Effect of a Commercial Active Dry Yeast (CNCM I-1079) on Productive and Metabolic Measures during the Periparturient Transition

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Authorization to Submit Thesis

This thesis of Maeghan Raphaelle Steelreath, submitted for the degree of Master of Science with a Major in Animal Science and titled "Effect of a Commercial Active Dry Yeast (CNCM I-1079) on Productive and Metabolic Measures during the Periparturient Transition," has been reviewed in final form. Permission, as indicated by the signatures and dates below, is now granted to submit final copies to the College of Graduate Studies for approval.

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Abstract

The transition period is a metabolically demanding time for dairy animals because of the increased nutrient requirements for milk production. The objective of this study was to investigate the effect of feeding a commercial direct-fed microbial (DFM) supplement in multiparous and primiparous dairy cows on productive measures, blood metabolites, and immune status markers during the transition period. Primiparous heifers, (n=33) and multiparous cows (n=35) were fed a close-up TMR before calving and a lactation TMR postpartum. Three weeks before expected calving, all animals were blocked to balance parity and body weight, then randomly assigned to either control group (CTRL; n=34) or an active dry yeast (ADY; n=34). The ADY animals received a top-dressed ADY (*Saccharomyces cerevisiae boulardii*, CNCM I-1079) fed daily at 12.5 g per head. Dry matter intake (DMI) was measured by subtracting the weight of refusals from the amount of feed given daily during both pre- and post-calving periods. All animals were weighed weekly for the duration of the study. Blood samples were collected weekly and were analyzed for glucose, non-esterified fatty acid (NEFA), and β – Hydroxybutyrate (BHBA) concentrations. Colostrum samples were collected at calving and analyzed for IgG, IgA, and IgM content and somatic cell count (SCC). Milk samples were collected once per week postpartum, and all of the milk samples were analyzed for protein percentage, fat percentage, lactose percentage, urea nitrogen (MUN), and somatic cell count. All results were analyzed using PROC MIXED in SAS with significance defined as $P \leq 0.05$. All covariate models were selected based on the lowest AIC value. Results showed that the interaction of treatment, parity, and time affected DMI ($P < 0.01$). Supplemented cows were maintained a heavier body weight overall ($P = 0.05$). The supplementation of the DFM had a

significant effect on milk yield as the ADY animals produced more milk overall ($P = 0.04$); also, there was a tendency for ADY multiparous animals to produce more energy corrected milk (ECM) over time ($P = 0.06$). Also, there was a tendency for ADY animals to be more efficient (kg ECM/kg DMI) over time ($P = 0.06$). There was a significant interaction of treatment, parity, and time on milk protein percentage as the multiparous ADY animals had a greater percentage of protein at day 0 ($P = 0.03$). There was also a significant interaction of treatment, parity, and time on MUN as the DFM primiparous cows had a greater MUN at days 0 and 21 ($P < 0.01$). There was not, however, a significant difference in milk fat percentage ($P = 0.34$), milk lactose ($P = 0.54$), somatic cell count ($P = 0.98$), IgG concentration (0.47), IgA concentration (0.94), IgM concentration (0.92), colostrum somatic cell count (0.98), peak milk production (0.31), plasma glucose ($P = 0.73$), serum NEFA ($P = 0.89$), or plasma BHBA (0.96) by treatment effect. Supplemented animals also had a greater circulating concentration of haptoglobin ($P = 0.03$). Supplementation of an active dry yeast showed a significant effect on DMI, milk yield, gross feed efficiency, milk protein percentage, increased MUN, and increased haptoglobin concentration. Overall, feeding an ADY improved some productive measures, but there needs to be more research conducted to fully understand all of the mechanisms that occur during this time.

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Introduction

Historically, the periparturient period is one of the biggest challenges faced by the dairy cow. This period of time (three weeks prior to calving to three weeks post parturition) is known to be a difficult time for dairy cows. They will go from late gestation to parturition and immediately begin lactation. Due to this extreme shift in physiological state, the body is adapting at the metabolic level to meet demands imposed by lactation. Dairy cows are adjusting to the extreme metabolic demands imposed by lactation i.e. mobilizing lipids from adipose tissue to meet the energy deficit caused by the drastic shifts in physiological states. If a dairy cow fails to meet the energy demands associated with lactation, she is at great risk for developing metabolic disorders like fatty liver and ketosis.

Current research is focusing on methods and tools to attempt to ease dairy cattle through this period of time that sets the tone for the rest of the lactation cycle. This period of time is full of intricate details, like nutrient partitioning, and if any of those details becomes out of sync with the rest, a metabolic disorder will develop. These metabolic disorders can lead to decreased milk yield over the course of the lactation cycle. One of those methods is probiotic supplementation. Probiotics are live feed supplements with specific targets in the gastrointestinal tract. In addition, these feed supplements are known to promote healthy microflora to improve host intestinal health, keep pathogenic organisms from colonizing the gastrointestinal tract, increase digestive capacity, drop the pH, and aid in mucosal immunity (Uyeno et al., 2015).

Ideally, probiotic supplementation will ease dairy cows through the periparturient period by improving energy balance, positively affecting immune status, and increasing productive measure like dry matter intake. All of these changes would occur by targeting an

organ in the gastrointestinal tract like the rumen or the large intestine. Since the gastrointestinal tract is open to outside contaminants and pathogens, probiotic supplementation can aid in overall animal health by competitive exclusion (McAllister et al., 2011). Competitive exclusion is a biological principle that states that true competitors cannot occupy the same habitat. Therefore, feeding probiotics is a viable method to improving the periparturient period in dairy cattle.

Chapter 1: Review of Literature

1.1 Periparturient Period

1.1.1 Overview

The periparturient period (three weeks prior to parturition to three weeks postpartum) is a metabolically demanding time for dairy animals because of the increased nutrient requirements for milk production. Pregnancy and lactation are high metabolic priorities, even to the expense of other metabolic processes, such as establishing energy reserves (Bauman & Currie, 1980). The major challenge of the transition period is the increase in nutrient requirements concurrent with low dry matter intake (Drackley, 1999). Therefore, it is extremely important to note the extreme energy demands during periparturient period, and the intense metabolic shifts that occur during this time. The extreme demand on the body for lactation is the focus of researching methods to improve overall animal health and productivity during this period of time with probiotic supplementation being one of the methods. Probiotic supplementation has been known to have beneficial effects on the digestive system, and there is reason to believe that these supplements can improve animal health and productivity the periparturient period.

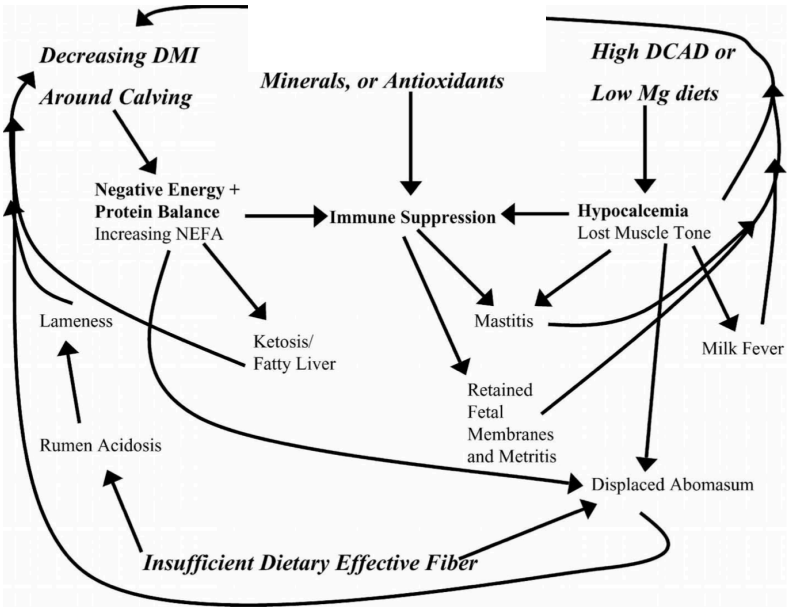

Figure 1.1 Illustration of the relationships among key aspects of the periparturient period (Adapted from Goff, 2006)

Parturition is challenging principally because, shortly after parturition, cows enter into a negative energy balance because milk production and maintenance energy demands exceeds nutrient intake as shown in Figure 1.1. Energy gained via dry matter intake will lag behind energy lost via milk production until after the peak milk yield of that lactation (Smith et al., 2005), approximately 10 weeks into lactation (Bauman & Bruce Currie, 1980). During negative energy balance, cows experience an energy deficit equivalent to milk yield of 9 kg/d (Bauman & Bruce Currie, 1980). To meet energy demands for milk production not provided by nutrient intake, cows mobilize body adipose stores to meet their energy demands. Changes in body condition can be measured in periparturient body weight or body condition score (**BCS**). Another nutrition management tool is the management of BCS. Body condition is scored on a 5-point scale with 0.25 point increments. A score of 1 is considered very thin; however, a score of 5 is considered obese. Average score of 3. If the

cow is over-conditioned (BCS > 3.25) at calving, the dry matter intake is limited, and the cows may be subjected to a greater rate of BCS loss. However, if the cows have a low BCS (BCS < 2.0) at parturition, they may endure some BCS loss but the BCS will remain low. Primiparous cows will take three weeks longer to conceive if they have a low BCS approximately 7-10 weeks postpartum (Wathes et al., 2007). There is a strong influence of BCS loss in the first six weeks of lactation on calving to conception interval (Westwood et al., 2002). Therefore, detail-oriented management of the periparturient period can have positive effects on breeding back dairy cows.

Major body condition loss can be defined as anywhere from 0 to -1.5 points on the 5-point BCS used in dairy cattle (Kim & Suh, 2003). During the first four weeks of lactation, cows will utilize body stores that are energetically equivalent to 33% of milk produced (Bauman & Bruce Currie, 1980). As the cows mobilize their lipid stores to meet the energy demand of lactation, they are susceptible to various diseases and metabolic disorders that impair health, productivity, and fertility. Therefore, it is extremely important to manage the metabolic demands imposed on periparturient dairy cattle to minimize the occurrence and severity of metabolic diseases.

1.1.2 Metabolic Diseases

The following diseases are prevalent at the postpartum stage: metritis, mastitis, displaced abomasum, laminitis, hypocalcemia, ketosis, and retained placenta. The financial loss per case for each of the aforementioned diseases are as follows: $162, $153, $340, $68, $146, $73, $244 respectively. The total financial loss per case from the seven diseases is $1698 (Yildiz, 2018). In addition, fatty liver disease costs an estimated $60 million annually in the United States (Bobe et al., 2004). The average incidence percentage in a 100-head

lactating dairy herd for endemic diseases is as follows: retained placenta (8.6), hypocalcemia (3.9), metritis (13.3), mastitis (7.0), ketosis (2.5), laminitis (4.3), and displaced abomasum (1.8; Yildiz, 2018). Fatty liver is extremely hard to diagnose as the clinical signs are not that specific because of other factors (anorexia, reduced rumen motility, weight loss, puerperal disease) associated with the periparturient period (Adewuyi et al., 2005). The occurrence of diseases and disorders during the periparturient period decreases total milk production by 850 kg, or 9% (Drackley, 1999). In all, periparturient diseases have strong negative effects on lactation and reproduction success of dairy cows.

Of the periparturient diseases, three principal metabolic diseases of the periparturient transition are fatty liver disease, ketosis, and milk fever (Leblanc, 2010). Cows that experienced ketosis during the first two weeks of lactation had a lower pregnancy rate until 140 days in milk (Leblanc, 2010) and also experience lower rates of conception due to the stress that accompanies metabolic disorders (Drackley, 1999).

The other major metabolic disorder associated with the periparturient period is fatty liver disease. This usually follows excessive plasma NEFA concentrations from adipose tissue. In ruminants, fatty liver develops when the liver is taking in more lipids than it is oxidizing and secreting. The excess lipids are stored in the liver, and they decrease metabolic functions within the liver (Bobe et al., 2004). When this disease occurs, milk production and feed intake are negatively affected and cows experience greater rates of dystocia, diseases, infections, and inflammation (Bobe et al., 2004). Fatty liver disease can be treated by decreasing triglyceride lipolysis, increasing hepatic oxidation of NEFAs and increasing the rate of VLDL export from the liver (Grummer, 2008). Other metabolic disorders, like ketosis, can develop as a result of fatty liver disease.

Another metabolic disorder associated with the periparturient period is milk fever. Milk fever, or hypocalcemia, occurs when there is not enough calcium in the blood to maintain bodily functions. The main preventative strategy is decreasing the dietary cation-anion difference (DCAD: milli-equivalents ($\frac{[(Na+K)-(Cl+S)]}{kg} of\ DM$; Seifi et al., 2010). Positive effects on calcium homeostasis are mediated by this controlled metabolic acidosis (Seifi et al., 2010). This particular metabolic disorder can lead to death, if not managed correctly. With that, the lactation cycle will also be negatively affected in that milk production will decrease.

1.1.3 Nutrition Management

During the transition period, nutrition and management are key to mitigate metabolic diseases. One way to manage periparturient metabolic diseases is through dietary cation-anion difference management during the dry period. Manipulating the dietary cation-anion difference will increase blood Ca in an attempt to maintain a healthy level of circulating calcium at the onset of lactation (Charbonneau et al., 2006; Lean et al., 2006). If a dairy cow has a low blood Ca level, she is extremely susceptible to hypocalcemia or milk fever, which can have detrimental effects on that lactation cycle. Feeding anionic salts improve calcium homeostasis by affecting the acid/base balance. The anionic salts induce a metabolic acidosis (Seifi et al., 2010) which results in retention and absorption of more calcium, thereby mitigating the likelihood of hypocalcemia.

1.2 Productivity During the Periparturient Period

1.2.1 Dry Matter Intake

Dry matter intake (DMI) is a crucial aspect that needs to be monitored as parturition approaches. In the last three weeks of pregnancy, DMI decreased by 32.2% with 88.9% of

that decrease occurring in the last week of pregnancy (Hayirli et al., 2002). Though cows will respond to the increased energetic demands by increasing their DMI, this response is typically delayed (Grummer et al., 2004). Due to the decreased DMI, dairy animals are more at risk of metabolic disorders due to an inconsistent influx of nutrients. Dairy cattle experience unparalleled stress imposed when transitioning from late gestation to early lactation, and this time is usually accompanied with depressed dry matter intake (Grummer et al., 2004). Decreased DMI exacerbates negative energy balance, thereby increasing the likelihood of metabolic diseases associated with the periparturient period. If a metabolic disease develops, the cows can enter a vicious cycle of developing a more severe negative energy balance due to decrease DMI.

1.2.2 Colostrum and Milk

The principal energy output of a cow during the periparturient period is the production of colostrum and milk. Colostrum is the first secretion produced by the mammary gland at the onset of lactation and will transition to mature milk during the first week of lactation. The key difference between colostrum and milk is the high concentrations of immunoglobulins and antimicrobial factors in colostrum that not only protect the calf. In addition, the mammary gland is extremely susceptible to infection because the teat opening provides a link to the external environment (Stelwagen et al., 2009).

Colostrum contains a high concentration of immunoglobulins. These proteins are produced by lymphocytes when pathogenic antigens are present in the mammalian body (Sordillo, 2016). There are five immunoglobulin classes: IgM, IgG, IgA, IgD, and IgE (Butler, 1969). IgG is the most highly (65-90% of total immunoglobulins) concentrated immunoglobulin in bovine colostrum (Puppel et al., 2019). This immunoglobulin

accumulates in the mammary gland prior to parturition by transfer across the mammary epithelium (Conneely et al., 2013). The majority of immunoglobulins enter the colostrum through a selective receptor-mediated intracellular route, and they may be blood-derived and synthesized by plasma cells in the mammary gland itself (Stelwagen et al., 2009). These different classes of immunoglobulins play a role in passive immunity in calves. Calves are born with only innate immunity, and these immunoglobulins are the building blocks for the developing immune system.

When a dairy cow begins lactating, her body will set 67.1% of total energy intake aside to support lactation (Boerman et al., 2015). This nutrient partitioning is driven by homeorhesis, the planned control of metabolic processes in body tissue to support a desired physiological state like pregnancy or lactation (Bauman & Currie, 1980). As cows begin lactogenesis, a significant amount of nutrient partitioning occurs, to the point where "the nutrient needs of the mammary gland are of such magnitude relative to total metabolism in a high producing dairy cow that the cow should be considered an appendage on the udder rather than the reverse" (Bauman & Currie, 1980). This matters because there are several extreme metabolic changes occurring in a short period of time, and it is crucial to be aware of the major metabolic demands imposed by the onset of lactation.

Some physiological functions for lactogenesis occur as a result of a metabolic change. Milk synthesis occurs partly because of an increased use of nutrients in the mammary gland. Lipid metabolism changes, with increased lipolysis and decreased lipogenesis in the adipose tissue. A dairy cow's milk yield per day follows a predictable curve that peaks at 6-9 weeks into lactation and then declines over time. In order to support lactation, the cow will utilize a combination of dietary energy and mobilized adipose tissue

(Nebel & McGilliard, 1993). Milk fat content will come from diet and de novo synthesis in the mammary epithelial cells. Milk protein is derived de novo synthesis in the rumen, and the amino acids will enter the mammary gland. The mammary gland will use the amino acids to synthesis milk specific proteins like casein. Lactose is the main fate of glucose metabolism in the body during lactation. Considering that lactose is an osmoregulatory factor for milk, milk yield is highly dependent on lactose concentration.

1.3 Metabolism During the Periparturient Period

The periparturient transition is a major metabolic shift to negative energy balance. When cattle cannot adapt to the metabolic requirements of the periparturient period, there is an increased risk of metabolic disorders like fatty liver and ketosis (Goff, 2006). The principal metabolic changes during the periparturient period can be seen in changes in blood energy metabolite concentrations. With the onset of lactation, dairy cattle will produce milk at the cost of their own lipid stores, which contributes to the presence of certain blood metabolites like non-esterified fatty acids (**NEFA**) and β - Hydroxybutyric acid (**BHBA**). NEFA and BHBA are commonly used to determine energy balance in transition cows (Ospina et al., 2010b).

Glucose is the primary energy source on the cellular level in ruminants; however, the majority of glucose is used by the mammary gland during lactation. When the mammary gland is producing large amounts of milk, the gland will use up to 80% of the total glucose turnover (Bauman & Currie, 1980). Glucose is also a precursor for lactose synthesis, so the available glucose is directly linked to the amount of milk produced because lactose is an osmoregulator for milk production (Miglior et al., 2007). During lactation, glucose requirements increase 4-fold in high-yielding lactating dairy cows in comparison to open or

non-lactating cows (Bell & Bauman, 1997). When the available glucose decreases, the cows will mobilize adipose tissue to address the energy deficit.

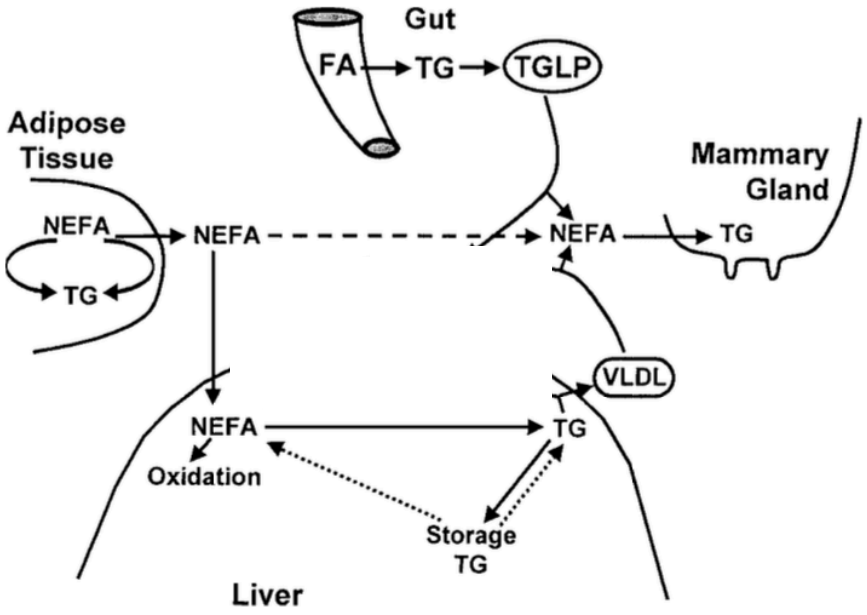

Figure 1.2. Illustration of how lipid mobilization occurs from different body tissues (adipose tissue, gastrointestinal tract, liver) and enters the mammary gland (Adapted from Drackley 1999)

Lipid mobilization in response to the onset of lactation involves triglycerides, non-esterified fatty acids (**NEFA**), and β-hydroxybutyric acid (BHBA). Lipids are released from the adipose tissue as NEFA, and fatty acids, from the diet, are absorbed in the small intestine as seen in Figure 1.2. Some NEFA will come from the adipose tissue and travel to the liver where some will undergo oxidation. The NEFA that is not oxidized will be converted to triglycerides (**TG**) and packaged into very low-density lipoproteins (**VLDL**). The VLDL will be converted back to NEFA and will enter the mammary gland. Some NEFA from the adipose tissue will go straight to the mammary gland as well. Some fatty acids will come from the gut. These fatty acids are converted to TG and transported to the mammary gland. Once the fatty acids are absorbed, they are placed into lipoproteins which will ultimately

transport dietary fatty acids to the peripheral tissues. The influx of dietary fatty acids leads to an increase in the concentration of NEFA because of the actions of lipoprotein lipase in the peripheral tissues. The completely oxidized NEFA will provide energy for the liver, while the partially oxidized NEFAs produce ketone bodies like acetone, acetoacetic acid, and β - hydroxybutyric acid (McArt et al., 2013). Some NEFA (prepartum NEFA > 0.3 to 0.5 mEq/L; postpartum NEFA 0.7 to 1.0 mEq/L; Wankhade et al., 2017) and BHBA (prepartum BHBA >0.6 to 0.8 mmol/L; postpartum BHBA 1.0 – 1.4 mmol/L; Wankhade et al., 2017) in the blood is a normal occurrence when the cows are in a negative energy balance in early lactation (McArt et al., 2013). Excessive amounts of NEFA (>0.7 mEq/L) and BHBA (>0.06 mmol/L) are associated with detrimental effects on animal health and production (McArt et al., 2013). Multiparous cows that develop fatty liver disease or ketosis suffer a greater decrease in production when compared to primiparous cows.

Fatty liver disease occurs when the liver takes in more lipids than it is oxidizing and secreting (Grummer, 1993), when the liver is high in NEFA (>0.72 mEq/L; Ospina et al., 2010b; McArt et al., 2012). Ruminant livers have a low capacity to reconvert NEFA back to triglycerides (Drackley et al., 2005). This buildup of lipids can develop into fatty liver disease, especially in transition dairy cattle (Grummer, 1993). Fatty liver disease is associated with overall decreased animal health and productivity (i.e. decreased milk yield) as well as decreased reproductive performance (Wensing et al., 1997). Fatty liver disease is usually a precursor to ketosis.

Another important metabolic disease of the periparturient transition is ketosis. Ketosis (BHBA > 3.0 mmol/L; Oetzel, 2007) develops after dairy cows enter a negative energy balance. Overall, cows with BHB greater than 1.8 mmol/L one week into lactation

had a greater than 300 kg lower projected production for the whole lactation (Leblanc, 2010). At a subclinical level (BHB > 1.2 -1.4 mmol/L), ketosis in the first two weeks post-parturition is associated with decrease in milk yield of 1.9 – 3.3 kg/d in the first two weeks of lactation (Duffield et al., 2009; LeBlanc et al., 2005; Ospina et al., 2010a) . After subclinical ketosis develops, cattle have increased odd of developing postpartum diseases like displaced abomasum and metritis (Duffield et al., 2009; Ospina et al., 2010a). There is also a significant reduction in immune function which increases the chances of developing mastitis and other infections (Trevisi et al., 2011). This matters because ketosis causes cows to decrease milk yield over the course of their lactation cycle while declining in overall health.

Transition cows also show an inflammatory response as a result of pregnancy and lactation without a determined pathology, and this inflammation can compromise the animal's immune response by increasing metabolic stress (Trevisi et al., 2012). This matters because these metabolic disorders have a major effect on milk production, fertility, and general animal health.

1.4 Immune Status During the Periparturient Period

The periparturient period not only impacts energy metabolism, but also immune status. Inflammation and dysregulated immune responses are linked to metabolic disorders in transition cows (Esposito et al., 2014; Wathes et al., 2009). The negative energy balance and associated increased NEFA lead to a variety of health disorders like fatty liver which leads to periparturient immunosuppression postpartum (Kehrli, Jr. et al., 1999; Lacetera et al., 2005). Negative energy balance upregulates pro-inflammatory genes (Wathes et al., 2009), whereas acquired immune responses genes are downregulated (Moyes et al., 2010).

Postpartum inflammation exacerbates negative energy balance, shown in Figure 1.1 (Trevisi et al., 2012), highlighting the interrelationship between negative energy balance and immune response.

The immune response involves major players like reactive oxygen species, neutrophils, cytokines, and acute phase proteins. Principal signaling molecules involved in the immune response during the periparturient period include cytokines (interleukin-1, interleukin-6, and tumor necrosis factor-α) and acute phase proteins (serum amyloid A, haptoglobin) which are part of the acute phase response. During the periparturient period, the immune response follows a trajectory of insult, leading to inflammation, then to an acute phase response.

The entire process begins with some form of inflammatory trigger (i.e. infection or tissue injury), which leads to inflammation. First, there is an increase in the production of reactive oxygen species (ROS; Sordillo et al., 2009). The reactive oxygen species can trigger lipid peroxidation and lead to cellular damage (Sordillo, 2018). Immune cells are extremely sensitive to oxidative stress as their membranes are full of polyunsaturated fatty acids (PUFA; Esposito et al., 2014). The PUFA also produce a large quantity of ROS when stimulated (Spears and Weiss, 2008). This matters because the cellular damage serves as a stressor that leads to inflammation which has other consequences.

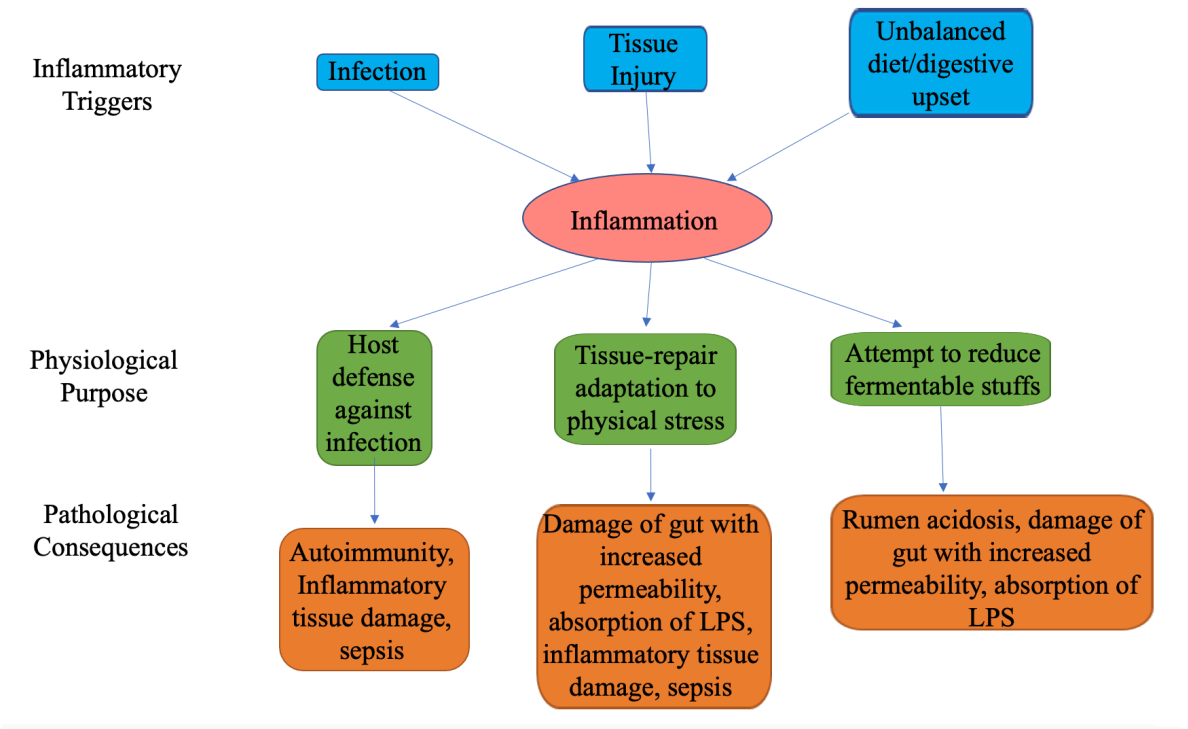

Figure 1.3 Cause-and-effect relationship of immunity and inflammation beginning with inflammatory triggers leading to pathological consequences (Adapted from Trevisi et al., 2011)

In response to the inflammation, there are pathological consequences (i.e. autoimmunity, tissue damage, sepsis), illustrated in Figure 1.3. Inflammation signaling involves a number of cytokines. Both leukocytes and non-leukocyte cells can produce pro-inflammatory cytokines like interleukin-1, interleukin-6, and tumor necrosis factor-α. These pro-inflammatory cytokines promote local and systemic responses (Bertoni et al., 2015). The cells can also produce anti-inflammatory cytokines to inhibit the pro-inflammatory cytokines in order to avoid detrimental side effects (Medzhitov, 2008).

Cytokines initiate a cascade of inflammatory mediators that target the endothelium. This will activate cyclo-oxygenase-2, which causes eicosanoids (prostaglandins, leukotrienes, and lipoxins) to be released (Trevisi et al., 2011). Eicosanoids have anti-inflammatory effects, and they aid in resolving inflammatory processes (Serhan et al., 2008).

Leukotrienes are powerful vasoactive mediators that aid in resolving inflammation (Trevisi et al., 2011). This matters because all of these different components of the acute phase response are essential to overall animal health. At the same time, there are many different checks and balances to keep the response at a healthy level.

The cytokines are responsible for the clinical symptoms, specifically fever, anorexia, and weight loss (Gabay & Kushner, 1999). Also, cytokines activate target cell receptors that lead to systemic inflammatory reactions. These reactions lead to increased adrenocorticotrophic hormones and glucocorticoids, activation of the blood coagulation system, decreased serum concentrations of calcium, zinc, iron, and vitamin A, and changes in the concentrations of some plasma proteins (Gruys et al., 2005) The most important metabolic change is the production of acute phase proteins by the liver (Gabay & Kushner, 1999). This matters because dairy cows will show these symptoms during particularly stressful times such as the periparturient transition.

The next phase of the immune response in dairy cows is the acute phase response. This process includes immediate events at the damaged sites and the acute phase response initiated by cytokines (Trevisi et al., 2011). Acute phase proteins are designed to protect and defend the host organism against pathological damage, to assist in the restoration of homeostasis and in the regulation of different stages of inflammation (Petersen et al., 2004). Cows in the periparturient period utilize acute phase proteins in an attempt to restore a new balance due to an extreme change in physiological status post parturition. Some of the acute phase proteins (α_1-anti-trypsin, α_2-macroglobulin) have anti-protease activity designed to inhibit proteases released by phagocytes or pathogens to minimize damage to normal tissues. Others (α_1-acid glycoprotein) are characterized by anti-bacterial activity and by the ability

to influence the course of the immune response (Fournier et al., 2000). Acute phase proteins are meant to restore a new homeostasis that supports lactation.

1.5 Prebiotics & Probiotics

Prebiotics and probiotics are supplements that can potentially improve metabolic challenges of the periparturient period, depending on the target of the specific supplement. Unlike probiotics, prebiotics are non-living, selectively fermented feed additives that lead to specified changes in the gut microbe populations that benefit the host in overall health (Gibson et al., 2004). Supplementation of prebiotics can have immunological effects and can improve milk production and feed conversion efficiencies in dairy cattle (Baines et al., 2011). Prebiotic supplementation slowed down the progression of an *E. coli* infection in dairy cattle (Baines et al., 2011). Therefore, fermented prebiotics can aid animal health through several mechanisms.

Probiotics, on the other hand, are viable feed supplements. Exact definitions of probiotics have evolved over the last hundred years. Currently, probiotics are defined as a live microbial feed supplement meant to benefit the host animal by changing and improving the microbial balance in the intestine (Fuller, 1992). This definition put a needed emphasis on the fact that a probiotic needs to be viable. Probiotics are capable of promoting the development of healthy microflora in order to improve the intestinal health of the host, inhibiting pathogens from colonizing the intestine, increasing digestive capacity, dropping the pH, and improving mucosal immunity (Uyeno et al., 2015). In addition, probiotics are capable of competitive exclusion by keeping pathogenic organisms, like *E.Coli*, from flourishing in the digestive tract (McAllister et al., 2011). This matters for the same reasons as prebiotics. Therefore, these microbial supplements have had some success with

improving a number of aspects of the periparturient period based on a few proposed mechanisms of action.

There are two main types of probiotics: direct-fed microbials (**DFM**) and active dry yeasts (**ADY**). All ADY are DFM, but not all DFM are ADY. Direct fed microbials include bacteria, like *Lactobacillus* and *Acidophilus*, as well as active dry yeasts. Active dry yeasts are characterized by containing at least 10 billion cfu/g, and the most common species is *Saccharomyces cerevisiae* (Chaucheyras-Durand et al., 2008). Probiotics are most effective when they are included in the diet while the animals are going through a significantly stressful period of time such as weaning, onset of lactation, or diet transition (Chaucheyras-Durand & Durand, 2010). Many probiotic studies have attempted to determine plausible modes of action by focusing on the modification of ruminal digestion. These modifications occur as a result of rumen acid production modulation, establishing favorable microbial populations, and enhancing fiber digestion in the rumen (McAllister et al., 2011). Therefore, direct fed microbials have been proven to aid in the digestion and fermentation processes which has beneficial effects on the adult ruminant.

The most commonly studied DFM are as follows: *Saccharomyces* yeasts, *Aspergillus* bacteria, *Bidifobacterium*, lactic acid bacteria, *Bacillus*, *Enterococcus*, and *Faecalibacterium* (Raabis et al., 2019). In calves, administering lactate-utilizing bacterium (*Megasphaera elsdenii*) increased feed intake and butyrate production in the rumen (Muya et al., 2015); however, supplementing *Lactobacillus plantarum* and *Bacillus subtilis* did not change rumen fermentation (Zhang et al., 2017). These bacterial DFM are not all the same, and the aforementioned studies prove that they have different effects in the gastrointestinal tract bacterial DFM are one of the subsets of direct fed microbials.

The most commonly used type of probiotic used in dairy cattle nutrition is some form of yeast, primarily *Saccharomyces cerevisiae* (Uyeno et al., 2015). *Saccharomyces cerevisiae* is capable of metabolizing lactic acid; however, they are aerobic organisms so their ability to break down lactic acid in the rumen is debatable (McAllister et al., 2011); however, yeasts are recognized as facultative anaerobes meaning they can survive with or without oxygen (Dashko et al., 2014). The rumen ecosystem is extremely diverse in terms of microbiota like anaerobic bacteria, ciliate protozoa, anaerobic fungi, and archaea. These microbes are responsible for degradation and fermentation of the majority of ingesta (Chaucheyras-Durand & Durand, 2010). Feeding a certain strain of *Saccharomyces cerevisiae* does not increase or decrease dry matter intake (AlZahal et al., 2014b). Supplemented a DFM led to greater DMI in cows prepartum, but there was not a change in DMI postpartum (Nocek & Kautz, 2006; Oetzel et al., 2007). This matters because direct fed microbials and active dry yeasts can have different targets in the digestive system, which accounts for the energy input.

1.6 Summary and Knowledge Gap

The use of probiotics is varied, and their efficacy is controversial in industry; however, probiotics have been proven to support DMI to minimize the risk of metabolic dysbiosis and disease. In addition, there is speculation that probiotics are capable of mitigating metabolic risks. Each probiotic species and strain is unique in their modes of action which leads to unpredictable efficacy of each product. Some of the modes of action are modulation of digestibility, inflammation, and microbial competitive exclusion. Different probiotics target different areas of the gastrointestinal tract in order to assist in

digestibility. With that being said, our study is looking at how a probiotic targeting the hind gut affects productivity measure sand immune response during the transition period.

1.7 Hypothesis and Objective

Feeding an active dry yeast (*S. cerevisiae boulardii*; CNCM, I-1079) will increase productive measures and positively effect blood metabolites. The objective of the present research was to investigate the effect of feeding an active dry yeast (**ADY**: *Saccharomyces cerevisiae boulardii*; CNCM, I-1079) on dairy cow productivity during the periparturient period. This research focused on productive measures, blood metabolites, and immune status markers.

Chapter 2: Effect of a Commercial Active Dry Yeast (CNCM I-1079) on Productive and Metabolic Measures during the Periparturient Transition

2.1 Materials and Methods

2.1.1 Animals and Treatments

All animals were randomly selected out of the calving pool. The number of lactation cycles was considered. All animals were entering either their first, second, or third lactation cycle. Thirty-four primiparous and thirty-four multiparous cows were designated to receive dietary active dry yeast (ADY, n = 34) or control (n = 34) in a completely randomized blocked design, in which the animals were blocked by body weight and parity. Supplementation with the active dry yeast (*Saccharomyces cerevisiae boulardii* (CNCM I-1079), Lallemand Animal Nutrition, Milwaukee, WI, USA), which is a rumen protected active dry yeast, began on day 1 at 12.5 g/day/head as per the manufacturer recommendation. Control animals did not receive any active dry yeast supplement. The primiparous animals were fed a close-up total mixed ration (TMR) ad libitum until parturition. The multiparous animals were fed a close-up TMR ad libitum with anionic salts (0.68 kg/head/day) until parturition (Table 2.1). After parturition, all animals were fed a lactation TMR ad libitum (Table 2.1). Ration compositions are shown in Table 2.2. All animals had access to fresh, clean water at all times. All animals were housed in tie-stalls from 3 ± 1 weeks prior to parturition to 8 ± 1 weeks postpartum. All animals were fed twice per day at 06:00 h and 18:00 h. After parturition, all cows were milked four times per day at 06:45 h, 10:30 h, 19:30 h, and 22:30 h for 28 days. From day 29 onward, all cows were milked twice per day at 06:45 h and 19:30 h. All animal experimentation was approved by the University of Idaho Animal Care and Use Committee (protocol 2017-63).

2.1.2 Sample Collection and Analysis

Feed intake was measured twice per week and averaged for weekly intake. Feed samples were collected once per week to calculate dry matter content. A portion of each feed sample, 75-100 g, was dried at 55°C in a forced air-drying oven for 72 hours. All of the dried feed samples were ground using a 2mm sieve, and chemical analysis was conducted on each dried, ground feed sample (Dairy One, Ithaca, New York, USA). Colostrum samples were collected after parturition and frozen at -20ºC. Milk samples were collected once per week from each cow and analyzed for fat, protein, milk urea nitrogen (MUN), somatic cell count (SCC), and lactose (Dairy One, Ithaca, New York, USA). Each milk sample underwent Fourier Transform Infrared Spectroscopy (FTIR) for determining the protein percent, fat percent, lactose percent, and MUN. SCC was determined using flow cytometry. Somatic Cell Score (SCS) was determined using the following equation: SCS = log base 2 (SCC / 100,000) + 3; where somatic cell count is somatic cells per mL (Wiggins and Shook, 1987). Blood samples were collected once per week pre- and postpartum via the coccygeal vein. Blood samples for plasma were collected in 10 mL sodium heparin tubes, and blood for serum was collected in 10 mL plain tubes. All blood samples were centrifuged at 1700 g at 4°C for twenty minutes to isolate the serum or plasma. After centrifugation, both the plasma and serum were aliquoted and frozen in either -20°C or -80°C. All animals were weighed once per week for the duration of the study.

Colostrum samples were analyzed for IgG, IgA, IgM content using radial immunodiffusion plates (Triple J Farms, Bellingham, WA). Every sample was diluted 1:9 with dH_2O, and 5 μL of each dilution was placed in the wells on the radial immunodiffusion plates. Every sample was run in duplicate. All plates were read at 18 h and 72 h using a

jeweler's loupe. The diameters of the precipitin rings were plotted on a reference curve. The diameters were used to back calculate the Ig concentrations. Undiluted colostrum samples were also analyzed for somatic cell count via a somatic cell counter (DeLaval Somatic Cell Counter).

Blood plasma was analyzed for glucose using an established protocol (Keston, 1956). The color solution consisted of a single one-gram capsule of PGO enzyme (Sigma Cal. No. P7119-10CAP) and 1.6mL rehydrated dianisidine dihydrochloride (Sigma Cal. No. F5803-50MG). Each capsule of PGO enzyme contained 500 units of glucose oxidase (*Aspergilus niger*), 100 purpurogallin units of peroxidase (horseradish), and buffer salts. Five μL of each standard, sample, and blank were used in each well. Then 150 μL of the color solution was added to each well. Every plate was incubated at room temperature for 45 min and read immediately at 450 nm on a Spectramax I3x Plate Reader (Molecular Devices, LLC, San Jose, California, USA). All standards, samples, and blanks were run in triplicate and read at room temperature. The intra- and inter- assay variations were 5.22% and 5.34%, respectively.

Blood plasma was analyzed for β - Hydroxybutyric Acid (BHBA) using a colorimetric assay following an established protocol with modifications (Williamson et al., 1962). Enzymic determination of D (−)-β-hydroxybutyric acid and acetoacetic acid in blood. This assay included a buffer comprised of 0.1M Tris, 0.1M oxalic acid, 2 mM EDTA, and 2.5 mM NADH. The buffer pH was 8.5 and it was stored at 4°C. Before starting the assay, 25 mg NAD (Sigma Aldrich, N7004) was added to 15 mL of the buffer (15mL was the amount needed for one plate). Also, 100 μL of 3-hydroxybutyrate dehydrogenase was centrifuged at 12,000 g for 10 minutes. The supernatant was discarded, and the pellet was

resuspended in the buffer/NAD solution. The calibrator for this assay was the 300 μM standard from Wako Diagnostics (Richmond, Virginia, USA, #412-73791). Twenty-five μL of each standard, sample, and blanks were used in each well. After adding the standard, sample, or blank, 150 μL of the buffer/NAD/enzyme solution was added to each well. Every plate was shaken in the plate reader for 60 seconds at 37°C and read immediately after at 340 nm followed by a read at 1 and 2 minutes using a Spectramax I3 Plate Reader. All calibrators, samples, and blanks were run in triplicate. Any samples that were more than 1000 μM BHBA were diluted with 0.9% saline and reanalyzed. Depending on the initial reading, samples were diluted in either 1:1, up to 1:10. The equation used to determine the BHB concentration in each sample was as follows:

$$\text{BHBA concentration } (\mu M) = \frac{mean\ ABS\ change\ in\ sample}{mean\ ABC\ change\ in\ standard} \times concentration\ of\ standard$$

The intra- and inter- assay variations were 3.13% and 4.24%, respectively.

Serum total non-esterified fatty acid (NEFA) were determined using a colorimetric assay (Fujifilm, Wako Diagnostics U.S.A.) following manufacturer's protocol with minor modifications. Two and a half μL of each standard, sample, and pool was used in place of 5 μL. All standards, samples, and blanks were run in triplicate and read at 37°C at 550 nm using a Spectramax I3 Plate Reader. The intra- and inter- assay variations were 6.18% and 3.16%, respectively.

Blood serum was analyzed for haptoglobin via a colorimetric assay (TriDelta Development, Ltd, Maynooth, County Kildare, Ireland) following a modified protocol. 6.7 μL of standards and samples were used plus 89.3 μL of reagent I and 125 μL of reagent II was used for this assay. All standards, samples, and blanks were run in duplicate and read at

room temperature at 600 nm using a Spectramax I3 Plate Reader. The intra- and inter- assay variations were 10.3% and 3.88%, respectively.

2.1.3 Statistical Analysis

All data were analyzed using MIXED procedure in SAS (V. 9.4, SAS Institute Inc., Cary, NC). The model included the main effects of treatment, time, parity, and the interactions of treatment × time, treatment × parity, time × parity, and treatment × time × parity. The Shapiro-Wilk test was used for normality on all data. No covariates were used as there was not significant difference in the measured variables. Because the animals were blocked by body weight, there was no covariate used. Block and cow were random effects. Weeks were repeated measures, and the experimental unit was each animal. Significance was defined as $P \leq 0.05$. A trend was defined as $0.05 \leq P \leq 0.10$. All data was reported as LSMeans ± SEM.

2.2 Results & Discussion

There is a large volume of research regarding the supplementation of probiotics, especially active dry yeasts. Feeding these supplements is known to have diverse effects in several species including dairy cattle, but the mechanisms behind these effects is not well understood. Further, there is an interest in maximizing milk production and energy status during the periparturient period, which is where the probiotic supplementation can play a major role. Therefore, our objective was to determine the effect of feeding an active dry yeast on dairy cattle production performance and blood metabolites during the periparturient period.

2.2.1 Cow Productivity

Dry matter intake (**DMI**) and body weight (**BW**)was analyzed from three weeks prior to expected parturition to nine weeks postpartum. As seen in Figure 2.1, there was a significant three-way interaction of treatment, parity, and time prepartum ($P = 0.01$) in DMI. Multiparous cows consumed more dry matter across time compared with primiparous cows, which was to be expected. At day -7 relative to expected calving date, primiparous ADY-fed cows consumed more dry matter when compared to that for other groups, prepartum. There was a significant treatment effect on BW in that the ADY-fed cows consistently maintained a heavier BW for the duration of the study ($P = 0.05$) as seen in Figure 2.2. There was also a tendency for an interaction between time and parity ($P = 0.08$) in that the ADY multiparous animals were heavier over time.

Dry matter intake is directly correlated with BW. Hayliri et al. (2002) observed a positive correlation between parity and DMI as well as energy intake and parity. The correlation between energy intake and parity was related to the multiparous animals

consuming more dry matter when compared to that for the primiparous animals. Oetzel et al. (2007) observed a greater DMI in the direct fed microbial (**DFM**) supplemented group prepartum; however, there was not a significant effect of the DFM treatment on DMI postpartum. Alzahal et al. (2014b) observed no significant treatment effects on DMI; however, there was a main effect of parity as well as an interaction between parity and time. Nocek and Kautz (2006) observed an increased DMI in DFM group prepartum when compared to that for the control group. In addition, the DFM supplemented groups consumed more dry matter postpartum. In comparison, the present study had a significant three-way interaction among treatment, time, and parity. This matters because the ADY supplemented cows consumed more dry matter in both parities over time.

As previously stated, DMI is directly related to BW and BCS. Oetzel et al. (2007) observed a BCS loss in all animals before calving with no treatment effect, which can be extrapolated to body weight (BW). In addition, Alzahal et al. (2014b) did not see a treatment effect on BW or change in BW. Nocek and Kautz (2006) observed no treatment effect on BW or BCS both pre- and postpartum. In comparison, there was a treatment effect in the present study in that the ADY supplemented cows were significantly heavier for the duration of the study. This matters because it can be expected for BW or BCS to decrease around parturition as DMI decreases. The aforementioned studies all supplemented a probiotic, and reported no significant changes in BW or BCS, which means that the probiotics did not affect any of the associated processes like lipid mobilization. However, our probiotic did affect BW meaning that the supplemented animals consistently maintained a heavier body weight meaning that maybe the supplemented cows are in a less negative energy balance.

Dry matter intake and BW are directly related to energy balance. Higher producing dairy cows will need to mobilize more adipose tissue to meet metabolic demands with early lactation (McArt et al., 2013). Because we detect no difference in DMI and a higher BW in ADY groups, cows may have been utilizing the nutrients they are taking in or that they have been more efficient with using their mobilized lipids. Feeding out the ADY used in this study for 3-4 weeks prior to parturition to 7-9 weeks post-parturition might indirectly improve energy status via DMI and MY, which would significantly improve the periparturient period in dairy cattle.

Milk yield (**MY**) data were analyzed from parturition to nine weeks postpartum. There was a significant treatment effect on MY in that the ADY-fed cows produced approximately 2 kilograms more milk per day ($P = 0.04$; Figure 2.3). There were no significant interactions among any of the main effects. Energy corrected milk (**ECM**) was derived from total milk yield (ECM [kg/d] = [milk yield (kg/d) × 0.327] + [milk fat (kg/d) × 12.86] + [milk true protein (kg/d) × 7.65]) and was measured from parturition to nine weeks postpartum. There was a tendency for ADY-fed cows of both parities to produce more ECM over time ($P = 0.06$; Figure 2.4). Peak milk production data were collected when all animals reached peak production at approximately 90 days in milk (DIM). As reported in Table 2.3, there was no main effect of treatment ($P = 0.31$). There was, however, a significant effect of parity on peak production ($P < 0.01$), which was to be expected. First lactation animals produced 2.2 kg/day less milk when compared with later lactation cycles, and fifth lactation animals produced the most milk per day (30.24 ± 0.58 kg/day). The multiparous cows produced more milk at peak production (43.5 ± 1.8 kg) when compared to that for the primiparous cows (34.7 ± 1.8).

Milk yield was significantly affected by our ADY; however, peak milk was not affected by the ADY. In another study feeding ADY, milk yield was not different between treatment groups; however, lactose content was significantly greater in cows receiving the probiotic (4.53%) when compared to that for control cows (4.40%; AlZahal, et al., 2014a). Cows fed DFM produced 2.3 kg/d more milk than cows not fed DFM (Nocek and Kautz, 2006). Contrary to our results, feeding a DFM in this study did not affect 3.5% FCM; however, there was a tendency for ADY-fed cows of both parities to produce more ECM over time (Nocek and Kautz, 2006). Fat-corrected milk (**FCM**) can be extrapolated to predict ECM. That being said, in other studies FCM was not affected by treatment, but supplemented primiparous and multiparous cows tended to produce more ECM over time (Nocek and Kautz, 2006). The ADY in our study was targeting the hindgut, so there likely is not an effect on rumen fermentation modulation, instead it is thought that nutrient partitioning is shifting towards milk production (Nocek and Kautz, 2006). Competitive exclusion with regards to ADY supplementation may also affect milk yield in that pathogenic organisms are not as likely to colonize the hindgut impacting milk yield (McAllister, 2011). If a probiotic can prevent pathogenic organisms from colonizing the hindgut, the animal would not develop an infection or illness associated with the hindgut. Therefore, that method of prevention ultimately leads to increased milk production when compared to an illness affecting milk yield. In our study, ADY may have increased MY by influencing nutrient utilization toward milk synthesis.

Gross feed efficiency is an important tool to measure the animals' ability to convert feed consumed into milk produced. Gross feed efficiency was derived from ECM and DMI and was calculated from parturition to nine weeks postpartum. As shown in Figure 2.5, there

was a tendency for ADY-fed cows to be more feed efficient over time ($P = 0.06$) as compared with that for the control group. There was not a significant change in feed efficiency between treatment groups as the control cows had a feed efficiency of 1.59 kg/kg and the treatment cows had a feed efficiency of 1.62 kg/kg (Oh et al., 2019). On the contrary, a study that focused on supplementation of a yeast culture in mid-lactation cows observed a higher feed efficiency in the supplemented cows when compared to that for the control cows (1.59 kg/kg vs. 1.49 kg/kg; Schingoethe et al., 2004). Our ADY tended to increase feed efficiency over time while other studies reported either a positive or negative influence on efficiency (Oh et al., 2019; Schingoethe et al., 2004). One reason for this could be that our ADY was fed in early lactation. The cows' physiology would be different because of the timing in the lactation cycle. Another reason could be that not all strains of DFMs and ADYs are created equal. Cows consuming our ADY tended to have a better feed efficiency, so the ADY in our study may be improving efficiency.

2.2.2 Milk Components

Milk protein percent showed a three-way interaction of treatment, time, and parity at day 1 (Figure 2.6). Both primiparous and multiparous supplemented cows had a greater protein percentage on day 1 ($P = 0.03$). Active dry yeast (ADY) primiparous cows produced 3.47 ± 0.09% protein, and ADY multiparous cows produced 3.59 ± 0.08% protein. There was no main effect of treatment ($P = 0.34$) or parity ($P = 0.18$), and there were not any significant interactions detected, as seen in Table 2.3. The milk fat percentages had negligible differences between treatment groups. The ADY cows produced 2.98 ± 0.35% fat, and the control cows produced 3.06 ± 0.35% fat. Milk lactose percent data were analyzed from parturition to nine weeks postpartum. There was no main effect of treatment

($P = 0.54$) detected. There was, however, a tendency for primiparous cows to have a greater lactose percentage ($P = 0.08$), as seen in Table 2.3. Primiparous cows produced 4.81 ± 0.09 %, and multiparous cows produced 4.74 ± 0.09 %.

Milk urea nitrogen (**MUN**) data were analyzed from parturition to nine weeks postpartum. There was a significant three-way interaction of treatment, time, and parity ($P < 0.01$; Figure 2.7). The ADY-fed primiparous cows had a greater MUN concentration at day 0 and at day 21 compared with that for the control group on day 0 and day 21. Milk somatic cell count (**SCC**) data were analyzed from parturition to nine weeks postpartum. There was no main effect of treatment ($P = 0.98$) or parity ($P = 0.53$; Table 2.3); there were also no significant interactions detected. The differences were not significant among treatment groups. Active dry yeast (ADY) cows had 71.7 ± 24.3 × 10^3 cells/mL, and control cows had 71.2 ± 24.3 × 10^3 cells/mL. Colostrum somatic cell count (**CSCC**) data were analyzed at parturition. There was no main effect of treatment ($P = 0.98$); however, there was a significant effect of parity ($P = 0.03$) as shown in Table 2.3. Multiparous cows had a significantly lower CSCC when compared to that for the primiparous cows.

This ADY increased milk yield, but it did not change the proportion of milk components produced. Milk fat was very low in both treatment groups. The silage quality for the ruminant herd was notably worse during our study, and it was seen in a spike of off-study herd cow hospitalizations of cows fed the same silage. Because of the fact that milk fat was very low across both treatment groups, the results should be taken cautiously. In comparison with our study, AlZahal et al. observed milk fat numbers that were 3.68 – 3.96% (2014a). Other studies have shown decreased milk fat percentage in DFM cows (4.44% vs. 4.76%) and increased lactose percentage (4.65% vs. 4.59%; Nocek and Kautz, 2006). In

addition, supplementation of an ADY did not affect milk fat percent or milk protein percent (Li et al., 2016).

Milk protein is derived from ingested nutrients that go through de novo synthesis in the rumen and enter milk. The proteins pass into the abomasum and are subsequently broken down into amino acids that travel through the body including the mammary gland. There was not a treatment effect on milk protein percent when comparing DFM cows (3.12%) to control cows (3.13%; Nocek and Kautz, 2006). Why there was likely no effect on milk protein is probably related to the probiotic mode of action. The ADY targeted the lower gut, and it does not have an adequate opportunity to affect milk protein.

We did not see a significant treatment effect on the lactose percentage; however, there was a tendency for the ADY-fed cows to have a decreased lactose percentage. Considering that lactose is one of the major osmoregulators (lactose, prolactin, glucocorticoids, and IGF-1) in milk, it is interesting that the ADY increased milk production as the lactose concentration did not change in our study. Our results showed a transient response during week 1 of milk protein percent unlike other studies using similar ADY (AlZahal et al., 2014a; Nocek and Kautz, 2006; Li et al., 2016).

Milk urea nitrogen had a transient response in our study. The primiparous animals had a greater concentration of MUN because of growth. Growing animals have a higher rate of protein turnover, so the protein that is broken down in this process increases free ammonia in the body. The ammonia is transported to the liver for detoxification in the form of urea, which is excreted via milk, urine, and rumen fluid (Spek et al., 2013). Regarding the primiparous MUN concentrations, growth calls for a greater protein intake, which directly affects urinary total nitrogen excretion (Jonker et al., 1998; Kebreab et al., 2002). The

greater protein intake contributes to protein turnover leading to an increase in urinary total nitrogen excretion. This also leads to an elevated concentration of MUN (Spek et al., 2013). Our results mirrored other studies in that the primiparous cows had a greater concentration of MUN, which is to be expected (Spek et al., 2013; Jonker et al., 1998; Kebreab et al., 2002). Nocek and Kautz (2006) did not observe any significant differences in milk urea nitrogen (MUN) between DFM cows (12.0 mg/dL) and control cows (12.5 mg/dL). This matters because our ADY did not have a consistent effect on MUN, so it could be speculated that the ADY did not increase excreted nitrogen.

Our study observed no significant treatment effects on somatic cell count. Somatic cell count is affected by disease state and environment. The main indicator of a mastitic infection is an elevated somatic cell count (Norman et al., 2000). The main source of environmental pathogens like coliforms is the environment of the cow specifically beddings, manure, and soil to a certain extent (Harmon, 1994). In addition, there was not any significant difference in somatic cell count (cells/mL $\times 10^3$) between DFM cows (154 vs. 210) and control cows (Nocek and Kautz, 2006). The ADY did not affect any of the aforementioned items, and that is likely why we did not see a change in SCC in milk or colostrum.

Our results mirrored other studies in that our ADY did not affect infection status or environment. Feeding this ADY does not affect somatic cell count in milk or colostrum and should not be fed for that purpose. Somatic cells enter the milk in a stepwise pattern. There is an internal stressor on the mammary gland such as infection or change in the environment. As a result of this, there is an inflammatory response in the form of immune cells, which

leads to elevated somatic cells in milk. However, this process is not controlled at the level of the small intestine, which was the target of our ADY.

2.2.3 Colostrum

Colostrum is the way to pass immunity on to calves just after parturition; therefore, quality is very important. Immunoglobulin concentration is one of the methods of grading colostrum quality. Supplementing *Enterococcus faecium* and *Saccharomyces cerevisiae* did not affect the IgG and IgA production in multiparous cows (Ort et al., 2018). In a study focused on sows, colostrum immunoglobulins G and A were measured in hours after parturition, and both immunoglobulins were observed in greater concentrations in the DFM-fed sows (Laskowska et al., 2019). The DFM-fed sows had a greater concentration of IgG across all measured time points postpartum in comparison to the control sows. The DFM-fed sows had a greater concentration of IgA at all measured time points, and IgA concentration increased at 48 hours postpartum after dropping at 24 hours postpartum.

Similarly, supplementing *Saccharomyces cerevisiae boulardii* did not seem to affect IgG, A and M production and concentration in our study. As shown in Table 2.3, there was no main effect of treatment on any immunoglobulin concentrations ($P = 0.47$ for IgG, $P = 0.94$ for IgA, and $P = 0.92$ for IgM). There was no main effect of parity on IgG and IgM concentrations (IgG $P = 0.92$, IgM $P = 0.89$); however, there was an effect of parity on IgA concentration ($P < 0.01$). Multiparous cows had a significantly greater concentration of IgA in the colostrum when compared to that for the primiparous cows. A reason as to why is that our ADY is not affecting the lymphocytes synthesizing immunoglobulins.

Maternal immunoglobulins are the main source of immunity for calves, so it is important to be aware of the immunoglobulin concentrations in colostrum. Other studies

reported no change in Ig concentrations when supplementing Saccharomyces cerevisiae, which is similar to our results. Lymphocytes produce immunoglobulins and feeding an ADY does not affect these cells. The immunoglobulins (**Ig**) enter the mammary gland in a process known as colostrogenesis. These immunoglobulins require two separate entities to induce successful transfer (Larson et al., 1980). There must be receptors for specific Ig on the basal plasma membrane of the secretory cells that are able to catch the Ig from the extracellular fluid, and the epithelial cells in the mammary gland must be able to move the Ig to the lumen of the mammary gland (Larson et al., 1980). As previously stated, our ADY targeted the lower gut, and the ADY does not have an effect on colostrogenesis.

2.2.4 Blood Metabolic Measures

Plasma glucose, serum NEFA, and plasma BHBA are energy status metabolites that were analyzed at from three weeks prior to expected parturition to nine weeks postpartum. As shown in Table 2.4, there was no main effect of treatment on glucose ($P = 0.73$); however, there was a significant effect of parity ($P < 0.01$). Primiparous cows had a greater plasma glucose concentration (68.9 ± 4.5 mg/dL) when compared to that for the multiparous cows (64.0 ± 4.5 mg/dL). Serum NEFA data were analyzed at from three weeks prior to expected parturition to nine weeks postpartum. As shown in Table 2.4, there was no main effect of treatment on NEFA ($P = 0.89$); however, there was a significant effect of parity ($P < 0.01$). Multiparous cows had a greater serum NEFA concentration (0.36 ± 0.03 mEq/L) when compared to that for the primiparous cows (0.24 ± 0.03 mEq/L). There was no main effect of treatment on BHBA ($P = 0.96$); however, there was a significant effect of parity (P = 0.01), as shown in Table 2.4. Multiparous cows had a greater plasma BHBA concentration (795.0 ± 59.3 μM) when compared to that for the primiparous cows (669.0 ± 59.3 μM). In a

DFM supplementation study, there was not a significant difference in glucose until seven days postpartum in DFM-fed cows, while there was not a significant difference in NEFA at any time points between treatment groups (Nocek and Kautz, 2006). Also, DFM-fed cows had a significantly lower circulating BHBA one day prior to calving and one day post calving when compared to the control cows (Nocek and Kautz, 2006). A reason as to why we did not see results that mirror the previous studies is that our ADY targeted the lower gut which could have affected the rate of uptake and removal from the blood stream. In addition, blood metabolite concentrations are constantly changing as they are the difference between production/absorption and removal/metabolism.

Because there was not a significant treatment effect on glucose, NEFA, or BHBA, it could be postulated that the ADY did affect energy balance in transition dairy cattle under our experimental conditions. The energy input (DMI) did not change; however, milk yield increased. Assuming that energy balance = dietary input – fecal output – milk output, it could be said our ADY lead to a more negative energy balance. However, feeding this ADY had an effect on DMI and BW, but not on the aforementioned energy markers. There is likely an unknown mechanism occurring for this result to happen. Keeping in mind that the ADY affects production measures but not metabolic measures, it could be related to the fact that the ADY directly affects the lower gut but not the metabolic processes (lipolysis, lipogenesis, gluconeogenesis, glycolysis, etc.) in the liver and adipose tissue.

In our study, a higher haptoglobin concentration was observed in the treatment cows. There was a main effect of treatment on haptoglobin concentration ($P = 0.03$). The ADY-fed cows had a greater serum haptoglobin concentration compared with that for the control group (Figure 2.8). Haptoglobin is an acute phase protein produced by liver cells as a result

of the presence of cytokines secreted by immune cells during inflammation, tissue damage, and infection (Murata et al., 2004). In a DFM study, haptoglobin levels were highest across treatment groups during week 1 postpartum (AlZahal et al., 2014b). Another study reported increased haptoglobin within the first ten days postpartum (Trevisi et al., 2012). It is important to keep in mind that there is an immune reaction occurring when feeding this ADY that may or may not be a positive response. These probiotic supplements may affect microbial activity in the lower gut, but most of the studies have focused on competitive exclusion in order to prevent pathogenic bacteria from flourishing (Brashears et al., 2003). The probiotic mechanism is not well understood so it is unclear why the supplemented cows had a consistently greater concentration of haptoglobin.

2.2.5 Conclusion

Feeding an ADY (*Saccharomyces cerevisiae boulardii*, CNCM I-1079) to primi- and multiparous cows during the periparturient period resulted in an increased milk yield; however, there was not an increase in DMI. In addition, there was no major change in blood metabolites between treatment groups. This could be explained by a change in energy balance. Also, there was a significant increase in haptoglobin concentration in the ADY cows meaning that there was some inflammatory response occurring longer than the normal time frame.

In our study, we anticipated in increase in some productive measures, like milk yield and dry matter intake. However, supplementation of this probiotic (*Saccharomyces cerevisiae boulardii*) resulted in greater milk yield without having an effect on dry matter intake. In addition, there was a grander immune response than initially realized.

There were also some limitations to our experimental design which may have had an effect on our results. For example, we used the lowest recommended dosage of the probiotic as per manufacturer instructions. This could have affected results because the proportion of the probiotic compared to body weight in the cows was not addressed. Also, we were not able to study nutrient utilization which would have been useful information regarding body weight and body condition.

While keeping some of these limitations in mind, future research studies can address and prepare for the aforementioned boundaries. We conducted a small-scale study using 68 animals. A large-scale study would be able to provide more statistical power with stronger estimates. Also, we only tested a couple supplementation levels: no supplementation or 12.5g/head/day. Furthermore, there is great potential for testing different inclusion rates of this probiotic to see the affected outcomes. For probiotic supplementation in general, there is a strong possibility for a better understanding of applied use of probiotics. Developing this field of study could allow for more industry usage, which could improve the periparturient period as a whole.

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Tables and Figures

Table 2.1. Ingredients in each ration fed to prepartum primiparous animals (standard close-up ration [n = 33]), while prepartum multiparous animals received the same close-up ration supplemented with anionic salts (0.68 kg/head/day). Immediately postpartum all animals received a standard lactation ration.

	Diet (DMB)	
Ingredients	Close-Up	Lactation
Grass	18.9	2.0
Dairy Alfalfa	-	5.0
Alfalfa Silage	29.8	3.0
Triticale Silage	39.7	33.4
Corn	0.9	4.3
Barley	2.6	15.5
Canola	1.8	9.5
Corn DDGS[1]	0.9	13.1
Soybean Meal	-	4.7
MagnaFat[2]	-	3.9
Liquid Mineral Mix[3]	4.9	5.0
Vitamin ADE	0.3	0.5
Salt	0.1	0.1

[1]Corn Dried Distillers' Grain; [2]Energy source in lactation ration; 82.5% Fat, 9.0% Calcium; [3]Liquid mineral mix contained the following on a dry matter basis: 6.0% Ca, 0.16% P, 1.07% Mg, 3.07% K, 0.43% S, 1.63% Na, 2.47% Cl, 277.84 ppm Fe, 592.84 ppm Mn, 950.21 ppm Zn, 179.10 ppm Cu, 11.43 ppm Co, 11.36 ppm I, 4.57 ppm Se, 51,838.97 IU/lb vitamin A, 11,339.77 IU/lb vitamin D, 285.77 IU/lb vitamin E, 225.00 ppm organic Zn

Table 2.2. Composition of rations (on a dry matter basis) for prepartum primiparous (n = 33) animals receiving a common close-up ration, while prepartum multiparous (n = 35) animals received the same close-up ration supplemented with anionic salts. Immediately postpartum, all animals received a standard lactation ration.

	Ration			
	Close-Up (n = 10)		Lactation (n = 21)	
	Mean	SE_m	Mean	SE_m
Dry Matter, %	89.28	0.24	89.60	0.24
Crude Protein, %	15.85	1.92	18.15	0.42
Soluble Protein, %CP	41.33	3.84	39.17	1.25
ADF, %[1]	33.87	1.70	25.58	0.56
aNDF, %[2]	49.55	1.81	41.18	0.80
Lignin, %	5.40	0.32	4.72	0.35
NFC, %[3]	21.40	2.63	26.35	0.84
Starch, %	3.47	1.17	12.95	0.33
Crude Fat, %	2.53	0.14	5.02	0.33
Ash, %	10.67	0.63	9.30	0.21
TDN, %[4]	58.83	0.79	66.67	0.67
NE_l, Mcal/kg[5]	1.34	0.02	1.57	0.02
NE_m, Mcal/kg[6]	1.21	0.03	1.53	0.02
NE_g, Mcal/kg[7]	0.65	0.03	0.94	0.02

Analyses performed by Forage One (Ithaca, NY). [1]Acid Detergent Fiber; [2] Neutral Detergent fiber;
[3]Nonfibrous Carbohydrates; [4]Total Digestible Nutrients; [5]Net Energy for Lactation; [6]Net Energy for Maintenance; [7]Net Energy for Gain

Table 2.3. Treatment and parity effects on various measures including milk fat percent, milk lactose percent, milk somatic cell count, colostrum immunoglobulins (IgG, IgA, IgM), colostrum somatic cell count, and peak milk production of multiparous (n=35) and primiparous (n=33) cows consuming a common ration top-dressed by active dry yeast (ADY) at 12.5 g/d per head (n=34) or control (no added yeast, n=34).

	Treatment			Parity			P – value	
	CTRL	ADY	SE_m	P	M	SE_m	ADY	Parity
Milk Fat, %	3.06	2.98	0.35	2.96	3.08	0.35	0.34	0.18
Lactose, %	4.79	4.77	0.09	4.81	4.74	0.09	0.54	0.08
SCC (× 1000/mL)	71.2	71.7	24.3	65.2	77.7	24.2	0.98	0.53
IgG, mg/dL	6196	6770	580	6440	6526	571	0.47	0.92
IgA, mg/dL	883	879	39	749	1012	39	0.94	< 0.01
IgM, mg/dL	567	563	27	562	568	26	0.92	0.89
Colostrum SCC (× 1000/mL)	1256	1248	204	1578	926	204	0.98	0.03
Peak Milk Production, kg	38.3	39.8	1.8	34.7	43.5	1.8	0.31	< 0.01

Table 2.4. Treatment effect on various measures including plasma glucose, serum NEFA, plasma BHBA, and body weight of multiparous (n=35) and primiparous (n=33) cows consuming a common ration top-dressed by active dry yeast (ADY) at 12.5 g/d per head (n=34) or control (no added yeast, n=34).

	Treatment			Parity			P – value	
	CTRL	ADY	SE_m	P	M	SE_m	ADY	Parity
Plasma Glucose, mg/dL	66.7	66.2	4.5	68.9	64.0	4.5	0.73	<0.01
Serum NEFA, mEq/L	0.30	0.30	0.03	0.24	0.36	0.03	0.89	<0.01
Plasma BHBA, μM	733.4	730.7	59.6	669.0	795.0	59.3	0.96	0.01

Figure 2.1. Dry matter intake (3 wk prepartum through 9 wk postpartum) of multiparous (n = 35) and primiparous (n = 33) cows consuming a common ration top-dressed by active dry yeast (ADY) at 12.5 g/d per head (n = 34) or control (no added yeast, n = 34).

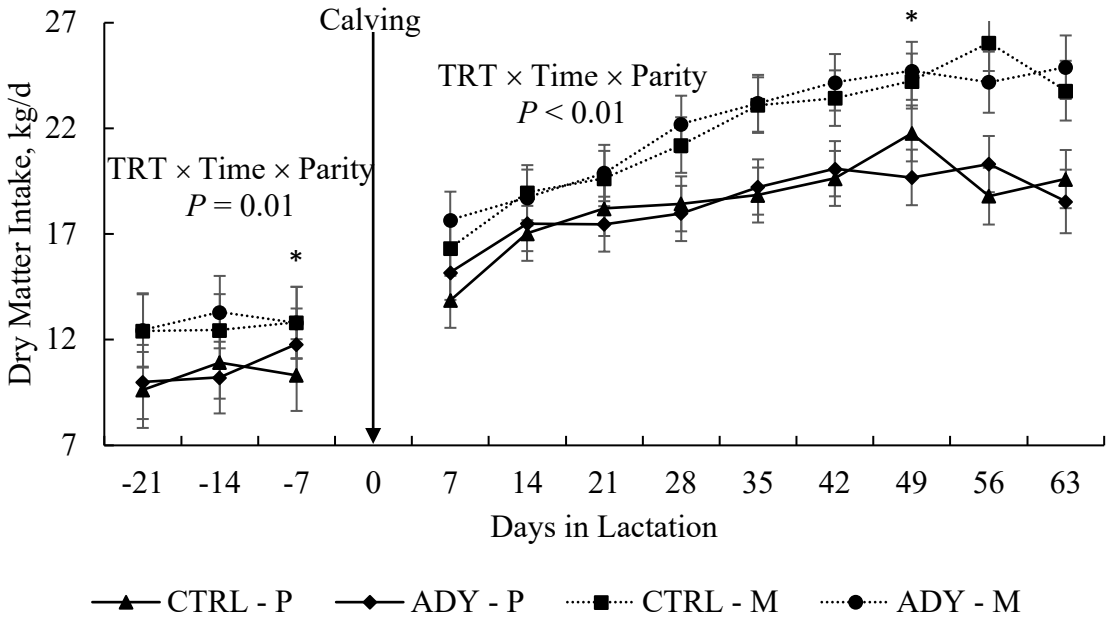


Figure 2.2. Body weight, kg (3 wk prepartum through 9 wk postpartum) of multiparous (n = 35) and primiparous (n = 33) cows consuming a common ration top-dressed by active dry yeast (ADY) at 12.5 g/d per head (n = 34) or control (no added yeast, n = 34).

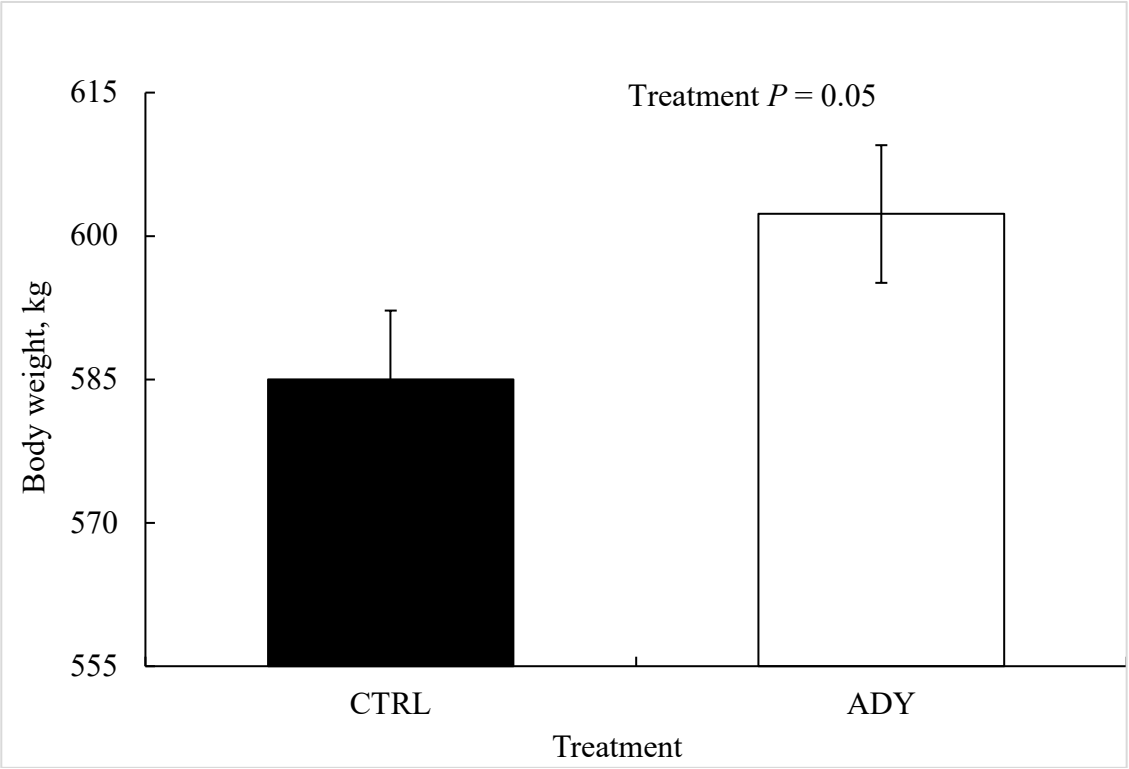


Figure 2.3. Milk yield (kg/d; wk 1 through 9 postpartum) of multiparous (n=35) and primiparous (n=33) cows consuming a common ration top-dressed by active dry yeast (ADY) at 12.5 g/d per head (n=34) or control (no added yeast, n=34).

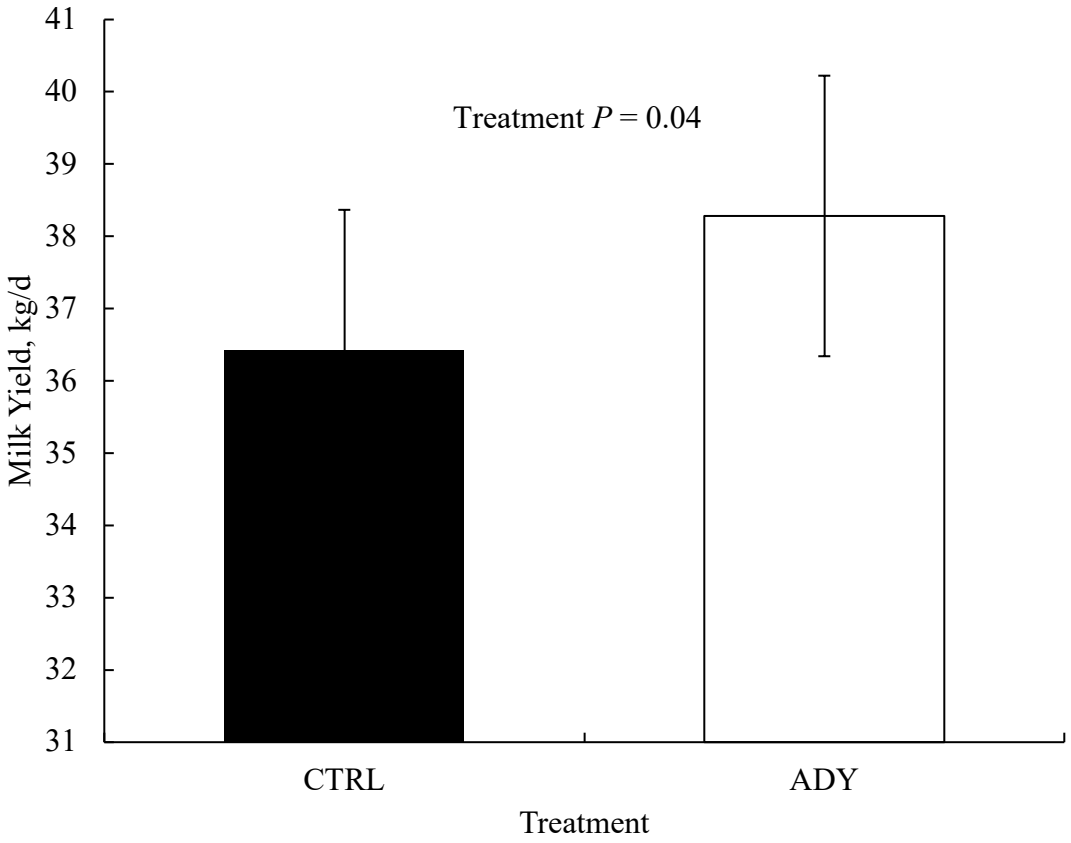


Figure 2.4. Energy corrected milk production (kg) of multiparous (n=35) and primiparous (n=33) cows consuming a common ration top-dressed by active dry yeast (ADY) at 12.5 g/d per head (n=34) or control (no added yeast, n=34).

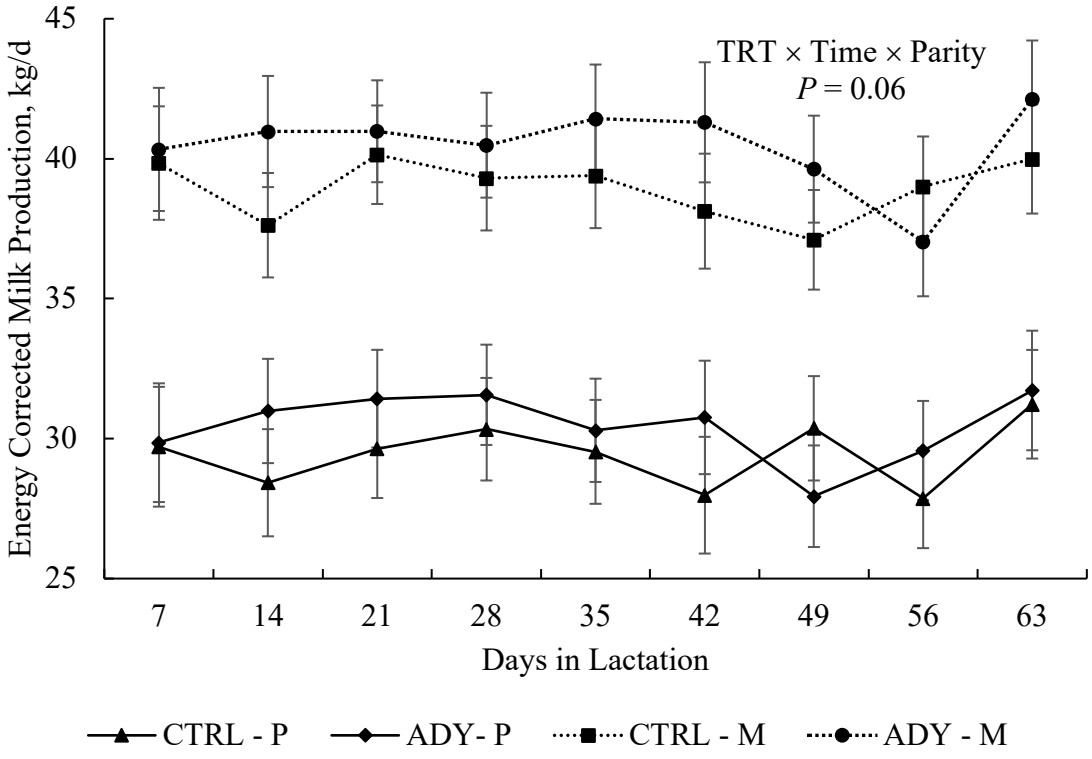


Figure 2.5. Gross feed efficiency (kg energy-corrected milk/kg dry matter intake) of multiparous (n=35) and primiparous (n=33) cows consuming a common ration top-dressed by active dry yeast (ADY) at 12.5 g/d per head (n=34) or control (no added yeast, n=34).

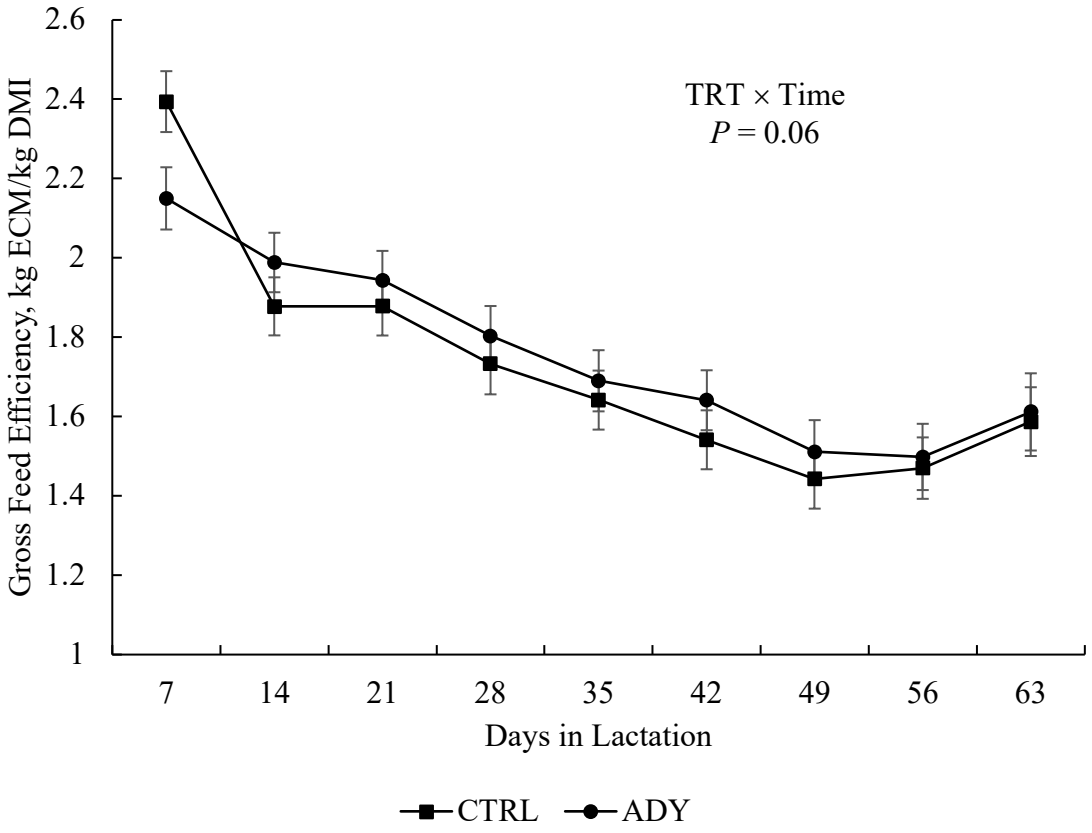


Figure 2.6. Milk protein (%, wk 1 through 9 postpartum) of multiparous (n=35) and primiparous (n = 33) cows consuming a common ration top-dressed by active dry yeast (ADY) at 12.5 g/d per head (n = 34) or control (no added yeast, n = 34).

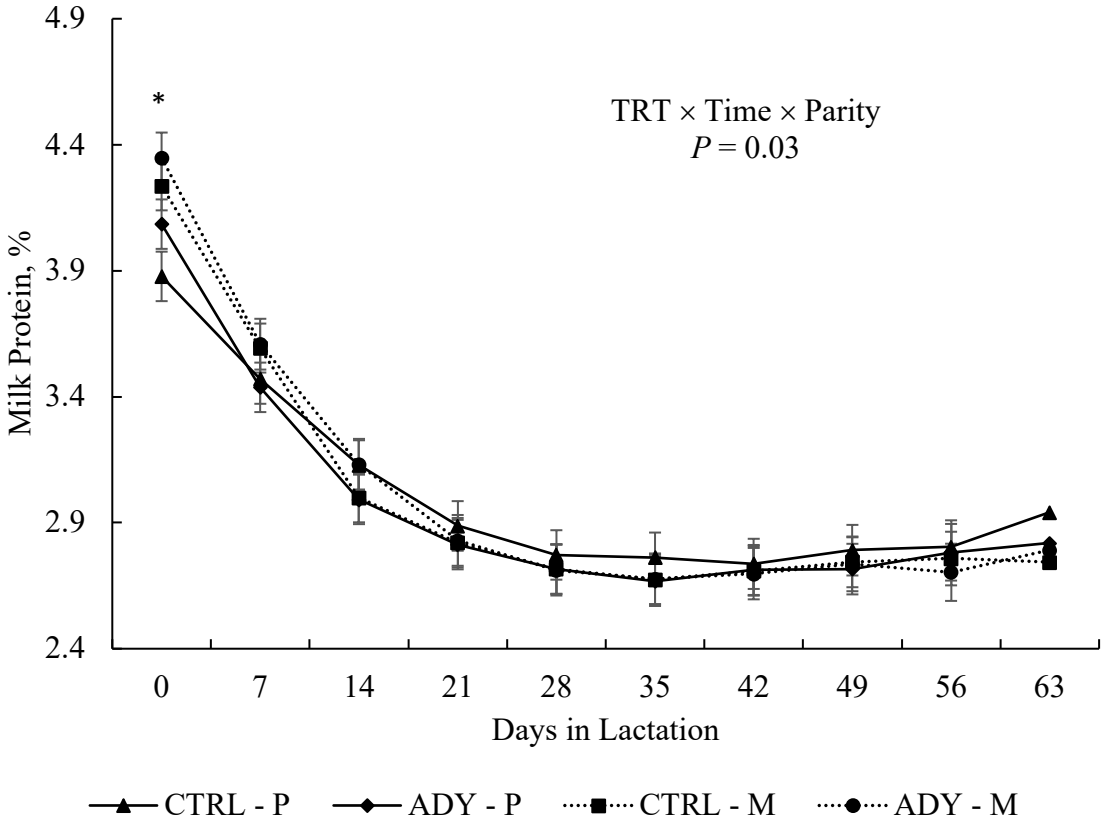


Figure 2.7. Milk urea nitrogen (mg/dL; wk 1 through wk 9 postpartum) of multiparous (n = 35) and primiparous (n = 33) cows consuming a common ration top-dressed by active dry yeast (ADY) at 12.5 g/d per head (n = 34) or control (no added yeast, n = 34).

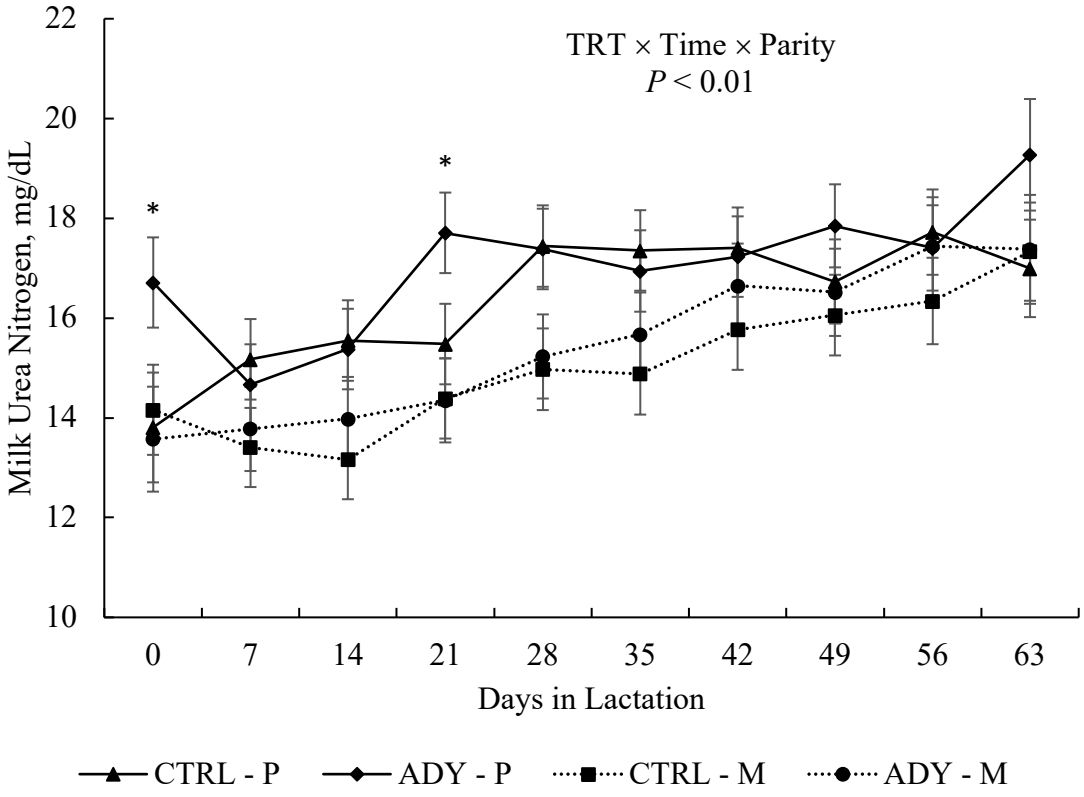
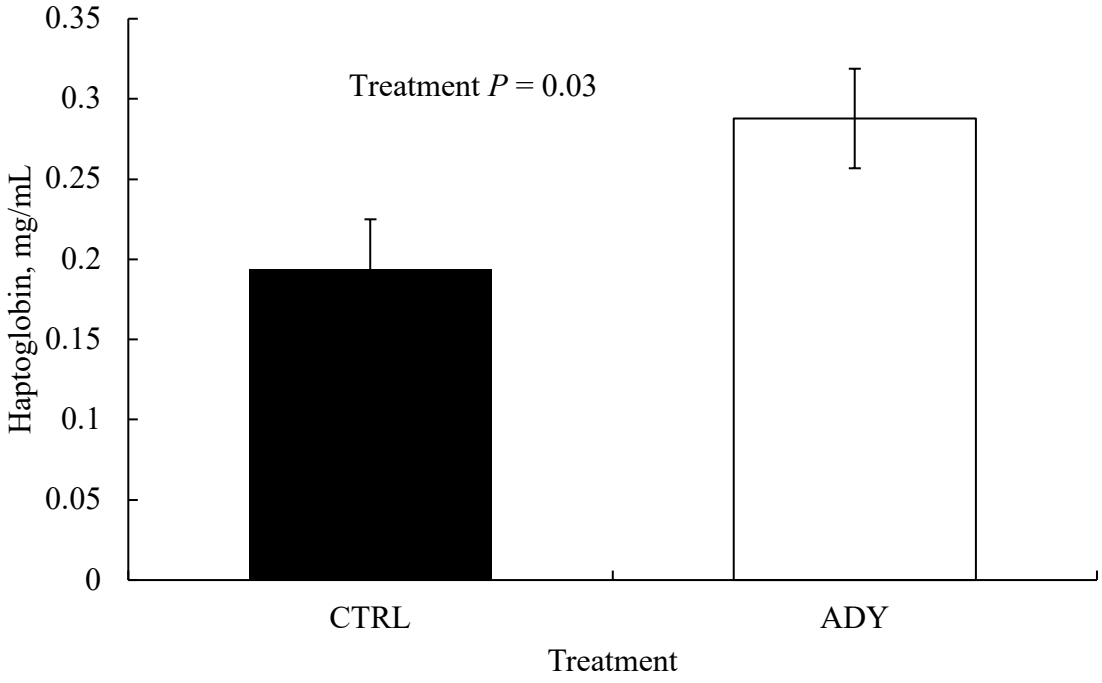


Figure 2.8. Serum haptoglobin (mg/mL; wk 1 through wk 9 postpartum) of multiparous (n = 35) and primiparous (n = 33) cows consuming a common ration top-dressed by active dry yeast (ADY) at 12.5 g/d per head (n = 34) or control (no added yeast, n = 34).



Appendix 1. Research Protocol Approval

University of Idaho
Institutional Animal Care and Use Committee

Date: September 04, 2018
To: Dr. Anne Hermen Laarman
From: University of Idaho
Institutional Animal Care and Use Committee
Re: Approval of personnel amendment request for Protocol
IACUC-2017-63 *Impact of* [Commercial Yeast Product] *on Inflammation Status in Periparturient Cows*

Your personnel amendment request, 003840, submitted on 08/29/2018 10:37:30 AM PDT to the animalcare and use protocol listed above was administratively reviewed and approved by the Institutional Animal Care and Use Committee on 09/04/2018.

The original approval date for this protocol was:
01/02/2018 This protocol approval will remain in effect
until: 01/02/2019
The protocol may be continued by annual updates until: 01/02/2021

Currently approved internal personnel on this protocol are: Ahmadzadeh, Amin; Anderson, Ashly; Bennett, Madeline; Bilton-Smith, Ashalynn; Bledsoe-Healy, Mikaela ; Degenshein, Maddison; Hiltz,Rebecca; Hung, Hao-Che; Laarman, Anne Hermen; Norseth, Jared; Roberts, Rayne ; Smith, Jennifer;Steelreath, Maeghan; Weber, Tanya

Federal laws and guidelines require that institutional animal care and use committees review ongoing projects annually. For the first two years after initial approval of the protocol you will be asked to submitan annual update form describing any changes in procedures or personnel. The committee may, at its discretion, extend approval for the project in yearly increments until the third anniversary of the original approval of the project. At that time, the protocol must be replaced by an entirely new submission.

Craig McGowan, IACUC Chair