2020

Physiological Responses to Feeding a Yeast Culture Supplement to Transition Dairy Cows

Nathaly Ana Carpinelli
South Dakota State University

PHYSIOLOGICAL RESPONSES TO FEEDING A YEAST CULTURE SUPPLEMENT TO TRANSITION DAIRY COWS

BY

NATHALY ANA CARPINELLI

A thesis submitted in partial fulfillment of the requirements for the

Master of Science

Major in Biological Sciences

Specialization in Dairy Science

South Dakota State University

2020

THESIS ACCEPTANCE PAGE

Nathaly Ana Carpinelli

This thesis is approved as a creditable and independent investigation by a candidate for the master's degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Johan Osorio
Advisor Date

Vikram Mistry
Department Head Date

Nicole Lounsbery, PhD
Director, Graduate School Date

ACKNOWLEDGMENTS

First and foremost, I would like to start by thanking my family for their love, patience, and support over the years. My parents have allowed me to follow my dreams and have been behind me every step of the way. Thank you Marilei and Antoninho, for your unconditional love and always reminding me that I am capable of anything. Additionally, my extended family has been so supportive during these years living abroad. I cannot express how much I love them.

I would like to express my sincere gratitude to my advisor Dr. Johan S. Osorio. I would not have accomplished this if not for his support and patience. Thank you for believing in me when I thought I was doubted myself. I find it hard to describe how much gratitude I feel toward him. Thank you for the life-changing opportunity!

Thank you to Dr. Mistry, Dr. Chase, and Dr. Salfer for serving as members of my graduate committee. Thank you, Dr. Bowser, for accepting to serve on my committee as the faculty representative. I greatly appreciate for the time spent reviewing my thesis and for all the valuable suggestions.

Thank you to the SDSU DRTF farm crew, especially Pete Linke, Melissa Schmitt and Danielle Tews for their assistance with the husbandry and care of the cows and calves during the farm trial, maintaining the facility, and their help whenever I needed to solve issues at the farm.

Thank you, Phibro Animal Health Corporation, for their financially support of my study and creation of this production. In particular, to Jim Chapman, Derek McLean, and Evin Sharman.

Thank you Jéssica Halfen for your help during this trial. Thank you to be by my side every day. The trial would not have been the same without an awesome colleague and friend. You are appreciated more than you know! We rock, girl!

Thank you to my fellow graduate students Chelsea Schossow, Karla Hernandez-Rodriguez, Lauren Shearer, Lydia Olaqunju, Michaela Joy Della, Nirosh Senevirathne, Rhea Lawrence, Roshin Mohan, Sonia Rodriquez-Jimenez, Tainara Michelotti. Their help and guidance on the farm, in the lab, and in our classes have been invaluable. Especially, I would like to thank Fernanda Rosa. Even prior to my arrival at SDSU, Fernanda helped, and as soon as I joined SDSU, we became great friends. Thanks for teaching me in the lab and for all your help during the trial. My gratitude for her friendship during this process.

Last but not least, to my lovely friends, I would like to express my sincere gratitude. Thank you for always encouraging me to be the best that I can be. Especially, to my Brazilian friends I met in the United States, Camila, Jossué, Luan, Maíra, Patricia, Reinaldo, Rhuan, Saulo, and Samara. Thanks for being my family in South Dakota!

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LIST OF ABBREVIATIONS

DMI	Dry matter intake
BCS	Body condition score
BHB	Beta hydroxybutyrate
BW	Body weight
cDNA	Complementary DNA synthesis
CON	Control
d	Days
DIM	Days in milk
DM	Dry matter
DNA	Deoxyribonucleic acid
EB	Energy balance
ECM	Energy-corrected milk
FA	Fatty acids
NEB	Negative energy balance
NEFA	Non-esterified fatty acids
PCR	Polymerase chain reaction
PMNL	Polymorphonuclear neutrophils
RNA	Ribonucleic acid
ROS	Reactive oxygen species
SCC	Somatic count cell
TG	Triglycerides

TLRs	Toll-like receptors
TMR	Total mix ratio
VFA	Volatile fatty acids
VLDL	Very-low density lipoprotein
YC	Yeast culture

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ABSTRACT

PHYSIOLOGICAL RESPONSES TO FEEDING A YEAST CULTURE SUPPLEMENT TO TRANSITION DAIRY COWS

NATHALY ANA CARPINELLI

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The transition period is the dynamic stage in the lactation cycle, where the most metabolic changes can occur. The use of nutritional strategies, such as yeast culture, can help cows during the transition from pregnancy to early lactation, improving health and decreasing the incidence of metabolic disorders. The first objective of this research was to evaluate the effects of a commercial yeast culture product (YC; Cellerate Culture Classic HD, Phibro Animal Health, Teaneck, NJ) on performance, blood biomarkers, and rumen fermentation and bacterial population in dairy cows during the transition period through 50 days in milk (DIM). Overall, the results showed that yeast culture products could promote positive responses on performance, such as milk yield, somatic cell count (SCC), and energy corrected milk (ECM). In the rumen, yeast culture product contributed to increased valerate, which was accompanied by improvements in rumen bacteria populations such as cellulolytic and lactic acid-utilizing bacteria. In addition, the results suggest that yeast may influence lipid metabolism in transition dairy cows, based on nonesterified fatty acid (NEFA) and beta-hydroxybutyrate (BHB) markers. The second objective was to assess the effects of yeast-based products on metabolism, inflammation, liver function, and immune function through blood biomarkers and gene expression in polymorphonuclear leukocytes (PMNL) in dairy cows during the transition period until 50 DIM. Yeast supplementation might stimulate a mild inflammatory response in

transition dairy cows, based on blood biomarker and PMNL mRNA expression. In summary, we observed that yeast culture could promote improvements in performance, rumen bacteria population, and stimulate the immune system in transition dairy cows through 50 DIM.

INTRODUCTION

The transition period is associated with the peak incidence of production problems, metabolic disorders, and infectious diseases in dairy cows (Drackley, 1999). During late pregnancy and particularly in early lactation, cows enter a condition called negative energy balance (NEB) due to the increase in energy requirements. The NEB is associated with a large mobilization of fatty acids stored in adipose tissue, thus, causing marked elevations in blood concentrations of non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHBA) (Drackley et al., 2006). Prolonged periods with high blood NEFA and BHB concentrations in the bloodstream will eventually cause further complications, such as metabolic disorders. In addition, during the transition period, the cow's immune system is suppressed. This is linked with the metabolic challenge associated with the onset of lactation, which can affect the immune function.

The advancements in dairy cow nutrition enable the development of nutritional strategies to help cows during the transition from pregnancy to early lactation. The positive effects of yeast-based products from *Saccharomyces cerevisiae* in transition dairy cows have been demonstrated (Dann et al., 2000; Yuan et al., 2015b; Olagaray et al., 2019). The response to yeast supplementation has been reported to improve the growth and activities of ruminal microbial communities and, consequently, promoting positive effects on milk or meat production (Fonty and Chaucheyras-Durand, 2006).

Our general hypothesis is that yeast culture supplementation during the transition period through 50 DIM will alleviate metabolic challenges associated with the onset of lactation, and consequently improve health and performance in transition dairy cows. The

objective of this study was to determine the effects a commercial yeast culture product (YC; Cellerate Culture Classic HD, Phibro Animal Health, Teaneck, NJ) on health and performance and correlate these with metabolism and immune function during the transition period of dairy cows.

CHAPTER 1. LITERATURE REVIEW

Transition period

Dairy cows have been genetically selected for the purpose of producing more milk during their reproductive life. Because of this genetic selection, cows require adequate management and nutrition during their reproductive life in order to sustain such enhanced milk yield. In dairy cows, the transition period spans from 3 weeks before through 3 weeks after parturition (Drackley, 1999), and it is the most critical period during the cow's life. It is characterized by dramatic changes in metabolism and physiology, where most infectious diseases and metabolic disorders can occur (Grummer, 1995; Drackley, 1999). According to Trevisi and Minuti (2018), five critical points are associated with the transition period: negative energy balance (NEB) due to the increase in energy requirements and decrease in feed intake, which leads to mobilization of adipose tissue; reduction of immune competence; systemic inflammatory condition; oxidative stress; and hypocalcemia.

Physiology and metabolism

Negative energy balance

During late pregnancy and particularly in early lactation, the energy demands increased in cows due to fetal development and milk production. At the same time, cows' energy intake cannot meet those energy demands. A decline in energy intake is commonly observed around parturition (Ingvartsen and Andersen, 2000). After parturition, dry matter intake (DMI) tends to increase if the cow is healthy. However, the health status will vary from cow to cow, which will be reflected in DMI. At the same

time, major changes in metabolism are commonly reported in cows in order to cope with the increase in nutrient requirements (Ingvartsen and Andersen, 2000).

Postpartum NEB is universal in mammals, and it is associated with an imbalance between decreased energy intake and increased energy requirements for milk synthesis. During this condition, cows mobilize large amounts of NEFA from adipose tissue to cope with the energy deficit to sustain milk production (Ingvartsen and Moyes, 2013). Lipid mobilization occurs when adipose tissue becomes predominantly lipolytic (Contreras and Sordillo, 2011). This is a normal physiological adaptation due to the reduced energy intake, coupled with an increase in energy demands after calving (Contreras and Sordillo, 2011).

Canonical metabolism

In early lactation, changes in endocrine signals and tissue metabolism have been observed (Ingvartsen, 2006). The changes in tissue metabolism during the transition period comprise liver, adipose tissue, and mammary gland. In adipose tissue, a lipolytic state is predominant due to low insulin during NEB, while the liver needs to maintain gluconeogenesis (Ingvartsen (2006). A summary of the metabolic adaptations across adipose tissue, liver, and mammary gland during the peripartum is presented in Figure 1.1. During NEB, an increase in blood NEFA mobilization from adipose tissue is commonly observed, which may exceed the capacity of the mammary gland to pack these excess NEFA into milk (Drackley, 1999). The liver is the most important site to remove and metabolize NEFA from circulation (Van den Top et al., 2005). During an extreme rate of lipid mobilization, it will lead to increased uptake of NEFA by the liver (Drackley, 1999). Once taken up by the liver, NEFA can be 1) complete oxidized through the

tricarboxylic acid cycle (TCA), providing energy for the liver; 2) partially oxidized to ketone bodies, that will serve as an energy source for tissues; 3) TG synthesis for storage in the liver; 4) TG synthesis to be exported into the bloodstream as a very-low density lipoprotein (VLDL); 5) secreted in bile (Emery et al., 1992; Ingvartsen, 2006; White, 2015). Prolonged periods with high blood NEFA concentrations in the bloodstream will eventually impair the liver's ability to oxidize NEFA, causing further complications, such as impaired liver function and fatty liver.

NEFA metabolism

Once in the liver, NEFA is converted to fatty acyl-CoA by the enzyme long-chain-fatty-acid- acyl CoA synthetase. Fatty acyl-CoA is transported into the mitochondria by the coordinated effort of carnitine palmitoyltransferase I (CPT-I) and II (CPT-II) enzymes (Champe et al., 2005). Once inside the mitochondria, fatty acids (FA) undergo β-oxidation (Ingvartsen, 2006). The enzyme CPT-I is located in the outer mitochondrial membrane that converts fatty acyl-CoA in acylcarnitine that can be transported over the internal mitochondrial membrane. Then, the acylcarnitine is transported into the mitochondria by CPT-II, which converts acylcarnitine into fatty acyl-CoA that is oxidized via β-oxidation. The product of β-oxidation of fatty acyl-CoA is Acetyl-CoA and can be further oxidized in the TCA cycle (Champe et al., 2005). In some instances, the TCA cycle cannot oxidize the Acetyl-CoA generated via β-oxidation product of low availability of TCA cycle intermediates such as oxaloacetate. In that case, acetyl-CoA is then used to synthesize ketone bodies (Drackley, 1999). Ketone bodies comprise acetoacetate (ACAC), BHB, and acetone (nonmetabolized product). The ACAC and BHB are transported to peripheral tissues and can be used as an energy source.

Excessive circulation of blood ketone bodies may negatively affect animal health and performance, causing metabolic disorders such as ketosis (White, 2015).

In addition to mitochondrial β-oxidation of FAs, peroxisomal β-oxidation plays an alternative role in FA oxidation when these are in excess in the liver (Ingvartsen, 2006). The pathway is very similar to mitochondrial β-oxidation, except for the first step. The initial oxidation is mediated by acyl-CoA oxidase, producing hydrogen peroxidase (H_2O_2) instead of reduced NAD (Drackley, 1999). Although the peroxisome lacks of respiration chain and the ability to produce ATP, its activity has been related to oxidative stress and inflammation because of the production of H_2O_2, which is one of a family of reactive oxygen species (ROS)(Champe et al., 2005). The peroxisomal β-oxidation of FA is a significant source of ROS in transition dairy cows, however, most ROS found in healthy tissues are a product of an increase in cellular metabolism and energy generation by mitochondria (Drackley, 1999; Sordillo, 2016).

Fatty acids that are not oxidized are re-esterified with glycerol to form TG. A portion of these TG is packaged into VLDL and exported into the bloodstream to be used by extra-hepatic tissues (Champe et al., 2005). The mammary gland can use the FA contained in VLDL for milk fat synthesis; however, these need to be uptaken with the help of lipoprotein lipase, a VLDL receptor that hydrolyzes TG (Osorio et al., 2016). In ruminants, it is well known that VLDL secretion is very limited (Grummer, 2008). The low rate of synthesis and secretion of VLDL in ruminants is insufficient to remove considerable amounts of accumulated TG in hepatocytes (Drackley et al., 2006). In transition dairy cows, the low secretion of VLDL from liver could be explained by the hepatic downregulation of mRNA expression of Apo-B100 (*APOB*, gene symbol),

contributing to the accumulation of TG in liver (Bernabucci et al., 2004; Bernabucci et al., 2009). Together, the oxidation of FAs, TG accumulation, and the low synthesis of VLDL in the liver play a central role in the development of fatty liver syndrome (Ingvartsen, 2006). The fatty liver syndrome implicates a limited capacity to secrete TG within VLDL, coupled with an increased rate of FA esterification (McFadden, 2020). According to Overton and Waldron (2004), almost all high-producing dairy cows accumulate TG in liver during the first few weeks postpartum. Grummer (1993) observed that fatty liver syndrome and ketosis are common metabolic disorders related to lipid metabolism during the transition period.

Metabolic disorders

Fatty liver syndrome

The fatty liver syndrome is one of the major metabolic disorders that occur in the peripartal dairy cow, compromising the cow's metabolism (Bobe et al., 2004). White (2015) described that during the transition between pregnancy and lactation, around 60% of dairy cows develop fatty liver syndrome, which is a direct response to NEB. The rapid decrease in prepartum DMI has been linked with greater postpartal lipid mobilization of adipose tissue, increased NEFA, and accumulation of TG in liver (Drackley, 1999; Grummer et al., 2004).

The fatty liver syndrome commonly occurs in the early postpartum period, when an excess of TG accumulate in the liver. In dairy cows developing fatty liver syndrome, the hepatic TG deposition is due to increased esterification of lipolytic-derived FA, and the process is exacerbated by inadequate mitochondrial β-oxidation of FA (McFadden, 2020) or low production of VLDL to transport TG. The lipid accumulation causes

significant alterations in the liver, affecting the normal hepatic function, cow's health, milk production, and reproductive performance (Bobe et al., 2004; White, 2015).

According to Grummer (2008), there are strategies that can be used to prevent or treat fatty liver syndrome including: 1) decrease TG lipolysis in adipose tissue and, as a consequence, reduced blood NEFA; 2) increase complete hepatic oxidation of NEFA; 3) increase the rate of VLDL export from the liver. In this context, nutritional strategies have been used to prevent fatty liver syndrome, such as adjustments on prepartal plane of nutrition (Grum et al., 1996; Janovick et al., 2011; Osorio et al., 2013b), restricted diets (Van den Top et al., 2005) and nutritional supplements (i.e., rumen-protected choline and propylene glycol) (Grummer, 2008).

Ketosis

Ketosis is marked by an elevated circulation of ketone bodies associated with increased NEFA infiltration in the liver. Peripheral tissues can be use ketone bodies as an energy source; however, if the rate of ketones uptake by peripheral tissues is lower than the hepatic ketone production, an increase in ketone bodies will be observed, also known as hyperketonemia (Ingvartsen, 2006). Ketosis can be classified as a sub-clinical (SCK) or clinical (≥1.4 mmol/L) based on blood BHB concentrations.

According to Oetzel (2004), cows with a clinical ketosis condition had higher blood BHB concentrations (>3.0 mmol/L) than the BHB threshold used to determine SCK. During SCK, it is observed an increase in blood, urine, or milk ketone bodies without clinical symptoms; and in the clinical conditions, cows presented an increase in blood, urine, or milk ketone bodies associated with clinical signs, such as inappetence, weight loss, and dry manure (Gordon et al., 2013). The incidence of SCK varies widely

across herds, but it has been reported to be around 60%, while clinical ketosis occurs between 2% to 15% (Duffield, 2000). Ketosis or fatty liver syndrome conditions have been negatively associated with immune function (Overton and Waldron, 2004). In a review paper, Bobe et al. (2004) suggested that fatty liver syndrome is indirectly associated with immune function because metabolic and hormonal changes attributed to this syndrome may interfere with immune function. Ketosis is a metabolic condition that can alter immune function in transition dairy cows (Zhu et al., 2019), regulate genes involved in lipid metabolism (Mezzetti et al., 2019), impair liver function, and lead to oxidative stress conditions (Rodriguez-Jimenez et al., 2018).

Immune system

The immune system comprises lymphoid organs, cells, and humoral factors that work in a communicative network to recognize, resist, and eliminate pathogens (Bertoni et al., 2015). The immune system is divided into innate and adaptive components, but these two are integrated (Daha, 2011). The innate immunity is described by an early and rapid response that can occur in seconds to combat a wide variety of potentially infectious pathogens (Sordillo, 2016). In contrast, the adaptative immune system adapts the response specifically to each infectious agent encountered and is delayed compared to the innate immune system (Sordillo, 2016).

Innate immune system

The innate immune system is rapidly activated during the early stages of infection. The major components of innate immunity include physical barriers, pattern recognition receptors, complement activation, cytokines, oxylipids, endothelial cells, and immune cells (Sordillo, 2016). The immune cells in the innate immune system are

leukocytes [i.e., polymorphonuclear neutrophils (PMNL), monocytes, macrophages, lymphocytes, eosinophils, basophils], dendritic cells, and natural killer cells. These cells play an important role against pathogens as well as providing communication between innate and adaptive immune systems.

The pattern recognition receptors (PRR) on the surface of the immune cells are responsible for recognizing specific molecules on the surface of pathogens. These receptors detect microbial factors associated with infectious pathogens and stimulate the innate immune response (Sordillo, 2016). The PRR recognize two classes of molecules: pathogen-associated molecular patterns (PAMPs) associated with pathogen products; and damage-associated molecular patterns (DAMPs), which are related to host cell components released during cell damage or death. This recognition by PRR stimulate a number of responses, including cellular activity such as phagocytosis and initiation of the inflammatory response (Trevisi and Minuti, 2018). For instance, toll-like receptors (TLRs) recognize various PAMPs, which can initiate a signal pathway that induces gene transcription and activation of inflammatory mediators, such as cytokines and chemokines (Lee and Hwang, 2006). These mediators can affect endocrinal signals, which coordinates an inflammatory response (Ballou, 2011).

During the early stages of the immune response, the goal is to localize and eliminate the infection by recruiting immune cells, mainly neutrophils, to the site of infection. The innate immune system combats 95% of infections through combined mechanisms, including the action of different cell types (e.g., PMNL, monocytes, macrophages), humoral factors (i.e., complement, lysozyme), and network of cytokines (Daha, 2011).

Neutrophils

Granulocytes are a subcategory of leukocytes, including PMNL, eosinophils, and basophils, with different cytoplasmic characteristics and cellular morphologies. The PMNL are the major circulating leukocytes (50-70%) and present cytoplasmatic granules and multilobed nucleus that can be highly differentiated from the other leukocytes (Paape et al., 2003). The PMNL cells are originated from myeloid lineage progenitor cells, synthesized, and maturated in the bone marrow. The synthesis and maturation take around 7 to 10 days until neutrophils release into the blood circulation (LeBlanc, 2020). The PMNL circulate for a short time (6 to 8 h half-life in humans; 8.9 h in cows), but the lifespan is extended once activated, although still to less than ∼1 day (Paape et al., 2003; LeBlanc, 2020). A recent study in humans suggests that neutrophils may traffick through more complex cycles and sites, and perhaps longer lifespans (Hidalgo et al., 2019). In response to an infection, PMNL are considered the first line of defense from the innate immune system against pathogens, mainly bacteria and virus. The inflammatory process initiates with PMNL migrating to the site of infection. After migration, PMNL interact with damaged cells or bacteria through a variety of mechanisms, including phagocytosis, intracellular digestion by oxidation (e.g., oxidative burst in lysosomes), and extracellular release of oxidants from neutrophil granules (e.g., myeloperoxidase) (LeBlanc, 2020). Additionally, PMNL may undergo programmed apoptosis, culminating with casting neutrophil extracellular traps (NETs) of DNA (LeBlanc, 2020).

The receptors in PMNL are classified according to their function, including chemotactic, adhesion, cluster of differentiation (CD14), and opsonin receptors (Paape et al., 2003). Chemotactic receptors are activated to recognize chemoattractants that recruit PMNL to the site of infection. Examples of chemoattractants include chemokine (C-X-C

motif) ligand 8 (CXCL8) (also called IL-8), platelet-activating factor, complement component C5a, tumor necrosis factor α (TNFα), and PRR (Bassel and Caswell, 2018). Adhesion receptors provide the attachment of PMNL to the endothelium and play an important role in allowing PMNL to move across the endothelium adjacent to inflamed tissue (Paape et al., 2003). L-selectin is one example of an adhesion receptor that mediating the rolling of PMNL over the inflamed endothelium and subsequently adherence (Raffler et al., 2005). The CD14 receptors bind bacterial toxins, such as lipopolysaccharide (LPS) and cytokines that promote gene regulation related to the host defense (Paape et al., 2003). Opsonin receptors are characterized by immunoglobulins (e.g., IgM and IgG2) and complement components that promote bacteria recognition and allow PMNL to initiate phagocytosis (Paape et al., 2003).

Pathogen recognition by neutrophils leads to phagocytosis, a process that involves engulfing and killing pathogens. During the respiratory burst in PMNL, large volumes of ROS (O_2 and H_2O_2) are produced by stimulation of NADPH oxidase to destroy invading pathogens (Sordillo et al., 2009). The accumulation of ROS can damage the host's cells and tissues and lead to oxidative stress in transition dairy cows. During the transition period, the oxidative stress may be a major underlying cause of inflammatory and immune dysfunction in dairy cattle, associated with parturition and a loss in antioxidant potential (Sordillo and Aitken, 2009). In periparturient dairy cows, different variables can affect neutrophil functional capacity. The main factors that influence neutrophil function are fat mobilization (BHB, NEFA, oxidative stress), nutrient supply (antioxidant, glucose, calcium), social stressors, and exogenous immune modulators (LeBlanc, 2020). In addition, mRNA expression has been correlated with neutrophil function (Moyes et al.,

2014; Batistel et al., 2017; Vailati-Riboni et al., 2019). Metabolic status alters the expression of genes associated with inflammation in bovine blood PMNL (Moyes et al., 2010).

Macrophages

Premonocytes are synthesized in the bone marrow and released into the bloodstream, where they differentiate in monocytes (MO) and have a half-life of 1-2 d (Ziegler-Heitbrock, 2014). Once in the bloodstream, they can migrate into the tissues and differentiate into tissue-specific macrophages, such as intestinal macrophages (gut), osteoclasts (bone), kupffer cells (liver), and adipose tissue macrophages (adipose tissue). Macrophages form a line of defense in tissues, like a barrier against infection. Important functions of macrophages and monocytes include phagocytosis, ROS production, and secretion of pro-inflammatory cytokines during an inflammatory response (Calder, 1995). Macrophages are involved in the innate and adaptative immune responses because they can also behave as antigen-presenting cells, recruiting other immune cells to the site of infection through antigen presentation to T cells (Calder, 1995).

In transition dairy cows, adipose tissue macrophages (ATM) are a key component during lipolysis in adipose tissue due to NEB. During lipolysis, the role of ATM is to remove lipolytic products (e.g., FA, diglycerides, and monoglycerides), recruitment of new adipocyte progenitors by secreting chemotactic proteins, and activation of NF-κB transcription factor pathways and inflammatory gene transcription (Contreras et al., 2017).

Lymphocytes

Lymphocytes are produced in the bone marrow and newly release lymphocytes are termed naïve, virgin, or unprimed. They become activated due to local antigenic stimulation responses and are termed memory or effector cells (Sordillo and Streicher, 2002). Lymphocytes consist of T cells (helper or cytotoxic) and B cells (secrete antibody). The T cells can be subdivided into T-helper and T-cytotoxic (CTL) lymphocytes. The T-helper cells produce cytokines, such as interleukin-2 (IL-2) and interferons (IFN), crucial for an effective cell-mediated immune response. The B lymphocytes differentiate to produce proteins called antibodies or immunoglobulins (Ig) and effector B cells or plasma cells (Sordillo et al., 1997). Plasma cells are central to specific immunity, which have a short half-life and produce and secrete antibodies.

The innate immune system in transition dairy cows

Peripartal dairy cows are often regarded as immunocompromised animals because functions related to the immune system seem to be depressed. In a recent review paper, Trevisi and Minuti (2018) reported that peripartal cows have a high probability of experiencing events that challenge their immune system and expose any immunocompetence. Such effects are related to the NEB characterized by critical reduction of feed intake around parturition, severe body fat mobilization, production of ROS, and incidence of metabolic disorders.

Before calving, the immune dysfunctions can be due to a combination of metabolic and endocrine factors. However, it is not clear if the reduction in immunocompetence is a physiological condition of transition dairy cows or an early signal of disease induced by other events (Trevisi and Minuti, 2018). It is well established

that some cellular processes essential in the immune system are depressed around calving, including phagocytosis by neutrophils, antibody synthesis by lymphocytes, and immunoglobulins concentration in plasma (Goff and Horst, 1997; Lacetera et al., 2005; Trevisi and Minuti, 2018). After parturition, the development of health disorders, such as mastitis, ketosis, retained placenta, metritis, and displaced abomasum, can contribute to immune dysfunction (Sordillo, 2016). In addition, metabolic changes related to energy and calcium supply in support of lactation can also impair the innate immune system (Ingvartsen and Moyes, 2013).

Immune dysfunction has been correlated with specific factors associated with the transition period. For example, after calving, blood glucose is prioritized to the non-insulin-dependent glucose transporters, which are only expressed on immune cells and the mammary gland. This is particularly important during systemic inflammation when activated immune cells have higher glucose requirements to sustain phagocytic and killing capacity (i.e., oxidative burst) activities (Kvidera et al., 2017). Changes in steroid hormones can contribute to an immune dysfunction during the transition period, mainly by altering the function of neutrophils (Sordillo and Mavangira, 2014). Glucocorticoids are known to have a potent immunosuppressive effect on blood neutrophil, while prepartal estradiol and progesterone were reported to affect the functional capabilities of immune cells (Sordillo and Mavangira, 2014).

Inflammatory response during the transition period

The inflammatory response comprises localized and systemic responses that involve physiological and pathological alterations that help the body adapt or eliminate adverse stimulus to return tissues to normal function or homeostasis (Bradford et al.,

2015; Sordillo, 2016). The most common event to occur during the inflammatory responses is a systemic or acute phase response (APR). The mechanisms include fever, pain, leukocytosis, and the over- or under-expression of a large family of structurally unrelated proteins, the acute phase proteins (APP) (Ceciliani et al., 2012).

The liver plays an important role during APR, coordinating the synthesis of key metabolites to face an inflammatory condition. The primary stimulators of the APR are the pro-inflammatory cytokines, including TNFα, IL1β, and IL6 (Gauldie et al., 1987). These cytokines can activate leukocytes and endothelial cells (Bannerman et al., 2009) and alter the normal synthesis of proteins in hepatocytes. These proteins for which their hepatic synthesis is affected during APR are called acute phase proteins (APP). The APP play major roles during APR, and they are classified as positive (increase) or negative (decrease) APP depending on their change in blood concentration over the course of an inflammatory condition (Ceciliani et al., 2012). The blood concentration of positive APP increases during inflammation, and haptoglobin, serum amyloid A, LPS binding protein, ceruloplasmin, and fibrinogen are common examples of positive APP. Oppositely, negative APP includes paraoxonase, albumin, lipoprotein, and retinol binding protein. As mentioned previously, the peripartal dairy cow is an immunocompromised animal characterized by inflammatory conditions such as infectious diseases and metabolic disorders that often result in pro-inflammatory cytokine release, e.g., TNFα, IL1β, and IL6 (Bertoni et al., 2008). In turn, any significant increment in the severity or length of exposure time to these pro-inflammatory cytokines will likely induce a change in the hepatic activity related to the synthesis of APP.

Nutritional strategies during the transition period

In past years, valuable information about the condition of cows undergoing the transition from pregnancy to early lactation has been reviewed, including nutritional and management approaches to minimize the incidence of postpartal metabolic and reproductive related diseases (Drackley, 1999; Ingvartsen, 2006; Sordillo, 2016). Prepartal DMI has a major effect on postpartal DMI and peripartal lipid metabolism, which, in turn, influence the incidence of health disorders (Drackley, 1999). Ingvartsen (2006) reported a link between body fatness at calving and subsequent degree of lipid mobilization, underscoring the importance of managing dairy cows to calve at the optimum body fatness to decrease the risk of developing health disorders. Later, Sordillo (2016) focused more on the connection between nutritional strategies and the immune system, suggesting that the development of nutritional-based strategies that can enhance the immune response will have a prominent role in food animal agriculture.

Dairy cattle research has demonstrated the potential of nutritional strategies such as manipulation of prepartal energy intake (Grum et al., 1996; Janovick et al., 2011; Zhou et al., 2015), supplementation with amino acids (Osorio et al., 2013a; Zhou et al., 2016; Abdelmegeid et al., 2018), and yeast-based products (Zaworski et al., 2014; Yuan et al., 2015b; Stefenoni et al., 2020) to improve the health as well as preventing metabolic and reproductive disorders in transition dairy cows.

Yeast-based products

Yeast-based products have been used in livestock animals for many years due to its beneficial effects, with the most common yeast species being *Saccharomyces cerevisiae*. The effects of yeast products in ruminants have been previously reviewed

from rumen characteristics and animal performance point of view (Fonty and Chaucheyras-Durand, 2006; Chaucheyras-Durand et al., 2008). Among the main effects observed, yeast improves growth and activities of ruminal microbial communities and, consequently, promotes positive effects on milk or meat production (Fonty and Chaucheyras-Durand, 2006). Yeast can be beneficial to balance digestive microflora, promoting better animal health (Chaucheyras-Durand et al., 2008). However, the use of yeast appears to be more relevant when both animal and its rumen microbiota are challenged, for example, during a sudden nutritional change or stress period (Chaucheyras-Durand et al., 2008). Furthermore, yeast-based products from *S. cerevisiae* are commercially available in different forms: live, dead, a combination of both forms (live and dead), or yeast culture.

Yeast culture

Yeast culture is a complex fermentable yeast-based product that contains yeast biomass and fermentation metabolites, including vitamins, proteins, peptides, amino acids, nucleotides, lipids, organic acids, oligosaccharides, esters, and alcohols (Jensen et al., 2008). Yeast culture can also provide fermentable metabolites, minerals, and enzymes that can change the profile and physiology of ruminal bacteria (Chaucheyras-Durand et al., 2008; Ghazanfar et al., 2017). In addition, yeast culture may contain residual amounts of yeast biomass, providing mannooligosaccharides and glucans, which can promote an immunomodulatory effect (Jensen et al., 2008).

Main effects and mode of action of yeast-based products

Several mechanisms have been proposed to explain the mode of action of yeast in animal nutrition; however, the overall positive effect is the modulation and improvement

of the rumen microbial population. The main factors implicated in the beneficial effects of yeast are 1) oxygen uptake; 2) micronutrient supply; and 3) ruminal lactate metabolism; that will promote microbial establishment (Chaucheyras-Durand et al., 2008). Figure 1.2 summarizes the mode of action of yeast products at the rumen and animal level.

Live yeast can scavenge oxygen, and as a result, promote a better anaerobic ruminal environment. Live yeast can interact with ruminal bacteria in a "micro-consortium structure", by promoting a localized anaerobiosis in freshly ingested particles (e.g., forages and starch) that may contain oxygen, thus benefiting bacterial digestion (Jouany, 2006). Live yeast uses oxygen to metabolize sugars and oligosaccharides, which results in end-products (e.g., ethanol, glycerol, and peptides) utilized by ruminal bacteria to stimulate growth and fermentation (Jouany, 2006; Ghazanfar et al., 2017). The anaerobic environment promoted by live yeast improves growth and metabolism of cellulolytic species, given their high sensitivity to the presence of oxygen (Chaucheyras-Durand and Fonty, 2009; Ghazanfar et al., 2017). Oxygen scavenging by live yeast can increase cellulolytic bacteria populations and the flow of microbial protein to the lower gastrointestinal tract, which will contribute to the metabolizable protein (Fonty and Chaucheyras-Durand, 2006). In support of the oxygen uptake theory, Newbold et al. (1996) reported that wild-type strains of *S. cerevisiae* were able to consume oxygen and stimulate bacterial activity in rumen-simulating fermenters. Lactate can accumulate rapidly in the rumen due to sudden changes to high carbohydrate diets. Volatile fatty acids (VFA) are one the end-products of ruminal fermentation. Lactate is considered a stronger acid than VFA (pK_a 3.9 versus 4.7, respectively), and the accumulation of lactate

in rumen can lead to a more pronounced decrease in pH (Russell, 2002). The decrease in pH can render cows at risk of developing subacute ruminal acidosis (SARA). The effect of yeast supplements on maintain ruminal pH and preventing SARA has been attributed to a reduction in ruminal lactate production (Dias et al., 2018) while stimulating growth of lactate-utilizing bacteria (Callaway and Martin, 1997; Fonty and Chaucheyras-Durand, 2006). Lactate-associated bacteria such as *Megasphaera elsdenii, Anaerovibrio lipolytica,* and *Selenomonas ruminantium* can utilize lactate as an energy source and produce a variety of end-products (Russell, 2002; Puniya et al., 2015). The lactate reduction caused by yeast supplementation has been reported by Pinloche et al. (2013), who demonstrated that live yeast supplementation was associated with greater lactate-utilizing bacteria. This, in turn, promoted greater total VFA and ruminal pH while reducing lactate. In addition, the increase in lactic acid utilizer bacteria has been associated with better animal efficiency due to its metabolic pathway that utilizes lactate to produce propionate (Pinloche et al., 2013; AlZahal et al., 2014; Shabat et al., 2016).

Taking together, the effects of live yeast on oxygen uptake, micronutrient supply, and pH stabilization may depend on the type of yeast or the diet of the animals. However, most of the yeast products can stabilize rumen pH (Desnoyers et al., 2009) by promoting an increase in cellulolytic (*Fibrobacter and Ruminococcus*) and lactate-utilizing bacteria (*Megasphaera and Selenomonas*) (AlZahal et al., 2014; Jiang et al., 2017b). Little is known about the positive effects of yeast-based products on the immune system in dairy cows. The effects of yeast cell wall components such as β-glucans and mannooligosaccharides have been reported as the main mechanisms to interact with the immune system. This has been well established since these two yeast cell wall

components can promote an immunomodulatory response (Goodridge et al., 2009; Korolenko et al., 2019). The β -glucan can activate macrophages, dendritic cells, neutrophils, natural killer cells, B and T lymphocytes, and increase phagocytosis and cytokine production (Kim et al., 2011). Mannooligosaccharides can regulate the inflammatory response by modulating the level of molecules released into the circulation during inflammation (Korolenko et al., 2019). Yeast can modulate gene expression in porcine intestinal epithelial cells, by decreased the transcript and protein abundance of pro-inflammatory genes, including IL-6 and IL-8, during *Escherichia coli* exposure (Zanello et al., 2011).

The mechanism behind the interaction between supplemented yeast, rumen epithelium, and innate immunity still unclear. This interaction can be partially attributed to better energy status promoted by positive effects on digestion or activation of the immune system by sensing yeast components (Lopreiato et al., 2020). A recent *in vitro* study using ovine ruminal epithelial cells demonstrated that β-glucan could activate the immune system via Dectin-1 signaling pathway, suggesting a potential mechanism to explain this activation (Zhang et al., 2019). This mechanism was explained by β-glucan from *S. cerevisiae* upregulating β-defensin 1 (*SBD1*) in ovine ruminal epithelial cells via the Dectin-1–Syk–NF-κB axis, which can release a variety of cytokines, thereby promoting the innate immune function. Similar to β-glucan, the effects of mannan derived from *S. cerevisiae* have been reported in ovine cell culture models. For instance, mannan can induce β-defensin-1 expression via Dectin-2-Syk-p38 pathway in ovine ruminal epithelial cells (Jin et al., 2019). According to these findings *in vitro*, the immune activation by β-glucans and mannooligosaccharides in ovine ruminal epithelial cells is

due to the C-type lectin receptor involved in fungal recognition (Jin et al., 2019; Zhang et al., 2019). To our knowledge, there are no studies evaluating C-type lectin receptor on ruminal epithelium or immune cells in transition dairy cows. In transition dairy cows, Bach et al. (2018) reported that live yeast supplementation resulted in upregulation of genes (e.g., *TLR4*) related to inflammation and epithelial barrier in the ruminal epithelium, leading to a quicker response by innate and adaptive immunity.

Yeast-based products in dairy cow performance

In dairy cows, the effects of different yeast products have been reported across performance (Jiang et al., 2017a; Dias et al., 2018), rumen characteristics (AlZahal et al., 2014; Jiang et al., 2017b; Zhu et al., 2017) and methane emission (Meller et al., 2019; Welty et al., 2019). However, there is a lack of consistency in response to yeast supplementation in dairy cows, which might be associated with the wide variety of configurations of yeast products and metabolites that can be incorporated in animal feeds. In addition, Piva et al. (1993) suggested that the stage of lactation, type of forage, feeding strategy, and the forage-to-concentrate ratio can affect the response to yeast supplementation in dairy cows.

In a meta-analysis Robinson and Erasmus (2009) examined 22 lactation experiments with different *S. cerevisiae*-based yeast products and observed a 0.9 kg/d increase in milk production due to yeast supplementation. Desnoyers et al. (2009) summarized the findings from 110 papers and 157 experiments in ruminants, representing a broad range of yeast products and feeding conditions, reporting effects of yeast supplementation on dry matter intake, milk yield, rumen pH, rumen VFA, and organic matter digestibility. In a more recent meta-analysis, Poppy et al. (2012) reported that

yeast culture from *S. cerevisiae* could increase milk yield, milk fat, and milk protein in dairy cows. In the same meta-analysis, it was observed that yeast culture supplementation during early lactation increased DMI by 0.62 kg/d and energy-corrected milk (ECM) by 1.65 kg/d.

Yeast-based products in peripartal dairy cows

Live yeast or yeast-based products supplementation has been evaluated in peripartal or early lactation dairy cows in terms of performance (Dann et al., 2000; Nocek et al., 2011; Shi et al., 2019; Stefenoni et al., 2020), rumen characteristics (Al Ibrahim et al., 2010; Bach et al., 2019), metabolic profile (Zaworski et al., 2014; Faccio-Demarco et al., 2019; Olagaray et al., 2019) and immune system function (Yuan et al., 2015b; Knoblock et al., 2019).

Dann et al. (2000) observed that yeast culture improved DMI during the last 7 d before calving through the first 42 d of lactation, suggesting a potential benefit to the common decline in DMI during the transition period. Later, Shi et al. (2019) also observed effects on DMI, where the use of yeast culture increased feed intake around calving and feed efficiency postpartum. In early lactation dairy cows, yeast culture plus enzymatically hydrolyzed yeast improved production performance and mammary gland health (Nocek et al., 2011). In a recent study, Stefenoni et al. (2020) described that the use of yeast culture plus enzymatically hydrolyzed yeast did not affect DMI or milk production; however, they observed positive effects on milk components coupled with a decrease in days to first estrus. In contrast, other experiments did not observe improvements in performance, using live yeast or yeast culture (Al Ibrahim et al., 2010; Olagaray et al., 2019).

During the peripartal period, the rumen experiences a transient but important loss of microbial diversity, followed by a recolonization process due to diet, physiological, and environmental changes (Bach et al., 2019). The recolonization process occurs after calving, and it is characterized by a shift in bacterial populations that will affect the rumen kinetics and VFAs profile (Minuti et al., 2015). In addition, Minuti et al. (2015) observed changes in rumen characteristics, such as increased butyrate and decreased ruminal pH and acetate, which were partially explained by alterations in ruminal microbial species. Such alterations corresponded to increased *Anaerovibrio lipolytica*, *Prevotella bryantii*, and *Megasphaera elsdenii*, while decreased fibrolytic bacteria (*Fibrobacter succinogenes* and *Butyrivibrio proteoclasticus*). Al Ibrahim et al. (2010) did not observe major alterations in rumen parameters due to live yeast supplementation; however, they observed an increase in rumen protozoal counts, acetate molar proportion, and a reduction in rumen ammonia. In a recent study focused on rumen microbiota, Bach et al. (2019) reported that most of the effects of live yeast supplementation on rumen population were observed before calving and the taxonomic diversity after calving was more associated with feed intake, milk yield, and feed efficiency.

Metabolic shifts during the transition period are common because of the adaptations associated with the transition period, such as NEB, reduced immunocompetence, and systemic inflammatory condition. Yeas culture supplementation can decrease serum cortisol concentrations and tended to increase serum concentrations of calcium, glucose, urea, and serum amyloid A during the first day after calving (Zaworski et al., 2014). This suggests that yeast culture may affect the metabolic and immunologic status of dairy cows in early lactation. Recent findings reported that yeast

culture supplementation led to shifts in cholesterol metabolism, tending to decrease hepatic cholesterol and increase plasma cholesterol (Olagaray et al., 2019), while other studies did not report the same effects (Piva et al., 1993). In a semi-extensive production system, supplementation with yeast culture plus enzymatically hydrolyzed yeast had no impact on the metabolic profile of dairy cows, even when these cows produced more milk (Faccio-Demarco et al., 2019).

The connections between the ruminal effects of yeast products and subsequent systemic responses (e.g., immunometabolism) in transition dairy cows remain unclear. Yuan et al. (2015b) reported that yeast culture plus enzymatically hydrolyzed yeast supplemented at rates of 0, 30, 60, or 90 g/d from -21 to 42 d relative to calving linearly increased plasma anti-ovalbumin IgG levels following 3 ovalbumin challenges, which, in turn, indicates enhanced humoral immunity. Yuan et al. (2015b) also suggested that yeast supplementation in transition dairy cows could enhance mucosal immunity. Together, these effects could modulate uterine inflammatory signals and mammary gland health. Recently, Knoblock et al. (2019) reported the effects of yeast culture supplementation in dairy cows fed either a low-starch (22.1% starch) or high-starch (28.3% starch) diets during early lactation. They observed that yeast culture supplementation had an anti-inflammatory effect, indicated by the lower serum haptoglobin, but no effects on adaptive immunity were detected. Knoblock et al., 2019 also concluded that a combination of yeast culture and low-starch diets during early lactation might reduce inflammation. In lactating dairy cows, yeast cell wall supplementation could increase mRNA expression of cytokines in peripheral blood mononuclear cells and enhance immune function (Aung et al., 2020).

RATIONALE AND OBJECTIVES

The overall goal of this study was to understand the physiological responses to feeding a yeast culture supplement to transition dairy cows. Therefore, the objectives of this study were to evaluate the effects of a commercial yeast culture product on a) performance, blood biomarkers, rumen fermentation, and bacterial population; b) metabolism, inflammation, liver function, and immune function through blood biomarkers and gene expression in PMNL.

CONCLUSION

The success of the transition period will determine the profitability of the cow during that lactation (Drackley, 1999). Adequate nutrition and good management practices are essential for a successful transition period in dairy cows (Drackley et al., 2006). The transition period is commonly associated with metabolic and immunologic challenges. For instance, factors associated with NEB, such as the rapid decrease in DMI before parturition and lipid mobilization from adipose tissue, are important points to consider when designing a dry cow management. During early lactation, cows are more susceptible to develop metabolic disorders, and the approaches of management should focus on it.

Nutritional approaches have been developed through research to help cows achieve a smooth transition between late pregnancy to lactation. The interest in using yeast-based products derived from *S. cerevisiae* in dairy cows' rations has increased in recent years. The utilization of these products appears to be relevant to improve performance and health. Yeast products are commercially available in different forms,

such as live, dead, a combination of both (live and dead), or yeast culture. Various mechanisms have been proposed to explain the mode of action of yeast in animal nutrition; however, the overall positive effect is the modulation and improvement of the rumen microbial population. The main factors implicated in the beneficial effects of yeast are 1) oxygen uptake; 2) micronutrient supply; and 3) ruminal lactate metabolism; that will promote microbial establishment (Chaucheyras-Durand et al., 2008). Besides the ruminal effects of live yeast, yeast cell wall components can be highly degradable in the rumen and promote an immunomodulatory effect (Jensen et al., 2008).

Based on the above, the use of alternative nutritional strategies, such as yeast culture, can promote beneficial effects in dairy cows, especially during stressful periods such as the peripartal period. However, the optimal utilization of yeast-based products to confer the best physiological conditions to dairy cows for a smooth transition from pregnancy to lactation remains to be elucidated. The extensive amount of research and plethora of commercial yeast products and their combinations highlights the tremendous potential of yeast to fine-tune the metabolism and immune system of livestock animals. If decipher correctly, yeast technology applied to solve the inheritely complex immunometabolic challenges in the transition dairy cow model will provide the largest gains in productivity and profitability in commercial dairy farms.

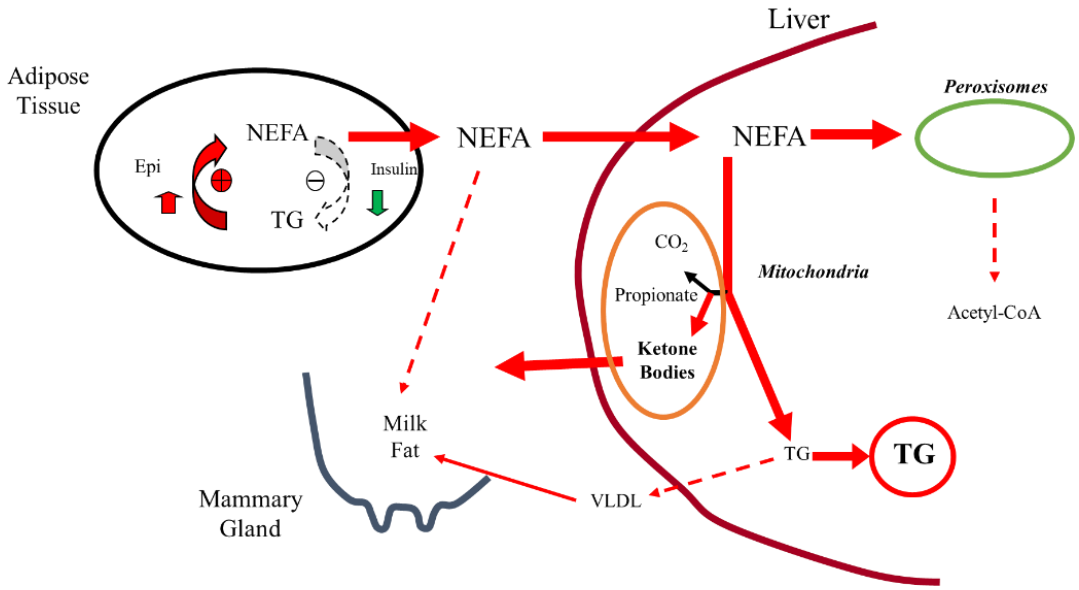

Figure 1.1 - Schematic representation of relationships among lipid metabolism in adipose tissue, liver, and mammary gland. Plus signs (+) indicate stimulatory effects, minus signs (−) indicate inhibitory effects. Dashed lines indicate processes that occur at low rates or only during certain physiological states. Abbreviations: Epi = epinephrine, NEFA = non-esterified FAs; TG = triglyceride, VLDL = very-low-density lipoproteins. Source: Adapted from (Drackley, 1999).

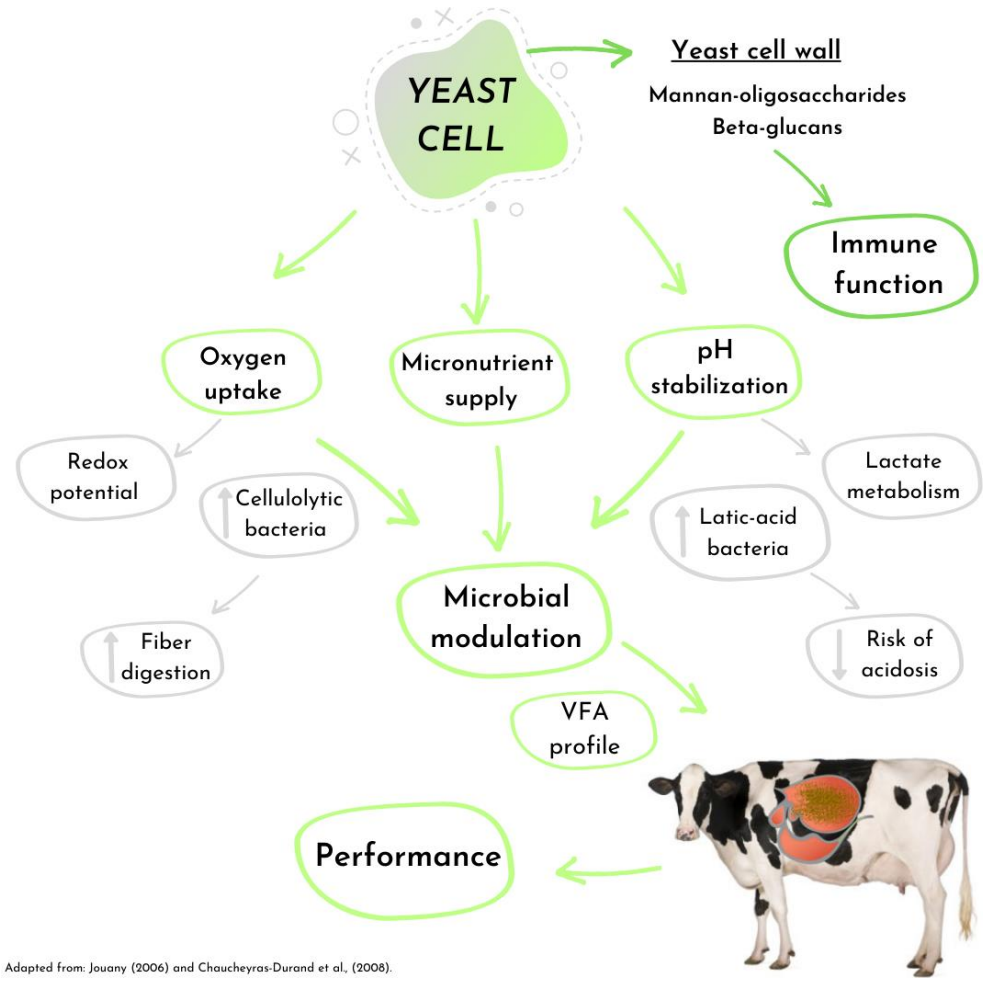

Figure 1.2 - Schematic representation to explain the action of yeast in the rumen. Adapted from (Jouany, 2006, Chaucheyras-Durand et al., 2008).

CHAPTER 2. EFFECTS OF FEEDING YEAST CULTURE PRODUCT ON PERFORMANCE, BLOOD BIOMARKERS, RUMEN FERMENTATION, AND RUMEN BACTERIA SPECIES IN PERIPARTAL DAIRY COWS

ABSTRACT

Feeding yeast culture fermentation products has been associated with improved feed intake and milk yield in transition dairy cows. The objective of this study was to evaluate the effects of a commercial yeast culture product (YC; Culture Classic HD; Phibro Animal Health, Teaneck, NJ) on performance, blood biomarkers, rumen fermentation and bacterial population in dairy cows during the transition period until 50 DIM. Forty Holstein dairy cows were enrolled in a randomized complete block design from -30 to 50 DIM and blocked according to expected calving day, parity, previous lactation milk yield, and genetic merit. At -30 DIM, cows were assigned to either a basal diet plus 114 g/d of ground corn (CON; n = 20) or basal diet plus 100 g/d of ground corn and 14 g/d YC (n = 20), fed as a top-dress. Cows received the same close-up diet from -30 until calving (1.39 Mcal/kg DM and 12.3% CP) and lactation diet from calving to 50 DIM (1.60 Mcal/kg DM and 15.6% CP). Blood samples and rumen fluid were collected at various time points from -30 to 50 d relative to calving. Cows fed YC compared with CON had trend for an increased milk yield (+2.5 kg/d) and ECM (+3.2 kg/d). Lower SCC was observed in YC cows than CON. There was a diet × time interaction in NEFA and BHB that could be attributed to a trend for greater NEFA and BHB in YC cows than CON at 7 DIM, followed by a lower NEFA in YC cows than CON at 14 and 30 DIM. Similarly, BHB tended to be lower in YC cows than CON at 30 DIM. This suggests yeast supplementation have a potential effect on postpartal metabolism. In the rumen, YC product contributes to mild changes in rumen fermentation, mainly increasing postpartal

valerate while decrease isovalerate. This was accompanied by alterations in rumen microbiota, including a greater abundance of cellulolytic (*Fibrobacter succinogenes*) and lactate-utilizing bacteria (*Megaspheara elsdenii* and *Anaerovibrio lipolytica*). These results show an overall beneficial effect of supplementing yeast culture during the late pregnancy through early lactation, at least in terms of rumen environment and performance.

Key words: transition cow, yeast culture, rumen bacteria

INTRODUCTION

The transition period has been defined as a critical period for dairy cows, characterized as 3 wk before and 3 wk after parturition (Drackley, 1999). This is a challenging period for dairy cows because of the lactogenesis, uterine involution, and metabolic and endocrine changes associated with negative energetic balance (Bradford et al., 2015) and often impairs the immune response (Trevisi and Minuti, 2018). Direct-fed microbials can be used as a nutritional alternative to improve health and performance during the transition between pregnancy and early lactation (Roche et al., 2013; Lopreiato et al., 2020).

Yeast culture is a complex fermentable feed additive that contains: yeast cell walls (i.e., beta-glucans and mannan-oligosaccharides), vitamins, proteins, peptides, amino acids, nucleotides, lipids, organic acids, oligosaccharides, esters, and alcohols (Jensen et al., 2008). According Chaucheyras-Durand et al. (2008), the main effects of yeast products are related to rumen fermentation, benefiting key microbial populations and their metabolism, and consequently increasing fiber degradation and stabilizing rumen pH.

The beneficial effects of different live yeast or yeast culture-based products have been reported on the performance of dairy cows supplemented throughout the transition period or during early lactation only (Dann et al., 2000; Nocek et al., 2011; Shi et al., 2019). These effects have been further described in terms of rumen characteristics (Al Ibrahim et al., 2010; Bach et al., 2019), metabolic profile (Zaworski et al., 2014; Faccio-Demarco et al., 2019; Olagaray et al., 2019) and immune system function (Yuan et al., 2015b; Knoblock et al., 2019; Lopreiato et al., 2020). However, these cumulative data on yeast supplementation across transition cow studies are inconsistent and might be associated with the wide variety of configurations of yeast cultures or yeast fermentation metabolites used in such studies.

The yeast culture evaluated in the current study was a concentrated form of yeast culture and fermentation metabolites originating from a *S. cerevisiae* yeast strain that is grown on a medium and dried on beneficial plant-based carriers. The hypothesis was that feeding this complex product during the transition period would affect performance and ruminal characteristics. The objective of this study was to evaluate the effects of a commercial yeast culture product (Cellerate Yeast Solutions®, Culture Classic HD; Phibro Animal Health, Teaneck, NJ) on performance, blood biomarkers, rumen fermentation, and bacterial population in dairy cows during the transition period until 50 DIM.

MATERIAL AND METHODS

Experimental Design and Dietary Treatments

The Institutional Animal Care and Use Committee (IACUC) of the South Dakota State University approved all the procedures for this study. Forty late pregnant Holstein

dairy cows, including 34 multiparous and 6 primiparous cows, were used in a randomized complete block design and were blocked according to calving day, parity, previous lactation milk yield, and genetic merit.. During the dry period, the cows received the same close-up diet (1.39 Mcal of NE_L/kg and 12.3% CP; Table 2.1) and at -30 d before expected calving date cows were assigned to the treatments. The treatments were top-dressed as a control (CON; 114g/h/d ground corn; n = 20) or CON plus Culture Classic HD (YC; 100 g ground corn + 14g YC/h/d; n = 20). After parturition, all cows were fed the lactation basal diet (1.60 Mcal of NE_L/kg and 15.56% CP; Table 2.1), as a total mixed ration, and the supplementation continued through 50 d postpartum.

Animal Management

Cows were enrolled in the experiment from mid-September 2018 to early April 2019. Cows were fed using an individual gate system (American Calan, Northwood, NH), and intakes were recorded daily. Diets were formulated using the NDS Professional (Nutrition Dynamic System; RUM&N Sas.) to meet requirements of the average cow in the group according to the NRC (2001) (Table 2.1). Dry matter (DM) of individual feed ingredients was determined weekly and diets were adjusted accordingly to maintain formulated DM ratios of ingredients in the total mix ratio (TMR). The cows were fed once daily before morning milking using a Data Ranger Tool (http://americancalan.com/code/template.cfm?page=3). The feed offered was adjusted daily to achieve a 5 to 10% refusals the following days.

During the dry period, cows were housed in a bedded pack pen, and 3 d before expected calving date, cows were reallocated in individual pens bedded with straw until

parturition. On day 3, after calving, cows were moved to a lactation free-stall barn where they were receiving the same lactation basal diet until 50 d postpartum.

Body weight was measured weekly for each cow in the afternoon at 13:00h. Body condition score (BCS) (scale 1= thin to 5= obese) was assigned by two individuals and the average score was used for statistical analysis.

Feed and Milk Samples

Individual samples of ingredients and TMR were collected weekly were frozen at -20°C after DM analysis and until further nutrient analysis. Composited samples for ingredients and TMR were analyzed for contents of DM, CP, NE_L, NDF, ADF, using wet chemistry methods (Dairy One, Ithaca, NY).

Cows were milked twice a day and milk yield was recorded at each milking until 50 DIM. Consecutive morning and evening milk samples were collected 1 d/wk during the experimental period. Composite milk samples were performed in proportion to milk yield at each milking, preserved (Broad Spectrum Microtabs II, Advanced Instruments, Norwood, MA), and analyzed for fat, protein, lactose, solids, milk urea nitrogen (MUN) and somatic cell count (SCC).The ECM was calculated based on milk yield and milk sample analysis as follows: ECM = [12.82 × fat yield (kg)] + [7.13 × protein yield (kg)] + [0.323 × milk yield (kg)] (Hutjens, 2010). Equations from NRC (2011) were used to calculate energy balance (EB) for each cow. The energy intake was determined using daily DMI multiplied by NE_L density of the diet. Net energy of maintenance was calculated as $BW^{0.75} \times 0.080$. Requirements of NE_L were calculated as $NE_L = (0.0929 \times$ fat % + 0.0547 × protein % + 0.0395 × lactose %) × milk yield. The net energy requirement for pregnancy (NE_P; Mcal/d) was calculated as $NE_P = [(0.00318 \times$ day of

gestation − 0.0352) × (calf birth weight/45)]/0.218. The equation used to calculate prepartal EB (EB_{PRE}; Mcal/d) was $EB_{PRE} = NE_I - (NE_M + NE_P)$ and EB_{PRE} (as % of requirements) = $[NE_I/ (NE_M + NE_P)] \times 100$. The equation used to calculate postpartal EB (EB_{POST}) was EB_{POST} (Mcal/d) = $NE_I - (NE_M + NE_L)$ and EB_{POST} (as % of requirements) = $[NEI/(NE_M + NE_L)] \times 100$.

Blood Collection and Analyses

Blood was sampled from the coccygeal vein before morning feeding using a 20-gauge vacutainer needle (Becton Dickinson, Franklin Lakes, NJ) at -30, -15, 7, 14, and 30 d relative to parturition from a subset of multiparous cows (n=8 multiparous cows/treatment). Blood was collected into evacuated tubes (5 mL, BD Vacutainer, Becton Dickinson, Franklin Lakes, NJ) containing either serum clot activator or lithium heparin. After collection, tubes that contained lithium heparin were placed on ice and tubes with serum clot activator were kept at 21°C until centrifugation. Serum and plasma were obtained by centrifugation at 1,300 × *g* for 15 min at 21°C and 4°C, respectively. The aliquots of plasma were frozen at -80°C until further analysis.

Plasma samples were analyzed for biomarkers of muscle body mass [e.g., creatinine and urea], metabolism [e.g., glucose, NEFA and BHB] and oxidative stress [e.g., reactive oxygen metabolites (ROM) and ferric reducing anti-oxidant power (FRAP)] using kits purchased from Instrumentation Laboratory (Lexington, MA) and the procedures were described previously (Trevisi et al., 2012; Batistel et al., 2016; Jacometo et al., 2016).

Rumen Fluid Collection and Analyses

Rumen fluid (~50 mL) was sampled on -59, -29, 5, and 30 d relative to parturition from a subset of multiparous cows (n = 8 cows/treatment) via esophageal tubing. After discarding the first 200 mL of fluid to minimize saliva contamination, approximately 50 mL of rumen fluid was collected. After collection, the rumen pH was immediately measured using a pH meter (Waterproof pH Test 30, Oakton Instruments, Vernon Hills, IL). Two aliquots of 10 mL were saved in a bottle containing either 200 µL of 50% sulfuric acid or 2 mL of 25% metaphosphoric acid and stored at -20°C until analysis of ammonia (NH3) and VFA respectively. Furthermore, 2 mL of rumen fluid sample was immediately frozen in liquid nitrogen followed by storage at -80°C until DNA isolation followed by relative abundance of bacteria species quantification via qPCR method.

Ammonia and VFA

Rumen fluid samples preserved with sulfuric acid and 25% meta-phosphoric acid were thawed and transferred into a 2mL microcentrifuge tube and centrifuged at 30,000 × *g* for 20 min at 4°C (Model 5403, Eppendorf, Hamburg, Germany). The supernatant of rumen fluid sample with sulfuric acid was used to analyze Ammonia-N concentration using the assay described by Chaney and Marbach (1962). VFA concentrations were measured in samples contained 25% metaphosphoric acid using an automated gas chromatograph (Model 689, Hewlett-Packard, Palo Alto, CA) equipped with a 0.25 mm i.d ×15 m capillary column (Nukol 24106-U, Sulpeco, Inc., Bellefonte, PA) and the internal standard used was 2-ethylbutyrate.

Ruminal Bacteria DNA Isolation and qPCR Amplification of 16S rDNA Genes

Ruminal bacteria DNA was isolated using the QIAmp Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany) with modifications to the protocol described by Henderson et al. (2013). Briefly, 1mL of rumen fluid was centrifuged at 12,000 × *g* for 5 min and the supernatant was discarded. Pellet was resuspended in 1 mL of buffer EX, vortex, incubated in a heat block at 95°C for 5 min and centrifuged at 20,000 × *g* for 1 min. Then, 600 μL of supernatant was transferred to a new microcentrifuge tube contained 25 μL of Qiagen Proteinase K, followed by addition of 600 μL of Buffer AL. The mixture was vortexed for 15 s and incubated at 70 °C for 10 min. After incubation, 600 μL of 96% molecular ethanol was added and vortexed. The mixture was transferred into a QIAamp mini spin column, and the subsequent steps were performed according to manufacturer's procedures (Qiagen, Hilden, Germany). The DNA quantity and purity were measured using a NanoDrop spectrophotometer (ND 1000, NanoDrop Technologies Inc., Wilmington, DE) and the extracted DNA was standardized to 8 ng/μL for qPCR.

The primer sets used in this study have been previously reported and validated (Table 2.2). The qPCR analysis was performed using 10 μL of qPCR mixture containing 4 μL of sample DNA, 5 μL of 1 x SYBR Green master mix (Applied Biosystems, Waltham, MA), 0.4 μL of 10 μM each for forward and reverse primers, and 0.2 μL of DNase-RNase-free water in a MicroAmp Optical 384-well reaction plate (Applied Biosystems). Each sample was run in triplicate and the qPCR reactions were performed in a QuantStudio 6 Flex Real-Time PCR System (Applied Biosystems) using the same conditions described by Grazziotin et al. (2020). A geometrical mean of two universal

bacteria primers was used to calculate the relative abundance of bacterial species (Abdelmegeid et al., 2018).

Statistical Analysis

Data were analyzed as repeated measures with the MIXED procedure of SAS according to the following model:

$$Y_{ijklm} = \mu + D_i + P_j + DP_{ij} + B_k + C_{ijkl} + T_m + DT_{im} + DPT_{ijm} + e_{ijklm}$$

Where $Y_{ijklm}$is the dependent, continuous variable; μ is the overall mean; D_i is the fixed effect of the *i*th diet (*i* = 1 and 2); P_j is the fixed effect of the *j*th parity (*j* = 1, 2, 3); B_k is the random effect of the *k*th block (*k* = 1, …20); C_{ijkl} is the random effect of *l*th cow nested within the *i*th treatment, the *j*th parity, and the *k*th block (*l* = 1,…, n_{ijk}); T_m is the fixed effect of the *m*th time (d or wk) of the experiment (*m* = 1,… n); DT_{im} is the fixed effect of the *i*th treatment by the *m*th time of the experiment interaction; DPT_{ijm} is the fixed effect of the *i*th treatment by the *j*th parity by the *m*th time of the experiment interaction; and e_{ijklm} is the residual error. Parity effect was removed from the model any time it was nonsignificant ($P > 0.05$). Blood biomarkers and rumen fluid data, including pH, VFA, NH_3, and relative abundance of microbial species were analyzed at various time points that were not equally spaced; therefore, an exponential correlation covariance structure SP (POW) was used for repeated measures. Blood biomarkers and relative abundance of microbial species were log-scale transformed if needed to comply with normal distribution of residuals. Blood biomarkers and rumen fluid data on -30 and -59 d, respectively, were used as a covariate. The covariate of previous 305-d milk yield was maintained in the model for all variables for which it was significant ($P < 0.05$). Statistical significance was declared at $P \leq 0.05$ and tendencies at $P < 0.15$.

RESULTS

Peripartal DMI, BW, and BCS

Main effects and interactions for prepartal and postpartal BW, BCS, DMI, DMI as percent BW, and EB are presented in Table 2.3. Neither the prepartal nor postpartal BW, BCS, and DMI, DMI as %BW, and EB were affected ($P \geq 0.23$) by dietary treatments.

Milk Production and Composition

Main effects and interactions for postpartal production variables and milk efficiency in terms of Milk/DMI and ECM/DMI are presented in Table 2.4 and Figure 2.1. The SCC was lower ($P < 0.01$) in YC fed cows than CON. A trend for a diet effect was observed in milk yield ($P = 0.13$) and ECM ($P = 0.07$), where YC cows produced 2.5 kg/d and 3.2 kg/d more than CON, respectively. Also, similar to milk yield, a trend ($P = 0.11$) was observed for a greater milk fat yield in YC cows when compared to CON. Milk efficiency in terms of milk/DMI was not affected ($P = 0.52$) by dietary treatments, and milk efficiency in terms of ECM/DMI even though it was ca. 0.10 greater in YC cows than CON, this effect did not reach statistical significance ($P = 0.18$)

Rumen Fermentation

Main effects and interactions for rumen fermentation characteristics are presented in Table 2.5. There was a D × T interaction observed in VFA concentrations of isovalerate ($P = 0.05$) and valerate ($P = 0.04$) (Figure 2.2). The D × T in valerate was mainly associated with greater ($P = 0.01$) concentration in YC cows than CON at 5 DIM. In contrast to valerate, the D × T in isovalerate was reflected in lower ($P < 0.01$) concentrations in YC cows than CON at -29 DIM. The latter was reflected in lower ($P = 0.02$) isovalerate in YC cows in comparison to CON.

Abundance of Ruminal Bacteria

Main effects and interactions for the relative abundance of microbial species in rumen fluid are presented in Table 2.6. There was a D × T ($P \leq 0.05$) on the relative abundance of *Prevotella albensis*, *Prevotella bryantii, Prevotella ruminicola*, and *Succinivibrio dextrinosolvens* (Figure 2.3). Similarly, a trend for a D × T was observed in *Selenomonas ruminantium* ($P = 0.11$) and *Succinimonas amylolytica* ($P = 0.12$) (Figure 2.3). The D × T observed in *P. albensis* was associated with a greater ($P = 0.03$) abundance of this bacterium in YC cows than CON at 30 DIM. The D × T in *P. ruminicola* resulted in lower ($P = 0.01$) abundance of this bacterium in YC cows in comparison to CON at 5 DIM. Similar to *P. ruminicola*, *S. ruminantium* tended ($P = 0.12$) to be lower in YC cows in comparison to CON at 5 DIM. The D × T in *S. dextrinosolvens* ($P = 0.02$) and *P. bryantii* ($P = 0.04$) resulted in a similar pattern, where lower in YC cows than CON at -29 DIM. Contrary to *S. dextrinosolvens* and *P. bryantii*, *S. amylolytica* had a greater ($P = 0.01$) abundance in YC cows than CON at -29 DIM. There was a greater relative abundance of *Megaspheara elsdenii* ($P = 0.05$) and *S. amylolytica* ($P = 0.03$) in YC cows than CON (Table 2.6). In contrast to *M. elsdenii* and *S. amylolytica*, *P. brevis* abundance was lower ($P < 0.01$) in YC cows in comparison to CON. In addition, there was a trend for greater abundance of *Anaerovibrio lipolytica* ($P = 0.12$) and *Fibrobacter succinogenes* ($P = 0.09$) in YC cows than CON (Figure 2.4).

Blood Biomarkers

Main effects and interactions for blood biomarkers of muscle body mass, metabolism, and oxidative stress are presented in Table 2.7. There was a D × T ($P = 0.02$) in NEFA and a trend (P = 0.13) in BHB (Figure 2.5). The D × T in NEFA resulted in

lower NEFA in YC cows than CON at -15 (P = 0.05), 14 (P = 0.05), and 30 d (P = 0.02) relative to parturition, while a tendency (P = 0.12) for greater NEFA was observed in YC cows than CON at 7 DIM. The latter effects can be attributed to a trend for overall lower NEFA (P = 0.10) in YC cows than CON. Similar to NEFA, the D × T in BHB was associated with a tendency for a greater (P = 0.10) BHB in YC cows than CON at 7 DIM, followed by a trend for lower (P = 0.11) BHB in YC cows than CON at 30 DIM.

DISCUSSION

Effects on DMI, BW, and BCS

Peripartal dairy cows commonly experience a decline in DMI around parturition. (Drackley, 1999). This decline in DMI results in low energy intake, which has been associated with BCS and hormonal changes around parturition (Ingvartsen and Andersen, 2000). As a result of this decrease in energy intake, dairy cows enter a negative energy balance (NEB) that is exacerbated by the substantial increase in energy demands to sustain milk production after calving. Dairy cows respond to NEB by mobilizing lipid from adipose tissue in the form of NEFA, to cope with energy demands in early lactation (Contreras and Sordillo, 2011). Because of these reasons, it is typical to observe a decrease in BCS and BW in dairy cows during early lactation.

Yeast culture has supplemented to dairy cows with the aim of improving performance and health (Poppy et al., 2012). Yeast culture supplementation has been reported to affect DMI in transition dairy cows(Olagaray et al., 2019; Shi et al., 2019; Stefenoni et al., 2020). For instance, Shi et al. (2019) reported a trend for an increase in DMI during the first week after calving in dairy cows supplemented with 19 g/d of *S.*

cerevisiae fermentation product. Similarly, Dann et al. (2000) observed improvements in DMI during the first 42 DIM in peripartal Jersey dairy cows fed 60g/d of a yeast culture product. In the current study, yeast culture supplementation did not statistically affect peripartal DMI, and by extension, no effect on BW or BCS was observed around parturition.

Milk Production and Milk Components

There is a lack of consistency on the milk yield response to yeast supplementation in dairy cows. This is predicated on the multiple forms (e.g., active and dead), packing, and metabolites that can be incorporated in commercial yeast supplements. For instance, feeding yeast culture products has been observed to increase milk yield between 3.3 and 5.2 kg/d in transition and mid-lactation dairy cows (Zaworski et al., 2014; Dias et al., 2018). Robinson and Erasmus (2009) reviewed several yeast culture studies in dairy cows and reported that overall yeast supplementation has a positive effect on milk yield (~ 0.9 kg/d). In contrast, some studies reported a lack of effect on milk production in transition dairy cows (Yuan et al., 2015a; Olagaray et al., 2019; Stefenoni et al., 2020). A positive effect on milk production was observed in YC cows with an increase of 2.5 kg/d, which was within the range of milk yield improvement observed in previous studies feeding yeast supplements.

Yeast culture supplementation has been observed to effect milk fat yield in early and mid-lactation dairy cows (Dias et al., 2018; Perdomo et al., 2020). Olagaray et al. (2019) observed 0.13kg/d increase in milk fat yield at 4 and 5 wk postpartum, feeding 19 g/d of yeast culture during the peripartal period, which was similar to the 0.16 kg/d increase in milk fat yield observed in YC cows in comparison to CON. Diets containing

relatively high levels of unsaturated fats and starch have been associated with milk fat depression in dairy cows due to increased flow of trans FAs from ruminal fermentation (Bradford and Allen, 2004). In this context, Longuski et al. (2009) reported that lactating dairy cows supplemented with a yeast culture had a higher degree of resistance to milk fat depression during a short-term dietary challenge with fermentable starch. This can be partially ascribed to yeast maintaining a favorable rumen fermentation for cellulolytic bacteria which is the primary source of ruminal acetate. Therefore, yeast supplementation may indirectly promote a stable supply of acetate for milk fat synthesis by maintaining a favorable population of cellulolytic bacteria (Ghazanfar et al., 2017).

The benefits of yeast culture supplementation on milk performance paramenters, in the present study were reflected in ECM and milk efficiency in terms of ECM/DMI. These results agree with those of Perdomo et al. (2020) and Dias et al. (2018), where yeast products caused an increase in ECM. Perdomo et al. (2020) observed that under heat stress conditions, lactating cows fed with 1g of live yeast had an increase in ECM and feed efficiency measured as ECM/DMI, and such effects were attributed to improvements in crude protein and NDF digestibility. According to Dias et al. (2018), supplementation of yeast culture from *S. cerevisiae* could increase ECM in lactating cows fed diets varying in starch content. In contrast, others did not observed positive effects in ECM in transition dairy cows supplemented with yeast derived from *S. cerevisiae* (Yuan et al., 2015a; Stefenoni et al., 2020). The fermentable products present in the YC product may have improved nutrient utilization by ruminal bacteria and consequently allowed a higher milk efficiency in YC fed cows.

Improvements in udder health, in terms of SCC, have been observed when feeding yeast culture products to dairy cows. For instance, Nocek et al. (2011) tested a yeast culture product alone or in combination with an enzymatically hydrolyzed yeast fed to dairy cows from calving to 14 wk postpartum, and this combination resulted in lower SCC through the first 14 weeks of lactation.(Zaworski et al., 2014). reported positive effects on SCC during the first 4 wk postpartum, when supplementing different dosages of *S. cerevisiae* fermentation product. Those effects were attributed to a potential activation of the animal's immune system at the gut level by antigenic exposure in the form of β-glucans and mannan-oligosaccharides presented in some yeast products. Besides a systemic activation of the immune system as described by Nocek et al. (2011), the action of β-glucans has been tested at the local level during a mastitis induction (Waller et al., 2003). Waller et al. (2003) evaluated the effects of an intramammary infusion of β-1,3-glucan in dairy cows under a chronic subclinical mastitis model induced by *Staphylococcus aureus*. They did not observe any benefical effects of β-1,3-glucan on reducing subclinical mastitis. However a tendency to increase milk lymphocytes after β-1,3-glucan infusion was observed, suggesting that, to some extent, β-1,3-glucan may stimulate a better local inflammatory response by increasing immune cell recruitment.

Rumen Fermentation Parameters

Dietary carbohydrates are fermented by ruminal bacteria, fungi, and protozoa into end products, including VFA (e.g., acetate, propionate, and butyrate) that make up for nearly 50% of the energy requirements for ruminants (Puniya et al., 2015). Desnoyers et al. (2009) reported in a meta-analysis that yeast supplementation increased total VFA concentration in domesticated ruminant species. Zhu et al. (2017) reported a positive

effect on total VFA when dairy cows were supplemented with *S. cerevisiae* fermentation, which was associated with a stimulatory effect on rumen microbial population, including cellulolytic bacteria. In the current study, total VFA production was not affected by yeast culture supplementation.

The normal transition of dairy cows from late pregnancy into lactation is predicted on the cow's ability to adapt to a postpartal lactation diet, which leads to increase ruminal butyrate and valerate, while other VFAs such as isobutyrate and isovalerate remained unchanged (Minuti et al., 2015). The primary ruminal VFAs (i.e., acetate, propionate, and butyrate) seem to be less responsive to yeast supplementation in transition dairy cows (Robinson and Garrett, 1999; Kumprechtova et al., 2019). Like in transition dairy cows, yeast culture and live yeast culture supplementation in mid-lactation dairy cows did not elicit an effect on acetate, propionate, and butyrate (Dias et al., 2018, Meller et al., 2019). In agreement with those data, our results showed supplementing YC during the transition period did not influence VFA concentrations.

Ruminal isoacids comprised of isobutyrate, 2-methylbutyrate, isovalerate, and valerate have been found to increase in response to yeast culture supplementation (Andries et al., 1987). For instance, in an experiment testing incremental levels of concentrate plus yeast culture supplementation (1g/kg of TMR as fed), yeast culture was shown to increase the concentration of isobutyrate and isovalerate regardless of concentrate level in the diet in cannulated Holstein dairy heifers (Lascano and Heinrichs, 2009). Lactating dairy cows fed with a high dose of inactivated yeast increased the molar proportion of valerate, compared with control cows (Jiang et al., 2017a), which is consistent with the increase in valerate in YC cows at 5 DIM in the current study. An

alternative explanation for the increase in valerate could be associated with the greater *M. elsdenii* in YC cows, which was more evident postpartum (Figure 2.4 C). The latter is based on the fact that *M. elsdenii* can ferment L-lactate into propionate and subsequently valerate (Weimer and Moen, 2013). In addition, live yeast supplementation increased the concentration of isovalerate in early lactation dairy cows under heat stress conditions (Garcia Diaz et al., 2018; Perdomo et al., 2020). Similar findings for isovalerate were observed in the current study, where this isoacid increased in YC cows in early lactation. However, others have observed a lack of response of valerate and isovalerate concentration to supplementation of active dry *S. cerevisiae* in cannulated lactating dairy cows (AlZahal et al., 2014). Interestingly, Harrison et al. (1988) and coworkers observed a decrease in molar proportion of isovalerate while valerate increased in lactating cows fed a yeast culture supplement, and this effect was attributed to lower ruminal pH. The positive effects of yeast in ruminal valerate concentrations can be associated with changes in metabolic activities of ruminal bacteria, including amylolytic, cellulolytic, and lactate-utilizer populations. For instance, ruminal degradation of proteins by amylolytic bacteria commonly leads to production of isoacids (Dawson et al., 1990), while the fermentation pathway of lactate by ruminal bacteria may lead to the production of valerate (Weimer and Moen, 2013). These isoacids are required for most cellulolytic bacteria to degrade fiber, underscoring the essential role of isoacids for fiber digestibility (Liu et al., 2009).

Yeast Culture and Ruminal Bacteria Species

In transition dairy cows, rumen microbial populations are expected to change around parturition, which can be attributed to a natural adaptation of rumen microbes to a

lactating cow diet after calving. Minuti et al. (2015) reported diet changes after calving could result in a decrease in fiber digesting bacteria. According to Lima et al. (2015), the main differences between prepartal and postpartal bacterial populations encompass changes in cellulolytic and amylolytic bacteria followed by variations in other uncultured bacteria species for which a ruminal function remains unknown.

The rumen environment is malleable, to some extent, and positive modifications can affect bacteria population and, by extension, its fermentation products, leading to improved performance and nutrient utilization in ruminants (Ghazanfar et al., 2017). Different mechanisms have been proposed to explain the action of yeast in the rumen. According to Jouany (2006), live yeast can interact with ruminal bacteria in a "micro-consortium structure", by promoting a localized anaerobiosis in freshly ingested particles (e.g., forages and starch) that may contain oxygen, thus benefiting bacterial digestion. Yeast uses oxygen to metabolize sugars and oligosaccharides, which results in end-products (e.g., ethanol, glycerol, peptides, and amino acids) utilized by bacteria associated with yeast (Jouany, 2006). In addition, yeast culture products provide mannooligosaccharides and glucans present in the yeast cell wall that can be highly degradable in the rumen, as well as fermentable metabolites, minerals, and enzymes that can change the profile and physiology of ruminal bacteria, stimulating the growth of cellulolytic, amylolytic, and proteolytic bacteria (Chaucheyras-Durand et al., 2008; Ghazanfar et al., 2017; Jiao et al., 2019).

The impact of yeast culture products on ruminal bacteria sbudance have been evaluated in lactating dairy cows (AlZahal et al., 2014; Zhu et al., 2017; Jiao et al., 2019) and transition dairy cows (Bach et al., 2019). There were positive effects in bacterial

populations in YC cows in the current study, mainly by increasing lactate and fiber digesting bacteria. Similar to the current study, early lactating dairy cows supplemented with live yeast presented a greater abundance of lactate-utilizing bacteria (*Megasphaera)* and fiber digesting bacteria (*Fibrobacter)* (Pinloche et al., 2013).

Minuti et al. (2015) observed a decrease in postpartal *F. succinogenes* abundance in transition dairy cows, primarily driven by high grain content in the lactation diet. Strains of *S. cerevisiae* have been reported to increase the abundance and activity of fiber digesting bacteria in rumen, such as *Fibrobacter spp.* (Jiang et al., 2017b). A trend (P = 0.09) for a greater *F. succinogenes* abundance in YC cows compared to CON was observed, and such effect was more evident at 30DIM (Figure 2.4B). A more pronounced effect for greater *F. succinogenes* was observed by AlZahal et al. (2014) in cannulated lactating dairy cows fed with active dry *S. cerevisiae*. A similar response was reported by Zhu et al. (2017), where *F. succinogenes* increased linearly in lactating dairy cows receiving incremental amounts (0, 60, 120, and 160 g/d) of *S. cerevisiae* fermentable products. In contrast, other studies *in vitro* and *in vivo* did not observe a yeast effect in *F. succinogenes* abundance (Mullins et al., 2013; Jiao et al., 2019). Yeast culture used in the current experiment contained live yeast, which could have promoted localized anaerobiosis, and, in turn, this could partially explain the trend for a greater abundance of *F. succinogenes*.

Lactate-associated bacteria such as *Megasphaera elsdenii* and *Anaerovibrio lipolytica* can utilize lactate as an energy source and, consequently, slower growth rates of these bacteria are observed in low carbohydrate diets (Russell, 2002; Puniya et al., 2015). Increased abundance of *M. elsdenii* and *A. lipolytica* has been observed in

transition dairy cows after calving, which was attributed to greater DMI and starch digestibility (Minuti et al., 2015). Similarly, live yeast supplementation has been reported to increase ruminal abundance of *M. elsdenii* (Ogunade et al., 2019) and *A. lipolytica* (AlZahal et al., 2014).In the present study, lactate was not evaluated, but *M. elsdenii* and *A. lipolytica* increased after calving, which is consistent with greater starch content in lactation diets. Additionally, the increase in the abundance of these bacteria in YC cows was more pronounced at 30 DIM (Figure 2.4A and 2.4C), suggesting that these bacteria were affected by yeast supplementation beyond the expect increased due to greater starch content in the lactation diet.

Abdelmegeid et al. (2018) observed a higher abundance of *S. dextrinosolvens* during prepartum than postpartum, and *S. amylolytica* tended to follow the same pattern. In contrast, we observed no change over time in both bacteria, regardless of treatment (Figure 2.3E and 2.3F). These data are conflicting with these bacterias's known starch-degrading function (Russell, 2002), which implies that such bacteria abundance should increase postpartum. In contrast to these findings, AlZahal et al. (2014) did not report a change in population of either *S. amylolytica* and *S. dextrinosolvens* when supplementing lactating dairy cows with active dry *Saccharomyces cerevisiae*. There was a prepartal increase of *S. amylolytica* with a contrasting decrease of *S. dextrinosolvens* in YC cows compared to CON. These effects on *S. amylolytica* and *S. dextrinosolvens* took place within 24 hours after initial supplementation with YC (-30 DIM), which render these effects as short-term and potentially transient as the rumen adapted to the YC supplementation.

Prevotella species can use a variety of substrates, such as starch, hemicellulose, pectin, β-glucans, and proteins, to produce acetate, propionate, succinate, and formate (Russell, 2002). The presence of *Prevotella spp.* in rumen across a variety of diets indicate the substantial metabolic diversity of this genus (Zeineldin et al., 2018). According to Stevenson and Weimer (2007), bacteria population of the genus *Prevotella spp.* are the most dominant bacteria in rumen, but only a small percentage is composed of *P. bryantii*, *P. brevis*, and *P. ruminicola* in lactating dairy cows fed with a normal TMR diet. This was later confirmed by Lima et al. (2015), showing that *Prevotella* is the most abundant bacterium in transition cows.

In the current study, different responses due to YC supplementation were observed for *P. albensis*, *P. bryantii*, *P. brevis*, and *P. ruminicola*, confirming the metabolic diversity of this genus in the rumen. Supplementation of active dry *S. cerevisiae* reduced the population of *P. albensis*, and no effects were observed in *P. bryantii* and *P. brevis* in cannulated lactating dairy cows (AlZahal et al., 2014). Our findings showed a greater abundance of *P. albensis* in YC cows at 30DIM (Figure 2.3 A), suggesting that YC supplementation coupled with an eventual adaptation to the lactation diet promoted an increase of this bacterium. *P. bryantii* was lower in cows supplemented with YC at -29 DIM, which seems to be similar to *S. amylolytica* and *S. dextrinosolvens*, where such effect might be transient as a 24 h response to YC supplementation. Overall, *P. bryantii* increased postpartum regardless of dietary treatments (Figure 2.3 B), which was a similar pattern observed by Minuti et al. (2015). Subacute ruminal acidosis induced via grain or alfalfa pellets cause a differential response among the *Prevotella* genus, specifically, *P. ruminicola* and *P. albensis*, which further underscore the metabolic

diversity of this genus (Khafipour et al., 2009). Our results suggest that *Prevotella* spp. evaluated in this study behave differently due to the YC supplementation, which is in line with previous differential response across *Prevotella* spp. An alternative explanation for the differential abundance among *Prevotella* spp. can be related to their ability to use signaling molecules such as N-Acyl homoserine lactones to communicate between bacteria in an orchestrated effect to coordinate bacterial population density, also known as quorum sensing (Won et al., 2020). Interactions through quorum sensing have been evaluated between yeast and bacteria (Proust et al., 2020). However, the specific mechanisms and interactions between YC products and ruminal bacteria such as *Prevotella* spp. remains unclear.

Taken together, we observed that yeast culture supplementation promoted changes in rumen bacteria species across cellulolytic, amylolytic, and lactate-utilizer populations. These effects seem to have promoted beneficial rumen fermentation, at least in terms of valerate, which might have promoted better fiber digestion. The researchers speculate that the presence of lactate-utilizer bacteria can be helpful during the periods where the diet changes abruptly, such as the transition period. The abrupt change from a high-fiber diet prepartum to a lactation diet postpartum containing large amounts of fermentable carbohydrates can commonly lead to decreased pH, altering ruminal bacteria population and predisposing transition dairy cows to metabolic disorders.

Blood Biomarkers

During the transition period, dairy cows experience a reduction in energy balance, and lipid mobilization becomes a physiological adaptation mechanism to offset or compensate the NEB (Contreras and Sordillo, 2011). Lipid mobilization is associated

with hormonal changes around parturition, and it is commonly characterized by an excessive lipolysis rate over lipogenesis (Bradford et al., 2015). The main product of triglyceride hydrolysis in adipose tissue during lipid mobilization is NEFA, and it and serves as an alternative energy substrate for most tissues (Contreras and Sordillo, 2011). Blood NEFA reflects the level of NEB in transition dairy cows, and greater NEFA is commonly associated with fatty liver syndrome and increased production of ketone bodies. (Ingvartsen and Andersen, 2000; Ingvartsen, 2006). During NEB, the liver exceeds its capacity for oxidizing NEFA, which leads to partial oxidation of NEFA and, consequently, increasing BHB synthesis (Ingvartsen and Andersen, 2000). The latter is released into the bloodstream and serves as fuels for other tissues (Ingvartsen and Andersen, 2000). Minuti et al. (2015) detected lower prepartal NEFA and BHB, followed by a rapid increase after parturition, with the highest concentrations at 10 and 20 DIM.

Inconsistent responses on blood NEFA and BHB have reported when feeding yeast culture products to transition dairy cows (Al Ibrahim et al., 2010; Yuan et al., 2015a; Faccio-Demarco et al., 2019). For instance, Al Ibrahim et al. (2010) supplemented transition dairy cows with live yeast, and no effects were observed in NEFA and BHB. Similarly, Faccio-Demarco et al. (2019) supplemented 28 g/d of yeast culture plus enzymatically hydrolyzed yeast of *S. cerevisiae* to transition dairy cows and they did not observe differences in NEFA and BHB levels. Transition dairy cows fed with *S. cerevisiae* in combination with two *Enterococcus* strains from -21 to 70 d relative to parturition had lower postpartal NEFA and BHB concentrations after 8 and 22 DIM, respectively (Nocek et al., 2003). Yuan et al. (2015a) reported that a combination of live yeast culture plus enzymatically hydrolyzed yeast at the rate of 0, 30, 60, and 90 g/d from

-21 to 42 d relative to parturition cause an overall postpartal increase in BHB in yeast fed cows regardless of the level of supplementation.

A postpartal increase in NEFA and BHB in YC cows was observed with the highest concentrations at 7 DIM. These responses were similar to those observed in transition dairy cows diagnosed with subclinical ketosis (Mezzetti et al., 2019) with maximal NEFA and BHB values at 7 DIM. However, the levels of NEFA and BHB (0.77 and 0.72 mmol/L, respectively) in YC cows were much lower than those observed by Mezzetti et al. (2019). The latter suggests that the level of lipid mobilization in YC cows was not conducive to subclinical ketosis development. In addition, we observed that NEFA and BHB in YC cows declined more sharply after 7 DIM than CON cows. The reasons underlying this observation are unclear but suggest a potential effect of yeast product on lipid metabolism in transition dairy cows.

CONCLUSIONS

Yeast culture products have been fed to peripartal dairy cows for many years to improve milk yield and milk efficiency. The findings of this study revealed that YC promoted positive responses on performance, such as milk yield, SCC, and ECM. In the rumen, YC contributed to increased valerate soon after calving. The latter was accompanied by increments in rumen microbiota populations such as cellulolytic and lactic acid-utilizing. The supply of the yeast culture product and its fermentation metabolistes in rumen, could be a source of substrates for ruminal bacteria that can promote improvements in fermentable characteristics and optimize the relative abundance of microbial species. In addition, the yeast culture product may influenced

lipid metabolism in transition dairy cows, but this mechanism remains to be elucidated. The results describe an overall beneficial effect of supplementing YC during the transition period through early lactation, at least in terms of rumen environment and performance.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Phibro Animal Health (Teaneck, NJ, USA) for financial support of this research. The authors also thank South Dakota Agricultural Experiment Station (Brookings, SD, USA), and we gratefully acknowledge the help from all the staff at the South Dakota State University Dairy Research and Training Facility (Brookings-SD) for animal care and handling.

Table 2.1 - Ingredient composition of diets during the close-up (-30d to expected calving) and early lactation periods.

	Diet	
Component	Close-up	Lactation
Ingredient, % DM[1]		
Corn silage	37.5	36.31
Alfalfa silage	----	8.71
Alfalfa hay	----	15.97
Grass Hay	23.9	----
Cotton seed	----	8.25
Wheat straw	12.6	----
Soy best	10.5	----
Corn grain ground fine	3.1	16.07
Soybean meal	4.67	3.65
Distillers grains dry	----	3.66
Animate[3]	3.72	----
Wheat midds	----	3.65
Calcium carbonate	2.61	1.00
Calcium phosphate Di	0.52	----
Sodium bicarbonate	----	0.91
Energy booster 100[2]	----	0.91
Salt white	0.19	0.34
Magnesium oxide	0.21	0.16
Urea 281% CP	----	0.11
JPW Dairy Vitamin Premix[4]	0.13	0.10
JPW Dairy TM Premix[5]	0.10	0.10
Dairy VTM	0.14	----
Mepron	----	0.04
Vitamin E, 20000 UI/lb	0.10	0.04
Biotin 1%	----	0.01
Rumensin 90	0.01	0.01
Chemical analysis		
DM, %	49.81	50.61
NE_L, Mcal/ kg DM	1.54	1.60
CP, % DM	12.31	15.56
NDF, % DM	46.39	38.55
ADF, % DM	28.78	25.83
DCAD, mEq/100 g	-9.70	--

[1]Ingredients included in the ration formulated by using Spartan Dairy Ration Evaluator 3.0

[2] Animate was used as the sole DCAD anion source.

[3] Energy Booster 100 (MSC, Carpentersville, IL)

[4]Contained: 25.8 % Ca (DM basis) 1,545 IU/kg of Vitamin A, 387 IU/kg of Vitamin D, and 4,826 IU/kg of Vitamin E (JPW Nutrition, Sioux Falls, SD).

[5]Contained: 11.7 % Ca (DM basis), 1.96 % S, 10,527 mg/kg of Fe, 63,158 mg/kg of Zn, 12,632 mg/kg of Cu, 63,158 mg/kg of Mn, 325 mg/kg of Se, 632 mg/kg of Co, and 1,053 mg/kg of I (JPW Nutrition, Sioux Falls, SD).

Table 2.2 - Species-specific primers used in real-time qPCR assay for the quantification of selected rumen bacteria population.

Target bacterial species		Primer sequence (5'- 3')	Reference
Anaerovibrio lipolytica	F[a]	GAAATGGATTCTAGTGGCAAACG	(Abdelmegeid et al., 2018)
	R[b]	ACATCGGTCATGCGACCAA	
Butyrivibrio fibrisolvens	F	ACACACCGCCCGTACCA	(Klieve et al., 2003)
	R	TCCTTACGGTTGGGTCACAGA	
Butyvibrio proteoclasticus	F	GGGCTTGCTTTGGAAACTGTT	(Abdelmegeid et al., 2018)
	R	CCCACCGATGTTCCTCCTAA	
Eubacterium ruminantium	F	CTCCCGAGACTGAGGAAGCTTG	(Abdelmegeid et al., 2018)
	R	GTCCATCTCACACCACCGGA	
Fibrobacter succinogenes	F	GCGGGTAGCAAACAGGATTAGA	(Abdelmegeid et al., 2018)
	R	CCCCCGGACACCCAGTAT	
Megaspheara elsdenii	F	AGATGGGGACAACAGCTGGA	(Abdelmegeid et al., 2018)
	R	CGAAAGCTCCGAAGAGCCT	
Prevotella albensis	F	GCGCCACTGACGCTGAAG	(Khafipour et al., 2009)
	R	CCCCAAATCCAAAAGGACTCAG	
Prevotella brevis	F	GGTTTCCTTGAGTGTATTCGACGTC	(Stevenson and Weimer, 2007)
	R	CTTTCGCTTGGCCGCTG	
Prevotella bryantii	F	AGCGCAGGCCGTTTGG	(Abdelmegeid et al., 2018)
	R	GCTTCCTGTGCACTCAAGTCTGAC	
Prevotella ruminicola	F	GAAAGTCGGATTAATGCTCTATGTTG	(Stevenson and Weimer, 2007)
	R	CATCCTATAGCGGTAAACCTTTGG	
Rumicoccus albus	F	ACGTCRTCCMCACCTTCCTC	(Koike and Kobayashi, 2001)
	R	CCTCCTTGCGGTTAGAACA	
Rumicoccus flavefaciens	F	CGAACGGAGATAATTTGAGTTTACTTAGG	(Denman and McSweeney, 2006)
	R	CGGTCTCTGTATGTTATGAGGTATTACC	
Ruminobacter amylophilus	F	CTGGGGAGCTGCCTGAATG	(Stevenson and Weimer, 2007)
	R	GCATCTGAATGCGACTGGTTG	
Selenomonas ruminantium	F	CAATAAGCATTCCGCCTGGG	(Abdelmegeid et al., 2018)
	R	TTCACTCAATGTCAAGCCCTGG	
Succinimonas amylolytica	F	CGTTGGGCGGTCATTTGAAAC	(Abdelmegeid et al., 2018)
	R	CCTGAGCGTCAGTTACTATCCAGA	
Succinivibrio dextrinosolvens	F	TAGGAGCTTGTGCGATAGTATGG	(Abdelmegeid et al., 2018)
	R	CTCACTATGTCAAGGTCAGGTAAGG	
Streptococcus bovis	F	TTCCTAGAGATAGGAAGTTTCTTCGG	(Abdelmegeid et al., 2018)
	R	ATGATGGCAACTAACAATAGGGGT	
Treponema bryantii	F	AGTCGAGCGGTAAGATTG	(Tajima et al., 2001)
	R	CAAAGCGTTTCTCTCACT	
Bacteria general 1	F	GGATTAGATACCCTGGTAGT	(Abdelmegeid et al., 2018)
	R	CACGACACGAGCTGACG	
Bacteria general 2	F	GTGSTGCAYGGYTGTCGTCA	(Abdelmegeid et al., 2018)
	R	ACGTCRTCCMCACCTTCCTC	

[a]F: forward primer

[b]R: reverse primer

Table 2.3 - Effects of supplementing cows with a control diet (CON) or control diet plus yeast culture (YC; Cellerate Yeast Solutions®, Culture Classic HD; Phibro Animal Health, Teaneck, NJ) in dairy cows during the peripartal period until 50DIM on BW, BCS, DMI, and energy balance.

	Treatment			*P-value*			
Parameter	CON	YC	SEM[3]	Diet	Parity[4]	Time	D x T[5]
Prepartum[1]							
BW, kg	773	785	16.4	0.61	<0.01	0.36	0.36
BCS	3.12	3.19	0.08	0.56	--	0.12	0.81
DMI, kg/day	12.2	12.7	0.61	0.59	--	<0.01	0.21
DMI, % BW	1.5	1.6	0.07	0.95	--	0.02	0.24
EB, Mcal/day	4.01	3.50	1.02	0.65	0.02	0.02	0.36
Postpartum[2]							
BW, kg	688	688	13.75	0.99	<0.01	<0.01	0.92
BCS	2.79	2.81	0.04	0.77	--	<0.01	0.28
DMI, kg/day	19.9	20.5	0.47	0.40	<0.01	<0.01	0.87
DMI, % BW	2.8	2.9	0.07	0.54	--	<0.01	0.97
EB, Mcal/day	-4.30	-5.93	1.26	0.23	0.27	0.17	0.98

[1]Prepartum parameters were analyzed from -30 d to calving.
[2]Postpartum parameters were analyzed from calving to 50 DIM.
[3]Largest standard error of the mean.
[4]Interaction of treatment and days in milk.

Table 2.4 - Effects of supplementing cows with a control diet (CON) or control diet plus yeast culture (YC; Cellerate Yeast Solutions®, Culture Classic HD; Phibro Animal Health, Teaneck, NJ) in dairy cows during the peripartal period until 50DIM on milk composition.

	Treatment			*P-value*			
Parameter	CON	YC	SEM[1]	Diet	Parity	Time	D × T[2]
Milk yield, kg/d	34.3	36.8	1.15	0.13	<0.01	<0.01	0.78
Milk composition							
Milk fat, %	3.31	3.28	0.14	0.88	--	<0.01	0.59
Milk fat yield, kg/d	1.30	1.46	0.07	0.11	<0.01	0.19	0.97
Milk protein, %	3.18	3.14	0.05	0.52	--	<0.01	0.71
Milk protein yield, kg/d	1.06	1.12	0.03	0.26	<0.01	<0.01	0.95
Milk lactose, %	4.92	4.94	0.02	0.55	--	<0.01	0.58
SCC[3]	1.84	1.55	0.05	<0.01	<0.01	0.58	0.75
MUN, mg/dL	10.46	10.72	0.57	0.74	--	0.02	0.96
ECM, kg/day	35.2	38.4	1.22	0.07	<0.01	<0.01	0.97
Milk/DMI	1.90	1.95	0.05	0.52	--	<0.01	0.78
ECM/DMI	1.89	1.99	0.05	0.18	--	0.03	0.96

[1]Largest standard error of the mean
[2]Interaction of treatment and days in milk
[3]Somatic cell counts were transformed to Log10

Table 2.5 - Effects of supplementing cows with a control diet (CON) or control diet plus yeast culture (YC; Cellerate Yeast Solutions®, Culture Classic HD; Phibro Animal Health, Teaneck, NJ) in dairy cows during the peripartal period until 50DIM on VFA percentages.

	Treatment			*P-value*			
Parameter	CON	YC	SEM[1]	Diet	Parity	Time	D × T[2]
pH	6.2	6.4	0.08	0.22	--	< 0.01	0.90
NH_3, mg/dL	10.4	9.9	0.78	0.68	<0.01	0.11	0.52
Total VFA, n*M*	94.1	91.9	4.82	0.74	--	0.40	0.37
VFA (mol/100 mol)							
Acetate, %	64.6	64.7	0.84	0.93	--	<0.01	0.88
Propionate, %	24.3	24.1	0.77	0.85	--	<0.01	0.77
Butyrate, %	8.6	9.0	0.26	0.26	0.01	0.02	0.79
Isovalerate, %	1.5	1.4	0.04	0.02	--	<0.01	0.05
Valerate, %	0.9	1.0	0.04	0.24	<0.01	<0.01	0.04
Acetate:Propionate	2.7	2.7	0.09	0.94	--	<0.01	0.92

[1]Largest standard error of the mean.
[2]Interaction of treatment and days in milk.

Table 2.6 - Relative abundance (%) of target bacterial species mixed ruminal fluid from peripartal dairy cows supplemented with control diet (CON) or control diet plus yeast culture (YC; Cellerate Yeast Solutions®, Culture Classic HD; Phibro Animal Health, Teaneck, NJ).

	Treatment			*P-value*		
Species[2]	CON	YC	SEM[1]	Diet	Time	D × T[3]
Anaerovibrio lipolytica	1.36×10^{-3}	1.83×10^{-3}	0.19	0.12	0.26	0.39
Butyrivibrio fibrisolvens	8.53×10^{-4}	9.92×10^{-4}	0.15	0.32	0.27	0.87
Butyvibrio proteoclasticus	1.28×10^{-1}	1.28×10^{-1}	0.15	0.98	0.37	0.48
Eubacterium ruminantium	8.51×10^{-3}	9.04×10^{-3}	0.22	0.78	0.25	0.39
Fibrobacter succinogenes	5.52×10^{-2}	7.27×10^{-2}	0.15	0.09	0.04	0.47
Megaspheara elsdenii	3.65×10^{-4}	5.53×10^{-4}	0.22	0.05	<0.01	0.49
Prevotella albensis	2.61×10^{-3}	4.58×10^{-3}	0.65	0.40	<0.01	0.01
Prevotella brevis	8.74×10^{-2}	7.12×10^{-2}	0.04	<0.01	0.04	0.70
Prevotella bryantii	2.96×10^{-2}	2.30×10^{-2}	0.58	0.66	<0.01	0.05
Prevotella ruminicola	8.20×10^{-1}	7.43×10^{-1}	0.11	0.36	0.99	0.02
Rumicoccus albus	2.59×10^{-3}	2.59×10^{-3}	0.23	0.99	0.19	0.98
Rumicoccus flavefaciens	6.38×10^{-4}	6.51×10^{-4}	0.38	0.96	0.08	0.26
Ruminobacter amylophilus	1.29×10^{-3}	2.63×10^{-3}	0.82	0.39	0.42	0.45
Selenomonas ruminantium	2.32×10^{-1}	2.39×10^{-1}	0.10	0.73	0.04	0.11
Succinimonas amylolytica	1.85×10^{-3}	4.63×10^{-3}	0.39	0.03	0.13	0.12
Succinivibrio dextrinosolvens	5.22×10^{-3}	6.78×10^{-3}	0.53	0.63	0.66	0.05
Streptococcus bovis	9.62×10^{-4}	9.54×10^{-4}	0.27	0.97	0.02	0.42
Treponema bryantii	4.37×10^{-3}	5.62×10^{-3}	0.33	0.46	0.18	0.76

[1]Largest standard error of the mean is shown.
[2]Data were log-transformed before statistics. The standard errors of the means associated with log-transformed data are in log scale.
[3]Interaction of treatment and days in milk.

Table 2.7 - Effects of supplementing cows with a control diet (CON) or control diet plus a yeast culture supplement (YC; Cellerate Yeast Solutions®, Culture Classic HD; Phibro Animal Health, Teaneck, NJ) in blood biomarkers related to muscle body mass, metabolism and oxidative stress in dairy cows during the peripartal period until 50DIM.

	Treatment			*P-value*		
Parameter	CON	YC	SEM[1]	Diet	Time	D × T[3]
Muscle body mass						
Creatinine, µmol/L	88.1	89.6	1.04	0.30	< 0.01	0.56
Urea, mmol/L	4.60	4.58	0.14	0.92	0.19	0.39
Metabolism						
Glucose, mmol/L	4.24	4.24	0.05	0.98	< 0.01	0.84
NEFA, mmol/L[2]	0.31	0.25	0.12	0.10	< 0.01	0.02
BHB, mmol/L[2]	0.43	0.42	0.06	0.83	< 0.01	0.13
Oxidative stress						
ROM, mg H_2O_2/100 mL	14.0	15.0	0.52	0.19	0.03	0.40
FRAP, µmol/L[2]	142.0	136.3	0.04	0.28	< 0.01	0.48

[1]Largest standard error of the mean is shown.
[2]Data were log-transformed before statistics. The standard errors of the means associated with log-transformed data are in log scale.
[3]Interaction of treatment and days in milk.

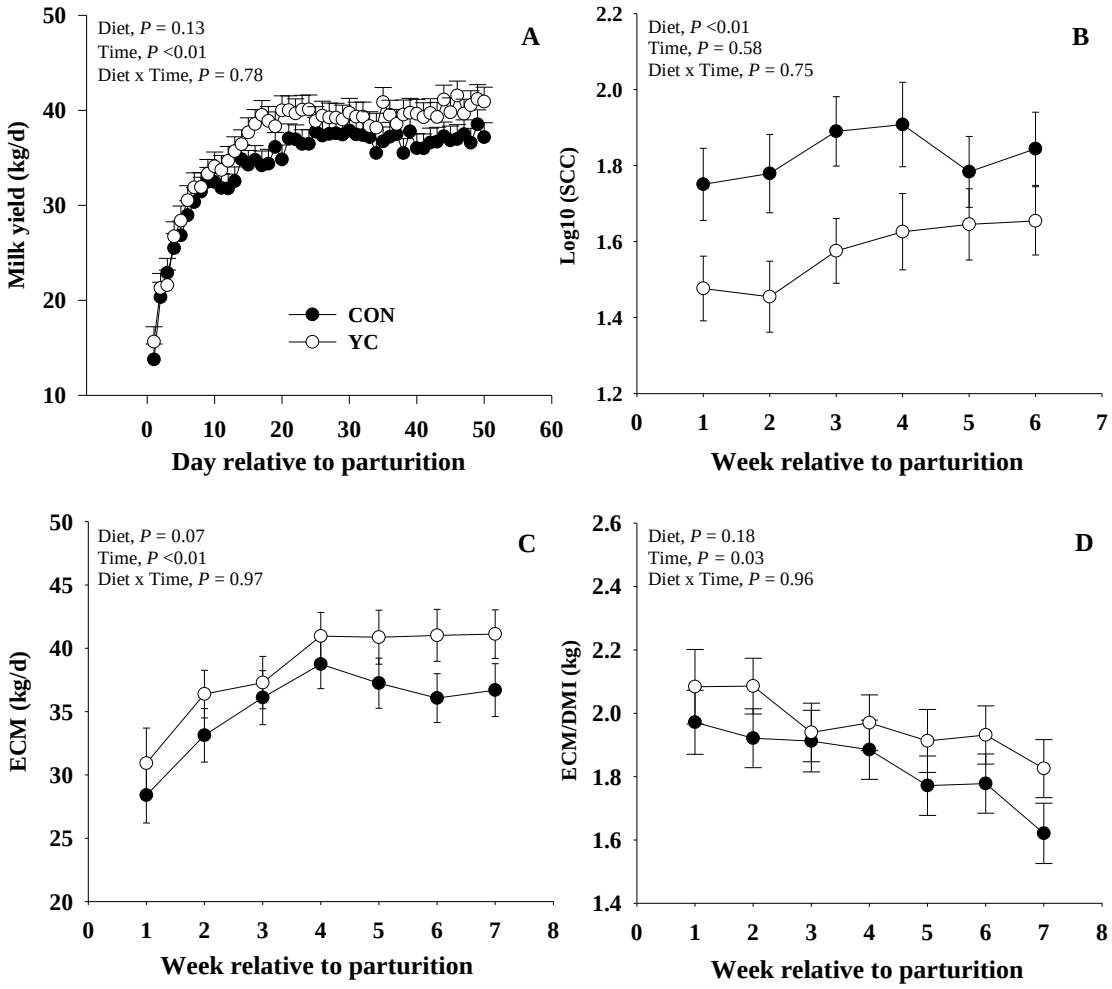

Figure 2.1 - Milk yield (A), SCC (B), ECM (C) and ECM/DMI (D) in cows supplemented with a control diet (CON) or control diet plus yeast culture (YC; Cellerate Yeast Solutions®, Culture Classic HD; Phibro Animal Health, Teaneck, NJ) in dairy cows during the transition period until 50DIM. Values are means and the standard errors are represented by vertical bars.

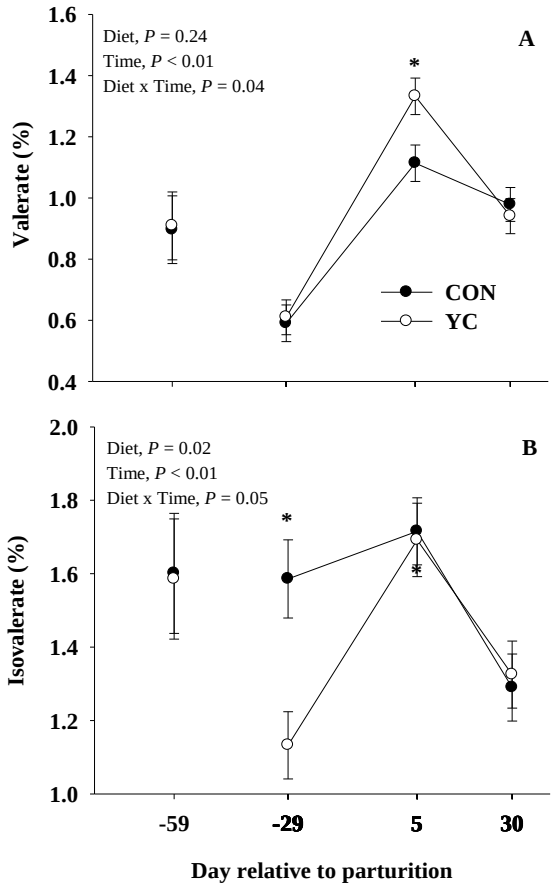

Figure 2.2 - Effects of control diet (CON) or control diet plus a yeast culture supplement (YC; Cellerate Yeast Solutions®, Culture Classic HD; Phibro Animal Health, Teaneck, NJ) on ruminal valerate and isovalerate in dairy cows during the transition period until 50DIM. Values are means and the standard errors are represented by vertical bars.

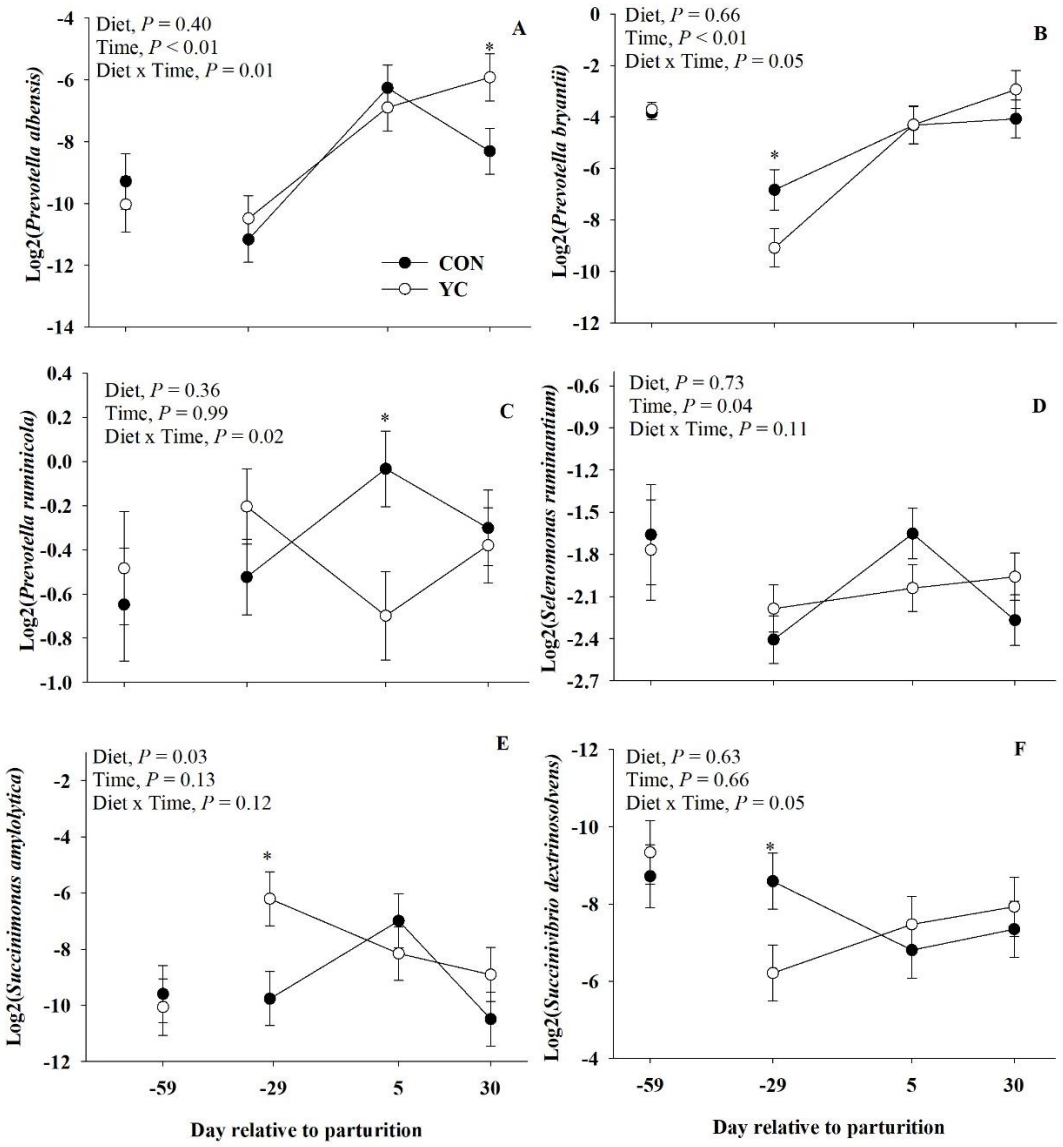

Figure 2.3 - Effects of control diet (CON) or control diet plus a yeast culture supplement (YC; Cellerate Yeast Solutions®, Culture Classic HD; Phibro Animal Health, Teaneck, NJ) on relative abundance (%) of microbial species in rumen fluid in dairy cows during the peripartal period until 50DIM. Values are means and the standard errors are represented by vertical bars.

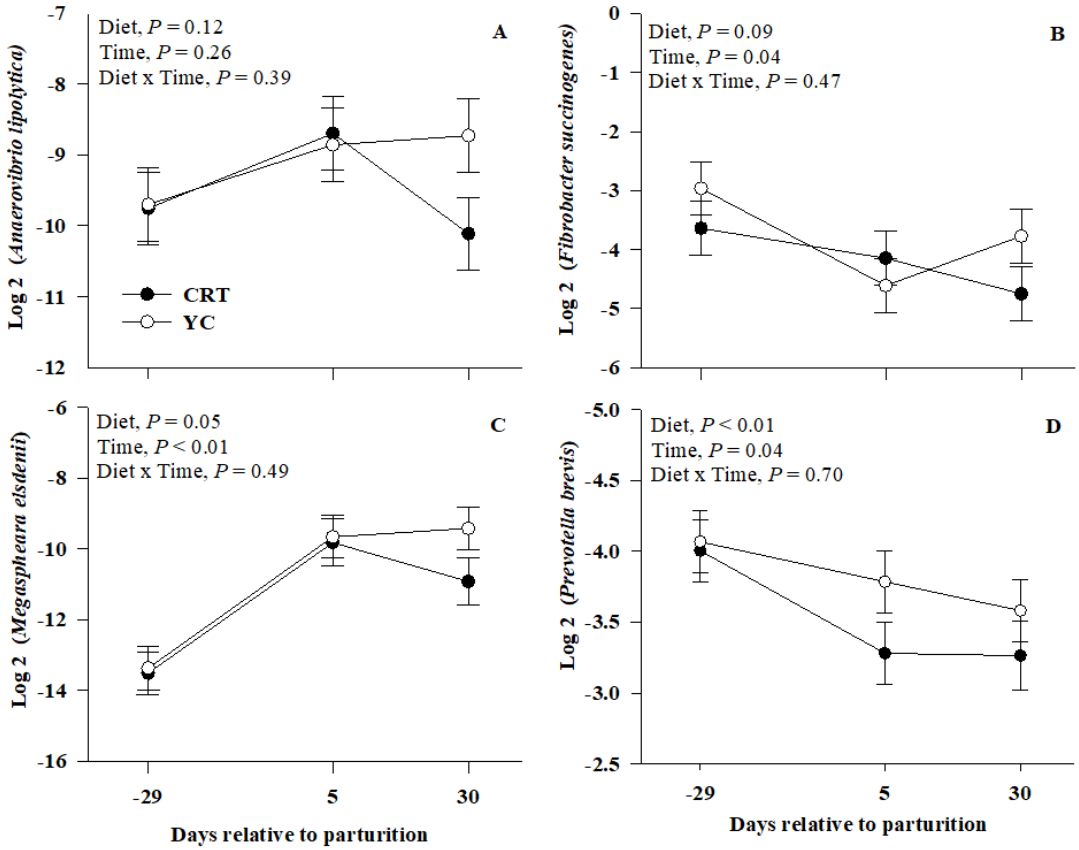

Figure 2.4 - Effects of control diet (CON) or control diet plus a yeast culture supplement (YC; Cellerate Yeast Solutions®, Culture Classic HD; Phibro Animal Health, Teaneck, NJ) on relative abundance (%) of microbial species in rumen fluid in dairy cows during the peripartal period until 50DIM. Values are means and the standard errors are represented by vertical bars.

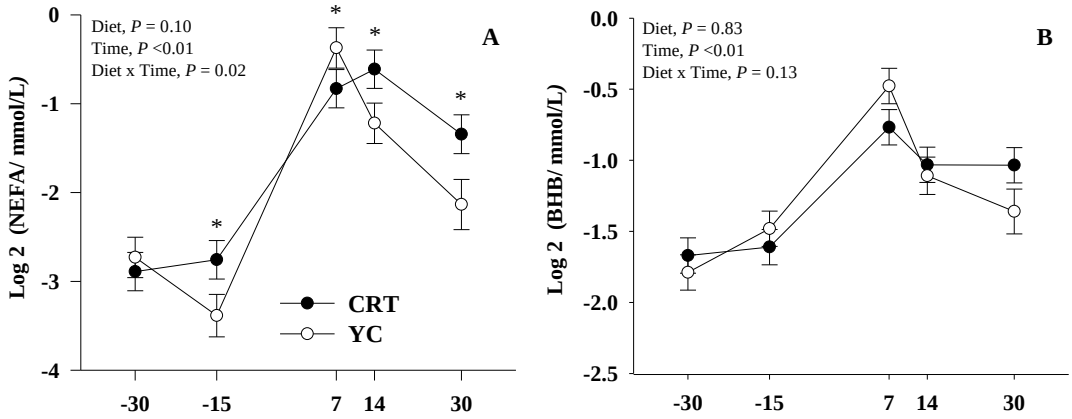

Figure 2.5 - Effects of control diet (CON) or control diet plus a yeast culture supplement (YC; Cellerate Yeast Solutions®, Culture Classic HD; Phibro Animal Health, Teaneck, NJ) on blood NEFA (A) and BHB (B) in dairy cows during the transition period until 50DIM. Values are means and the standard errors are represented by vertical bars.

CHAPTER 3. YEAST CULTURE SUPPLEMENTATION EFFECTS ON SYSTEMIC AND POLYMORPHONUCLEAR LEUKOCYTES MRNA BIOMARKERS OF INFLAMMATION AND LIVER FUNCTION IN PERIPARTAL DAIRY COWS.

ABSTRACT

Feeding yeast culture additives has been attributed to health benefits during the transition period of dairy cows. The objective of this study was to evaluate the effects of feeding a commercial yeast culture on blood biomarkers of inflammation and liver function, and gene expression in polymorphonuclear leukocytes (PMNL) in dairy cows during the transition period until 50 d postpartum. Forty Holstein dairy cows were used in a randomized complete block design from -30 to 50 d and blocked according to parity, previous lactation milk yield, and genetic merit. At -30 d, cows were assigned to a basal diet plus a top-dressed of either 114 g/d of ground corn (CON; n = 20) or 100 g/d of ground corn and 14 g/d yeast culture product (YC; n = 20; Cellerate Culture Classic HD; Phibro Animal Health, Teaneck, NJ). Cows received the same close-up diet from -30 to calving (0.63 Mcal/kg DM and 12.3% CP) and lactation diet after calving (0.73 Mcal/kg DM and 15.6% CP). Blood samples were collected at -30, -15, 7, 14, and 30 DIM to evaluate liver function and inflammation biomarkers. Blood samples for PMNL gene expression were collected at -30, -15, 5, 10, and 30 d. The mRNA in PMNL was analyzed for genes associated with inflammation, cellular receptor, and oxidative stress. A trend for a Diet × Time interaction was observed in GGT, where greater GGT was observed in YC than CON cows at 30 d. Negative acute-phase proteins (APP), albumin, and paraoxonase tended to be lower in YC than CON cows, while the positive APP, haptoglobin, tended to be higher in YC than CON. In PMNL mRNA expression, the *NFKB1* was upregulated in

YC cows in comparison to CON. A trend for upregulation of *TNFA, IL10, SELL,* and *TLR2* was observed in cows supplemented with YC. The present study showed that yeast culture supplementation might stimulate a mild inflammatory response in transition dairy cows, based on blood biomarker and PMNL mRNA gene expression.

Key words: transition period, inflammation, yeast culture

INTRODUCTION

In dairy cows, the transition period is defined as 3 weeks before through 3 weeks after parturition (Drackley, 1999), and it is the most critical period during the cow's life. The metabolic stress and altered immune function may contribute to the noticeable incidence of health disorders in early lactation in dairy cows (Goff and Horst, 1997). The development of nutritional-based strategies that can modulate an immune response will positively affect dairy cows' health. Yeast-based products have been used in livestock for many years due to the beneficial effects on animals, with the most common species being *S. cerevisiae*.

During the transition period, yeast-based products have been shown to improve DMI (Dann et al., 2000; Shi et al., 2019) and milk yield (Nocek et al., 2011; Zaworski et al., 2014), while others did not observe the same effects (Olagaray et al., 2019; Stefenoni et al., 2020). Recent findings also suggest that yeast may influence immune function. However, the mechanism behind the interaction between supplemented yeast and innate immunity still unclear. According to Lopreiato et al. (2020), the responses can be attributed to a better energy status promoted by positive effects on digestive function or activation of the immune system by sensing yeast components.

Jensen et al. (2008) demonstrate that yeast culture provides antioxidant, anti-inflammatory, and immuno-modulatory activities *in vitro*. Yuan et al. (2015b) reported that yeast culture plus enzymatically hydrolyzed yeast could enhance humoral and mucosal immunity and modulated uterine inflammatory signals and mammary gland health in transition dairy cows. Recently, Knoblock et al. (2019) evaluated the effects of *S. cerevisiae* fermentation product in dairy cows fed diets with different starch content during the early lactation and observed that yeast supplementation coupled with low-starch diets might reduce inflammation. In lactating dairy cows, yeast cell wall supplementation increased mRNA expression of cytokines in peripheral blood mononuclear cells, which underscores yeast's ability to mediate immune function (Aung et al., 2020).

The objective of this study was to assess the effects of yeast-based products on metabolism, inflammation, liver function, and immune function through blood biomarkers and gene expression in PMNL in dairy cows during the transition period until 50 DIM.

MATERIAL AND METHODS

Animals, Experimental Design and Dietary Treatments

The Institutional Animal Care and Use Committee (IACUC) of the South Dakota State University approved all the procedures for this study. Forty Holstein dairy cows were used in a randomized complete block design and were blocked according to lactation, calving date, and previous lactation milk yield. There were 6 primiparous and 34 multiparous enrolled in this study. Cows received the same close-up diet (1.32 Mcal of

NE_L/kg and 14.15% CP) 7 d prior to starting dietary treatments as an adaptation period. Then, at -30 d before expected calving, cows were assigned to the treatments. The treatments were top-dressed a control (CON; 114g/h/d ground corn; n = 20) or CON plus yeast culture product (YC; 100g ground corn + 14g YC/h/d; n = 20). After parturition, all cows were fed the lactation basal diet (1.72 Mcal of NE_L/kg and 16.05% CP) as a total mixed ration, and the supplementation continued through 50d postpartum.

The cows were enrolled in the experiment from mid-September 2018 to early April 2019. Cows were feed using an individual gate system (American Calan, Northwood, NH), and the intake of dry matter (DM) was recorded daily. Diets were formulated using the NDS professional (Nutrition Dynamic System; RUM&N Sas) to meet the requirements of the average cow in the group according to the NRC (2001) (Table 2.1). Dry matter of individual feed ingredients was determined weekly accordingly, and diets were adjusted accordingly to maintain formulated DM ratios of ingredients in the TMR. Cows were fed once daily at 0500 h before morning milking, and feed offered was adjusted daily to achieve 5 to 10% refusals.

During the close-up period, the cows were housed in a bedded pack pen, and three days before the expected calving date, the cows were reallocated in individual pens bedded with straw until parturition. On day 3, after calving, cows were moved to a lactation free-stall barn where they were receiving the same lactation basal diet until 50 DIM. Body weight was measured weekly for each cow at the same time in the afternoon. Body condition score (BCS) (scale 1= thin to 5= obese) was assigned by two individuals, and the average score was used for statistical analysis.

Blood Collection and Analyses

Blood was sampled from the coccygeal vein before morning feeding using a 20-gauge BD Vacutainer needle (Becton Dickinson, Franklin Lakes, NJ) at -30, -15, 7, 14, and 30 d relative to parturition from a subset of cows (n=8 cows/treatment). Blood was collected into Vacutainer (5 mL, BD Vacutainer, Becton Dickinson, Franklin Lakes, NJ) containing either serum clot activator or lithium heparin. After collection, tubes containing lithium heparin were placed on ice, and tubes with serum clot activator were kept at 21°C until centrifugation. Serum and plasm were obtained by centrifugation at 1,300 × *g* for 15 min at 21°C and 4°C, respectively. The aliquots of plasma were frozen at -80°C until further analysis.

Samples were analyzed for biomarkers of liver function [e.g., total bilirubin, aspartate aminotransferase (AST/GOT), γ-glutamyl transpeptidase (GGT), cholesterol and paraoxonase (PON)], inflammation [e.g., albumin, ceruloplasmin, haptoglobin and interleukin 1β (IL-1β)], using kits purchased from Instrumentation Laboratory (Lexington, MA) and the procedures were described by (Trevisi et al., 2012; Batistel et al., 2016; Jacometo et al., 2016).

PMNL Isolation

Neutrophils were isolated based on procedures described by Osorio et al. (2013b). Briefly, blood (~100 mL) was collected into evacuated tubes (8 mL, BD Vacutainer, Becton Dickinson, Franklin Lakes, NJ) containing solution A of trisodium citrate citric acid and dextrose (ACD) from the coccygeal vein prior to morning feeding at -30, 5, 15, and 30 days relative to parturition from a subset of cows (n=8 cows/treatment). Tubes were mixed by inversion immediately after collection and placed on ice (~4 °C) until

isolation. Samples were centrifuged at 600 × *g* for 30 min at 4 °C. The plasma, buffy coat, and approximately one-third of the red blood cells were discarded. The remaining sample was poured into a 50-mL conical tube (Fisher Scientific, Pittsburgh, PA). Red blood cells (RBC) were lysed with twenty-five milliliters of deionized water at 4 °C was added into the tube, followed by the addition of 5 mL of 5 × PBS at 4 °C to restore an iso-osmotic environment. Samples were centrifuged at 900 × *g* for 10 min at 4°C, and the supernatants were decanted. The cell pellet was washed with 10 mL of 1 × PBS, and the cell suspension was centrifuged at 900 × *g* for 5 min at 4°C, and the supernatants were decanted. The remaining RBC were lysed with 8 mL of ice-cold deionized water, homogenized gently by inversion and 2 mL of 5 × PBS at 4°C was added. The samples were centrifuged at 900 × *g* for 5 min at 4°C, and the supernatant was discarded. Subsequently, samples were washed twice using 10 mL of 1 × PBS at 4°C were performed, followed by centrifugation 900 × *g* for 5 min at 4°C. The PMNL pellet was homogenized with 1.5 mL of 1 × PBS at 4°C, transferred to a 2-mL RNase-DNase-free microcentrifuge tube and centrifuged at 6,000 × *g* (Sorvall Legend Microcentrifuge 21R) for 5 min at 4°C. The final PMNL pellet was homogenized in 1 mL of Trizol reagent (Invitrogen, Carlsbad, CA) and stored at -80°C until further gene expression analysis.

RNA Isolation, cDNA Synthesis, and Real-Time Quantitative PCR

Total RNA was extracted from blood PMNL using Trizol (Invitrogen, Carlsbad, CA) reagent in combination with the RNeasy® Plus Mini Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions with some modifications. Briefly, the cell pellet immersed in Trizol was transferred to a 2-mL RNase-free O-ring tube, containing one stainless steel bead, 5 mm (Qiagen, Hilden, Germany) and homogenized

in a Beadbeater (BioSpec Products, Bartlesville, OK) for 1 min. After homogenization, the lysate was transferred to a 2-mL RNase-free microtube and 200 µL of Phenol:chloroform (Invitrogen, Carlsbad, CA) at 4 °C was added in order to isolate the RNA from the organic phase. After centrifugation at 13,000 × *g* for 15 min at 4 °C, the upper phase supernatant was transferred into a new 2-mL RNase-free microtube. The total RNA was purified using RNeasy® Plus Mini Kit and eluted in 50 µL of RNase-free water. The RNA quantity for all PMNL samples was 327.17 ± 292.99 ng/µL and purity (260/280) was 2.02 ± 0.10 determined using NanoDrop spectrophotometer (ND 1000, NanoDrop Technologies Inc., Wilmington, DE).

RNA isolated from neutrophils was used for qPCR analysis. Complementary DNA (cDNA) synthesis was performed according to Osorio et al. (2013a) with modifications. cDNA was synthesized using 100 ng RNA, 1 µL random primers (Invitrogen Corp., CA), and 9 µL DNase/RNase free water (HyClone, UltraPure™). The mixture was incubated at 65 °C for 5 min and kept on ice for 5 min. A total of 9 µL of master mix composed of 4 µL 5X Reaction Buffer, 1 µg dT18 (Operon Biotechnologies, AL), 2 µL 10 mmol/L dNTP mix (Invitrogen Corp., CA), 0.25 µL (50 U) of RevertAidTM Reverse Transcriptase (Fermentas Inc., MD), 0.125 µL of RNase Inhibitor (10 U, Promega, WI), and 1.625 µL of DNase/RNase free water (HyClone, UltraPure™) was added. The reaction was performed in an Eppendorf Mastercycler® Gradient using the following temperature program: 25 °C for 5 min, 42 °C for 120 min and 70 °C for 5 min.

The cDNA was diluted 1:4 with RNase-free water (HyClone, UltraPure™). The quantitative PCR was performed in a MicroAmp Optical 384-Well Reaction Plate

(Applied Biosystems, Grand Island, NY). In each well, 4µL diluted cDNA combined with 6 µL of a mixture composed of 5 µL 1 × SYBR Green master mix (Applied Biosystems, CA), 0.4 µL each of 10 µM forward and reverse primers, and 0.2 µL DNase/RNase free water (HyClone, UltraPure™). Samples were run in triplicate, and a 6-point relative standard curve plus a non-template control were used. The reaction was performed in QuantStudio™ 6 Flex Real-Time PCR System (Applied Biosystems, Walthan, MA) following the conditions: 2 min at 50°C, 10 min at 95°C, 40 cycles of 15 s at 95°C (denaturation) and 1 min at 60°C (annealing + extension). A dissociation curve was performed (gradient from 95°C to 60°C to 95°C for 15s) to check the amplicon quality.

Primers sequence information and sources can be found in Table 3.1. In this study, the genes Golgin A5 (*GOLGA5*), Oxysterol Binding Protein Like 2 (*OSBPL2),* and Single-Strand-Selective Monofunctional Uracil-DNA Glycosylase 1 (*SMUG1*) were used as internal control genes (ICGs) as described by Moyes et al. (2010). The final gene expression data were normalized with the geometric mean of the 3 internal control genes. The stability of the ICG was assessed using the geNorm software (Vandesompele et al., 2002) with a favorable final pairwise variation of 0.17.

Target genes measured are described in Table 3.2, and these were related to inflammation [tumor necrosis factor alpha (*TNFA*), nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (*NFKB1*), interleukin 1B (*IL1B*) and interleukin 10 (*IL10*), interleukin 6 (*IL6*)] and cell receptors [toll like receptor 2 (*TLR2*), toll-like receptor 4 (*TLR4*), selectin-L (*SELL*) and interleukin 1 receptor associated kinase 1 (*IRAK1*)].

Statistical Analysis

Blood biomarkers and gene expression data were analyzed using the MIXED procedure of SAS 9.4 (SAS Institute Inc., Cary, NC) using diet, time (day or week), and their interactions as fixed effects and cow nested within treatment as a random effect. Parity effect was removed from the model any time it was nonsignificant ($P > 0.05$).

The exponential correlation covariance structure SP for repeated measures was used for the analysis of gene expression and blood biomarkers data with the following model:

$$y_{ijk} = \mu + D_i + T_j + DT_{ij} + \alpha_k + e_{ijk}$$

where y_{ijk} is the dependent, continuous variable; μ is the general mean; D_i is the fixed effect of the diet (i =1 or 2, namely, CON or YC); Tj is the fixed effect of the time (j = 1, 2, 3 or 4, namely, -30, 5, 15, and 30 DIM); DT_{ij} is the fixed effect of ith treatment by jth time of the interaction.; α_k is the random effect of cow; e_{ijk} is the random residual.

Blood metabolites data were log-scale transformed if needed to comply with normal distribution of residuals and subsequently back-transformed. Gene expression data were log-transformed prior to statistical analysis. Data on day -30 was used as a covariate for all the analyses. The covariate of previous 305-d milk yield was maintained in the model for all variables for which it was significant ($P < 0.05$). Statistical significance was declared at $P \leq 0.05$ and tendencies at $P < 0.15$.

RESULTS

Blood Biomarkers

Main effects of diet, time, and their interactions for blood metabolites associated with liver function and inflammation are presented in Table 3.3 and Figure 3.1. There was a trend (P= 0.07) for a D × T in GGT, which resulted in greater concentrations of GGT in YC fed cows than CON at 30 DIM ($P < 0.01$), and this effect was reflected in an overall greater ($P = 0.03$) GGT in YC cows. Similar to GGT, haptoglobin tended ($P = 0.12$) to be greater in YC cows in comparison to CON. In contrast to GGT and haptoglobin, there was a trend for lower PON ($P = 0.12$) and albumin ($P = 0.06$) in YC fed cows when compared to CON. Total bilirubin, GOT, cholesterol, ceruloplasmin and IL-1β were not affected by diet, time, or their interactions.

Gene Expression

Main effects of diet, time, and their interactions for PMNL mRNA gene expression are present in Table 3.4 and Figure 3.2. There was a trend ($P = 0.11$) for an upregulation of *TNFA* in YC cows than CON cows. The mRNA expression of *NFKB1* was greater ($P = 0.04$) in YC than CON cows. Similar to *TNFA*, there was a tendency for greater ($P = 0.07$) mRNA expression of *IL10* in cows supplemented with YC than CON (Figure 3.2). There was a trend ($P = 0.11$) for an upregulation of *SELL* in YC fed cows in comparison to CON (Figure 3.2). Similar to *SELL*, there was a trend ($P = 0.14$) for a greater expression of *TRL2* in YC than CON group. The expression of *IL1B*, *TLR4*, and *IRAK1* was not affected by diet, time, or their interaction.

DISCUSSION

Effects on performance parameters, blood biomarkers (i.e., muscle body mass, metabolism, and oxidative stress) and rumen fermentation and bacteria populations for this study have been evaluated in a companion manuscript. In summary, we observed a trend for a diet effect in milk yield, where YC cows (36.8 ± 1.15 kg/d) produced 2.5 kg/d more milk than CON cows (34.3 ± 1.15 kg/d). The latter was reflected in a trend for greater energy corrected milk (ECM) and milk fat yield in YC cows than CON. Yeast culture supplementation also promoted alterations in rumen fermentation, increasing valerate by optimizing the abundance of cellulolytic and lactate-utilizing bacteria. The current companion manuscript focused on systemic and PMNL mRNA indicators of inflammation and liver function during the transition period of dairy cows supplemented with a yeast culture.

Inflammation

During the transition period, dairy cows are more susceptible to health disorders such as mastitis, ketosis, retained placenta, metritis, and displaced abomasum. A common factor associated with the development of these disorders is the immune dysfunction (Sordillo, 2016), which, in turn, is caused by metabolic changes during the transition period.

Inflammation is a component of the immune response that involves physiological and pathological responses that helps the body adapt or eliminate an adverse stimulus to return tissues to normal function or homeostasis (Bradford et al., 2015; Sordillo, 2016). The most common event to occur during the inflammation is a systemic or acute phase response (APR). The primary stimulators of the APR are the pro-inflammatory cytokines,

including TNFα, IL1β, and IL6 (Gauldie et al., 1987). These cytokines can activate leukocytes and endothelial cells (Bannerman et al., 2009) and alter the normal protein synthesis in hepatocytes. These proteins for which their hepatic synthesis is altered during APR are called acute phase proteins (APP). According to Ceciliani et al. (2012), during the acute phase response, the concentration of APP change dramatically, and because of this, they were classified as positive (increase) or negative (decrease) APP. The most common positive APP are serum amyloid A, haptoglobin, and ceruloplasmin, and the negative APP include albumin, PON, and apolipoproteins.

During the transition period, alterations in APP have been used to monitor inflammation and liver function (Trevisi et al., 2010). In a retrospective analysis, Bertoni et al. (2008) classified early lactation dairy cows according to a liver activity index (LAI) and evaluated APP concentrations based on this classification. The albumin levels from cows classified as a high LAI was 34.5 g/L, while low LAI cows had 29.0 g/L. In the current study, albumin reached nadir levels at 7 DIM, regardless of treatment, at which point the albumin levels in YC and CON cows were 34.4 and 35.6 g/L, respectively. These data indicate that while YC cows tended ($P = 0.06$) have lower albumin concentration than CON, this was comparable to that in cows classified with a high LAI in Bertoni et al. (2008). The levels of albumin are constant during the pregnancy (35-36 g/L) followed by a reduction soon after calving and returning to prepartal levels (36-37 g/L) as cows progress into their lactation (Bertoni and Trevisi, 2013). The same effect was observed in our study. The latter suggests that it is less likely that YC cows were under a significant inflammation level, and perhaps the impact on liver function was minimal.

The positive APP, haptoglobin, has been observed to increase during stress periods promoting an APR; therefore, low LAI cows have been observed to have greater haptoglobin than high LAI cows (Bertoni et al., 2008). The trend for overall greater haptoglobin in YC cows than CON seems to be driven by the greater haptoglobin in YC cows at 7 and 14 DIM (Figure 3.1). However, the maximal haptoglobin ca. 0.32 g/L in YC cows at 7 DIM reached similar levels than cows classified as high LAI (0.3 g/L) at 7 DIM (Bertoni et al., 2008). After 7 DIM, YC cows presented an evident decline in haptoglobin, similar to that observed in Bertoni et al. (2008). Maximal haptoglobin commonly occurs within the first week postpartum. For instance, at 7 DIM, a ~0.8 g/L haptoglobin concentrations have been reported (Bertoni and Trevisi, 2013). Our data suggest that YC cows experienced a mild activation of the immune system that was transient as this group of cows did not present common inflammation patterns of low albumin coupled with high haptoglobin. In contrast to our results, yeast supplementation tended to reduce haptoglobin levels in different conditions (Dias et al., 2018; Perdomo et al., 2020). For instance, under heat stress, early lactation dairy cows supplemented with up to 1g the dose of live yeast tended to reduce haptoglobin (Perdomo et al., 2020). Dias et al. (2018) evaluated the effects of a culture of *S. cerevisiae* in lactating dairy cows fed diets with varying starch content. The supplementation of yeast culture attenuated the inflammatory stimulus, tended to reduce the concentration of haptoglobin in high-starch diets. The authors further discussed that yeast can minimized the risk of cows developing subacute ruminal acidosis and systemic inflammation because of the translocation of inflammatory mediators from rumen to blood. However, the yeast effect attenuating inflammation can be due the conditions that the lactating dairy cows were subjected to,

like heat stress or high-starch diets. In transition cows, due to the metabolic changes associated to NEB, the haptoglobin levels can vary more, especially post-calving.

Liver Function

The low levels of PON during the early postpartum period in dairy cows have been associated with metabolic disorders such as excessive fat mobilization and triglyceride accumulation (Turk et al., 2004). Bionaz et al. (2007) performed a retrospective analysis by classifying cows based on their blood PON concentration with the aim to test the feasibility of using this metabolite as a marker for liver function. The overall PON values for cows classified as high PON activity group were 92 U/mL, and PON concentration increased significantly in this group during the first 2wk of lactation. In our study, the PON levels for YC (95.0 U/mL) and CON (102.5 U/mL) cows were greater than those reported by Bionaz et al. (2007), followed by an increase postpartum. Similar to albumin, the lowest PON levels in YC cows were observed at 7 DIM (Figure 3.1); however, this PON level in YC cows was similar to that in cows designated as high PON activity group in Bionaz et al. (2007). Our results suggest that both YC and CON cows were at a greater level of PON, indicating that cows were not in an inflammatory condition. During inflammatory conditions, negative APP, such as PON, albumin, lipoproteins and retinol-binding protein, typically decrease (Bionaz and Loor, 2007; Trevisi et al., 2012). The reduction of PON observed at 7 DIM in YC cows can happen in transition dairy cows due to a reduction in liver-derived HDL, which plays an important role in PON's availability in circulation, particularly before calving (Bionaz et al., 2007). Our study is limited in that we cannot ascribe the observed effect in liver-derived HDL because we did not measure this metabolite.

The GGT is a membrane-associated enzyme involved in hepatic AA metabolism, and its plasma concentration has been associated with liver cell damage (Bionaz et al., 2007). In the current study, we observed an increase in GGT in YC cows, and this effect was more pronounced at 30 DIM, where GGT levels for YC cows were 94.9 U/L. Bertoni et al. (2008) showed that GGT levels were not different across different LAI-based groups, suggesting that LAI does not account for liver cell damage. According to Trevisi et al., (2001), impaired liver function is not equal to lower liver activity due to liver damaged, but rather a diverted synthesis of proteins (i.e., APP) during inflammation. Although we can conclude that YC cows had greater GGT than CON by 30 DIM, these levels did not surpass those observed in Bertoni et al. (2008), which indicates that YC cows in the current study were less prone to develop liver cell damage. Our results are also in agreement with others, where transition dairy cows presented high concentrations of GGT at 30DIM (Rodriguez-Jimenez et al., 2018; Mezzetti et al., 2019). Other authors have reported the effects of yeast supplementation in GGT levels in dairy cows (Al Ibrahim et al., 2010; Aung et al., 2019). In lactating dairy cows, yeast cell wall supplementation did not affect the GGT concentration, but the levels (27.38 U/L) were higher than those observed in our study (Aung et al., 2019). Live yeast supplementation did not affect the GGT levels in early lactating dairy cows; however, the concentration (10.36 U/L) was lower than we observed in this study (Al Ibrahim et al., 2010). Taken together, our results suggest that YC cows were in a mild inflammatory condition, meaning that inflammation was not enough to cause negative effects on liver activity.

Gene Expression

The immune system relies on the innate immune system components to detect and mount an early response to infectious microorganisms. In the cell surface of immune cells, there are receptors known as pattern recognition receptors that recognize two classes of molecules: pathogen-associated molecular patterns, associated with pathogens products, and damage-associated molecular patterns, which are related to components of host's cells that are released during cell damage or death (Sordillo, 2016). When activated, these receptors initiate a signal transduction pathway that results in gene transcription and activation of pro-inflammatory cytokines, playing an important role in triggering and regulating the immune responses (Lee and Hwang, 2006).

The antioxidant, anti-inflammatory, and immunomodulatory properties of yeast culture have been demonstrated *in vitro.* Such characteristics can mediate interactions between different immune cell populations, including neutrophils, monocytes, dendritic cells, B cells, T cells, and natural killer cells (Jensen et al., 2008). In the same study, Jensen et al. (2008) indicated that the mode of action of yeast culture remains unknown; however, this could be partially supported by an initial cellular interaction in gut mucosal tissue, resulting in changes in blood metabolites and cytokine profile. In our study, we observed that yeast culture altered blood biomarkers related to inflammation and liver function, and gene expression in PMNL, suggesting a cellular interaction between immune cells and yeast.

The immunomodulatory effects of yeast have been reported on genes related to inflammation across different species (Zanello et al., 2011; Yuan et al., 2015b; Adeyemi et al., 2019). Zanello et al. (2011) showed that *S. cerevisiae* (strain CNCM I-3856) exerts

an immunomodulatory effect in porcine intestinal epithelial cells, activating genes related to inflammation. In weaning pigs supplemented with *S. cerevisiae* fermentation products, Weedman et al. (2011) reported an upregulation of *TLR2* and *TLR4* in mesenteric lymph nodes on 1 d after weaning, suggesting that yeast culture had primed immune cells for the changes that occur during the weaning period. In transition dairy cows, Yuan et al. (2015b) reported that yeast culture plus enzymatically hydrolyzed yeast magnified humoral and mucosal immunity, causing an overall improvement in uterine and mammary gland health. In newly weaned beef steers, supplementation with *S. cerevisiae*-based product was attributed with upregulating gene networks responsible for promoting the animal's immune response to pathogens (Adeyemi et al., 2019).

To the best of our knowledge, there are no published data evaluating gene expression in bovine PMNL responsiveness of yeast culture supplementation. In the current study, we observed that *NFKB1*, *TNFA,* and *TLR2* were upregulated to a lesser extent. The *NFKB1* is a transcription factor associated with inflammation. An inflammatory response commonly causes an upregulation of *NFKB1*, which, in turn, upregulates cytokines such as *TNFA* and *IL10*, as well as several other genes related to inflammation (Trevisi et al., 2011; Moyes et al., 2014). The upregulation of *NFKB1* in cows supplemented with YC can be associated with increased blood biomarkers related to inflammation and liver function, previously discussed. The effects on blood metabolites indicating mild inflammatory condition in YC cows could be reflected in the upregulation of *NFKB1* and, consequently, activation of other cytokines. Due to *S. cerevisiae*-based supplementation, Adeyemi et al. (2019) observed an upregulation of genes involved in the immune response, such as *TNFA* and *TLR2* detected in whole-blood

RNA gene expression. In mice, yeast cell wall supplementation induced a release of proinflammatory cytokines, such as *TNFA* (Walachowski et al., 2016), similar to our findings.

Neutrophils are targeted by IL-10, a cytokine commonly associated with the resolution of the inflammation (Moore et al., 2001; Bazzoni et al., 2010). This cytokine expression has been reported in transition dairy cows in different conditions (Zhou et al., 2015; Batistel et al., 2017). Transition dairy cows fed with a prepartal high-energy diet upregulated the anti-inflammatory *IL10* in PMNL, and such upregulation was found at −14 and 7 DIM (Zhou et al., 2015). In our study, an upregulation of proinflammatory cytokines was observed in YC cows, coupled with the upregulation of *IL10*. The production of proinflammatory cytokines is inhibited by *IL-10*, which helps to limit the duration and pathology of inflammatory responses (Moore et al., 2001). Yeast improving the production of anti-inflammatory cytokines in transition dairy cows may indicate a pivotal effect of this feed supplement by activating the immune response aiding on the resolution of inflammation since cows experience an immune dysfunction during this period. The alterations of immunity in transition dairy cows, mainly after parturition, can be exacerbated by metabolic disorders (Sordillo, 2016).

In a recent study, Aung et al. (2020) isolated peripheral blood mononuclear cells from lactating dairy cows supplemented with yeast cell wall and observed that supplementation increased the leukocyte population and upregulated *IL8* and *IL12* in mononuclear cells, which might enhance the cellular immune function. The actual mechanism that yeast uses to stimulate an inflammatory response in ruminants still unclear, but the yeast cell wall fraction, medium used to grow the yeast, and yeast species

will likely play a fundamental role in this mechanism. In non-ruminants, β-glucan fragments present in the yeast cell wall have been suggested to stimulate the immune system (Alugongo et al., 2017). The Dectin-1 pathway is the most studied initial interaction of dietary β-glucans with the immune system, and the signaling cascade results in upregulation of *TNFA* and interleukins (e.g., *IL-2*, *IL-10*, *IL-12*, and *IL-4*), as well as the production of reactive oxygen species (ROS) (Willcocks et al., 2006). We did not evaluate the Dectin-1 pathway in our study; however, it would be interesting looking at this pathway in further studies.

The *SELL* gene encodes the L-selectin protein expressed in the cell surface of PMNL that mediates its initial attachment to inflammation sites in the endothelium (Paape et al., 2003). The L-selectin facilitates the interaction between leukocytes and endothelial cells to start the process called “rolling” (Raffler et al., 2005). After rolling, the PMNL binds to additional endothelial receptors such as intercellular adhesion molecule (ICAM), making tighter attachments between PMNL and endothelium. In the current study, we observed a trend for an upregulation of *SELL* in YC cows, meaning that the neutrophils might be stimulated due to yeast culture supplementation.

Based on gene expression results, it is plausible that yeast culture supplementation elicits an immune response in transition dairy cows. This is based on PMNL in YC cows being more stimulated than CON, suggesting that YC cows were exposed to an inflammatory stimulus. We can assume that yeast cell wall fragments such as β-glucans can bypass ruminal fermentation and promote PMNL gene expression alterations in the lower gastrointestinal tract. The exact mechanism that yeast may use to escape ruminal fermentation and interact with immune cells still inconclusive. We can also speculate that

β-glucans fragments can interact with membrane receptors in the ruminal epithelium, promoting the recruitment of immune cells and, in consequence, an immune response.

CONCLUSIONS

Supplementation of yeast culture derived from *S. cerevisiae* has been examined for many years to improve performance and health in dairy cows. In transition dairy cows, it is well known that the immune system is compromised, and this type of supplementation can be helpful during this challenging period. The present study showed that yeast supplementation might stimulate a mild inflammatory response in transition dairy cows, based on blood biomarker and PMNL mRNA expression. This can be associated with a natural response to yeast cell wall components, such as β-glucans, that can activate the immune system. In a companion paper, we reported the effects of yeast supplementation on performance, and we conclude that mild inflammatory conditions in YC did not cause a negative effect on DMI and milk yield. This result confirms that yeast culture could stimulate the immune system in transition dairy cows and promote a pronounced immune response against pathogen infection.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Phibro Animal Health (Teaneck, NJ, USA) for financial support of this research. The authors also thank South Dakota Agricultural Experiment Station (Brookings, SD, USA), and we gratefully acknowledge the help from all the staff at the South Dakota State University Dairy Research and Training Facility (Brookings-SD) for animal care and handling.

Table 3.1 - GenBank accession number, hybridization position, sequence and amplicon size of primers for Bos taurus used to analyze gene expression by qPCR.

Accession no.	Symbol	Primers	Primers (5'-3')	Amplicon size (bp)
EU276074.1	*IL10*	F.171	GAAGGACCAACTGCACAGCTT	98
		R.268	AAAACTGGATCATTTCCGACAAG	
NM_174093.1	*IL1B*	F.453	TCCACCTCCTCTCACAGGAAA	100
		R.552	TACCCAAGGCCACAGGAA	
NM_001040555.1	*IRAK1*	F.950	CCTCAGCGACTGGACATCCT	103
		R.1052	GGACGTTGGAACTCTTGACATCT	
NM_001076409.1	*NFKB1*	F.172	TCAACCGGAGATGCCACTAC	95
		R.266	ACACACGTAACGGAAACGAAATC	
NM_174182.1	*SELL*	F.588	CTCTGCTACACAGCTTCTTGTAAACC	104
		R.691	CCGTAGTACCCCAAATCACAGTT	
NM_174197.2	*TLR2*	F.1201	AAGAGTCACAATAGAAAG	102
		R.1302	GCTATTTATGACACATCCAA	
NM_174198.6	*TLR4*	F.102	GCTGTTTGACCAGTCTGATTGC	102
		R.203	GGGCTGAAGTAACAACAAGAGGAA	
EU276079.1	*TNFα*	F.438	TCTCAAGCCTCAAGTAACAAGCC	100
		R.537	CCATGAGGGCATTGGCATAC	

Table 3.2 - Genes selected for transcript profiling in bovine PMNL.

Gene Name	Symbol	Function
Interleukin 10	*IL10*	Inhibits the synthesis of a number of cytokines, including IFN-gamma, IL-2, IL-3, TNF and GM-CSF produced by activated macrophages and by helper T-cells.
Interleukin 1 B	*IL1B*	Produced by activated macrophages, IL-1 stimulates thymocyte proliferation by inducing IL-2 release, B-cell maturation and proliferation, and fibroblast growth factor activity. Potent pro-inflammatory cytokine.
Interleukin 1 Receptor Associated Kinase 1	*IRAK1*	This gene encodes the interleukin-1 receptor-associated kinase 1, one of two putative serine/threonine kinases that become associated with the interleukin-1 receptor (IL1R) upon stimulation. This gene is partially responsible for IL1-induced upregulation of the transcription factor NF-kappa B. Involved in Toll-like receptor (TLR) and IL-1R signaling pathways. Is rapidly recruited by MYD88 to the receptor-signaling complex upon TLR activation.
Nuclear Factor Of Kappa Light Polypeptide Gene Enhancer In B-Cells 1	*NF-KB1*	NF-kappa-B is a pleiotropic transcription factor present in almost all cell types and is the endpoint of a series of signal transduction events that are initiated by a vast array of stimuli related to many biological processes such as inflammation, immunity, differentiation, cell growth, tumorigenesis, and apoptosis.
Selectin-L	*SELL*	Mediates the adherence of lymphocytes to endothelial cells of high endothelial venules in peripheral lymph nodes. Promotes initial tethering and rolling of leukocytes in endothelia.
Toll-like receptor 2	*TLR2*	The protein encoded by this gene is a member of the Toll-like receptor (TLR) family which plays a fundamental role in pathogen recognition and activation of innate immunity.
Toll-like receptor 4	*TLR4*	The protein encoded by this gene is a member of the Toll-like receptor (TLR) family which plays a fundamental role in pathogen recognition and activation of innate immunity. This receptor has been implicated in signal transduction events induced by LPS found in most gram-negative bacteria. Mutations in this gene have been associated with differences in LPS responsiveness
Tumor Necrosis Factor Alpha	*TNFα*	Secreted by macrophages and can induce cell death of certain tumor cell lines. It is potent pyrogen causing fever by direct action or by stimulation of interleukin-1 secretion and is implicated in the induction of cachexia, under certain conditions it can stimulate cell proliferation and induce cell differentiation.

Table 3.3 - Effects of supplementing cows with a control diet (CON) or control diet plus a yeast culture supplement (YC; Cellerate Yeast Solutions®, Culture Classic HD; Phibro Animal Health, Teaneck, NJ) in blood biomarkers related to liver function and inflammation in peripartal dairy cows until 50DIM.

	Treatment			*P-value*		
Parameter	CON	YC	SEM[1]	Diet	Time	D × T[3]
Liver function						
Total bilirubin, µmol/L[2]	1.88	1.98	0.28	0.81	< 0.01	0.70
GOT, U/L	93.3	94.6	3.99	0.81	< 0.01	0.72
GGT, U/L[2]	18.3	20.3	0.04	0.03	< 0.01	0.07
Cholesterol, mmol/L	3.72	3.53	0.11	0.25	< 0.01	0.96
Paraoxonase, U/mL	102.5	95.0	3.4	0.12	< 0.01	0.21
Inflammation						
Albumin, g/L	35.6	34.4	0.40	0.06	< 0.01	0.54
Ceruloplasmin, µmol/L	2.44	2.48	0.06	0.69	0.03	0.15
Haptoglobin, g/L[2]	0.28	0.36	0.15	0.12	0.09	0.59
IL-1β, pg/mL[2]	32.3	31.2	0.21	0.86	< 0.01	0.17

[1]Largest standard error of the mean is shown.
[2]Data were log-transformed before statistics. The standard errors of the means associated with log-transformed data are in log scale.
[3]Interaction of treatment and days in milk.

Table 3.4 - Effects of supplementing cows with a control diet (CON) or control diet plus a yeast culture supplement (YC; Cellerate Yeast Solutions®, Culture Classic HD; Phibro Animal Health, Teaneck, NJ) in PMNL mRNA gene expression in peripartal dairy cows until 50DIM.

	Treatment			*P-value*		
Gene[1]	CON	YC	SEM[2]	Diet	Time	D × T[3]
Inflammation						
TNFA	1.41	2.34	1.22	0.11	< 0.01	0.16
NFKB1	0.99	1.17	0.23	0.04	0.33	0.42
IL1B	3.24	2.32	1.69	0.32	0.28	0.62
IL10	1.15	2.19	1.13	0.07	< 0.01	0.17
Cell receptors and signaling						
TLR2	1.14	1.40	0.48	0.14	0.05	0.44
TLR4	0.94	1.11	0.15	0.33	0.82	0.45
SELL	0.93	1.22	0.29	0.11	0.80	0.53
IRAK1	1.07	1.20	0.25	0.36	0.24	0.73

[1]Fold change data were log-transformed before statistics
[2]Largest standard error of the mean is shown.
[3]Interaction of treatment and days in milk.

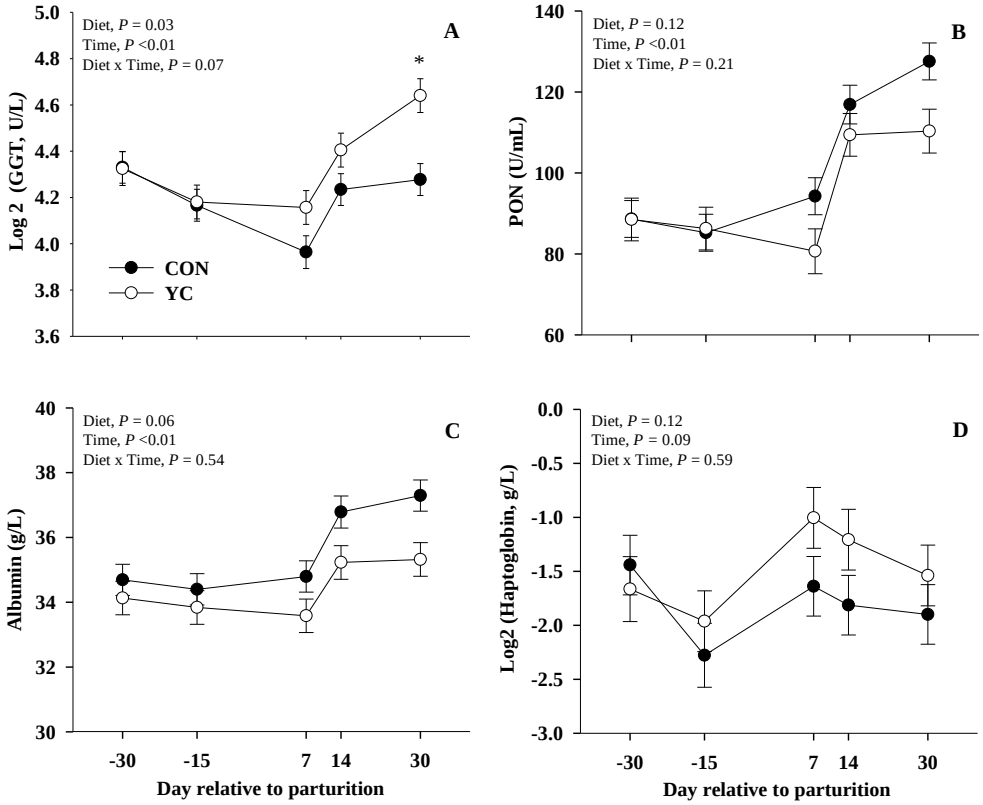

Figure 3.1 - Effects of control diet (CON) or control diet plus a yeast culture supplement (YC; Cellerate Yeast Solutions®, Culture Classic HD; Phibro Animal Health, Teaneck, NJ) on blood GGT (A), Paraoxonase (B), Albumin (C) and Haptoglobin (D) in peripartal dairy cows until 50DIM. Values are means and the standard errors are represented by vertical bars. The *P*-values for main effect of diet (D) and time (T) and D × T are shown. Significant differences between D x T ($P < 0.10$) are denoted by an asterisk.

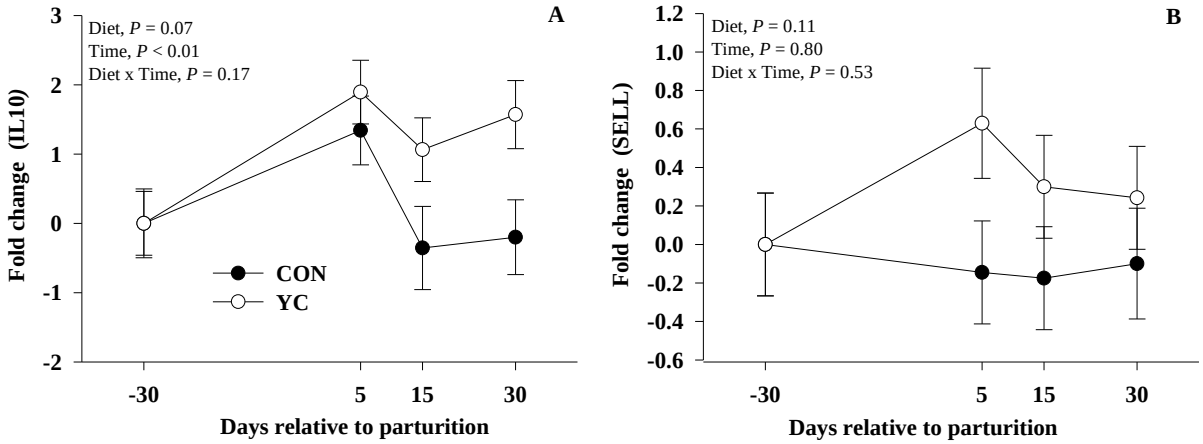

Figure 3.2 - Expression of genes associated to inflammation (A) and cell receptor and signaling (B) in PMNL in dairy cows fed with control diet (CON) or control diet plus a yeast culture supplement (YC; Cellerate Yeast Solutions®, Culture Classic HD; Phibro Animal Health, Teaneck, NJ) in peripartal dairy cows until 50DIM. Data are from -30d (prepartum), 5d, 15d and 30d (postpartum). The *P*-values for main effect of diet (D) and time (T) and D × T are shown.

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