexia, subclinical ketosis, FCMI and CDEVT can be found in Table 2. There were no differences in RCMI (DEX = 28.09±8.87%; SAL = 34.5±9.27%; CON = 12.64±5.77%; p = 0.13) and FCMI (DEX = 18.90±6.15%; SAL = 20.21±6.21%; CON = 5.72±3.36%; p = 0.11) incidences between treatment groups (Table 2). Regardless of treatment, PRIM cows had higher RCMI (PRIM = 37.11±7.48%; MULT = 14.00±5.23%; p = 0.01) and FCMI (PRIM = 22.70±5.57%; MULT = 7.39±3.12%; p = 0.01) incidences compared to MULT cows (Table 2). Cows treated SAL tended to have higher incidence of subclinical ketosis compared with CON cows (SAL = 49.84±9.90%; CON = 21.64±7.19%; p = 0.08; Table 2). Interestingly, regardless of treatment, MULT cows had a higher incidence of subclinical ketosis compared to PRIM cows (PRIM = 22.70±5.57%; MULT = 7.39±3.12%; p = 0.01; Table 2).

For daily milk yield, the variables that remained in the model were day, treatment, day by treatment interaction, parity and CDEVT. There was no difference in daily milk yield between treatment groups (DEX = 39.63±1.42 kg/day; SAL = 39.37±1.26 kg/day; CON = 41.53±1.14 kg/day; p = 0.36). However, there was a tendency for a day by treatment interaction (p = 0.10), where cows in the CON group had higher milk yields in several days compared to DEX and SAL cows (Figure 2). There was no difference on DIMC (DEX = 120 d; 104-138 95% CI; SAL = 133 d; 116-153 95% CI; CON = 126 d; 108-147 95% CI; p = 0.20), SPC (DEX = 2.14±0.26 srv.; SAL = 2.42±0.25 srv.; CON = 2.38±0.27 srv.; p = 0.38) or PAI (DEX = 67.75±8.51 %; SAL = 51.35±8.35 %; CON = 58.34±8.35 %; p = 0.41) between treatment groups.

Table 2: Percentage (LSM ± SEM) of clinical diseases recorded by research team and farm personnel in cows treated with an intrauterine 50% dextrose infusion (DEX), or with an intrauterine saline infusion (SAL) or that remained untreated (CON) at 4±1 days after calving.

Variable	Treatment				Parity		
	DEX (n = 44)	SAL (n = 45)	CON (n = 45)	*P - value*	Primiparous (n = 65)	Multiparous (n = 70)	*P - value*
RCMI[1]	28.09±8.87	34.5±9.27	12.64±5.77	0.13	37.11±7.48[a]	14.00±5.23[b]	0.01
Pyrexia[2]	10.33±5.70	9.98±5.52	25.68±7.53	0.17	.	.	.
Subclinical ketosis[3]	38.31±9.85[ab]	49.84±9.90[a]	21.64±7.19[b]	0.08	20.62±6.12[a]	54.2±7.06[b]	0.002
FCMI[4]	18.90±6.15	20.21±6.21	5.72±3.36	0.11	22.70±5.57[a]	7.39±3.12[b]	0.01
CDEVT[5]	36.36±7.32	33.32±7.11	26.66±6.66	0.61	.	.	.

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

[1] Percentage of cows with clinical metritis diagnosed with the Metricheck® device by the research team on study day 7. Cows that presented a watery, red-brownish vaginal discharge were categorized as having clinical metritis.

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

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

[4] Percentage of cows with clinical metritis recorded by farm personnel collected from on-farm computer records.

[5] Percentage of cows that had one or more clinical disease events recorded in on-farm computer records during the first 60 d after calving.

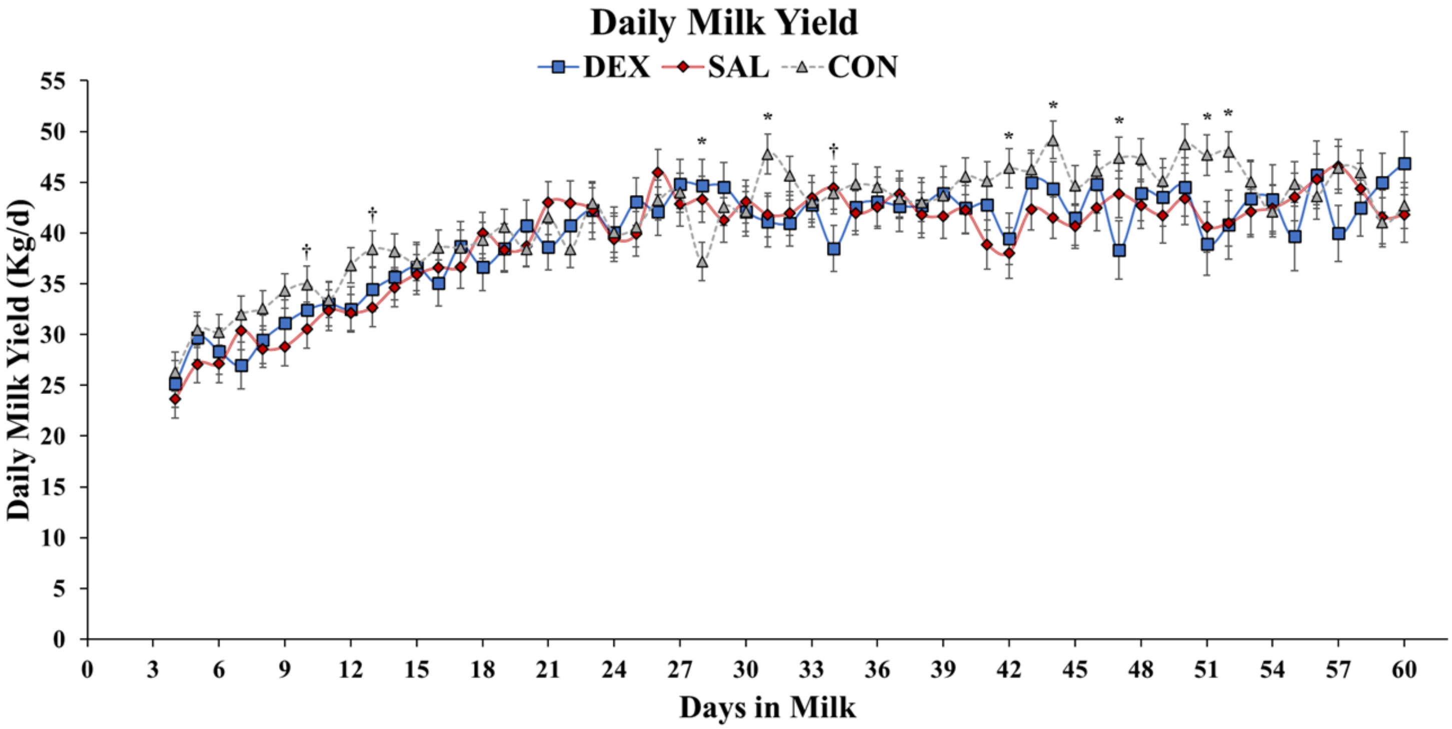

Figure 2: Daily milk yield (kg/day; LSM ± SEM) in cows treated with an intrauterine 50% dextrose infusion (DEX,), or with an intrauterine saline infusion (SAL;) or that remained untreated (CON;) at 4±1 days after calving, for the first 60 DIM. Day × treatment (P ≤ 0.10) interaction tendency was found. *P < 0.05; †0.05 < P ≤ 0.1.

Chapter 5

Discussion

The main findings of this study were: 1) Although not significant (RCMI p = 0.13; FCMI p =0.11), the RCMI and FCMI in DEX and SAL cows was on average 14.52 and 18.16 percentage points higher, respectively, compared to CON cows; 2) SAL cows had higher BHB and HP concentration and higher incidence of subclinical ketosis compared to CON cows on study d 7; and 3) produced more milk in certain days compared to DEX and SAL cows during the first 60 DIM.

Although there was not statistically difference in incidence of clinical metritis between study groups, it appears to be an obvious trend, where cows treated with either DEX or SAL had higher incidences of this disease. In our previous studies (Barragan et al., 2019), we reported that in the vaginal discharge of non-metritis cows there were large concentration of important inflammation metabolites such as HP and neutrophil-derived haptoglobin-matrix metalloproteinase-9. A possible explanation for the observed results could be that the administration of these solutions inside the uterus could have diluted these biomarkers and affected normal inflammatory processes needed for proper uterine involution and bacterial removal. Although the uterus is aseptic during pregnancy, most of dairy cows may experience uterine bacterial contamination during or after calving (Sheldon et al., 2009; LeBlanc et al., 2010). In addition, after calving, one of the main processes of uterine involution is formation and expulsion of a large amount lochia discharge, which in normal conditions this fluid is completed in the first 12 days after calving (Leslie, 1983). Another possible explanation could be that at 4±1 days after calving, when study cows were enrolled, there was still large amount of this fluid inside the reproductive tract, which often is contaminated with bacteria (Leslie, 1983) and the administrated solutions were not concentrated enough to kill contaminant bacteria (i.e., dextrose solution) or provided a fluid environment for bacteria to reproduce and thrive (i.e., saline solution).

The association between clinical metritis and systemic inflammation has been extensively proven. For instance, Barragan et al. (2018) reported, in a cross-sectional study, that cows diagnosed with clinical metritis had higher concentration of HP when comparing this group with non-metritis cows. Huzzey et al. (2009) found that cows that developed mild or severe clinical metritis in the first weeks of lactation had higher concentration of HP around calving compared to cows with a healthy uterus. Similarly, clinical metritis has been identified as an important risk factor for subclinical ketosis. Garro et al. (2014) reported that cows with metritis were 4.9% more likely of developing subclinical ketosis compared to cows without metritis. Lastly, there is an extensive scientific literature describing the negative effects of clinical metritis on milk yield in the first weeks after calving (Rajala and Gröhn, 1998; Fourichon et al., 2000; Gröhn et al., 2003; Huzzey et al., 2007; Machado et al., 2014). In this study SAL cows had the numerically highest RCMI (diagnosed on study d 7) compared to the other two groups. Perhaps, the increased inflammatory and metabolic stress observed on study d 7 could be related to clinical metritis rather than the actual treatment.

Chapter 6

Conclusions

Although not significant, CON cows had the numerically lowest RCMI and FCMI compared to DEX and SAL cows. Furthermore, CON cows had lower BHB and HP concentration on study d 7 compared to SAL cows and produced more milk in certain days compared to DEX and SAL cows during the first 60 DIM. Based on these findings, authors conclude that intrauterine dextrose infusion in postpartum cows with normal vaginal discharge may be prejudicial for cow health and performance.

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ACADEMIC VITA

Hailey D. Mattice

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Education
The Pennsylvania State University
Schreyer Honors College
Major: Veterinary and Biomedical Sciences
Anticipated graduation: May 2022

Experience

***Animal Care Volunteer*, **Centre Wildlife Care — January 2020- present
Port Matilda, PA 16870

- Provide care to injured wildlife
- Support the rehabilitation and release of wild animals

***Learning Assistant*, **Organic Chemistry Lab 213W — January 2021- December 2021
Pennsylvania State University

- Taught weekly concept workshops to enhance student learning.
- Held office hours to answer student questions on course materials.

Veterinary Assistant, Animal Health Clinic of Funkstown — Summer 2020 and Summer 2021
Funkstown, MD 21734

- Assisted veterinarians with animal treatments to ensure high level of care.
- Ran diagnostic tests to determine the cause of illness.
- Prepared prescriptions for animal patients.
- Performed preventative treatments such as vaccination and nail trims to support sustained health.

Loop Abroad Australia and Costa Rica — Summer 2019 and Summer 2021
Walkabout Wildlife Sanctuary and Kids Saving The Rainforest

- Learned from veterinarians via both classwork and clinical training about Australian and Costa Rica wildlife.
- Participated in 150 hours of hands-on veterinary experience.
- Practiced veterinary skills such as blood draws and catheter placement.
- Assisted with surgeries and necropsy.

Veterinary Assistant, Guilderland Animal Hospital — Summer 2019
Altamont, NY 12009

- Assisted with medical treatment and care of animals to promote health and recovery.
- Gained experience in how animal hospitals operate.
- Learned proper animal handling and restraint techniques to assist veterinarians during exams.
- Ensured cleanliness of kennels and exam rooms to deter the spread of disease.

Clubs

***Outstanding Senior Contest Chair*, Coaly (Honor) Society** November 2020-present
Pennsylvania State University

- Organized the outstanding senior contest to award a scholarship to a College of Agriculture student
- Exhibited leadership and scholarship in the College of Agriculture

***Member*, The National Society of Leadership and Success** April 2019 - present
Pennsylvania State University

- Attended leadership training.
- Attended speaker broadcasts to learn about leadership.

***Member,* Pre-vet Club** August 2018 - present
The Pennsylvania State University

- Attend bi-weekly meetings and listened to guest speakers to gain an understanding of the profession.
- Participate in hands-on labs and fundraisers.

***Member*, Small and Exotic Animal Club** August 2018 - present
2019-2020 Fundraising Chair, 2020-2021 Vice President, 2021-2022 President
The Pennsylvania State University

- Attend bi-weekly presentations and meetings to learn about various animal careers.
- Assist in animal-based volunteer opportunities and fundraisers.
- Arrange trips and speakers to inspire interest of the club members.
- Represent the club at community and recruitment events to obtain new members.

***Member*, Club Tennis** August 2019-May 2021
Pennsylvania State University

- Attended open practices.
- Attended online socials.

Awards and Certifications
Dean's List Fall 2018-Fall 2021

American Red Cross Lifeguard, CPR and First Aid Certified

Pennsylvania Department of Health EMT certification

2nd place in Gamma Sigma Delta Undergraduate Research Exposition- Animal Systems Division