2019

EFFECTS OF YEAST-DERIVED MICROBIAL PROTEIN ON TRANSITION DAIRY COW HEALTH AND PERFORMANCE

Gustavo Mazon Correa Alves
University of Kentucky, gustavo.mazon@uky.edu
Author ORCID Identifier:
https://orcid.org/0000-0002-5877-1997
Digital Object Identifier: https://doi.org/10.13023/etd.2019.241

STUDENT AGREEMENT:

I represent that my thesis or dissertation and abstract are my original work. Proper attribution has been given to all outside sources. I understand that I am solely responsible for obtaining any needed copyright permissions. I have obtained needed written permission statement(s) from the owner(s) of each third-party copyrighted matter to be included in my work, allowing electronic distribution (if such use is not permitted by the fair use doctrine) which will be submitted to UKnowledge as Additional File.

I hereby grant to The University of Kentucky and its agents the irrevocable, non-exclusive, and royalty-free license to archive and make accessible my work in whole or in part in all forms of media, now or hereafter known. I agree that the document mentioned above may be made available immediately for worldwide access unless an embargo applies.

I retain all other ownership rights to the copyright of my work. I also retain the right to use in future works (such as articles or books) all or part of my work. I understand that I am free to register the copyright to my work.

REVIEW, APPROVAL AND ACCEPTANCE

The document mentioned above has been reviewed and accepted by the student's advisor, on behalf of the advisory committee, and by the Director of Graduate Studies (DGS), on behalf of the program; we verify that this is the final, approved version of the student's thesis including all changes required by the advisory committee. The undersigned agree to abide by the statements above.

Gustavo Mazon Correa Alves, Student

Dr. Joao H. C. Costa, Major Professor

Dr. David L. Harmon, Director of Graduate Studies

EFFECTS OF YEAST-DERIVED MICROBIAL PROTEIN ON TRANSITION DAIRY COW HEALTH AND PERFORMANCE

THESIS

A thesis submitted in partial fulfillment of the

requirements for the degree of Master of Science in the

College of Agriculture, Food and Environment

at the University of Kentucky

By

Gustavo Mazon Correa Alves

Director: Dr. Joao H. C. Costa, Assistant Professor of Animal and Food Sciences

Lexington, Kentucky

2019

https://orcid.org/0000-0002-5877-1997

ABSTRACT

EFFECTS OF YEAST-DERIVED MICROBIAL PROTEIN ON TRANSITION DAIRY COW HEALTH AND PERFORMANCE

The transition period for dairy cows is defined as the three weeks pre and postpartum. During the transition period, dairy cows experience a myriad of metabolic, managerial, and nutritional requirement changes. These changes lead to stress and increased susceptibility to diseases which can negatively affect lactational performance in the short and long term. However, dietary amino acid availability can have a dramatic impact on the health and performance of dairy cows around parturition. Thus, the objective of the thesis was to evaluate the effects of supplementing yeast-derived microbial protein, as an alternative protein source for dairy cows during the transition period. This was accomplished by using visual observations and precision dairy monitoring technologies to record disease, feeding behavior, and performance of dairy cows from 21 days prepartum to 150 days postpartum. Yeast-derived microbial protein was found to decrease dry matter intake but not negatively affect milk production or health of the animals. Yeast-derived microbial protein may be used as an alternative protein source for transition dairy cows as it did not negatively affect milk production or health of the animals.

Keywords: protein supplement, bypass protein, DEMP

Gustavo Mazon Correa Alves

June 6th, 2019

EFFECTS OF YEAST-DERIVED MICROBIAL PROTEIN ON TRANSITION DAIRY COW HEALTH AND PERFORMANCE

By

Gustavo Mazon Correa Alves

Dr. Joao H. C, Costa

Director of Thesis

Dr. David L. Harmon

Director of Graduate Studies

06/06/2019

Date

ACKNOWLEDGEMENTS

If someone went back in time and told me that I was going to obtain a graduate degree from an American university, I'd probably laugh thinking that was a joke. Even though I always wanted to pursue a graduate degree, it never occurred to me that I could do that in such an accredited institution such as the University of Kentucky. Therefore, the first person I would like to thank for this amazing opportunity is Dr. Jeffrey Bewley. I met Dr. Bewley during a workshop in Brazil in 2012, I was just a sophomore in college and yet he saw a lot of potential in me. On my senior year, I had the opportunity to come to the University of Kentucky and work as an intern for the Southeast Milk Quality Initiative. I worked hard and learned a lot about the American dairy industry throughout that internship. One year after my internship had ended, Dr. Bewley contacted me and offered a research assistantship. I am tremendously grateful to Dr. Bewley for seeing my potential and investing in me.

Thank you Dr. Joao Costa for "adopting" me and bringing me into your research program when everything seemed lost. I have no words to describe how thankful I am for all your guidance, support, and friendship. I understand that we were in a race against time when you became my advisor and I thank you for helping me finish and succeed. I probably won't have much time to celebrate with a victory lap, since we'll soon start another journey towards my PhD. I am looking forward to working with you and becoming part of the select group of young professionals that is revolutionizing the dairy research.

I would like to thank Alltech for sponsoring my project and its employees for all the help throughout the past three years. Thank you Amanda Gehman that, despite all the

delays in the project and last minute requests, seemed to still trust and believe me from beginning to end. Thank you Amanda Lisembee and Megan Scholer for analyzing my feed samples and for not yelling at me when I bugged you with silly questions in the lab.

To all my friends and fellow graduate students. I would like to specially thank Liz Eckelkamp and Amanda Lee for keeping me sane, entertained, and for buying me drinks whenever I needed (or not). Thank you Matthew Borchers and Jenna Guinn for being the best officemates ever. Thank you Melissa Cantor and Shannon Dierking for the daily coffee walks. Thank you Anna Hawkins for all the hugs and encouragement words in the past few months. Thank you Derek Nolan for being around and providing support and encouragement, you're awesome. Thank you David Blanchard for buying me half-priced drinks at the bars and for never complaining when I got home covered in manure. Thank you Anna Meurer for buying me great food when I stayed late in the office. Thank you Rick, Maggie, Fernanda, and Bruno for being great friends and cooking great Brazilian food over the weekends.

To everyone that, somehow, helped me during my research project. Thank you Brittany for not cursing and yelling at me when I certainly deserved. Thank you to Joey, Matt, and Amelia for letting me use your cows for scientific purposes. Thank you Mackenzi Cooper, Megan Cave, and Nichole Thomas for helping me during the fresh cow checks (I hope you never forget the fresh scent of metritis in the morning). Thank you Clémence, Justine, and Mathilde for spending countless hours in the basement helping me with feed samples. Thank you Magnus Campler for helping me write a SAS code that is probably twice as long as this thesis. THANK YOU STARBUCKS!

To my mom and dad, I know that you all don't love the fact that I'm far from home but I thank you for supporting me, texting me on a daily basis to see if I ate, and helping me when my car breaks. I promise that one day I'll get a "real job" and you all will be able to brag about that your son became a professor in the United States. I love you.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

FREQUENTLY USED ABBREVIATIONS

AA = Amino acid

BHBA = ß-hydroxybutyrate

CP = Crude protein

DMI = Dry matter intake

MCP = Microbial protein

NEB = Negative energy balance

RUP = Rumen undegradable protein

YMP = Yeast-derived microbial protein

CHAPTER ONE

REVIEW OF LITERATURE

INTRODUCTION

The transition period is defined as the period from 3 weeks pre to 3 weeks postpartum (LeBlanc, 2010). During the transition period, most periparturient dairy cows have been found to be in negative energy balance (**NEB**) (Ospina et al., 2010). Negative energy balance is highly prevalent among transition dairy cows, and is caused by a combination of factors such as fetal growth, lactation, and decreased dry matter intake during the periparturient period (**DMI**) (Gerloff, 2000, Ingvartsen and Andersen, 2000). The causes for the drop in DMI are complex and includes a plethora of factors, such as decreased rumen volume (Reynolds et al., 2004), hormonal (Pushpakumara et al., 2003a) and managerial changes (Proudfoot et al., 2009, Garnsworthy and Topps, 2010).

The drastic changes in nutrient demands along with decreased DMI have been associated with the incidence of transition cow diseases, such as hypocalcemia (Reinhardt et al., 2011), hyperketonemia (Sheehy et al., 2017), and metritis (Huzzey et al., 2007). When surveying more than 1,000 dairy cows in 20 Canadian herds, LeBlanc et al. (2005) reported that the incidence of clinical and subclinical metabolic disease ranged from 30 to 50% within the first week postpartum. In addition, the incidence of transition diseases can have negative effects on long term lactation performance and reproduction (Wallace et al., 1996, Mulligan and Doherty, 2008).

Because of the relationship between lower DMI and transition cow diseases, a common strategy adopted is to adjust the nutrient density of prepartum diets to fulfill the animal's nutrient requirements and minimize the risk for transition diseases. For example,

increasing the energy density prepartum by increasing the amount of non-fiber carbohydrates allows the ruminal microbiota to adapt to the high energy diets fed postpartum (Dirksen et al., 1985). Furthermore, McNamara et al. (2003) reported that increasing the energy density in the last four weeks of the dry period did not result in loss of body condition score prepartum and cows increased milk production.

However, besides energy, protein seems to limit performance during lactation (Clark and Davis, 1980). The NRC (2001) suggests that the dietary crude protein (**CP**) concentrations in prepartum diets should be 12%. Studies feeding extra prepartum CP amounts have reported inconsistent results on postpartum DMI, milk yield, and transition cow diseases incidence (Santos et al., 2001, Pushpakumara et al., 2003b). The results of the previously cited studies will be discussed further in this review. However, as previously reviewed by Santos et al. (1998), the responsiveness to increases in protein amount might be related to the rumen undegradable protein (**RUP**) source as well as the amino acid (**AA**) profile provided by the feedstuffs.

The AA profile available for absorption in the small intestine should be similar to that of ruminal microbial protein (**MCP**) which is close to the AA profile required for milk and milk protein synthesis (NRC, 2001). However, responses to AA supply during the transition period depend on the CP concentration and intestinal digestibility of the AA source (Socha et al., 2005). Recently, a yeast-derived microbial protein (**YMP;** DEMP®; Alltech Inc., Nicholasville, KY) product has become available in the market. Yeast-derived microbial protein is a byproduct of yeast fermentation with AA profiles similar to MCP and that, presumably, flows with the liquid phase of the rumen fluid, which permits more AA to be absorbed in the small intestine (Sabbia et al., 2012).

Thus, an opportunity exists for increasing the amount of metabolizable protein to the transition dairy cow by improving the AA profile in the diet utilizing YMP. Previous research reported increases in ECM, FCM, and protein percentages without changes in metabolic status when transition cows were fed rumen-protected methionine (Zhou et al., 2016). The decreased DMI typically observed in early lactation dairy cows might cause insufficient propionate production to sustain the animal's increased glucose needs (Drackley et al., 2001). Therefore, AA from the diet can contribute for gluconeogenesis in the liver (Drackley et al., 2001, Reynolds et al., 2003). Improving energy status in transition dairy cows is key in decreasing the risk for transition cow disease and maximizing lactation and reproductive performance.

Therefore, the aim of this review is to highlight some of the key diseases affecting dairy cows during the transition period. We will also discuss possible nutritional management strategies that can be adopted to prevent or mitigate the negative effects caused by transition cow diseases. Because of their high prevalence in dairy farms and elevated associated costs we decided to highlight hypocalcemia, hyperketonemia, retained placenta and metritis. Furthermore, we will briefly explore the importance of protein and its fractions when feeding transition dairy cows and focus on the current research available on the inclusion of yeast-derived microbial protein in dairy cow diets. Finally, I will conclude with highlights of the findings exposed in this literature review and present the thesis objectives.

THE TRANSITION PERIOD

The transition period is defined as the time frame from 3 weeks pre to 3 weeks postpartum (LeBlanc, 2010) and is characterized as challenging period in a dairy cow's productive life. During the transition period, dairy cows experience a myriad of physical (Reynolds et al., 2004), metabolic (Pushpakumara et al., 2003a, Huzzey et al., 2009), immune (Goff, 2006, LeBlanc, 2008), social (Sepúlveda-Varas et al., 2013), and dietary (Mulligan et al., 2006, Lean et al., 2013) changes that affect the animals negatively.

During the transition period, most cows have been found to be in NEB (Ospina et al., 2010) which results in a very high incidence of infectious or metabolic diseases during the transition period (LeBlanc, 2010). Metabolic diseases such as hypocalcemia (Reinhardt et al., 2011), hyperketonemia (Sheehy et al., 2017), and metritis (Huzzey et al., 2007) are highly prevalent during the transition period and can cause productive (LeBlanc et al., 2002, Duffield et al., 2009) and economic (McArt et al., 2015, Liang et al., 2017) losses.

Because of the association between lower NEB and transition cow diseases, a common strategy adopted is to adjust the nutrient density of prepartum diets to fulfill the animal's nutrient requirements and minimize the risk for transition diseases (Ingvartsen, 2006, Lean et al., 2013). Thus, in the first section of this review I will first describe the NEB experienced by periparturient dairy cows, then discuss an array of factors that might cause NEB, and possible management strategies to mitigate its negative effects on production and health of dairy cows.

Negative energy balance

During the last three weeks of gestation, the nutrient requirements for fetal development reach their peak (Bell, 1995). At that same time, cows might decrease their average dry matter intake by 10 to 30% (Drackley et al., 2005, Huzzey et al., 2007). Although it might seem alarming, the decrease DMI is common within mammals (Friggens, 2003). Research has reported that the drastic change in hormonal concentrations around calving might be a factor contributing to the decrease in DMI (Grummer, 1995). In addition, rumen volumes are affected by fetal growth, which might limit DMI due to physical fill. For example, Reynolds et al. (2004) reported that the weight of the reticulorumen in dairy cows increases after calving but that the increase is minimal before 21 days postpartum. In addition, the same study reported that DMI followed the same patterns as reticulorumen weight. In summary, the regulation of DMI in transition dairy cows is tremendously complex and might be a result of multiple factors (Ingvartsen and Andersen, 2000, Grummer et al., 2004).

However, the increase in energy required for fetal growth is minimal when compared to the amount of energy required to commence and maintain lactation. For example, the net energy of lactation (NE_L) requirement for a 570 kg primiparous cow two days before calving is 58.5 MJ/d. However, the energy required for the same animal to produce 20kg of 4% fat milk two days after calving is 105.0 MJ/d (NRC, 2001). As previously mentioned, DMI post-calving is still depressed. Consequently, the total intake of energy via the diet is less than the energy required (Bell, 1995). Therefore, energy deficit between energy intake and requirements is known as negative energy balance.

Furthermore, with the poor energy status, the transition dairy cow might become immunosuppressed (Kehrli et al., 1989b, a, Goff, 2006). Other non-physiological factor might also contribute for the increase in stress levels during the transition period such as drastic dietary and group changes, housing conditions, and animal handling practices (Grant and Albright, 2001). Therefore, the combination of energy and immune status with environmental and management factors likely contributes to the high incidence of metabolic and infectious diseases during the transition period (Drackley et al., 2005). In fact, Ingvartsen et al. (2003) summarized data from 93,000 primiparous and 58,000 multiparous Danish cows and reported that the highest incidence of total disease occurred within the first 10 days of lactation. Furthermore, in a survey including more than 1,000 dairy cows divided in 20 Canadian herds, LeBlanc et al. (2005) reported that the incidence of clinical and subclinical metabolic disease ranged from 30 to 50% within the first lactation week.

Overall, NEB has been associated with many transition cow diseases such as hypocalcemia (Reinhardt et al., 2011), hyperketonemia (Sheehy et al., 2017), metritis (Huzzey et al., 2007), and mastitis (Rezamand et al., 2007) for example. Therefore, the following sections will review the association between NEB and a metabolic disease and how to use nutritional management strategies to prevent those diseases.

Hypocalcemia

In the adult dairy cow, blood Ca levels are generally between 8.5 and 10 mg/dL (Goff, 2008). At the beginning of lactation, cows experience a rapid decrease in blood Ca concentrations (Kimura et al., 2006). This decrease when beyond cows' capability of replacement can cause the metabolic disease known as milk fever or hypocalcemia (Goff,

2008). Hypocalcemia can be classified as clinical or subclinical. Clinical hypocalcemia is defined as blood Ca < 5.6 mg/dL followed by clinical signs like excitability, weakness, and flaccid paralysis (DeGaris and Lean, 2008, Oetzel, 2011). Subclinical hypocalcemia is defined as blood Ca between 5.6 and 8.0 mg/dL (DeGaris and Lean, 2008). However, studies have shown that the 8.0mg/dL threshold might be underestimated. Seifi et al. (2011) described cows with serum Ca < 8.8 mg/dL in the first week after parturition were more likely to be culled before 60 DIM. Martinez et al. (2012) found that cows with Ca < 8.6 mg/dL had 3.2 times higher risk of developing metritis than normocalcemic cows. In addition, Chapinal et al. (2011) reported that cows with Ca ≤ 8.8mg/dL post-calving were 3.1 times more likely to develop displaced abomasum.

The overall incidence of clinical hypocalcemia reported by the National Animal Health Monitoring System (NAHMS) 2002 dairy study was 5% (Reinhardt et al., 2011). When defining subclinical hypocalcemia as serum Ca < 8.0 mg/dL, the incidence of subclinical hypocalcemia was 25%, 41%, and 49% for cows of first, second, and third lactation, respectively (Reinhardt et al., 2011). Furthermore, subclinical hypocalcemia suppresses DMI (Martinez et al., 2014) and might predispose the cow to several metabolic disorders. As previously stated, decreased concentration of blood Ca beyond hypocalcemia, increased the odds of the animals developing other transition period disease, such as metritis (Martinez et al., 2012) and displaced abomasum (Chapinal et al., 2011), and also increases the risk of the cow being culled early (Seifi et al., 2011). Cows affected with clinical milk fever had their milk production reduced by 14% in the subsequent lactation (Oetzel, 2011). Furthermore, hypocalcemic cows have increased chances of developing mastitis, which is speculated to be cause by the increased immune suppression

(Kimura et al., 2006) and decreased smooth muscle contraction (Goff, 2008). Because of the increased culling and death risk, hypocalcemia is a costly disease. Liang et al. (2017) used a stochastic model to estimate hypocalcemia costs in multiparous cows, and calculated that the total disease cost $246.23 per clinical case.

The most common methods to control hypocalcemia are oral supplementation with a source of easily absorbed Ca after calving; feeding acidifying rations by anionic salt supplementation during the last weeks of pregnancy; feeding low Ca rations during the last weeks of pregnancy (Thilsing-Hansen et al., 2002). Providing free Ca ions increases the amount of Ca ions absorbed into the bloodstream (Thilsing-Hansen et al., 2002). Cows receiving oral Ca boluses after calving had increased blood plasma concentration (Sampson et al., 2009) and increased first test milk yield of 2.9kg (Oetzel and Miller, 2012). Feeding anionic salts alters the dietary cation-anion difference (**DCAD**) of the ration. Diets with negative DCAD bring the cow to physiological stage of compensated systemic acidosis (Thilsing-Hansen et al., 2002). To evaluate if the cow is responding to the negative DCAD ration, their urine pH should be between 5.5 and 6.2 (Horst et al., 1997). Thilsing-Hansen et al. (2002) evaluated the incidences of hypocalcemia in 11 experiments applying feed with different DCAD. When DCAD was positive, incidences up to 80% were reported, however hypocalcemia incidence was lower than 20% when the DCAD was negative. When cows are fed Ca deficient diets, blood Ca levels drop, stimulating parathyroid hormone secretion, which will stimulate calcium resorption (Goff, 2008). Thilsing-Hansen et al. (2002) evaluated the results of 13 studies focused on prepartum Ca intake and hypocalcemia incidence. Mean hypocalcemia incidence on cows

fed less than 20g of Ca per day was 1.7%, whereas cows fed more than 20g of Ca per day had a mean hypocalcemia incidence of 32.4%.

Overall, hypocalcemia has been associated to NEB, as hypocalcemic animals have been shown to have reduced DMI higher postpartum NEFA concentrations. Furthermore, hypocalcemia seems to be associated with an increased risk of other transition dieseases disease (Chapinal et al., 2011, Martinez et al., 2012), lower production (Oetzel, 2011), and elevated costs (Liang et al., 2017).

Hyperketonemia

To fulfill the increased energy requirements in early lactation, the animal will mobilize fat reserves to overcome the NEB experienced around parturition (Drackley et al., 2005, Ospina et al., 2013). When broken down, fat releases nonesterified fatty acids (**NEFA**) that should be completely oxidized in the liver (Drackley et al., 2005). However, if NEFA concentrations exceed the liver's oxidative capacity, NEFA will be partially broken down to form ketone bodies (Drackley et al., 2005, Grummer, 2008). Ketones bodies include acetoacetate, acetone, and ß-hydroxybutyrate (**BHBA**) and serve as energy source for other tissues (Herdt, 2000). The increase in concentration of ketone bodies in blood as ketosis or hyperketonemia. Hyperketonemia can be classified as clinical or subclinical. Subclinical hyperketonemia can be defined as blood BHBA concentration of 1.2 to 2.9 mmol/L. Animals with clinical hyperketonemia have BHBA $\geq$ 3.0 mmol/L and nervous clinical that include abnormal licking, chewing on pipes or concrete, abnormal gait, and aggression (Oetzel, 2004, Gordon et al., 2013).

The epidemiology of hyperketonemia has been evaluated in 1,717 early lactation dairy cows from 4 commercial herds (McArt et al., 2012b). Forty-three percent of the cows (n = 741) had at least one BHBA test of 1.2 to 2.9 mmol/L in the first two weeks of lactation. The subclinical hyperketonemia group was composed of 28.7% primiparous and 71.3% multiparous cows. Peak incidence and peak prevalence of hyperketonemia occurred at 5 days in milk, and was reported as 22.3%, and 28.9%, respectively. Suthar et al. (2013) conducted a study on 528 European farms, and reported overall hyperketonemia prevalence to be 21.8%, ranging from 11.2 to 36.6%.

Studies have reported the association between BHBA concentration, cow health and production. McArt et al. (2012b) reported BHBA levels above 1.2mmol/L on the first week of lactation were associated with increased risk of developing displaced abomasum, and being removed from the herd. In addition, increased BHBA concentration were associated with decreased milk production, and conception at first service. Duffield et al. (2009) described increased risk ratio of developing displaced abomasum, and metritis if BHBA concentration was above 1.2 mmol/L during the first week of lactation. Furthermore, the same study reported that increased BHBA had a negative effect on milk production, and milk protein percentage. In addition, no association between above normal BHBA levels and mastitis was found (Duffield et al., 2009). Because of the association with other diseases and negative effects on production and reproduction, an increased cost is associated with hyperketonemia. The total cost of the disease was reported to be $289.00 per case (McArt et al., 2015).

A commonly used treatment for hyperketonemia is oral administration of propylene glycol. In a study by McArt et al. (2011), cows with hyperketonemia received daily 300mL

propylene glycol drenches until BHBA concentrations were below 1.2 mmol/L. Treated cows were 1.5 times more likely to resolve hyperketonemia, and milk production increased by 0.69 kg per day when compared to untreated cows. Untreated cows were 1.6 times more likely to develop displaced abomasum, and 2.1 times more likely die or be sold in the first 30 days of lactation. In addition, cows receiving the propylene glycol treatment were 1.3 times more likely to conceive at first service (McArt et al., 2012a).

Hyperketonemia can be prevented by adapting nutritional management practices during the far-off dry and transition periods (Duffield, 2000). Managing body condition scores prepartum is important for hyperketonemia prevention. Cows that lose body condition score during the prepartum period have higher BHBA concentrations post-calving than cows maintaining body condition scores (Sheehy et al., 2017). Cows calving with body condition scores over 3.5 had decreased DMI and longer NEB after calving when compared to thinner cows (Garnsworthy and Topps, 2010). Therefore, it is important to manage BCS in dairy cows before dry off.

Overall, hyperketonemia has been directly associated to NEB, as elevated BHBA levels have been associated with an increased risk of disease (Duffield et al., 2009, McArt et al., 2011), lower production (Duffield et al., 2009), and increased costs (McArt et al., 2015). However, management practices such as prepartum body condition score management seem to be effective on the prevention of hyperketonemia (Garnsworthy and Topps, 2010, Sheehy et al., 2017).

Retained Placenta

Retained placenta is characterized as the failure of the cow to expel the placenta within 24 hours postpartum (Kelton et al., 1998). In a summary of 50 studies, Kelton et al. (1998) reported retained placentas incidences ranging from 1.3 to 39.2%, while the median incidence was reported to be 8.6%. Risk factors such as dystocia, twins, hypocalcemia, and season have been reported to be associated with retained placenta (Gröhn and Rajala-Schultz, 2000). However, the immunosuppression as a result of the negative energy balance seems to be the main cause of retained placenta, as the immune system fails to degrade the placentomes at the end of pregnancy, preventing the placenta to be expelled (LeBlanc, 2008).

LeBlanc (2008) reported that approximately 25 to 50% cows with retained placenta develop metritis. In addition, it is estimated that cows who failed to expel their placenta have a 1.4 kg/d loss in milk production during the first two weeks of lactation and up to 0.5 kg/d loss from 42 days in milk until dry off (Rajala and Gröhn, 1998). Furthermore, cows affected by retained placenta had 15% lower pregnancy rates when compared to non-affected cows (Fourichon et al., 2000). When considering the losses in milk production, treatment costs, and decreased reproductive performance, Guard (1994) estimated the cost of retained placenta to be $285/case, however, if adjusted for inflation, the cost per case might be even higher.

Common treatments for retained placenta include the administration of oxytocin or prostaglandin postpartum. However, researchers have reported inconsistent results when adopting hormonal treatments for retained placenta (LeBlanc, 2008). Although research has shown that the manual removal of the placenta is detrimental to reproductive

performance (Bolinder et al., 1988). Additionally, antibiotic treatments may be used when the affected animal has temperatures above 39.5^{o}C. However, blanket treating all animals with antibiotics does not seem to have a positive impacts on future reproductive performance (Drillich et al., 2006).

Since retained placenta and immune function are associated with the energy status of the animal, the key for prevention is to encourage feed intake around parturition (Cook and Nordlund, 2004). In addition, the inclusion of selenium, vitamin E, and chromium in the diets has shown to improve immune function and, therefore, reduce retained placenta occurrence (Miller et al., 1993, Villalobos-F et al., 1997, Allison and Laven, 2000).

In summary the association between retained placenta and NEB lies in the immunosuppression experienced by most cows during the transition period (LeBlanc, 2008). Furthermore, retained placenta has been associated with losses in productive performance (Rajala and Gröhn, 1998), lower pregnancy rates (Fourichon et al., 2000), and elevated costs (Guard, 1994). Additionally, management practices to encourage DMI and feeding appropriate amounts of minerals and vitamins from available sources seems to reduce retained placenta occurrence (Allison and Laven, 2000, Cook and Nordlund, 2004). Furthermore, retained placenta has been associated with metritis, which will be discussed in the following section.

Metritis

Metritis is a disease caused by the infection of the uterus with bacteria within 21 days after calving. The clinical signs of the disease are fetid watery red-brown uterine discharge, including signs of systemic infection such as dullness, decreased milk yield, and

body temperatures above 39.5 º C (Sheldon et al., 2006). In an observational study evaluating 450 calvings in a commercial dairy farm in Florida, the overall incidence of metritis was 21% (Benzaquen et al., 2007). Early detection of metritis can be made by periodic examination of vaginal mucus using a 3-point scoring system described by (Williams et al., 2005). In addition, it is important to pay attention to the cow's feeding behavior, as Neave et al. (2018) reported that cows that developed metritis had decreased DMI and visited the feedbunk less often on the 3 days prior to clinical diagnosis.

Bacteria is present in the uterus of more than 80% of dairy cows in the first two weeks postpartum (Sheldon et al., 2008). The immune system of the dairy cow should progressively eliminate the bacteria in the uterus. However, as cows are immunosuppressed during the transition period, the bacterial growth in the uterus may cause an infection (Williams et al., 2005). Similar to retained placenta, metritis also has negative effects in production and reproduction. For example, LeBlanc et al. (2002) reported that conception rates were 8% lower for cows with metritis. Similarly, the same study reported that cows with metritis experienced longer intervals from calving to conception and more cows were culled because of reproductive failure. In addition, Esslemont and Kossaibati (2002) observed an average production loss of 300 L/lactation when cows had metritis. When considering indirect (increased calving intervals, increased culling rates, extra inseminations) and direct (treatment, reduced milk yield, and infertility) from 21 dairy farms in the UK, Esslemont and Kossaibati (2002) reported the total cost of metritis to be €192/case.

Since it is a bacterial infection, few disagree that cows with metritis should be treated using systemic antibiotics (LeBlanc, 2008). Laboratory and field studies have

reported that the use of ceftiofur is effective in treating cows with metritis (Drillich et al., 2001, Sheldon et al., 2004, Drillich et al., 2006). Besides the inclusion of adequate amounts of minerals and vitamins to the diet discussed earlier in this review, Curtis et al. (1985) reported that increasing the protein content in the prepartum was beneficial for uterine health.

Similarly to retained placenta, metritis seems to be associated with the decrease in immune function caused by NEB (Overton and Waldron, 2004). Additionally, metritis has been associated with productive and economic losses (Esslemont and Kossaibati, 2002, LeBlanc et al., 2002). Furthermore, metritis occurrence seems to be associated with changes in DMI and feeding behavior (Huzzey et al., 2007, Neave et al., 2018). Furthermore, increasing protein content in prepartum diets might be beneficial for the reduction in metritis incidence (Curtis et al., 1985).

In conclusion the NEB experienced by dairy cows during the transition period is associated with a high incidence of metabolic diseases. Furthermore, the incidence of transition cow diseases seems to be associated with decreased production, poor reproduction, and increased production costs. However, dietary management strategies during the transition period seem to be effective in the prevention of transition cow diseases. Thus, the following section of this review will focus on nutritional strategies that focus on increasing the nutrient density of dairy cow diets during the transition period.

FEEDING PROTEIN TO THE DAIRY COW

The effects of dietary formulation during the transition period has been vastly associated with the energy balance and incidence of transition cow diseases (Overton and

Waldron, 2004, Ingvartsen, 2006). The current review will focus mainly on the importance of dietary protein for dairy cows during the transition period. However, other dietary components such as energy (Rabelo et al., 2005), vitamins (Spears and Weiss, 2008), and minerals (Overton and Yasui, 2014) are fundamental for the successful formulation of a transition cow diet that will guarantee adequate feed intake and minimize the risks for transition cow diseases. Besides energy, protein is one of the nutritional components limiting performance during lactation (Clark and Davis, 1980, Bach et al., 2005). In a recent review, Lean et al. (2013) reported that the efficacy of supplementing protein in transition cow diets might depend on, the protein source, exposure time, and amino acid balance. Thus, the aim of this section is to briefly describe how proteins are utilized in the rumen and briefly explore how protein source can affect amino acid availability for the transition dairy cow.

Protein in the rumen

The amount of crude protein (**CP**) in diets is defined based on its nitrogen content multiplied by 6.25. This correction factor assumes that the nitrogen content of proteins to be on average 16% (NRC, 2001). This calculated CP value tends not to be accurate once it includes protein and nonprotein nitrogen. In addition, it is known that different protein sources differ in nitrogen content and amino acid (**AA**) composition (Jones, 1931). The degradability of protein is a major factor influencing the AA supply for the dairy cow. Therefore, in dairy cattle nutrition, we typically divide CP in three major fractions: A, B, and C (Sniffen et al., 1992).

Fraction A represents the percent of CP that is completely degradable and readily available for the microbial population in the rumen. On the other hand, fraction C is considered to be undegradable and, therefore, unavailable for the animal. In addition, fraction B is considered to be potentially degradable in the rumen. Fraction B's degradability in the rumen will depend on its rate of degradability (k_d) and passage (k_p) (Sniffen et al., 1992, NRC, 2001).

Given its partial degradability in the rumen, fraction B is divided in fractions B_1, B_2, and B_3 (Sniffen et al., 1992). The rumen degradability of the B fractions is vastly different. Rumen degradability ranges are reported to be 120-400%/h, 3-16%/h, and 0.06-0.55%/h for fractions B_1, B_2, and B_3, respectively (NRC, 2001).

Understanding how CP is degraded in the rumen is extremely important to break it down in two categories: rumen degradable protein (**RDP**) and rumen undegradable protein (**RUP**). The Cornell Net Carbohydrate Protein System reports that RDP and RUP can be calculated using the following equations:

$$RDP = A + B_1 [k_d B_1 / (k_d B_1 + k_p)] + B_2 [k_d B_2 / (k_d B_2 + k_p)] + B_3 [k_d B_3 / (k_d B_3 + k_p)]$$

$$RUP = B_1 [k_p / (k_d B_1 + k_p)] + B_2 [k_p / (k_d B_2 + k_p)] + B_3 [k_p / (k_d B_3 + k_p)] + C$$

Rumen degradable and undegradable protein have completely different roles in dairy cattle nutrition. Protein degraded in the rumen will generate ammonia, AA, and peptides that will stimulate rumen microbial yield and synthesis of microbial protein (**MCP)** (Argyle and Baldwin, 1989). Microbial protein synthetized in the rumen will provide most of the AA supply to be absorbed in the small intestine. In addition to MCP, RUP is the second most important source of AA to the animal.

According to Clark et al. (1992), MCP has one of the best AA profiles compared to protein found in plants. It is reported that MCP is responsible for providing 50 to 80% of the absorbable protein to the animal (NRC, 2001). However, MCP production is limited once the rumen reaches the point of ammonia overflow (Satter and Slyter, 1974). Consequently, the animal's AA requirements cannot be fulfilled solely by MCP (Brito and Broderick, 2007). Therefore, to meet the animal's metabolizable protein requirements, we must provide both RDP and RUP sources for the animal (NRC, 2001, Agle et al., 2010).

The NRC (2001), states that when designing diets, we should provide adequate amounts of RDP in order to maximize MCP synthesis and enough RUP to optimize the profile and amounts of absorbable AA. However, it is challenging to provide adequate amounts of energy and protein for dairy cows during the transition period, as most of the animals are experiencing negative energy balance due to reduced feed intake. Therefore, feeding more RUP during the transition period might be an effective strategy to minimize the undesirable effects caused by excessive negative energy balance.

Sources of Rumen Undegradable Protein

The most common feedstuffs rich in RUP and used in dairy cow diets are fish meal, non-ruminant meat and bone meal, feather meal, blood meal, corn gluten meal, and dried distillers grains (Santos et al., 1998). What those feedstuffs have in common is that they contain protein that are classified as "rumen protected" protein. The definition of "rumen protected" by the Association of American Feed Control Officials is "a nutrient(s) fed in such a form that provides an increase in the flow of that nutrient(s) , unchanged, to the abomasum, yet available to the animal intestine" (Noel, 2000).

Other proteins that are not naturally "rumen protected", can be done so through processing methods. Processing methods to protect protein from being degraded in the rumen are: heat treatment, chemical treatment or a combination of both (Kaufmann and Lüpping, 1982, Schwab, 1995). In North America, heat treatment is the most used method to increase RUP. Although vastly adopted, the heat treating process to increase RUP in feedstuffs might not be successful if not well managed. Controlling heat intensity is essential to maximize the content of digestible RUP (Schwab, 1995). If excessive heat is applied during the heat treatment process, the intestinal digestibility of RUP can be reduced (Van Soest, 1994). On the other hand, under-heating will yield small increases in digestible RUP (Satter, 1986). Chemically treating feed proteins has not been vastly adopted in a commercial setting and one of the main reasons is the lower level of effectiveness for increasing RUP content when compared to heat treatment. Therefore, some try to combine heat and chemical treatment in order to increase RUP in feedstuffs (Schwab, 1995).

For example, soybean meal has a high concentration of essential AA and is the most used protein source in dairy rations. Soybean meal is a byproduct of the soybean oil industry and can be obtained mainly by three different methods: expeller processing (heat treatment), solvent extraction (chemical treatment), and pre-treatment with lignosulfonate followed by expeller processing (heat + chemical treatment) (NRC, 2001, Can and Yilmaz, 2002, Castro et al., 2007). According to the NRC (2001), the average RUP as a percentage of CP in heat, chemical, and heat + chemical treatment are 69.0, 34.6, and 79.4%, respectively. Even though the combination of heat and chemical treatments for soybean meal yields higher amounts of RUP, the chemical components used in the process might be a source of environmental pollution (Smook and Kocurek, 1982, Castro et al., 2007). In

addition, research has reported no differences in the availability of essential AA between expeller processed and lignosulfonate treated soybean meal (Borucki Castro et al., 2007). Consequently, no differences in milk yield were found when comparing chemically and heat treated soybean meal (Borucki Castro et al., 2008). Thus, we are still searching for highly available RUP sources to fed dairy cattle and other ruminants.

Recently, a yeast-derived microbial protein (**YMP;** DEMP®; Alltech Inc., Nicholasville, KY) product has become available in the market. Yeast-derived microbial protein is a byproduct of yeast fermentation with AA profiles similar to MCP and that, presumably, flows with the liquid phase of the rumen, which permits AA absorption in the small intestine (Sabbia et al., 2012). Thus, in the following section of this review I will first describe the composition of YMP, then explore the, still limited, literature available on the YMP effects on DMI, production, and metabolic status of dairy cows.

YEAST-DERIVED MICROBIAL PROTEIN

As previously discussed, microbial protein is considered to be the main source of absorbable protein to the dairy cow and its AA profile is similar to the AA profile required by the animal (Clark et al., 1992, Bach et al., 2005). Sabbia et al. (2012) analyzed the AA profile of YMP and compared it to AA profiles of soybean meal and MCP (Table 1.1). Briefly, YMP showed a similar AA profile when compared with YMP and had higher concentrations of methionine and lysine when compared to soybean meal.

Yeast-derived microbial protein is believed to flow with the liquid phase of the rumen providing a source of intestinal absorbable AA(Sabbia et al., 2012), and further investigation is required for our understanding of YMP during its phase in the rumen and intestinal absorption. In a dose-response study with mid-lactation cows, Sabbia et al. (2012)

reported that ruminal ammonia concentrations tended to decrease linearly as YMP inclusion was gradually increased (0, 1.14, 2.28, and 3.41% of DM). Therefore, Sabbia et al. (2012) concluded that the decrease in ruminal ammonia concentration was due to a greater passage rate of peptides in cows fed YMP. However, there is still lack of evidence available on the intestinal availability of the AA content in YMP. One study has evaluated plasma concentrations of AA when cows were fed YMP (Manthey et al., 2016).When feeding YMP at 2.25% of DM to mid-lactation cows in high and low forage diets, Manthey et al. (2016) did not observe any differences in the arteriovenous difference or extraction efficiency of essential, non-essential, and branched-chain amino acids between treatments regardless of the forage to concentrate ratio in the diets. All diets fed by Manthey et al. (2016) were similar in AA concentration and no differences in DMI were observed when YMP was fed. Previous research has shown that the excess of metabolic fuels can decrease DMI when intake is not limited by gut fill (Allen, 2000). Therefore, future research should focus on the bioavailability of YMP as the availability of nutrients seems to play an important role in productive responses such as DMI. Thus, the next session of this review aims to describe previous research findings on the effects of YMP on DMI.

Effects of yeast-derived protein on dry matter intake

Researchers have investigated the inclusion of YMP at different inclusion concentrations and at different stages of lactation on DMI of dairy cattle. In a dose-response study feeding YMP at 0, 1.14, 2.28, and 3.41% of DM to cows around peak lactation (93 ± 37 DIM), Sabbia et al. (2012) reported that increasing YMP as % of DM showed a cubic effect on DMI with the highest DMI observed in cows fed YMP at 1.14 and 3.41% of DM.

In contrast, another study with cows around peak lactation (88 ± 18 DIM) reported no differences in DMI when YMP was included at 2.25% of diets containing low (45 % of DM) or high forage (65% of DM) (Manthey et al., 2016). Effects of feeding YMP on DMI of dairy cows seem to be inconsistent in the literature currently available.

A study with cows in early lactation (46 ± 8 DIM) fed alfalfa-hay based diets, Neal et al. (2014) reported a decrease in DMI when YMP was added at 1.15% of DM. In addition, the same study reported a decrease in DMI when diets were supplemented simultaneously with YMP and slow-release urea at 1.15 and 0.49% of DM, respectively (Neal et al., 2014). Differently, a recent study feeding YMP to transition dairy cows found no differences in DMI when YMP feeding pre and postpartum at 0.42 and 1.25% of DM, respectively (Higginson et al., 2018). However, different than the studies previously reported, Higginson et al. (2018) fed YMP top dressing instead of incorporating it into the TMR like previous authors. Furthermore, dry cows in Higginson et al. (2018) had prepartum DMI restricted to 12kg/d of TMR plus free choice hay and were reported to consume all the TMR offered prepartum. However, changes in feeding behavior might help explain the lack of consistency in DMI when cows were fed YMP. Also, previous research has associated feeding behavior, such as time spent eating, meal frequency, and time spent ruminating, to DMI (DeVries et al., 2003, Schirmann et al., 2012). Furthermore, to the present date, there are no studies evaluating the effects of YMP on feeding behavior of dairy cows. Additionally, similar to DMI, the effects of YMP on milk yield and components were diverse between studies. Thus the next session of this review aims to describe previous research findings on the effects of YMP on milk yield and components.

Effects of yeast-derived microbial protein on milk yield and components

The inclusion of protein, rumen-protected AA, or energy during the transition period can result in little to no change in milk production and components as has been reviewed by Lean et al. (2013). Similarly, studies feeding YMP at different inclusion rates and to cows in varied stages of lactation have reported little to no effects of YMP on milk yield and components. In a dose-response study feeding YMP at 0, 1.14, 2.28, and 3.41% of DM to cows around peak lactation (93 ± 37 DIM), Sabbia et al. (2012) reported that increasing YMP as % of DM showed no effect of YMP on milk yield and milk protein percentage. However, 4% FCM, ECM, and milk fat percentage tended to increase quadratically as YMP inclusion increased (Sabbia et al., 2012). In contrast, another study with cows around peak lactation (88 ± 18 DIM) reported no differences in milk yield, ECM yield, milk fat percentage, and milk protein percentage when YMP was included at 2.25% of diets containing low (45 % of DM) or high forage (65% of DM) (Manthey et al., 2016).

Moreover, in a study with cows in early lactation (46 ± 8 DIM) fed alfalfa-hay based diets, Neal et al. (2014) reported that milk yield, 3.5% FCM yield, and ECM yield increased when YMP was added at 1.15% of DM. However, the same study did not report any changes in milk fat or protein percentages when YMP was added at 1.15% of DM (Neal et al., 2014). Furthermore, no effects of YMP on milk yield, 4% FCM yield, ECM yield, milk fat percentage, and milk protein percentage were found in a recent study top-dressing the TMR with YMP pre and postpartum at 0.42 and 1.25% of DM, respectively (Higginson et al., 2018) . Overall the inclusion of YMP in dairy cow diets seems to have little to no effect on milk yield and components. Furthermore, as previously mentioned, the effects of YMP on DMI diverge between studies. However, in order to understand the

effects of YMP on the energy status of the dairy cow, we should investigate blood metabolites associated with the mobilization of body reserves, such as NEFA and BHBA. Therefore, the next session aims to describe previous research findings on the energy status of the dairy cow focusing mainly on blood metabolites.

Effects of yeast-derived microbial protein on blood metabolites

It is well documented that dairy cows in NEB will mobilize body reserves in order to fulfill the requirements for milk production (Drackley et al., 2005, Ospina et al., 2013). Therefore, when evaluating the effects of a novel feed additive, besides evaluating production responses, it is important to evaluate its effects on the energy status via metabolites such as NEFA and BHBA of dairy cows. At this time, only two studies have reported the effects of YMP inclusion on NEFA and BHBA (Sabbia et al., 2012, Higginson et al., 2018). However, other authors have reported the effects of YMP on the energy status of the dairy cow by calculating the daily energy balance (Manthey et al., 2016) and net energy utilization for weight gain and milk production (Neal et al., 2014).

In a dose-response study feeding YMP at 0, 1.14, 2.28, and 3.41% of DM to cows around peak lactation (93 ± 37 DIM), Sabbia et al. (2012) reported that increasing YMP as % of DM showed no effect of YMP on plasma NEFA. However, plasma BHBA tended to increase linearly as YMP inclusion increased (Sabbia et al., 2012). In contrast, Higginson et al. (2018) reported an overall reduction in serum NEFA but no changes in serum BHBA during the transition period when top-dressing the TMR with YMP pre and postpartum at 0.42 and 1.25% of DM, respectively (Higginson et al., 2018).

Furthermore, in a study with cows around peak lactation (88 ± 18 DIM) reported that the calculated energy balance was improved when YMP was included at 2.25% of diets containing low (45 % of DM) or high forage (65% of DM) (Manthey et al., 2016). On the other hand, in a study with early lactation cows (46 ± 8 DIM) fed alfalfa-hay based diets, Neal et al. (2014) reported a decrease in the net energy utilization for weight gain and a tendency to increase the net energy utilized for milk production that milk yield, 3.5% FCM yield, and ECM yield increased when YMP was added at 1.15% of DM.

There is still a limited availability of research and inconsistent results on the inclusion of YMP on dairy cow diets. However, feeding YMP to transition dairy cows might be beneficial as previous research has shown benefits of YMP feeding on milk yield (Neal et al., 2014), DMI (Sabbia et al., 2012), and metabolic status (Higginson et al., 2018) of dairy cows in different stages of lactation. Furthermore, there seems to be no studies evaluating the long-term effects of YMP inclusion on diets and the effects of YMP on feeding behavior of dairy cows. Therefore, in the next chapter will investigate the effects of YMP feeding on production health, and behavior of transition dairy cows.

CONCLUSIONS

During the transition period, dairy cows experience a myriad of metabolic, management, and nutritional requirement changes. This combination of factors can result in a state of negative energy balance and immune suppression, which increases the susceptibility to a series of metabolic diseases which can affect health lactational and performance. However, increasing the availability of nutrients, such as protein in the transition period, has the potential to improve metabolic status, health, and performance of dairy cows in the short and long term. Furthermore, protein sources can help with the

mitigation of these problems, and yeast-derived microbial protein has shown potential to serve as an alternative protein source for dairy cow diets. Therefore, the goal of this thesis is to evaluate the effects of supplementing yeast-derived microbial protein as a partial substitution of soybean meal on feed intake, feeding behavior, milk yield, and metabolic status of transition dairy cows. Furthermore, the current thesis aims to evaluate if there are any long term effects on lactational performance when cows are fed yeast-derived microbial protein during the transition period.

Table 1.1. Essential amino acid (AA) composition of yeast-derived microbial protein (YMP), soybean meal (SBM), and microbial protein (MCP). Table adapted from Sabbia et al. (2012).

AA, % of total essential AA	YMP	SBM[1]	MCP[2]
Arginine	10.9	16.2	10.2
Histidine	5.1	6.1	4.0
Isoleucine	11.1	10.1	11.5
Leucine	17.6	17.2	16.3
Lysine	16.0	13.9	15.8
Methionine	3.6	3.2	5.2
Phenylalanine	9.6	11.6	10.2
Threonine	10.0	2.8	2.7
Tryptophan	2.9	2.8	2.7
Valine	13.4	10.2	12.5
Total essential AA, % of CP	44.0	45.3	-

[1] Table 2.5-10 (NRC, 2001)

[2] Clark et al. (1992) and NRC (2001)

CHAPTER TWO

Effects of yeast-derived microbial protein on transition dairy cow health and performance

G. Mazon, M. R. Campler, and J.H.C. Costa*

Department of Animal and Food Sciences, University of Kentucky, Lexington, USA, 40546

*Corresponding author: Joao HC Costa; email: costa@uky.edu

INTRODUCTION

The transition period is defined as the time period from 3 weeks pre- to 3 weeks postpartum (LeBlanc, 2010). During the transition period, most periparturient dairy cows have been found to be in negative energy balance (**NEB**) (Ospina et al., 2010). Negative energy balance is highly prevalent among transition dairy cows, and is caused by a combination of factors such as fetal growth, increase of energy requirements for lactation, and decreased dry matter intake during the periparturient period (**DMI**) (Gerloff, 2000, Ingvartsen and Andersen, 2000). The causes for the drop in DMI are complex and includes a plethora of factors, such as decreased rumen volume (Reynolds et al., 2004), hormonal- (Pushpakumara et al., 2003a) and management changes (Proudfoot et al., 2009, Garnsworthy and Topps, 2010).

The drastic changes in energy demands and decreased DMI have been associated with the incidence of transition cow diseases, such as hypocalcemia (Reinhardt et al., 2011), hyperketonemia (Sheehy et al., 2017), and metritis (Huzzey et al., 2007). Due to the relationship between decreased DMI and transition cow diseases incidence, a common nutritional management strategy is to adjust the nutrient density of prepartum diets to meet cows' nutritional requirements to minimize the risk of transition diseases.

Protein intake is one of the nutritional factors that limits performance during lactation (Clark and Davis, 1980, Bach et al., 2005). The NRC (2001) suggests that the dietary crude protein (**CP**) levels in prepartum diets should be 12 %. Studies feeding higher protein amounts have reported inconsistent results as far as the effects of feeding extra prepartum CP on postpartum DMI, milk yield, and transition cow diseases incidence (Santos et al., 2001, Pushpakumara et al., 2003a). In a study evaluating the effects of

increasing prepartum dietary CP and rumen undegradable protein (**RUP**) content (12.7 % CP and 36 % RUP vs 14.7 % CP and 40 % RUP), Santos et al. (2001) reported that primiparous cows fed higher protein amounts prepartum had improved lactational performance in the first 120 DIM, but increasing protein content did not affect disease incidence. However, no effects on milk yield and metabolic status for 15 weeks following calving when cows were fed and additional 750 g/d of a soybean based RUP source (Pushpakumara et al., 2003a). However, as previously reviewed by Santos et al. (1998), the responsiveness to increases in protein level might be related to RUP source as well as the amino acid (**AA**) profile provided by the feedstuff. Previous research investigating the effect of rumen protected methionine in transition cows found increased ECM, FCM and protein levels in milk without changes in blood BHBA compared to non-supplemented cows (Zhou et al., 2016). However, responses to AA supply during the transition period depend on the CP concentration and intestinal digestibility of the AA source (Socha et al., 2005).

A possible solution to combat insufficient AA absorption in transition dairy cows may be found in yeast-derived microbial protein (**YMP**), that can be used as an alternative protein source. Yeast-derived microbial protein is a byproduct of yeast fermentation with AA profiles similar to microbial protein (**MCP**) and that, presumably, flows with the liquid phase of the rumen, which permits more AA absorption in the small intestine (Sabbia et al., 2012). However, previous research feeding YMP to dairy cows have reported inconsistent results. In a dose-response study, Sabbia et al. (2012) reported that increasing YMP as % of DM showed a cubic effect on DMI but milk yield was not affected by YMP when fed to cows around peak lactation (93 ± 37 DIM). Moreover, Neal et al. (2014)

reported that early lactation cows (46 ± 8 DIM) tended to have an increased milk production and a decreased DMI when cows were fed YMP at 1.15 % of total DM. In contrast, Manthey et al. (2016) reported no effects of feeding YMP at 2.25% of total DM, on milk yield or DMI of cows around peak lactation (88 ± 18 DIM). However, none of the previous studies were conducted with cows during the transition period. In a recent study with transition dairy cows, Higginson et al. (2018) reported that feeding YMP during the transition period (50 g/d prepartum and 200 g/d postpartum) did not affect DMI or milk yield but reduced metabolic stress, as cows fed a YMP pellet as a top dress had lower NEFA compared to cows fed a control pellet.

Feeding YMP to transition dairy cows might be beneficial as previous research has shown benefits of YMP feeding on milk yield (Neal et al., 2014), DMI (Sabbia et al., 2012), and metabolic status (Higginson et al., 2018) of dairy cows in different stages of lactation. However, further investigation is needed, since information on the use of YMP in transition cow diets is still limited. The objective of this study was to evaluate the effects of partial substitution of soybean meal with YMP on transition dairy cow performance and health. We hypothesized that cows fed YMP would have higher DMI, higher milk yield, and would mobilize less fat than control cows during the transition period.

MATERIALS AND METHODS

Animal Housing and Diet

The experiment was conducted at the University of Kentucky Coldstream Dairy Farm in Lexington, KY, USA, between August 2017 and June 2018, under Institutional Animal Care and use Committee (IACUC) protocol number 2017-2646. Seventy-one dry Holstein dairy cows (1.9 ± 1.0 lactations; Primiparous: 24; Multiparous: 47) averaging 726

± 113 kg body weight were enrolled in this study from 29 ± 3 days (mean ± SD; nominal 28 days) before expected calving until 21 days in milk (DIM). At enrollment, cows were randomly assigned according to parity to a treatment diet with (treatment; **YMP**) or without (control; **CON**) yeast-derived microbial protein (**YMP**).

Cows were housed in a compost bedded-pack barn throughout the study, that was tilled twice/d (approximately 0520 to 0550 h, and 1450 to 1510 h). Each side of the barn was equipped with two 4.9 m fans (Powerfoil X3.0, Big Ass Fans, KY) and six 91 cm fans over the feeding alley. Cows in this study were housed in one pen (Transition Pen) from study enrollment until 21 DIM. The transition pen was equipped with 8 automatic intake feeders (Insentec, Hokofarm Group, Marknesse, Netherlands). Cows had *ad libitum* access to feed which was provided once daily at 0800 h and orts were removed daily prior to feeding.

At 22 DIM, cows were moved from the transition pen into the general herd pen equipped with headlocks and commingled with other cows. In the herd pen they were fed twice per day at 0630 and 1400 h and feed was pushed up in the feedbunk approximately 22 times/d using an automatic feed pusher (Lely Juno, Lely Robots, Maassluis, The Netherlands). All cows had unlimited access to fresh water throughout the study via a self-filling water trough located in the feeding alley. Milking occurred twice daily at approximately at 0700 and 1700 h.

Treatment diets were formulated following the National Research Council (NRC) guidelines (NRC, 2001) to meet or exceed the nutrients required by a 636 kg animal during late gestation and to sustain at least 38 kg of milk yield post-calving. Isonitrogenous and isoenergetic diets were formulated with corn silage, alfalfa silage, grass hay, whole

cottonseed, and a grain mix with or without YMP. The dry matter content of the forages was determined weekly by NIR spectroscopy (AgriNIR, Dinamica Generale, St. Charles, IL) and diets adjusted to maintain a consistent forage to concentrate ratio throughout the experiment. Treatment diets were formulated to supply animals with 100 and 300 g of YMP/d (DEMP®; Alltech Inc., Nicholasville, KY) during the dry and fresh periods, respectively. Diet formulation and nutrient composition are shown in Table 2.1.

Measurements and Sampling

Daily feed intake, number of visits to the feeder and time spent eating were recorded from enrollment until 21 DIM using the automatic intake feeders. These automatic intake feeders measure intake and feeding behaviors and have previously been validated by Chapinal et al. (2007). From enrollment to 21 DIM, rumination and resting time were recorded using a tag placed on the left ear of each cow (CowManager, Sensoor, Agis, Harmelen, the Netherlands). Each tag contained a 3-dimensional accelerometer that registered ear movements and has been previously validated (Bikker et al., 2014, Borchers et al., 2016).

Feed samples from each diet were taken weekly immediately after feeding and stored at -20ºC until composition analysis. Samples were dried for 48 h at 55ºC in a forced-air oven (Tru-Temp, Hotpack Corp., Philadelphia, PA) to determine the dry matter content of each diet, that was further used to calculate individual dry matter intake. Samples were ground using a 2-mm screen (Standard Model 3, Arthur H. Thomas Co., Philadelphia, PA) and composited by month for analysis of chemical composition. Composite samples were sent to Alltech Laboratories (Alltech Inc., Nicholasville, KY) for composition analysis.

Crude protein was measured using an automated nitrogen combustion analyzer (Rapid N cube, Elementar Analysensysteme GmbH, Hanau, Germany). Concentrations of NDF and ADF were determine using a fiber analyzer (Ankom 200, Ankom Technology, Macedon, NY). Ether extract was determined using an extractor with diethyl ether as a solvent (Ankom XT10 extractor, Ankom Technology). Starch was determined according to the methodology by Hall et al. (1999).

From study enrollment until 21 days in milk (DIM), cows' body condition scores (BCS) and gait scores were recorded once per week. Body condition scores were evaluated using a 5-point scale with 0.25-point increments previously described by Ferguson et al. (1994). Gait scores were recorded using a 3-point scale according to methodology used by Amory et al. (2006). All cows were evaluated by a single trained observer throughout the study to avoid inter-observer inconsistencies.

Urine samples were obtained once weekly via vulva stimulation during the prepartum period and pH determined using an electronic meter (S220, Mettler Toledo AG, Greifensee, Switzerland). In addition, cows had their body weights electronically recorded once weekly prepartum and twice daily postpartum using an automatic scale at the exiting alley from the milking parlor (AfiWeigh, AfiMilk, Kibbutz Afikim, Israel).

Milk yield and components were recorded from calving until 150 DIM. Daily milk yield was recorded using an automatic meter (AfiMilk, AfiMilk, Kibbutz Afikim, Israel). Milk fat and protein were measured twice daily using an in-line milk analyzer (AfiLab, AfiMilk, Kibbutz Afikim, Israel) validated by Kaniyamattam and De Vries (2014). Four-percent fat-corrected milk (4% FCM) and energy-corrected milk (ECM) were calculated using equations found in the NRC (2001). From 1 to 21 DIM, composite milk samples

from each cow were collected once weekly during the morning milking and analyzed for somatic cell count using an infrared analyzer (Bentley FTS, Bentley Instruments Inc., Chaska, MN). Monthly milk samples were also obtained by the Dairy Herd Information Association (DHIA). Somatic cell count was converted to somatic cell score (SCS) according to methodology by Ali and Shook (1980).

From 1 to 21 days in milk (DIM), cows were physically assessed thrice per week by a single observer prior to feeding. The assessment consisted of blood collection, vaginal discharge evaluation, and rectal temperature recording. A blood sample was collected from the coccygeal vein using vacuum sealed tubes with no anticoagulant (BD Vacutainer, Franklin Lakes, NJ). Blood β-hydroxybutyrate (BHBA) was measured using a handheld meter (PortaCheck, Moorestown, NJ) previously validated by Sailer et al. (2018). Serum was obtained spinning the blood samples for 25 minutes at 5,000 rpm (IECCentra-HN, Thermo Fischer Scientific, Waltham, MA). Serum samples were stored at -20^{o}C until analyzed for NEFA and Calcium. Serum NEFA was analyzed using a colorimetric assay (NEFA kit, code 434-91795; Wako Chemicals USA Inc., Richmond, VA) and a microplate reader (Cary 50 MPR, Varian Inc., Lake Forest, CA). Serum calcium was measured via colorimetric spectrophotometry (University of Illinois Veterinary Diagnostic Lab Champaign, IL). Vaginal discharges were collected using an insertion device (Metricheck Simcro Tech Ltd, Hamilton, New Zealand) and scored on coloration and consistency using a 3-point scale reported by Sheldon et al. (2006). Rectal temperature was recording using a digital thermometer (GLA Agricultural Electronics M700, San Luis Obispo, CA).

Disease Diagnosis and Treatment

Dystocia was recorded by farm personal and defined as a calving where assistance was deemed needed. Periparturient paresis was defined as weakness and flaccid paralysis postpartum according to Oetzel (2011) and subclinical hypocalcemia was defined as serum calcium < 8.6 mg/dL following the methodology used by Martinez et al. (2012). Retained placenta was defined as the failure of the cow to expel the placenta within 24 hours postpartum (Kelton et al., 1998). Metritis was defined as vaginal discharge score of 3 according to Sheldon et al. (2006). Subclinical hyperketonemia was defined as blood BHBA ≥ 1.2 and < 2.9 mmol/L and clinical hyperketonemia as blood BHBA ≥ 2.9 mmol/L following (McArt et al., 2012a). Cows were visually examined for clinical mastitis symptoms such as abnormal milk coloration or texture and udder inflammation twice daily prior to milking (Royster and Wagner, 2015). Cows with a gait score of 3 were considered lame (Amory et al., 2006).

At calving, all cows received an oral calcium bolus (Bovikalk, Boehringer Ingelheim, St. Joseph, MO) and a subcutaneous injection of pegbovigrastim (Imrestor, Elanco Animal Health, Greenfield, IN). Animals in periparturient paresis received 500 mL intravenous Ca solution (Henry Nova-Tech Inc., Grand Island, NE). Cows diagnosed with retained placenta or metritis were treated with an antibiotic (Ceftiofur, Excede, Zoetis, Kalamazoo, MI) and 5 mL of prostaglandin (Lutalyse; Zoetis, Florham Park, NJ). Cows diagnosed with subclinical hyperketonemia were treated with 300 mL of oral propylene glycol and 5 mL of subcutaneous vitamin B12 (VEDCO, Inc., St. Joseph, MO) daily for five consecutive days. Cows diagnosed with clinical hyperketonemia also received propylene glycol and vitamin B12 for five consecutive days plus 500 mL of intravenous

dextrose (Henry Nova-Tech Inc., Grand Island, NE) daily until blood BHBA was < 2.9 mmol/L. Clinical mastitis were treated with a daily intramammary infusion of an antibiotic (Ceftiofur, Spectramast LC, Zoetis, Parsippany-Troy Hills, NJ) until disappearance of clinical signs or eight consecutive days. Lame cows were had their hooves trimmed or wrapped using a bandaging wrap (VetRap, 3M Animal Care Products, St Paul, MN) when deemed necessary by the farm personal.

Statistical Analysis

All statistical analyses were performed using SAS (version 9.4; SAS Institute Inc., Cary, NC) and all data were checked for normality using the UNIVARIATE procedure in SAS and probability distribution plots. All descriptive statistics were determined utilizing the MEANS procedure in SAS. Observations above the 99th and below the 1st percentile were deemed as outliers and therefore removed from further analysis. Blood BHBA was deemed non-normal, therefore outliers were removed using the 95th and 5th percentile and data transformed to fit normality using a logarithmic scale and back-transformed for geometric means. Milk yield, 4% FCM, ECM, milk fat and protein percentages, DMI, number of visits to the feeder, time spent eating, resting or ruminating, blood BHBA, and serum calcium and NEFA were summarized by week pre- or post-calving. At posteriori, cows were classified in 4 groups according of the number of diseases they had at any point during the transition period (0, 1, 2, and ≥ 3 diseases). Disease groups and prevalence of dystocia, hypocalcemia, retained placenta, metritis, hyperketonemia, mastitis, and lameness are shown in Table 2.2.

The effect of feeding YMP on milk yield, milk composition, DMI, behavior (number of visits to the feeder, time spent eating, ruminating or resting), and blood

metabolites (calcium, NEFA, and BHBA) was determined by an analysis of variance (ANOVA) using mixed linear models (MIXED procedure) in SAS. The fixed effects in the model were analyzed by period (prepartum, fresh, post-fresh, and 150 DIM; -21 to 0, 1 to 21, 22 to 150, and 1 to 150 DIM, respectively) included treatment (**YMP** and **CON**), parity (primiparous and multiparous), body weight, disease group (0, 1, 2, and ≥ 3), temperature and humidity index (THI), lactation week, and the interaction between treatment and week. For the prepartum period analyses, DMI and urine pH were also included. For the fresh period, models also included milk yield, DMI, serum NEFA, rectal temperature. Finally, milk yield was also included in the post-fresh, and 150 DIM period.

The mixed linear models (MIXED procedure) had week as a repeated measure and cow as subject using an autoregressive (AR-1) covariance structure. Models were selected using the smallest AIC structure consistent with the variable structure of interest. Effects with a *p*-value above 0.30 were removed from the model using a stepwise backward elimination process starting with the least contributing effect. When analyzing prepartum data, treatment, parity, body weight, DMI, and the interaction between treatment and week prepartum remained as a fixed effect regardless of significance. When analyzing data during the fresh period, treatment, parity, body weight, milk yield, DMI, disease group and the interaction between treatment and lactation week remained as a fixed effect regardless of significance. When analyzing data for the post-fresh period and 150 DIM, treatment, parity, body weight, milk yield, disease group and the interaction between treatment and week remained as a fixed effect regardless of significance. Significance was declared at $P \leq 0.05$, and trends were defined as $0.05 < P \leq 0.10$. To test the influence of treatment in each week during the transition period, the PDIFF statement was used to compare the least

squares means of each week for treatment comparisons (-3 wk to 3 wk around parturition), and the P-values were corrected using a Bonferroni correction. For the post-fresh period, week effects were analyzed where the interaction between week and treatment was $P \leq 0.10$.

RESULTS

The descriptive data of YMP and CON cows during the prepartum and fresh period is listed in table 2.3.

Milk yield and composition

The effects of feeding YMP to transition dairy cows on average milk yield, 4% FCM yield, ECM yield, milk fat percentage, and milk protein percentage by period (fresh, post-fresh and 150 DIM) are reported in Table 2.4. When looking at the 150 DIM production, there were no treatment effects on average milk ($F_{1,59} = 0.01$; $P = 0.91$; Table 2.4), 4% FCM ($F_{1,58} = 0.05$; $P = 0.83$; Table 2.4), and ECM ($F_{1,58} = 0.02$; $P = 0.88$; Table 2.4) yields. Additionally, no differences were found in average milk fat ($F_{1,58} = 1.38$; $P = 0.24$; Table 2.4) and protein ($F_{1,55} = 0.26$; $P = 0.61$; Table 2.4) percentages between YMP and CON cows within 150 DIM.

We found no effects of treatment on average milk yield ($F_{1,54} = 2.12$; $P = 0.15$; Table 2.4) in the fresh period. No treatment effects were found on average ECM ($F_{1,65} = 1.32$; $P = 0.25$; Table 2.4) yield in the fresh period. However, cows fed YMP tended to have higher average 4% FCM yield ($F_{1,54} = 3.31$; $P = 0.07$; Table 2.4) during the fresh period. Furthermore, treatment had no effect on average milk fat percentage ($F_{1,65} = 0.59$;

$P = 0.44$; Table 2.4) or protein percentage ($F_{1,65} = 0.09$; $P = 0.76$; Table 2.4) during the fresh period. When looking at the effects of treatment by week during the fresh period, no differences were found for milk fat ($F_{2,118} = 0.55$; $P = 0.58$; Table 2.5) or protein percentage ($F_{2,122} = 0.32$; $P = 0.73$; Table 2.5) between YMP and CON cows.

Similarly, no treatment effects were found on average milk ($F_{1,64} = 0.32$; $P = 0.57$; Table 2.4), 4% FCM ($F_{1,64} = 0.72$; $P = 0.40$; Table 2.4), and ECM ($F_{1,64} = 0.58$; $P = 0.45$; Table 2.4) yields in the post fresh period. Likewise, no treatment effects were found on average milk fat ($F_{1,64} = 1.06$; $P = 0.31$; Table 2.4) or protein ($F_{1,64} = 0.02$; $P = 0.88$; Table 2.4) percentages during the post-fresh period. There was a tendency for an interaction between treatment and week for milk yield ($F_{18,1107} = 1.45$; $P = 0.10$; Figure 2.1) but there were no differences between treatments during any of the weeks in the post-fresh period ($t \leq 1.13$; $P \geq 0.26$; Figure 2.1). There was an interaction between treatment and week for 4% FCM yield ($F_{18,1106} = 1.81$; $P = 0.02$; Figure 2.2) but no differences between treatments were found in any of the weeks during the post-fresh period ($t \leq 1.35$; $P \geq 0.18$; Figure 2.2). There was an interaction between treatment and week for ECM yield ($F_{18,1108} = 1.70$; $P = 0.03$; Figure 2.3) but no differences between treatments were found in any week during the post-fresh period ($t \leq 1.26$; $P \geq 0.21$; Figure 2.3). No interaction between treatment and week was observed for milk fat ($F_{18,1120} = 1.15$; $P = 0.29$; Figure 2.4) and protein ($F_{18,1088} = 1.02$; $P = 0.43$; Figure 2.5) percentages during the post-fresh period.

DMI and Feeding behavior

The effects of feeding YMP on DMI, number of visits to the feeder, and time spent eating, ruminating, or resting by period (prepartum and fresh) are shown in Table 2.6.

During the prepartum period, cows fed YMP had lower DMI ($F_{1,61} = 4.10$; $P = 0.05$; Table 2.6) compared to CON cows. However, feeding YMP did not seem to affect feeding behavior in the dry period as a whole, as no differences in number of visits to the feeder ($F_{1,61} = 2.09$; $P = 0.15$; Table 2.6) and time spent eating ($F_{1,61} = 0.68$; $P = 0.41$; Table 2.6), ruminating ($F_{1,59} = 0.26$; $P = 0.61$; Table 2.6), or resting ($F_{1,59} = 0.47$; $P = 0.50$; Table 2.6) were found between YMP and CON cows. When analyzing the effects of treatment by week prepartum, no differences were found in DMI between treatments ($t \leq 1.80$; $P \geq 0.10$; Table 2.7) when compared to CON cows. There were no weekly differences between treatments in the prepartum period for the number of visits to the feeder ($t \leq 1.29$; $P \geq 0.10$; Table 2.7), for time spent eating ($t \leq 1.08$; $P \geq 0.10$; Table 2.7), ruminating ($t \leq 0.91$; $P \geq 0.10$; Table 2.7), or resting ($t \leq 0.98$; $P \geq 0.10$; Table 2.7) during the prepartum period.

Similar to the prepartum period, cows fed YMP had lower DMI in the fresh period when compared to CON cows ($F_{1,65} = 14.69$; $P < 0.01$; Table 2.6). Additionally, cows fed YMP tended to visit the feeder less compared to CON cows during the fresh period ($F_{1,65} = 3.23$; $P = 0.08$; Table 2.6). However, no differences between treatments were found time spent eating ($F_{1,62} = 2.65$; $P = 0.11$; Table 2.6), ruminating ($F_{1,64} = 2.23$; $P = 0.14$; Table 2.6), or resting ($F_{1,63} = 0.40$; $P = 0.53$; Table 2.6) in the fresh period. When analyzing the effects of treatment in each week during the fresh period, YMP cows had lower DMI compared to CON cows on the second ($t = 3.73$; $P < 0.01$; Table 2.7) and third week of lactation ($t = 3.48$; $P < 0.01$; Table 2.7). Furthermore, treatment did not affect the number of visits to the feeder ($t > 1.02$ $P > 0.10$; Table 2.7), time eating ($t > 0.35$; $P > 0.10$ Table 2.7), time ruminating ($t \geq 0.23$; $P > 0.10$; Table 2.7), and time spent resting ($t \leq 0.77$; $P \geq 0.44$; Table 2.7) in any of the weeks in the fresh period.

Blood Metabolites

When looking at the fresh period, cow fed YMP had a higher concentration of serum calcium compared to CON cows (Least square means ± SEM; CON = 9.27 ± 0.06, YMP = 9.44 ± 0.06 mg/dL; $F_{1,65} = 4.30$; $P = 0.04$). However, treatment effects were not found on concentration of serum NEFA (CON = 0.81 ± 0.04, YMP = 0.79 ± 0.04 mEq/L; $F_{1,65} = 0.16$; $P = 0.69$). No treatment by week effects were found for concentration of serum calcium ($t < 2.10$; $P > 0.56$; Table 2.8) and NEFA ($t < 0.89$; $P > 0.10$; Table 2.8) in any of the weeks in the fresh period.

Blood BHBA was not affect by treatment during the fresh period [geometric mean (95% CI); CON = 0.72 (0.65, 0.80), YMP = 0.77 (0.71, 0.85) mmol/L; $F_{1,58} = 1.30$; $P = 0.26$). When analyzing the effects of treatment by week, no treatment effect was found on blood BHBA between treatments on the first [CON = 0.60 (0.52, 0.70), YMP = 0.64 (0.56, 0.72) mmol/L; t = 0.66; $P > 0.10$], second [CON = 0.78 (0.69, 0.87), YMP = 0.82 (0.73, 0.91) mmol/L; t = 0.64; $P > 0.10$], or third [CON = 0.80 (0.71, 0.91), YMP = 0.90 (0.80, 1.00) mmol/L; t = 1.46; $P > 0.10$] week of lactation.

DISCUSSION

In this study, isoproteic and isoenergetic diets were formulated substituting soybean meal for yeast-derived microbial protein (**YMP**) during the transition period. This study builds upon previous work showing benefits of YMP feeding on milk yield (Neal et al., 2014), DMI (Sabbia et al., 2012), and metabolic status (Higginson et al., 2018) of dairy cows in different stages of lactation. Higginson et al. (2018) investigated the effects of YMP feeding as a top dress on performance and metabolic status of transition dairy cows.

However, in this study, we fed higher amounts of YMP and incorporate it into the TMR. This study is the first to investigate the effects of feeding YMP on long-term lactation performance. Additionally, this is the first study to investigate the effects of feeding YMP on feeding and resting behavior during the transition period.

Milk yield and composition

The inclusion of yeast-derived protein in the diet of transition dairy cows in this study had limited to no differences in milk yield and milk fat and protein percentages on all the periods studied. To our knowledge the present study is the first to look at the long-term effects of feeding YMP to transition dairy cows. Overall, during the 150 d experimental period and during the fresh period, lactational performance was not affected by YMP inclusion in the transition period diet. For the first 4 weeks of lactation, a recent study top-dress feeding a lower inclusion of YMP (50 prepartum and 200 g/ d postpartum) found no effect of YMP on milk production and composition Higginson et al. (2018). Similarly, no effects of YMP on milk yield and components were found by Manthey et al. (2016) when YMP was fed (2.25% of total DM) to post-peak cows using low and high forage diets. In a study evaluating the effects of increasing prepartum dietary CP and RUP content (12.7 % CP and 36% RUP vs 14.7% CP and 40% RUP), Santos et al. (2001) reported that primiparous cows fed higher protein amounts prepartum had improved lactational performance in the first 120 DIM. However, the purpose of our study was not to increase CP in the diet but evaluate YMP as an alternative protein source in transition cow diets. Therefore, there is an opportunity to feed YMP strategically to increase the CP and RUP content and density in transition cow diets. In the current study, prepartum diets

had higher CP contents than the 12% recommended by the NRC (2001). Thus, further research should investigate the effects of YMP inclusion in high and low CP diets during the transition period.

Regarding milk composition, no differences in milk fat and protein percentages were observed in this study when cows were fed YMP pre- or postpartum. Consequently, feeding YMP during the transition period did not affect average 4% FCM and ECM yields during the first 150 DIM. However, in this study, cows fed YMP tended to have higher 4% FCM yield during the fresh period. Previous studies feeding YMP to dairy cows have shown inconsistent results for milk components, 4% FCM, and ECM yields. In a dose-response study with 16 Holstein dairy cows in mid-lactation, Sabbia et al. (2012) reported that milk fat percentage, 4% FCM, and ECM tended to have a quadratic response as YMP gradually increased from 0 to 3.41% of total DM. At the same time, increasing YMP did not affect milk yield or milk protein percentage. In a study feeding YMP at 2.25 % of DM in high and low forage diets, Manthey et al. (2016) saw no effects of YMP on milk yield and components regardless of the amount of forage in the diet. Comparatively, in a study feeding rumen-protected methionine to transition cows, Zhou et al. (2016) reported increases in ECM, FCM, and protein percentages when cows were fed rumen-protected methionine. Therefore, further research should investigate the opportunity of utilizing YMP to better provide transition cows with a rumen protected AA profile that might be able to provide the animals with AAs such as lysine and methionine.

DMI and feeding behavior

In this study we found that cows fed YMP had a lower DMI during the prepartum and postpartum periods. The decrease in DMI caused by YMP supplementation is in contrast to the hypotheses of the study. However, another study feeding YMP to early lactation cows also found a decrease in DMI when YMP was added as 1.15% of DM (Neal et al., 2014). We speculated that YMP might help cows meet their nutrient needs at a lower DMI. As previously reported by Sabbia et al. (2012), YMP has a similar essential amino acid profile to microbial protein and higher concentrations of lysine and methionine than soybean meal. In addition, Sabbia et al. (2012) reported that YMP flows with the liquid phase of the rumen, allowing a greater AA absorption in the small intestine. Previous research has shown that the excess of metabolic fuels can decrease DMI when intake is not limited by gut fill (Allen, 2000). Therefore, future research should evaluate the availability of the amino acids from YMP and their possible contribution for gluconeogenesis during the transition period.

During the prepartum period, despite experiencing a decrease in DMI, feeding- or resting behaviors were not affected by the addition of YMP to their diets. Moreover, Higginson et al. (2018) reported no effects of YMP on prepartum DMI but, cows had DMI restricted to 12kg/d of TMR prepartum and were reported to consume all prepartum TMR offered. Whereas the cows enrolled in the present study had unlimited access to TMR throughout the study. To our knowledge, previous authors have not investigated the effects of feeding YMP on eating and resting behaviors during the dry period. Our DMI and feeding behavior variables results are within the range reported for transition dairy cows in the literature for both treatments (Proudfoot et al., 2009, Schirmann et al., 2013, Neave et

al., 2017). Furthermore, changes in feed intake and behavior during the prepartum have been associated with the occurrence of postpartum diseases such as metritis (Huzzey et al., 2007), hyperketonemia (Goldhawk et al., 2009), and lameness (Proudfoot et al., 2010).

During the postpartum period, DMI was lower for cows fed YMP. Additionally, cows fed YMP tended to visit the feeder fewer times during the transition period. Furthermore, feeding behaviors such as time spent eating and time spent ruminating were not affected by treatment. Our results are in contrast with a previous study that investigated YMP feeding during the transition period. When Higginson et al. (2018) offered 200 g of YMP as a top dressing pellet to transition dairy cows, DMI was not affected by treatment. The changes in the number of visits to feeder observed in this study could be explained by the decrease in DMI experienced by cows fed YMP. Meal frequency has been previously correlated with DMI (DeVries et al., 2003) and it is plausible that YMP cows in this study visited the feeder less often because of the lower DMI. However, further research should investigate if protein sources, such as YMP affect meal frequency and other feeding behavior patterns in dairy cows.

Blood Metabolites

In this study no differences in NEFA or BHBA were found between treatments. Our findings for NEFA and BHBA were slightly different from previous research done feeding YMP to transition dairy cows. Higginson et al. (2018) reported that cows fed 200 g/d of YMP as a top dress had lower serum NEFA concentrations compared to control cows while BHBA did not differ between treatments. However, it is important to point out that NEFA concentrations in the present study were high in both treatments. According to Ospina et al. (2013) serum NEFA concentrations above 0.70 mEq/L increases risk of

metabolic diseases and decreases milk production. It was expected that the decrease in DMI observed in cows fed YMP would cause NEFA and BHBA concentrations to increase. However, it is well known that as DMI increases, passage rate increases, and there is a decrease in dry matter digestibility (Tyrrell and Moe, 1975, Colucci et al., 1982, Potts et al., 2017). Therefore, the lack of difference in NEFA and BHBA concentrations between YMP and CON cows might have been caused by an increase in dry matter digestibility in the YMP cows. In fact, in an in vitro study, Sabbia et al. (2012) reported that when YMP inclusion in the diet was gradually increased from 0 to 3.41% of DM, dry matter apparent digestibility and dry matter total digestibility tended to increase quadratically. Future research should investigate the effects of YMP on in situ dry matter digestibility and how does that affect the nutrient partitioning in the transition dairy cow.

Cows fed YMP had higher serum calcium than compared to CON cows. However, the effects of YMP on serum calcium were not expected by the authors. The YMP composition analysis published by Sabbia et al. (2012) shows a numeric difference between the calcium content of YMP and soybean meal (0.07 and 0.55% of DM, respectively). Previous research investigating the effects CP and RUP content in prepartum diets reported that CP or RUP content did not affect serum calcium (Greenfield et al., 2000). Despite the high prevalence of subclinical hypocalcemia in the current study, average serum calcium was within normal ranges for both YMP and CON cows according to Goff (2008). Cows were normally below the subclinical hypocalcemia threshold on the two days postpartum, by the third day in lactation, most animals had serum Ca within normal ranges (data not shown). Numerically, we found no differences in the disease prevalence between

treatments. However, future research should investigate the effects of YMP feeding on transition cow disease incidence and acute phase proteins such as haptoglobin.

Yeast-derived microbial protein has the potential to serve as an alternative protein source for transition dairy cow diets as it doesn't seem to have negative effects on production or energy status of the animals. However, the research on YMP feeding to transition dairy cows is still limited. Previous studies conducted with cows past the transition period have investigated the effects of YMP feeding on ruminal environment and volatile fatty acids (**VFA**) kinetics (Sabbia et al., 2012, Neal et al., 2014, Manthey et al., 2016). However, it is known that rumen microbiome can be affected by diet, mainly during the transition period (Edwards et al., 2004, Pitta et al., 2014). Therefore, future research should investigate on the strategic use of YMP during the transition period and how it affects rumen environment, VFA kinetics, and ruminal microbiome.

CONCLUSIONS

Feeding YMP to transition dairy cows did not affect milk or ECM yield during the first 150 DIM. However, cows fed YMP tended to have higher FCM yields during the fresh period. The FCM yield at 150 DIM was not affected by feeding YMP during the transition period. Additionally, cows fed YMP had lower DMI during the dry and fresh periods. Despite the decrease in DMI feeding YMP to transition dairy cows did not seem to affect cows' metabolic status as NEFA and BHBA concentrations did not differ between treatments. However, the decrease in DMI from cows fed YMP might have affected feeding behavior as cows fed YMP tended to visit the feeder less often. The results from this study indicate that yeast-derived microbial protein might be used as an alternative

protein source on transition dairy cow diets as cows were able to maintain production without any negative effects on metabolic status. Future research should investigate the effects of YMP on rumen environment and VFA kinetics in transition dairy cows.

ACKNOWLEDGEMENTS

This study was sponsored by Alltech Inc. (Nicholasville, KY) through a research partnership with the Dairy Science Program at the University of Kentucky. The authors thank the staff and students of the University of Kentucky Coldstream Dairy Research Farm staff for their help with this study. We also thank Dr. Amanda Gehman, Dr. Donna Amaral-Phillips, and Dr. Michelle Arnold for their insight during the planning and execution of the project.

Table 2.1 Ingredients and chemical composition of the experimental diets fed during the prepartum (1 to 3 weeks prepartum), fresh (1 to 3 weeks postpartum), and post-fresh periods with (Control) or without yeast-derived microbial protein supplement (YMP).

	Prepartum Period		Fresh period		Post-fresh Period
Item	Control	YMP	Control	YMP	-
Ingredient (% of DM)					
Corn silage	40.4	40.4	25.2	25.3	23.8
Alfalfa silage	-	-	19.2	19.3	17.0
Grass hay	30.7	30.7	-	-	-
Alfalfa hay	-	-	-	-	3.0
Acidified fermentation by-product[1]	9.7	9.7	-	-	-
Whole cottonseed	7.6	7.6	13.2	13.2	8.6
Ground corn	3.4	3.4	11.4	11.4	22.1
Corn hominy	-	-	9.3	9.1	-
Soft wheat middlings	-	-	5.5	5.5	6.6
Corn gluten	-	-	4.6	4.6	5.5
Expeller pressed soybean meal[2]	-	-	2.9	2.9	3.4
Soybean meal 48.5% CP	-	-	-	-	1.3

Table 2.1 (continued)

Soybean meal 47% CP	-	-	2.6	2.6	-
Soybean meal 46% CP	-	-	-	-	2.3
Soybean meal 44% CP	5.2	4.3	1.7	-	-
Ground soybean hulls	-	-	-	-	2.5
Calcium carbonate	-	-	1.2	1.2	1.2
Yeast-derived microbial protein[3]	-	1.0	-	1.7	-
Ca salts of long chain fatty acids[4]	0.6	0.6	-	-	-
Sodium bicarbonate	-	-	0.8	0.8	0.8
Sugarcane molasses	-	-	0.6	0.5	0.6
Mineral and vitamin mix[5,6]	1.1	1.1	0.4	0.4	0.1
White salt	0.5	0.5	0.4	0.4	0.4
Rumen-protected choline[7]	-	-	0.3	0.3	-
Magnesium oxide	0.2	0.2	0.3	0.3	0.3
Magnesium sulfate	0.2	0.2	-	-	-
Mineral oil	0.2	0.2	-	-	-
Urea	-	-	0.4	0.2	0.2
Enzymatically hydrolyzed yeast[8]	-	-	0.1	0.1	-

Table 2.1 (continued)

Rumen-protected methionine[9]	0.1	0.1	0.1	0.1	0.1
Biotin	0.1	0.1	0.1	0.1	0.1
Chemical Composition					
DM (%)	53.1 ± 2.7	53.2 ± 1.93	52.2 ± 1.7	52.4 ± 1.9	-
CP (% of DM)	14.9 ± 0.9	16.0 ± 0.9	18.0 ± 2.3	16.9 ± 0.8	-
NDF (% of DM)	42.4 ± 4.6	40.7 ± 2.6	30.6 ± 2.4	30.8 ± 1.5	-
ADF (% of DM)	24.9 ± 3.0	23.9 ± 1.4	19.8 ± 1.7	20.3 ± 1.9	-
Starch (% of DM)	23.7 ± 8.8	20.9 ± 6.2	29.2 ± 6.5	28.4 ± 7.0	-
Ether extract (% of DM)	5.6 ± 0.6	5.6 ± 0.3	6.2 ± 0.4	6.0 ± 0.4	-
NFC (% of DM)	29.4 ± 4.7	30.0 ± 3.3	37.3 ± 3.9	37.9 ± 1.4	-
Ash (% of DM)	7.7 ± 0.9	7.7 ± 0.5	8.0 ± 0.5	8.3 ± 0.7	-

[1] Biochlor (Biovance Industries, Omaha, NE)

[2] Soyplus (West Central, Ralston, IA)

[3] DEMP® (Alltech Inc., Nicholasville, KY)

[4] Megalac (Church & Dwight Co. Inc., Princeton, NJ)

[5] The mineral and vitamin mix had the following composition: vitamin A (2,144.4 KIU/kg), vitamin D3 (536,1 KIU/kg), vitamin E (8,113.8 IU/kg), Zn (80,972 ppm), Mn (73683 ppm), Cu (13,341 ppm), I (1,726 ppm), Co (1,175 ppm), Se (524 ppm), Ca (106 g/Kg), P (2.1 g/kg), Mg (1.8 g/kg), and Na (0.5 g/kg).

[6] Diets were formulated to include 0.01% of DM of Monensin (Elanco Animal Health, Indianapolis, IN)

[7] Reashure (Balchem Encapsulates, Slate Hill, NY)

[8] Celmanax (Vi-COR, Mason, IA)

[9] MetaSmart (Adisseo NA, Alpharetta, GA)

Table 2.2 Number of diseases (cows diagnosed) and disease frequency (% of cows diagnosed) from 3 weeks prepartum to 3 weeks postpartum for cows (n = 71) fed diets with (Control, n = 35) or without yeast-derived microbial protein (YMP, n = 36).

Item	Number of cows (% of n)		
	Dietary treatment[1]		
	Control	Treatment	Overall
Number of diseases[2]			
0[3]	5 (14.3)	3 (8.3)	8 (11.3)
1[4]	8 (22.8)	8 (22.2)	16 (22.5)
2[5]	12 (34.3)	10 (27.8)	22 (31.0)
3+[6]	10 (28.6)	15 (41.7)	25 (35.2)
Disease			
Dystocia[7]	1 (2.9)	1 (2.8)	2 (2.8)
Clinical hypocalcemia[8]	3 (8.6)	1 (2.8)	4 (5.6)
Subclinical hypocalcemia[9]	28 (80.0)	29 (80.6)	57 (80.28)
Retained placenta[10]	1 (2.9)	4 (11.1)	5 (7.0)
Metritis[11]	11 (31.4)	16 (44.4)	27 (38.0)
Clinical hyperketonemia[12]	4 (11.4)	8 (22.2)	12 (16.9)
Subclinical hyperketonemia[13]	15 (42.9)	19 (52.8)	34 (47.9)
Mastitis[14]	1 (2.9)	0 (0)	1 (1.4)
Lameness[15]	5 (14.3)	2 (5.6)	7 (9.9)

[1] Control = Cows fed TMR without yeast-derived microbial protein supplement pre and post-partum; Treatment = Cows fed TMR containing 1% of yeast-derived microbial protein prepartum and TMR containing 1.7% yeast-derived microbial protein postpartum.

[2] Cows diagnosed with any disorder pre- or post-calving were considered sick for the entire study period.
[3] 0 disorder was classified as 0 disorder detected from 28 ± 3 days before expected calving until 21 days post-calving.
[4] 1 disorder was classified as any 1 disorder detected from 28 ± 3 days before expected calving until 21 days post-calving.
[5] 2 disorders were classified as any 2 disorders detected from 28 ± 3 days before expected calving until 21 days post-calving.
[6] 3+ disorders were classified as any 3 or more disorders detected from 28 ± 3 days before expected calving until 21 days post-calving.
[7] Dystocia was defined when farm personal assisted with calf delivery.
[8] Clinical hypocalcemia as weakness of flaccid paralysis postpartum.
[9] Subclinical hypocalcemia was defined as serum calcium < 8.6mg/dL.
[10] Retained placenta was determined as retained fetal membrane for more than 24 hours post-calving.
[11] Metritis was defined as vaginal discharge score of 3 (Sheldon et al., 2006).
[12] Clinical hyperketonemia was defined as blood BHBA ≥ 2.9 mmol/L.
[13] Subclinical hyperketonemia was defined as blood BHBA ≥ 1.2 and < 2.9 mmol/L.
[14] Mastitis was defined by abnormal milk coloration or texture.
[15] Lameness was defined a gait score = 3 (Amory et al., 2006)

Table 2.3 Mean, minimum, and maximum body weight, body condition score, gait score, urine pH, and somatic cell score for dairy cows (n = 71) fed TMR without (Control, n = 35) or with a yeast-derived microbial protein supplement (YMP, n = 36) during the prepartum (1 to 3 weeks prepartum) and fresh (1 to 3 weeks postpartum) periods.

	Dietary treatment[1]					
	Control (n = 35)			YMP (n = 36)		
	Mean ± SD	Minimum	Maximum	Mean ± SD	Minimum	Maximum
Prepartum (-21 to 0 DIM)						
Body weight, kg	717.5 ± 115.3	558.5	976.1	735.0 ± 112.9	474.0	944.3
Body condition score[2]	3.6 ± 0.3	2.75	4.0	3.6 ± 0.3	3.0	4.25
Gait Score[3]	1.2 ± 0.4	1.0	3.0	1.2 ± 0.4	1.0	3.0
Urine pH	6.6 ± 0.7	5.6	8.3	6.6 ± 0.9	5.2	8.3
Fresh period (1 to 21 DIM)						
Body weight, kg	630.3 ± 84.1	489.1	822.1	650.1 ± 80.4	494.6	821.3
Body condition score	3.3 ± 0.4	2.5	4.0	3.3 ± 0.3	2.75	4.0
Gait Score	1.5 ± 0.6	1.0	3.0	1.4 ± 0.5	1.0	3.0
Somatic cell score[4]	4.4 ± 1.9	0.8	7.8	4.2 ± 1.5	1.4	6.8

[1] Control = Cows fed TMR without yeast-derived microbial protein supplement pre and post-partum; Treatment = Cows fed TMR containing 1% of yeast-derived microbial protein prepartum and TMR containing 1.7% yeast-derived microbial protein postpartum.
[2] Body condition score was recorded using a 5-point scale previously described by Ferguson et al. (1994).
[3]Gait scores were recorded using a 3-point scale according to methodology used by Amory et al. (2006).
[4] Somatic cell score was calculated according to methodology by Ali and Shook (1980)

Table 2.4. Least square means (± SEM) of milk yield and composition for dairy cows (n = 71) fed TMR without (Control, n = 35) or with a yeast-derived microbial protein supplement (YMP, n = 36) during the prepartum (1 to 3 weeks prepartum) and fresh (1 to 3 weeks postpartum) periods.

Item	Dietary treatment[1]		SEM	*P-value*
	Control (n = 35)	YMP (n = 36)		
Overall (1 to 150 DIM)				
Milk, kg/d	40.3	40.2	0.93	0.91
4% FCM[2], kg/d	40.5	40.7	0.91	0.83
ECM[3], kg/d	43.5	43.7	0.96	0.88
Milk fat, %	4.0	4.1	0.04	0.24
Milk protein, %	3.2	3.2	0.04	0.61
Total milk yield, kg	6,049.8	6,028.8	139.42	0.91
Total 4% FCM yield, kg	6,068.2	6,107.3	136.21	0.83
Total ECM yield, kg	6,544.6	6,577.8	146.02	0.86
Fresh period (1 to 21 DIM)				
Milk, kg/d	29.0	30.9	0.96	0.15
4% FCM, kg/d	30.6	33.0	1.02	0.07
ECM, kg/d	32.9	34.6	1.13	0.25
Milk fat, %	4.3	4.4	0.06	0.44
Milk protein, %	3.2	3.2	0.04	0.76
Total milk yield, kg	611.5	654.4	21.95	0.14
Total 4% FCM yield, kg	657.1	688.7	22.77	0.29
Total ECM yield, kg	700.1	750.3	24.06	0.12

Table 2.4 (continued)

Post fresh period (22 to 150 DIM)				
Milk, kg/d	39.4	40.4	1.33	0.57
4% FCM, kg/d	39.3	40.7	1.25	0.40
ECM, kg/d	42.4	43.7	1.37	0.45
Milk fat, %	4.0	4.1	0.05	0.31
Milk protein, %	3.2	3.2	0.04	0.88
Total milk yield, kg	5,197.4	5,159.1	203.68	0.89
Total 4% FCM yield, kg	5,220.9	5,260.5	163.07	0.85
Total ECM yield, kg	5,596.7	5,658.8	208.34	0.82

[1] Control = Cows fed TMR without yeast-derived microbial protein supplement pre and post-partum (n = 35); Treatment = Cows fed TMR containing 1% of yeast-derived microbial protein prepartum and TMR containing 1.7% yeast-derived microbial protein postpartum (n = 36).

[2] Four-percent fat corrected milk FCM = [0.4 x milk yield] + [15 x fat yield]

[3] Energy corrected milk ECM = [0.327 x milk yield] + [12.95 x fat yield] + [7.20 x protein yield]

Table 2.5. Weekly least square means (± SEM) of milk yield and composition for the first 3 weeks of lactation for dairy cows (n = 71) fed a TMR without (Control, n = 35) or with a yeast-derived microbial protein supplement (YMP, n = 36) during the during the prepartum (1 to 3 weeks prepartum) and fresh (1 to 3 weeks postpartum) periods.

	Dietary treatment[1]			
Lactation week	Control (n = 35)	Treatment (n = 36)	SEM	*P-value*
Milk Yield, kg/d				
1	24.1	26.2	1.09	*NS*
2	30.1	31.8	1.06	*NS*
3	32.8	34.7	1.11	*NS*
4% FCM[2], kg/d				
1	25.1	27.7	1.16	*NS*
2	32.2	34.3	1.12	*NS*
3	34.4	37.1	1.17	*NS*
ECM[3], kg/d				
1	27.6	29.3	1.23	*NS*
2	34.2	35.8	1.22	*NS*
3	36.9	38.8	1.22	*NS*
Milk fat, %				
1	4.2	4.3	0.07	*NS*
2	4.4	4.5	0.07	*NS*
3	4.3	4.4	0.07	*NS*
Milk protein, %				
1	3.3	3.3	0.04	*NS*

Table 2.5 (continued)

2	3.1	3.2	0.04	*NS*
3	3.2	3.2	0.04	*NS*

[1] Control = Cows fed TMR without yeast-derived microbial protein supplement pre and post-partum (n = 35); Treatment = Cows fed TMR containing 1% of yeast-derived microbial protein prepartum and TMR containing 1.7% yeast-derived microbial protein postpartum (n = 36).

[2] Four-percent fat corrected milk FCM = [0.4 x milk yield] + [15 x fat yield]

[3] Energy corrected milk ECM = [0.327 x milk yield] + [12.95 x fat yield] + [7.20 x protein yield]

Table 2.6. Least square means (± SEM) of DMI, feeding, and resting behaviors from dairy cows (n = 71) fed a TMR without (Control, n = 35) or with a yeast-derived microbial protein supplement (YMP, n = 36) during the prepartum (1 to 3 weeks prepartum) and fresh (1 to 3 weeks postpartum) periods

Item	Dietary treatment[1]		SEM	*P-value*
	Control (n = 35)	Treatment (n = 36)		
Prepartum period (21 to 1 d before calving)				
DMI, kg/d	12.22	10.83	0.52	0.05
Visits to the feeder, visits/d	55.46	49.54	3.13	0.15
Time spent eating, h/d	133.65	126.15	6.94	0.41
Time spent ruminating, min/d	463.36	456.12	10.77	0.61
Time spent resting, min/d	403.84	416.34	13.82	0.50
Fresh period (1 to 21 DIM)				
DMI, kg/d	19.36	16.21	0.63	< 0.01
Visits to the feeder, visits/d	74.75	65.54	3.93	0.08
Time spent eating, h/d	121.82	110.68	5.24	0.11
Time spent ruminating, min/d	476.27	496.34	10.16	0.14
Time spent resting, min/d	400.80	411.38	12.45	0.53

[1] Control = Cows fed TMR without yeast-derived microbial protein supplement pre and post-partum; Treatment = Cows fed TMR containing 1% of yeast-derived microbial protein prepartum and TMR containing 1.7% yeast-derived microbial protein postpartum.

Table 2.7. Weekly least square means (± SEM) of DMI, feeding, and resting behaviors from dairy cows (n = 71) fed a TMR without (Control, n = 35) or with a yeast-derived microbial protein supplement (YMP, n = 36) during the prepartum (1 to 3 weeks prepartum) and fresh (1 to 3 weeks postpartum) periods.

	Dietary treatment[1]			
Lactation week	Control (n = 35)	Treatment (n = 36)	SEM	*P-value*
Prepartum period (-3 to -1 week before calving)				
DMI, kg/d				
-3	12.87	11.29	0.69	*NS*
-2	12.82	11.51	0.61	*NS*
-1	10.96	9.66	0.58	*NS*
Visits to the feeder, visits/d				
-3	56.18	49.68	3.92	*NS*
-2	57.56	52.15	3.56	*NS*
-1	52.63	46.8	3.39	*NS*
Time spent eating, min/d				
-3	150.23	137.66	9.11	*NS*
-2	135.37	133.61	8.17	*NS*
-1	115.33	107.17	7.40	*NS*
Time spent ruminating, min/d				
-3	477.88	462.58	14.15	*NS*
-2	460.57	458.74	12.61	*NS*
-1	451.63	447.05	12.30	*NS*
Time spent resting, min/d				
-3	395.80	416.97	16.76	*NS*
-2	404.90	415.76	15.33	*NS*
-1	410.83	416.29	15.02	*NS*
Fresh period (1 to 3 weeks post-calving)				
DMI, kg/d				
1	18.87	16.66	0.86	0.54

Table 2.7 (continued)

2	19.68	15.90	0.75	< 0.01
3	19.54	16.06	0.80	0.01
Visits to the feeder, visits/d				
1	68.51	62.30	4.93	*NS*
2	77.45	65.92	4.49	0.83
3	78.29	68.38	4.68	*NS*
Time spent eating, min/d				
1	116.95	114.17	6.42	*NS*
2	124.24	108.46	5.89	0.70
3	124.26	109.43	6.18	*NS*
Time spent ruminating, min/d				
1	474.35	478.03	13.43	*NS*
2	488.70	516.71	12.23	*NS*
3	465.76	494.27	12.63	*NS*
Time spent resting, min/d				
1	430.70	439.38	16.06	*NS*
2	392.07	406.76	13.80	*NS*
3	379.62	388.01	15.49	*NS*

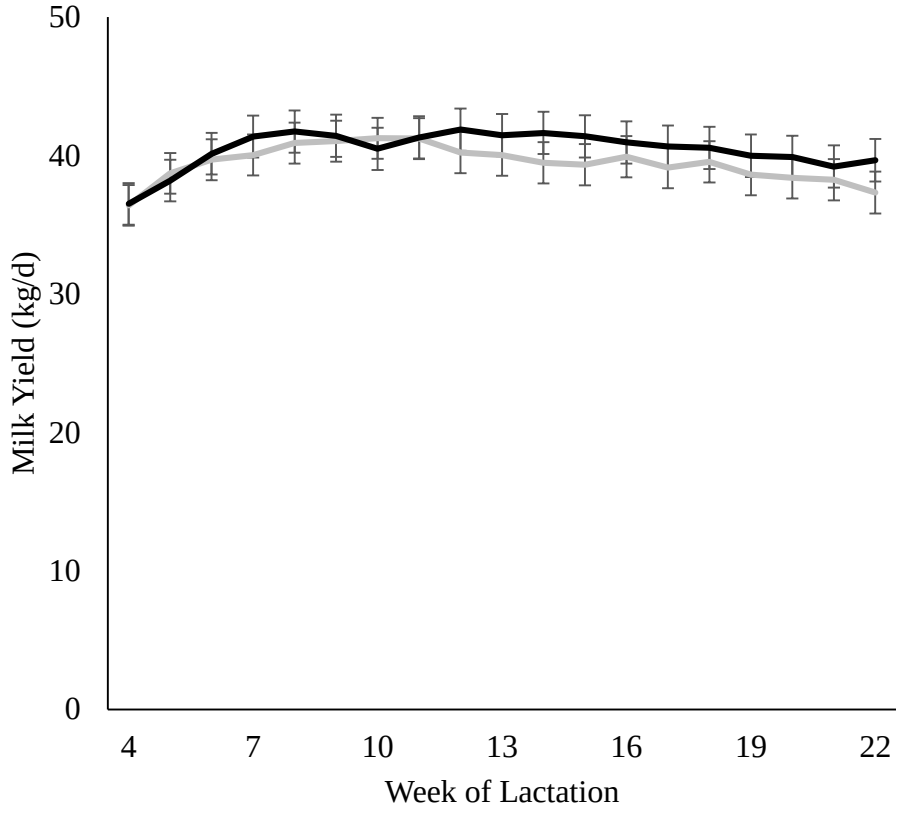
[1] Control = Cows fed TMR without yeast-derived microbial protein supplement pre and post-partum; Treatment = Cows fed TMR containing 1% of yeast-derived microbial protein prepartum and TMR containing 1.7% yeast-derived microbial protein postpartum.

Table 2.8. Weekly least square means (± SEM) of serum calcium and NEFA from dairy cows (n = 71) fed a TMR without or with a yeast-derived microbial protein supplement during the prepartum (1 to 3 weeks prepartum) and fresh (1 to 3 weeks postpartum) periods.

Lactation week	Dietary treatment[1]		SEM	*P-value*
	Control (n = 35)	Treatment (n = 36)		
Serum calcium, mg/dL				
1	9.14	9.23	0.09	*NS*
2	9.35	9.53	0.08	*NS*
3	9.33	9.55	0.08	0.56
Serum NEFA, mmol/L				
1	0.75	0.79	0.06	*NS*
2	0.84	0.79	0.05	*NS*
3	0.84	0.78	0.05	*NS*

[1] Control = Cows fed TMR without yeast-derived microbial protein supplement pre and post-partum; Treatment = Cows fed TMR containing 1% of yeast-derived microbial protein prepartum and TMR containing 1.7% yeast-derived microbial protein postpartum.

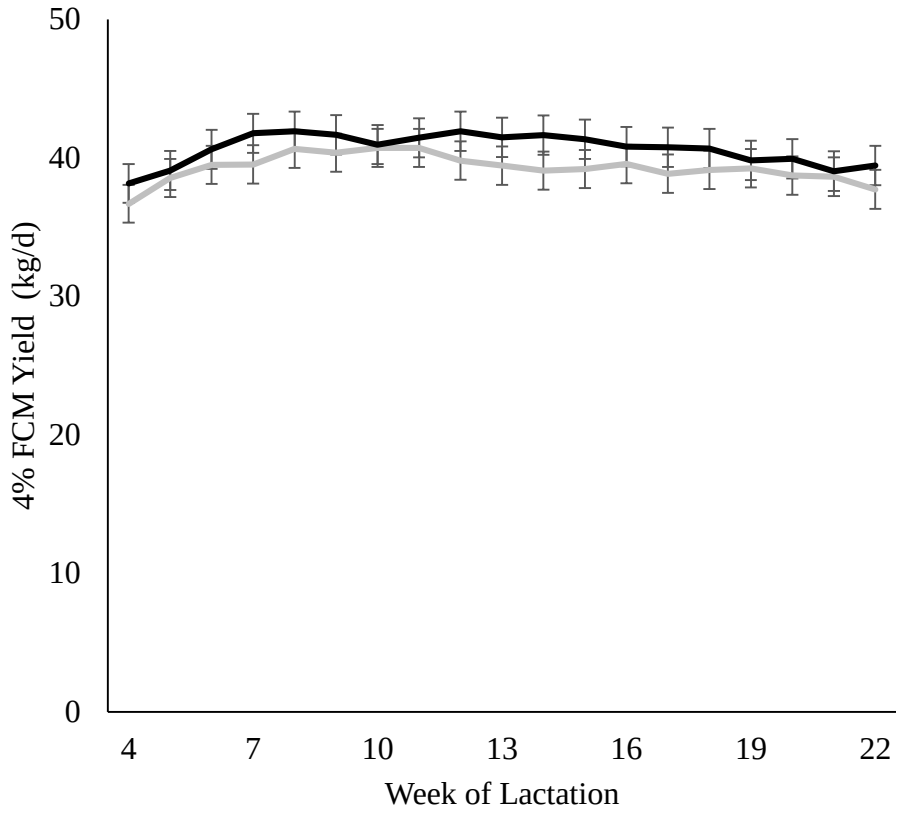
Figure 2.1. Differences in milk yield expressed as least square means ± SEM by lactation week in the post fresh (22 to 150 DIM) period in cows (n = 71) fed a TMR with (YMP, n = 36; black) or without (control, n = 35; gray) yeast-derived microbial protein during the prepartum (1 to 3 weeks prepartum) and fresh (1 to 3 weeks postpartum) periods.



* Indicates that treatments differed on that week ($P < 0.05$).

† Indicates that treatments tended to differ between treatments on that week ($0.05 < P < 0.10$).

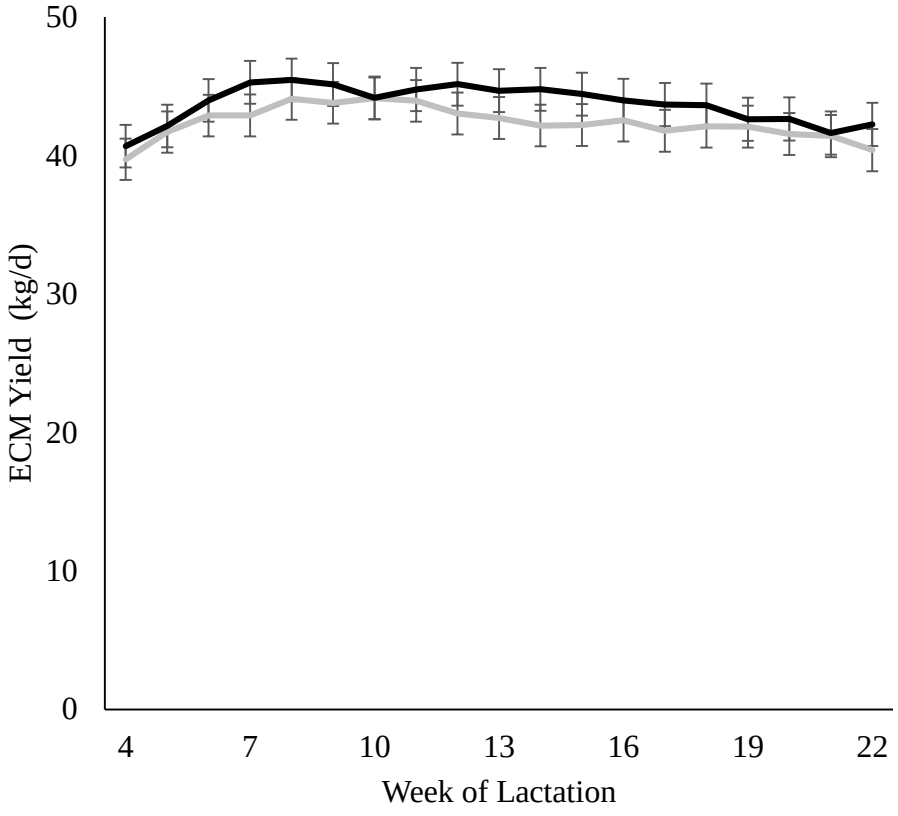
Figure 2.2. Differences in 4% fat corrected milk (4% FCM) expressed as least square means ± SEM by lactation week in the post fresh (22 to 150 DIM) period in cows fed a TMR with (black) or without (gray) yeast-derived microbial protein during the prepartum (1 to 3 weeks prepartum) and fresh (1 to 3 weeks postpartum) periods.



* Indicates that treatments differed on that week ($P < 0.05$).

† Indicates that treatments tended to differ between treatments on that week ($0.05 < P < 0.10$).

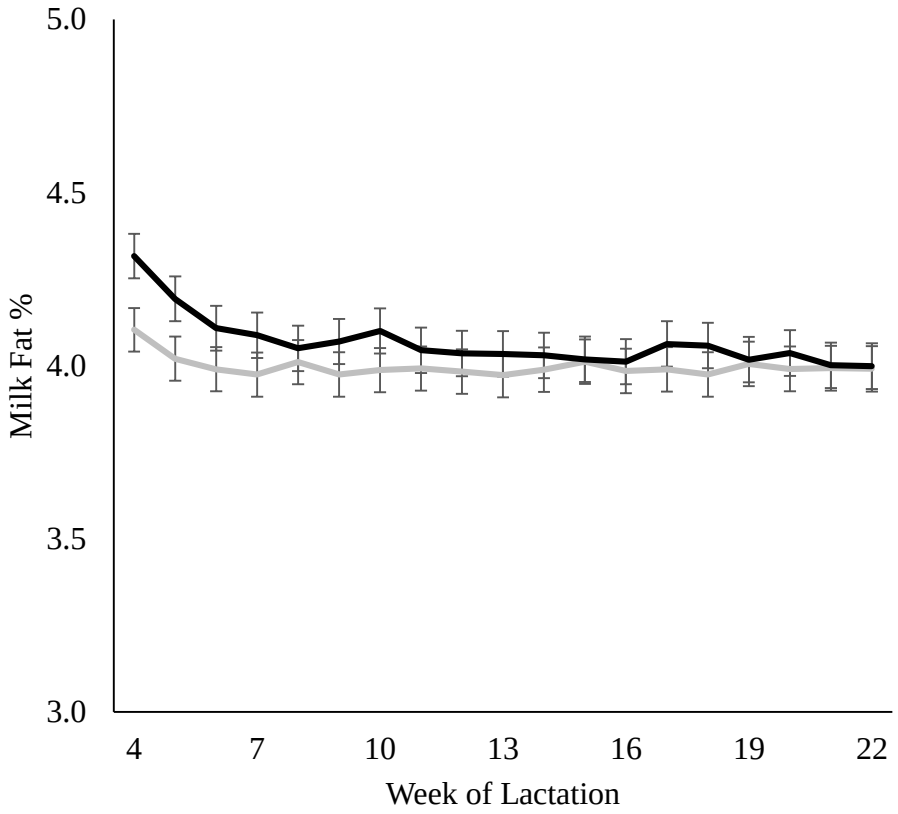
Figure 2.3. Differences in energy corrected milk (ECM) expressed as least square means ± SEM by lactation week in the post fresh (22 to 150 DIM) period in cows fed a TMR with (black) or without (gray) yeast-derived microbial protein during the prepartum (1 to 3 weeks prepartum) and fresh (1 to 3 weeks postpartum) periods.



* Indicates that treatments differed on that week ($P < 0.05$).

† Indicates that treatments tended to differ between treatments on that week ($0.05 < P < 0.10$).

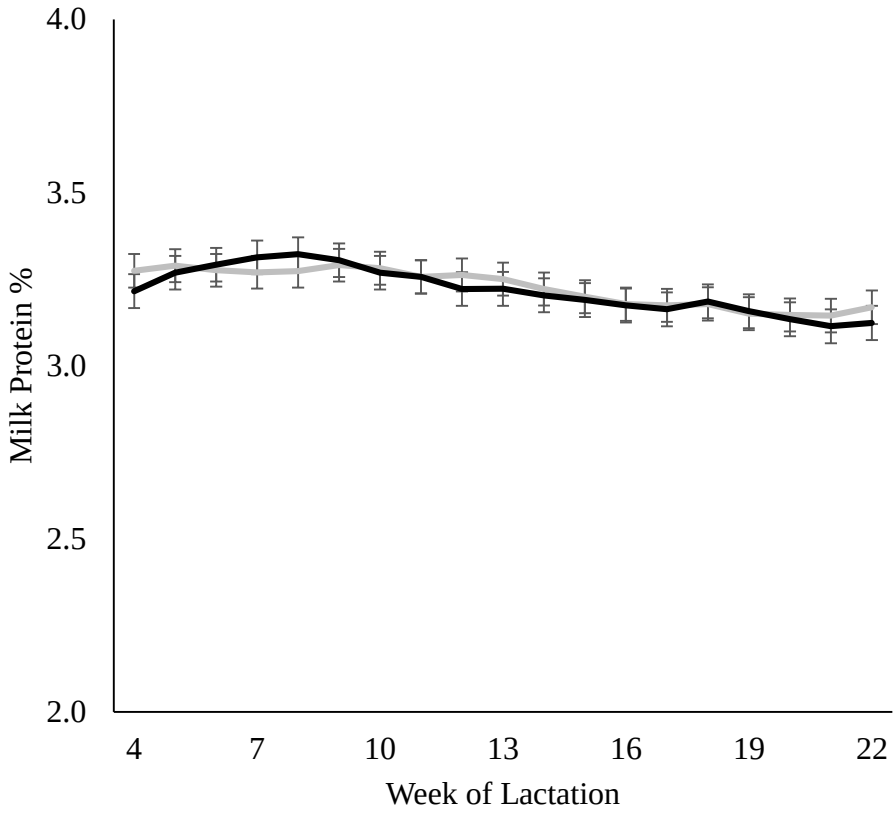
Figure 2.4. Differences in milk fat percentage expressed as least square means ± SEM by lactation week in the post fresh (22 to 150 DIM) period in cows fed a TMR with (black) or without (gray) yeast-derived microbial protein during the prepartum (1 to 3 weeks prepartum) and fresh (1 to 3 weeks postpartum) periods.



* Indicates that treatments differed on that week ($P < 0.05$).

† Indicates that treatments tended to differ between treatments on that week ($0.05 < P < 0.10$).

Figure 2.5. Differences in milk protein percentage expressed as least square means ± SEM by lactation week in the post fresh (22 to 150 DIM) period in cows fed a TMR with (black) or without (gray) yeast-derived microbial protein during the prepartum (1 to 3 weeks prepartum) and fresh (1 to 3 weeks postpartum) periods.



* Indicates that treatments differed on that week ($P < 0.05$).

† Indicates that treatments tended to differ between treatments on that week ($0.05 < P < 0.10$).

CHAPTER THREE

Summary of results

Effects of yeast-derived microbial protein on transition dairy cow health and performance

SUMMARY OF RESULTS

Dairy producers and nutritionists are commonly reluctant to try alternative protein sources in the dairy herd diets. Mostly because the inclusion of alternative protein sources, such as yeast-derived microbial protein in dairy cow diets in some cases are less cost effective or hard to source than using classic protein sources such as soybean meal. However, the inclusion of other sources of proteins, such as yeast-derived microbial protein, in dairy cow diets might be done in a strategic form to achieve some objectives, such as feeding a different amino acid profile to a sensitive animal, like during the transition period.

During the transition period, cows tend to have low dry matter intake, which, combined with an increase in nutrient requirements, leaves the animal in a negative energy balance state. Yeast-derived microbial protein is an alternative source of protein that partially bypasses the rumen, providing the animal with a good amino acid profile to be absorbed in the intestine. Providing a good source of amino acids for the transition dairy cow is essential to decrease the prevalence of metabolic disorders and increase performance throughout the lactation.

The strategic use of alternative protein sources, such as yeast-derived microbial protein in transition dairy cow diets might become vastly adopted in the dairy industry. The original research study presented in this thesis showed that cows fed yeast-derived microbial protein had lower dry matter intake than control-fed cows while maintaining similar milk yield and metabolic status. Therefore, we concluded that yeast-derived microbial protein has the potential to be strategically used used as an alternative protein source in transition dairy cow diets.

FUTURE RESEARCH

Future research should focus on the economic benefits of feeding yeast-derived microbial protein to transition dairy cows. Projects utilizing economic models should evaluate if the reduction in dry matter intake and maintenance of milk yield of transition cows fed yeast-derived microbial protein is economically viable. Furthermore, the mechanisms that caused the observed reduction in dry matter intake of transition cows fed yeast-derived microbial protein is still unknown. Therefore, studies investigating the effects of this alternative protein source on rumen VFA kinetics and dry matter digestibility should be conducted. In addition, studies investigating the bioavailability of the amino acids and how the amino acids from yeast-derived microbial protein play a role in the energy partitioning mechanisms in the transition dairy cow should be conducted.

REFERENCES

Agle, M., A. Hristov, S. Zaman, C. Schneider, P. Ndegwa, and V. Vaddella. 2010. The effects of ruminally degraded protein on rumen fermentation and ammonia losses from manure in dairy cows. Journal of dairy science 93(4):1625-1637.

Ali, A. and G. Shook. 1980. An optimum transformation for somatic cell concentration in milk. Journal of Dairy Science 63(3):487-490.

Allen, M. S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. Journal of dairy science 83(7):1598-1624.

Allison, R. and R. Laven. 2000. Effect of vitamin E supplementation on the health and fertility of dairy cows: a review. Veterinary Record 147(25):703-708.

Amory, J. R., P. Kloosterman, Z. E. Barker, J. L. Wright, R. W. Blowey, and L. E. Green. 2006. Risk Factors for Reduced Locomotion in Dairy Cattle on Nineteen Farms in The Netherlands. Journal of Dairy Science 89(5):1509-1515.

Argyle, J. and R. Baldwin. 1989. Effects of amino acids and peptides on rumen microbial growth yields. Journal of Dairy Science 72(8):2017-2027.

Bach, A., S. Calsamiglia, and M. D. Stern. 2005. Nitrogen Metabolism in the Rumen*. Journal of Dairy Science 88:E9-E21.

Bell, A. W. 1995. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. Journal of animal science 73(9):2804-2819.

Benzaquen, M. E., C. A. Risco, L. F. Archbald, P. Melendez, M. J. Thatcher, and W. W. Thatcher. 2007. Rectal Temperature, Calving-Related Factors, and the Incidence of Puerperal Metritis in Postpartum Dairy Cows. Journal of Dairy Science 90(6):2804-2814.

Bikker, J., H. Van Laar, P. Rump, J. Doorenbos, K. Van Meurs, G. Griffioen, and J. Dijkstra. 2014. Evaluation of an ear-attached movement sensor to record cow feeding behavior and activity. Journal of dairy science 97(5):2974-2979.

Bolinder, A., B. Seguin, H. Kindahl, D. Bouley, and D. Otterby. 1988. Retained fetal membranes in cows: Manual removal versus nonremoval and its effect on reproductive performance. Theriogenology 30(1):45-56.

Borchers, M., Y. Chang, I. Tsai, B. Wadsworth, and J. Bewley. 2016. A validation of technologies monitoring dairy cow feeding, ruminating, and lying behaviors. Journal of dairy science 99(9):7458-7466.

Borucki Castro, S. I., L. E. Phillip, H. Lapierre, P. W. Jardon, and R. Berthiaume. 2007. Ruminal Degradability and Intestinal Digestibility of Protein and Amino Acids in Treated Soybean Meal Products. Journal of Dairy Science 90(2):810-822.

Borucki Castro, S. I., L. E. Phillip, H. Lapierre, P. W. Jardon, and R. Berthiaume. 2008. The Relative Merit of Ruminal Undegradable Protein from Soybean Meal or Soluble Fiber from Beet Pulp to Improve Nitrogen Utilization in Dairy Cows. Journal of Dairy Science 91(10):3947-3957.

Brito, A. and G. Broderick. 2007. Effects of different protein supplements on milk production and nutrient utilization in lactating dairy cows. Journal of Dairy Science 90(4):1816-1827.

Can, A. and A. Yilmaz. 2002. Usage of xylose or glucose as non-enzymatic browning agent for reducing ruminal protein degradation of soybean meal. Small ruminant research 46(2-3):173-178.

Castro, S. B., L. Phillip, H. Lapierre, P. Jardon, and R. Berthiaume. 2007. Ruminal degradability and intestinal digestibility of protein and amino acids in treated soybean meal products. Journal of Dairy Science 90(2):810-822.

Chapinal, N., M. Carson, T. F. Duffield, M. Capel, S. Godden, M. Overton, J. E. P. Santos, and S. J. LeBlanc. 2011. The association of serum metabolites with clinical disease during the transition period. Journal of Dairy Science 94(10):4897-4903.

Chapinal, N., D. M. Veira, D. M. Weary, and M. A. G. von Keyserlingk. 2007. Technical Note: Validation of a System for Monitoring Individual Feeding and Drinking Behavior and Intake in Group-Housed Cattle. Journal of Dairy Science 90(12):5732-5736.

Clark, J., T. Klusmeyer, and M. Cameron. 1992. Microbial protein synthesis and flows of nitrogen fractions to the duodenum of dairy cows. Journal of dairy science 75(8):2304-2323.

Clark, J. H. and C. L. Davis. 1980. Some Aspects of Feeding High Producing Dairy Cows1. Journal of Dairy Science 63(6):873-885.

Colucci, P., L. Chase, and P. Van Soest. 1982. Feed intake, apparent diet digestibility, and rate of particulate passage in dairy cattle. Journal of Dairy Science 65(8):1445-1456.

Cook, N. B. and K. V. Nordlund. 2004. Behavioral needs of the transition cow and considerations for special needs facility design. Veterinary Clinics: Food Animal Practice 20(3):495-520.

Curtis, C. R., H. N. Erb, C. J. Sniffen, R. D. Smith, and D. S. Kronfeld. 1985. Path analysis of dry period nutrition, postpartum metabolic and reproductive disorders, and mastitis in Holstein cows. Journal of dairy science 68(9):2347-2360.

DeGaris, P. J. and I. J. Lean. 2008. Milk fever in dairy cows: A review of pathophysiology and control principles. The Veterinary Journal 176(1):58-69.

DeVries, T. J., M. A. G. von Keyserlingk, D. M. Weary, and K. A. Beauchemin. 2003. Measuring the Feeding Behavior of Lactating Dairy Cows in Early to Peak Lactation. Journal of Dairy Science 86(10):3354-3361.

Dirksen, G., H. Liebich, and E. Mayer. 1985. Adaptive changes of the ruminal mucosa and their functional and clinical significance. Bovine Pract 20:116-120.

Drackley, J. K., H. M. Dann, N. Douglas, N. A. J. Guretzky, N. B. Litherland, J. P. Underwood, and J. J. Loor. 2005. Physiological and pathological adaptations in dairy cows that may increase susceptibility to periparturient diseases and disorders. Italian Journal of Animal Science 4(4):323-344.

Drackley, J. K., T. R. Overton, and G. N. Douglas. 2001. Adaptations of glucose and long-chain fatty acid metabolism in liver of dairy cows during the periparturient period. Journal of Dairy Science 84:E100-E112.

Drillich, M., O. Beetz, A. Pfützner, M. Sabin, H.-J. Sabin, P. Kutzer, H. Nattermann, and W. Heuwieser. 2001. Evaluation of a systemic antibiotic treatment of toxic puerperal metritis in dairy cows. Journal of Dairy Science 84(9):2010-2017.

Drillich, M., U. Reichert, M. Mahlstedt, and W. Heuwieser. 2006. Comparison of Two Strategies for Systemic Antibiotic Treatment of Dairy Cows with Retained Fetal Membranes: Preventive vs. Selective Treatment. Journal of Dairy Science 89(5):1502-1508.

Duffield, T. 2000. Subclinical Ketosis in Lactating Dairy Cattle. Veterinary Clinics of North America: Food Animal Practice 16(2):231-253.

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

Edwards, J. E., N. R. McEwan, A. J. Travis, and R. J. Wallace. 2004. 16S rDNA library-based analysis of ruminal bacterial diversity. Antonie Van Leeuwenhoek 86(3):263-281.

Esslemont, R. and M. Kossaibati. 2002. The costs of poor fertility and disease in UK dairy herds: trends in DAISY herds over 10 seasons. Intervet.

Ferguson, J. D., D. T. Galligan, and N. Thomsen. 1994. Principal descriptors of body condition score in Holstein cows. Journal of dairy science 77(9):2695-2703.

Fourichon, C., H. Seegers, and X. Malher. 2000. Effect of disease on reproduction in the dairy cow: a meta-analysis. Theriogenology 53(9):1729-1759.

Friggens, N. 2003. Body lipid reserves and the reproductive cycle: towards a better understanding. Livestock Production Science 83(2-3):219-236.

Garnsworthy, P. C. and J. H. Topps. 2010. The effect of body condition of dairy cows at calving on their food intake and performance when given complete diets. Animal Science 35(1):113-119.

Gerloff, B. J. 2000. Dry Cow Management for the Prevention of Ketosis and Fatty Liver in Dairy Cows. Veterinary Clinics of North America: Food Animal Practice 16(2):283-292.

Goff, J. P. 2006. Major Advances in Our Understanding of Nutritional Influences on Bovine Health. Journal of Dairy Science 89(4):1292-1301.

Goff, J. P. 2008. The monitoring, prevention, and treatment of milk fever and subclinical hypocalcemia in dairy cows. The Veterinary Journal 176(1):50-57.

Goldhawk, C., N. Chapinal, D. M. Veira, D. M. Weary, and M. A. G. von Keyserlingk. 2009. Prepartum feeding behavior is an early indicator of subclinical ketosis. Journal of Dairy Science 92(10):4971-4977.

Gordon, J. L., S. J. LeBlanc, and T. F. Duffield. 2013. Ketosis treatment in lactating dairy cattle. Veterinary Clinics of North America: Food Animal Practice 29(2):433-445.

Grant, R. and J. Albright. 2001. Effect of animal grouping on feeding behavior and intake of dairy cattle. Journal of dairy science 84:E156-E163.

Greenfield, R. B., M. J. Cecava, T. R. Johnson, and S. S. Donkin. 2000. Impact of Dietary Protein Amount and Rumen Undegradability on Intake, Peripartum Liver Triglyceride, Plasma Metabolites, and Milk Production in Transition Dairy Cattle1. Journal of Dairy Science 83(4):703-710.

Gröhn, Y. T. and P. J. Rajala-Schultz. 2000. Epidemiology of reproductive performance in dairy cows. Animal Reproduction Science 60-61:605-614.

Grummer, R. R. 1995. Impact of changes in organic nutrient metabolism on feeding the transition dairy cow. Journal of Animal Science 73(9):2820-2833.

Grummer, R. R. 2008. Nutritional and management strategies for the prevention of fatty liver in dairy cattle. The Veterinary Journal 176(1):10-20.

Grummer, R. R., D. G. Mashek, and A. Hayirli. 2004. Dry matter intake and energy balance in the transition period. Veterinary Clinics: Food Animal Practice 20(3):447-470.

Guard, C. 1994. Costs of clinical disease in dairy cows. Page 100 in Proc. Proc. Ann. Cornell Conf. Vet. Cornell Univ., Ithaca, NY.

Hall, M. B., W. H. Hoover, J. P. Jennings, and T. K. M. Webster. 1999. A method for partitioning neutral detergent-soluble carbohydrates. Journal of the Science of Food and Agriculture 79(15):2079-2086.

Herdt, T. H. 2000. Ruminant Adaptation to Negative Energy Balance. Veterinary Clinics of North America: Food Animal Practice 16(2):215-230.

Higginson, V., B. Baurhoo, Y. Schuermann, R. Duggavathi, and A. Mustafa. 2018. Effects of yeast-derived microbial protein on lactation performance and metabolic status of transition dairy cows. Animal Feed Science and Technology 246:18-28.

Horst, R. L., J. P. Goff, T. A. Reinhardt, and D. R. Buxton. 1997. Strategies for Preventing Milk Fever in Dairy Cattle1,2. Journal of Dairy Science 80(7):1269-1280.

Huzzey, J., T. Duffield, S. LeBlanc, D. Veira, D. Weary, and M. Von Keyserlingk. 2009. Haptoglobin as an early indicator of metritis. Journal of dairy science 92(2):621-625.

Huzzey, J., D. Veira, D. Weary, and M. Von Keyserlingk. 2007. Prepartum behavior and dry matter intake identify dairy cows at risk for metritis. Journal of dairy science 90(7):3220-3233.

Ingvartsen, K. L. 2006. Feeding- and management-related diseases in the transition cow: Physiological adaptations around calving and strategies to reduce feeding-related diseases. Animal Feed Science and Technology 126(3–4):175-213.

Ingvartsen, K. L. and J. B. Andersen. 2000. Integration of Metabolism and Intake Regulation: A Review Focusing on Periparturient Animals. Journal of Dairy Science 83(7):1573-1597.

Ingvartsen, K. L., R. J. Dewhurst, and N. C. Friggens. 2003. On the relationship between lactational performance and health: is it yield or metabolic imbalance that cause production diseases in dairy cattle? A position paper. Livestock Production Science 83(2):277-308.

Jones, D. B. 1931. Factors for converting percentages of nitrogen in foods and feeds into percentages of protein. USDA Circ. 183:1-21.

Kaniyamattam, K. and A. De Vries. 2014. Agreement between milk fat, protein, and lactose observations collected from the Dairy Herd Improvement Association (DHIA) and a real-time milk analyzer. Journal of Dairy Science 97(5):2896-2908.

Kaufmann, W. and W. Lüpping. 1982. Protected proteins and protected amino acids for ruminants. Pages 36-75 in Protein contribution of feedstuffs for ruminants. Elsevier.

Kehrli, M. E., B. J. Nonnecke, and J. A. Roth. 1989a. Alterations in bovine lymphocyte function during the periparturient period. American Journal of Veterinary Research 50(2):215.

Kehrli, M. E., B. J. Nonnecke, and J. A. Roth. 1989b. Alterations in bovine neutrophil function during the periparturient period. American journal of veterinary research 50(2):207.

Kelton, D. F., K. D. Lissemore, and R. E. Martin. 1998. Recommendations for recording and calculating the incidence of selected clinical diseases of dairy cattle. Journal of dairy science 81(9):2502-2509.

Kimura, K., T. A. Reinhardt, and J. P. Goff. 2006. Parturition and Hypocalcemia Blunts Calcium Signals in Immune Cells of Dairy Cattle1. Journal of Dairy Science 89(7):2588-2595.

Lean, I. J., R. Van Saun, and P. J. DeGaris. 2013. Energy and Protein Nutrition Management of Transition Dairy Cows. Veterinary Clinics of North America: Food Animal Practice 29(2):337-366.

LeBlanc, S. 2010. Monitoring metabolic health of dairy cattle in the transition period. Journal of reproduction and Development 56(S):S29-S35.

LeBlanc, S., T. Duffield, K. Leslie, K. Bateman, G. P. Keefe, J. Walton, and W. Johnson. 2002. Defining and diagnosing postpartum clinical endometritis and its impact on reproductive performance in dairy cows. Journal of dairy science 85(9):2223-2236.

LeBlanc, S. J. 2008. Postpartum uterine disease and dairy herd reproductive performance: A review. The Veterinary Journal 176(1):102-114.

LeBlanc, S. J., K. E. Leslie, and T. F. Duffield. 2005. Metabolic Predictors of Displaced Abomasum in Dairy Cattle. Journal of Dairy Science 88(1):159-170.

Liang, D., L. M. Arnold, C. J. Stowe, R. J. Harmon, and J. M. Bewley. 2017. Estimating US dairy clinical disease costs with a stochastic simulation model. Journal of Dairy Science 100(2):1472-1486.

Manthey, A. K., K. F. Kalscheur, A. D. Garcia, and K. Mjoun. 2016. Lactation performance of dairy cows fed yeast-derived microbial protein in low- and high-forage diets. Journal of Dairy Science 99(4):2775-2787.

Martinez, N., C. A. Risco, F. S. Lima, R. S. Bisinotto, L. F. Greco, E. S. Ribeiro, F. Maunsell, K. Galvão, and J. E. P. Santos. 2012. Evaluation of peripartal calcium status, energetic profile, and neutrophil function in dairy cows at low or high risk of developing uterine disease. Journal of Dairy Science 95(12):7158-7172.

Martinez, N., L. Sinedino, R. Bisinotto, E. Ribeiro, G. Gomes, F. Lima, L. Greco, C. Risco, K. Galvão, and D. Taylor-Rodriguez. 2014. Effect of induced subclinical hypocalcemia on physiological responses and neutrophil function in dairy cows. Journal of dairy science 97(2):874-887.

McArt, J., D. Nydam, and G. Oetzel. 2012a. A field trial on the effect of propylene glycol on displaced abomasum, removal from herd, and reproduction in fresh cows diagnosed with subclinical ketosis. Journal of dairy science 95(5):2505-2512.

McArt, J. A. A., D. V. Nydam, and G. R. Oetzel. 2012b. Epidemiology of subclinical ketosis in early lactation dairy cattle. Journal of Dairy Science 95(9):5056-5066.

McArt, J. A. A., D. V. Nydam, P. A. Ospina, and G. R. Oetzel. 2011. A field trial on the effect of propylene glycol on milk yield and resolution of ketosis in fresh cows diagnosed with subclinical ketosis. Journal of Dairy Science 94(12):6011-6020.

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

McNamara, S., F. P. O'Mara, M. Rath, and J. J. Murphy. 2003. Effects of Different Transition Diets on Dry Matter Intake, Milk Production, and Milk Composition in Dairy Cows. Journal of Dairy Science 86(7):2397-2408.

Miller, J., E. Brzezinska-Slebodzinska, and F. Madsen. 1993. Oxidative stress, antioxidants, and animal function. Journal of dairy science 76(9):2812-2823.

Mulligan, F. J. and M. L. Doherty. 2008. Production diseases of the transition cow. The Veterinary Journal 176(1):3-9.

Mulligan, F. J., L. O'Grady, D. A. Rice, and M. L. Doherty. 2006. A herd health approach to dairy cow nutrition and production diseases of the transition cow. Animal Reproduction Science 96(3–4):331-353.

Neal, K., J. S. Eun, A. J. Young, K. Mjoun, and J. O. Hall. 2014. Feeding protein supplements in alfalfa hay-based lactation diets improves nutrient utilization, lactational performance, and feed efficiency of dairy cows1. Journal of Dairy Science 97(12):7716-7728.

Neave, H. W., J. Lomb, M. A. G. von Keyserlingk, A. Behnam-Shabahang, and D. M. Weary. 2017. Parity differences in the behavior of transition dairy cows. Journal of Dairy Science 100(1):548-561.

Neave, H. W., J. Lomb, D. M. Weary, S. J. LeBlanc, J. M. Huzzey, and M. A. G. von Keyserlingk. 2018. Behavioral changes before metritis diagnosis in dairy cows. Journal of Dairy Science 101(5):4388-4399.

Noel, R. 2000. Official feed terms. Association of American Feed Control Officials, Official Publication:187-200.

NRC. 2001. Nutrient requirements of dairy cattle: 2001. National Academies Press.

Oetzel, G. R. 2004. Monitoring and testing dairy herds for metabolic disease. Veterinary Clinics of North America: Food Animal Practice 20(3):651-674.

Oetzel, G. R. 2011. Diseases of Dairy Animals | Non-Infectious Diseases: Milk Fever A2 - Fuquay, John W. Pages 239-245 in Encyclopedia of Dairy Sciences (Second Edition). Academic Press, San Diego.

Oetzel, G. R. and B. E. Miller. 2012. Effect of oral calcium bolus supplementation on early-lactation health and milk yield in commercial dairy herds. Journal of Dairy Science 95(12):7051-7065.

Ospina, P. A., J. A. McArt, T. R. Overton, T. Stokol, and D. V. Nydam. 2013. Using Nonesterified Fatty Acids and β-Hydroxybutyrate Concentrations During the Transition Period for Herd-Level Monitoring of Increased Risk of Disease and Decreased Reproductive and Milking Performance. Veterinary Clinics of North America: Food Animal Practice 29(2):387-412.

Ospina, P. A., D. V. Nydam, T. Stokol, and T. R. Overton. 2010. Association between the proportion of sampled transition cows with increased nonesterified fatty acids and β-hydroxybutyrate and disease incidence, pregnancy rate, and milk production at the herd level. Journal of Dairy Science 93(8):3595-3601.

Overton, T. and M. Waldron. 2004. Nutritional management of transition dairy cows: strategies to optimize metabolic health. Journal of dairy science 87:E105-E119.

Overton, T. and T. Yasui. 2014. Practical applications of trace minerals for dairy cattle. Journal of animal science 92(2):416-426.

Pitta, D. W., S. Kumar, B. Vecchiarelli, D. J. Shirley, K. Bittinger, L. D. Baker, J. D. Ferguson, and N. Thomsen. 2014. Temporal dynamics in the ruminal microbiome of dairy cows during the transition period. Journal of Animal Science 92(9):4014-4022.

Potts, S. B., J. P. Boerman, A. L. Lock, M. S. Allen, and M. J. VandeHaar. 2017. Relationship between residual feed intake and digestibility for lactating Holstein cows fed high and low starch diets. Journal of Dairy Science 100(1):265-278.

Proudfoot, K. L., D. M. Veira, D. M. Weary, and M. A. G. von Keyserlingk. 2009. Competition at the feed bunk changes the feeding, standing, and social behavior of transition dairy cows. Journal of Dairy Science 92(7):3116-3123.

Proudfoot, K. L., D. M. Weary, and M. A. G. von Keyserlingk. 2010. Behavior during transition differs for cows diagnosed with claw horn lesions in mid lactation. Journal of Dairy Science 93(9):3970-3978.

Pushpakumara, P., N. Gardner, C. Reynolds, D. Beever, and D. Wathes. 2003a. Relationships between transition period diet, metabolic parameters and fertility in lactating dairy cows. Theriogenology 60(6):1165-1185.

Pushpakumara, P. G. A., N. H. Gardner, C. K. Reynolds, D. E. Beever, and D. C. Wathes. 2003b. Relationships between transition period diet, metabolic parameters and fertility in lactating dairy cows. Theriogenology 60(6):1165-1185.

Rabelo, E., R. L. Rezende, S. J. Bertics, and R. R. Grummer. 2005. Effects of Pre- and Postfresh Transition Diets Varying in Dietary Energy Density on Metabolic Status of Periparturient Dairy Cows. Journal of Dairy Science 88(12):4375-4383.

Rajala, P. J. and Y. T. Gröhn. 1998. Effects of Dystocia, Retained Placenta, and Metritis on Milk Yield in Dairy Cows. Journal of Dairy Science 81(12):3172-3181.

Reinhardt, T. A., J. D. Lippolis, B. J. McCluskey, J. P. Goff, and R. L. Horst. 2011. Prevalence of subclinical hypocalcemia in dairy herds. The Veterinary Journal 188(1):122-124.

Reynolds, C., P. Aikman, B. Lupoli, D. Humphries, and D. Beever. 2003. Splanchnic metabolism of dairy cows during the transition from late gestation through early lactation. Journal of dairy science 86(4):1201-1217.

Reynolds, C. K., B. Dürst, B. Lupoli, D. J. Humphries, and D. E. Beever. 2004. Visceral Tissue Mass and Rumen Volume in Dairy Cows During the Transition from Late Gestation to Early Lactation. Journal of Dairy Science 87(4):961-971.

Rezamand, P., T. A. Hoagland, K. M. Moyes, L. K. Silbart, and S. M. Andrew. 2007. Energy Status, Lipid-Soluble Vitamins, and Acute Phase Proteins in Periparturient Holstein and Jersey Dairy Cows With or Without Subclinical Mastitis. Journal of Dairy Science 90(11):5097-5107.

Royster, E. and S. Wagner. 2015. Treatment of Mastitis in Cattle. Veterinary Clinics of North America: Food Animal Practice 31(1):17-46.

Sabbia, J. A., K. F. Kalscheur, A. D. Garcia, A. M. Gehman, and J. M. Tricarico. 2012. Soybean meal substitution with a yeast-derived microbial protein source in dairy cow diets. Journal of Dairy Science 95(10):5888-5900.

Sailer, K. J., R. S. Pralle, R. C. Oliveira, S. J. Erb, G. R. Oetzel, and H. M. White. 2018. Technical note: Validation of the BHBCheck blood B-hydroxybutyrate meter as a diagnostic tool for hyperketonemia in dairy cows. Journal of Dairy Science 101(2):1524-1529.

Sampson, J., J. Spain, C. Jones, and L. Carstensen. 2009. Effects of calcium chloride and calcium sulfate in an oral bolus given as a supplement to postpartum dairy cows. Veterinary Therapeutics 10(3):131-139.

Santos, F. A. P., J. Santos, C. Theurer, and J. T. Huber. 1998. Effects of rumen-undegradable protein on dairy cow performance: A 12-year literature review. Journal of dairy science 81(12):3182-3213.

Santos, J. E. P., E. J. DePeters, P. W. Jardon, and J. T. Huber. 2001. Effect of Prepartum Dietary Protein Level on Performance of Primigravid and Multiparous Holstein Dairy Cows. Journal of Dairy Science 84(1):213-224.

Satter, L. and L. Slyter. 1974. Effect of ammonia concentration on rumen microbial protein production in vitro. British journal of nutrition 32(2):199-208.

Satter, L. D. 1986. Protein supply from undegraded dietary protein. Journal of Dairy Science 69(10):2734-2749.

Schirmann, K., N. Chapinal, D. M. Weary, W. Heuwieser, and M. A. G. von Keyserlingk. 2012. Rumination and its relationship to feeding and lying behavior in Holstein dairy cows. Journal of Dairy Science 95(6):3212-3217.

Schirmann, K., N. Chapinal, D. M. Weary, L. Vickers, and M. A. G. von Keyserlingk. 2013. Short communication: Rumination and feeding behavior before and after calving in dairy cows. Journal of Dairy Science 96(11):7088-7092.

Schwab, C. G. 1995. Protected proteins and amino acids for ruminants. Biotechnology in animal feeds and animal feeding 141.

Seifi, H. A., S. J. LeBlanc, K. E. Leslie, and T. F. Duffield. 2011. Metabolic predictors of post-partum disease and culling risk in dairy cattle. The Veterinary Journal 188(2):216-220.

Sepúlveda-Varas, P., J. Huzzey, D. Weary, and M. Von Keyserlingk. 2013. Behaviour, illness and management during the periparturient period in dairy cows. Animal production science 53(9):988-999.

Sheehy, M. R., A. G. Fahey, S. P. M. Aungier, F. Carter, M. A. Crowe, and F. J. Mulligan. 2017. A comparison of serum metabolic and production profiles of dairy cows that maintained or lost body condition 15 days before calving. Journal of Dairy Science 100(1):536-547.

Sheldon, I., M. Bushnell, J. Montgomery, and A. Rycroft. 2004. Minimum inhibitory concentrations of some antimicrobial drugs against bacteria causing uterine infections in cattle. Veterinary Record 155(13):383-387.

Sheldon, I. M., G. S. Lewis, S. LeBlanc, and R. O. Gilbert. 2006. Defining postpartum uterine disease in cattle. Theriogenology 65(8):1516-1530.

Sheldon, I. M., E. J. Williams, A. N. A. Miller, D. M. Nash, and S. Herath. 2008. Uterine diseases in cattle after parturition. The Veterinary Journal 176(1):115-121.

Smook, G. A. and M. J. Kocurek. 1982. Handbook for pulp & paper technologists. Canadian Pulp and Paper Association.

Sniffen, C., J. O'connor, P. Van Soest, D. Fox, and J. Russell. 1992. A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. Journal of animal science 70(11):3562-3577.

Socha, M. T., D. E. Putnam, B. D. Garthwaite, N. L. Whitehouse, N. A. Kierstead, C. G. Schwab, G. A. Ducharme, and J. C. Robert. 2005. Improving Intestinal Amino Acid Supply of Pre- and Postpartum Dairy Cows with Rumen-Protected Methionine and Lysine*,†. Journal of Dairy Science 88(3):1113-1126.

Spears, J. W. and W. P. Weiss. 2008. Role of antioxidants and trace elements in health and immunity of transition dairy cows. The Veterinary Journal 176(1):70-76.

Suthar, V. S., J. Canelas-Raposo, A. Deniz, and W. Heuwieser. 2013. Prevalence of subclinical ketosis and relationships with postpartum diseases in European dairy cows. Journal of Dairy Science 96(5):2925-2938.

Thilsing-Hansen, T., R. J. Jørgensen, and S. Østergaard. 2002. Milk Fever Control Principles: A Review. Acta Veterinaria Scandinavica 43(1):1-19.

Tyrrell, H. and P. Moe. 1975. Effect of intake on digestive efficiency. Journal of Dairy Science 58(8):1151-1163.

Van Soest, P. J. 1994. Nutritional ecology of the ruminant. Cornell university press.

Villalobos-F, J., C. Romero-R, M. Tarragó-C, and A. Rosado. 1997. Supplementation with chromium picolinate reduces the incidence of placental retention in dairy cows. Canadian Journal of Animal Science 77(2):329-330.

Wallace, R., G. McCoy, T. Overton, and J. Clark. 1996. Effect of adverse health events on dry matter consumption, milk production, and body weight loss of dairy cows during early lactation. J. Dairy Sci 79(Suppl 1):205.

Williams, E. J., D. P. Fischer, D. U. Pfeiffer, G. C. W. England, D. E. Noakes, H. Dobson, and I. M. Sheldon. 2005. Clinical evaluation of postpartum vaginal mucus reflects uterine bacterial infection and the immune response in cattle. Theriogenology 63(1):102-117.

Zhou, Z., M. Vailati-Riboni, E. Trevisi, J. K. Drackley, D. N. Luchini, and J. J. Loor. 2016. Better postpartal performance in dairy cows supplemented with rumen-protected methionine compared with choline during the peripartal period. Journal of Dairy Science 99(11):8716-8732.

VITA

Gustavo Mazon Correa Alves

EDUCATION

January 2016; **Bachelor of Science in Animal Sciences**
Federal University of Viçosa, Viçosa, Brazil

August 2014 – July 2015; **Exchange student**
University of Kentucky, Lexington, KY

PROFESSIONAL POSITIONS

2016 – Present; **Graduate Research Assistant**
Department of Animal and Food Sciences, University of Kentucky

December 2015 – July 2016; **Milk Quality Supervisor**
Tirolez Cheese, Quintinos, Brazil

SCHOLARSHIPS and AWARDS

2nd place College of Agriculture, Food and Environment Three-Minute Thesis
- April 2019

National Milk Producers Federation Scholarship Recipient
- August 2018

1st place American Dairy Science Association Three-Minute Thesis Challenge
- June 2018

2nd place American Dairy Science Association Southern Branch Dairy Production Oral Competition
- June 2018

3rd place MS oral presentation competition – Tri-State Dairy Nutrition Conference
- April 2018

Brazil Scientific Mobility Program Scholarship Recipient
- August 2014 – June 2015

CONFERENCE ABSTRACTS

Mazon, Gustavo, Magnus R. Campler, and Joao H. C. Costa. 2019. Effects of a yeast-derived microbial protein supplement on fresh cow performance and blood metabolites. Tri-State Dairy Nutrition Conference. Fort Wayne, IN.

Mazon, Gustavo and Joao H. C. Costa. 2018. Measuring reticulorumen pH and temperature in dairy cattle: How often is enough? Evaluation of different recording intervals from an automated reticulorumen bolus. American Society of Animal Science Annual Meeting, Vancouver, Canada.

Mazon, Gustavo, Cerina Holcomb, Jeffrey M. Bewley, and Joao H. C. Costa. 2018. Effects of a *Megasphaera elsdenii* oral drench on rumen pH, feed intake, and milk yield in lactating dairy cows. American Dairy Science Association Annual Meeting, Knoxville, TN.

Megan M. Woodrum, **Gustavo Mazon**, and Joao H. C. Costa. 2018. Automated temperature Reading systems to detect fever in dairy cattle. American Dairy Science Association Annual Meeting, Knoxville, TN.

Carrie P. Cecil, **Gustavo Mazon**, and Joao H. C. Costa. 2018. Comparison between non-dairy milk-like beverages and cow's milk. American Dairy Science Association Annual Meeting, Knoxville, TN.

Mazon, Gustavo, Cerina Holcomb, Jeffrey M. Bewley, and Joao H. C. Costa. 2018. Drenching cows with *Megasphaera elsdenii* improves reticulorumen pH. Tri-Sate Dairy Nutrition Conference, Fort Wayne, IN.

Mazon, Gustavo and Joao H. C. Costa. 2018. Evaluation of different recording intervals from an automated reticulorumen bolus. Animal and Food Sciences Graduate Student Association Poster Symposium, 2018, Lexington, KY.

Megan M. Woodrum, **Gustavo Mazon**, and Joao H. C. Costa. 2018. Automated temperature Reading systems to detect fever in dairy cattle. Southeast Regional American Dairy Science Meeting, 2018, Morgantown, WV.

Carrie P. Cecil, **Gustavo Mazon**, and Joao H. C. Costa. 2018. Comparison between non-dairy milk-like beverages and cow's milk. Southeast Regional American Dairy Science Meeting, 2018, Morgantown, WV.

Mazon, Gustavo, Jenna. Guinn, Derek Nolan, Peter Krawczel, Christina Petersson-Wolfe, Gina Pighetti, A. Stone, S. Ward, M. Marcondes, J. Bewley. 2017. Relationship between cow cleanliness, locomotion, and bulk tank somatic cell count in southeastern United States dairy farms. Abstract T187. Dairy Science Association Annual Meeting. Pittsburgh, PA.