Effects of Yeast-derived Microbial Protein and Live Yeast on the Lactational Performance and Metabolism of Transition Holstein Cows

Valerie Higginson, Department of Animal Science,
Macdonald Campus, McGill University, Montreal

August 2017

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Master of Science

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AA   Amino Acids

ADF   Acid detergent fiber

ADICP Acid detergent insoluble crude protein

AOAC Association of Official Analytical Chemists

AST   Aspartate aminotransferase

BCS   Body condition score

BHBA β-hydroxybutyrate

BUN   Blood urea nitrogen

CDC   Canadian Dairy Commission

CHO   Carbohydrate

CNCPS Cornell net carbohydrate and protein system

CP   Crude protein

DIM   Days in milk

DM   Dry matter

DMI   Dry matter intake

FCM   Fat corrected milk

GLDH Glutamate dehydrogenase

MUN   Milk urea nitrogen

NDICP Neutral detergent insoluble crude protein

NE$_L$ Net energy for lactation

NEB   Negative energy balance

NEFA Non-esterified fatty acids

NNB   Negative nutrient balance

NRC   National Research Council

SAS   statistical analysis method

SCM   Solids corrected milk

SNF   Solids-not-fat

TAG   Triacylglycerol

TMR   Total mixed ration

TS   Total solids

YMP Yeast microbial protein

YMPL Yeast microbial protein and live yeast

ABSTRACT

The current study evaluated the effects of a yeast-derived microbial protein (**YMP**) and live yeast on the health and lactational performance of Holstein cows during their transition period. Experiment 1 examined the effects of supplementing YMP alone, whereas Experiment 2 examined YMP fed in combination with live yeast (*Saccharomyces cerevisiae*). Both studies commenced 21 days prior to expected calving and ended 28 days postpartum. Cows were blocked according to parity and expected calving dates. Both studies evaluated the effects of treatments on milk production and composition, dry matter intake (**DMI**), body condition score (**BCS**) and serum biochemical parameters, macrominerals and proteins. In Experiment 1, twenty-seven primiparous and multiparous Holstein cows were randomly assigned to either a treated (**YMP**; 50 g YMP prepartum followed by 200 g YMP postpartum) or control (0 g YMP). In Experiment 2, cows were randomly assigned to either a **YMPL** (100g YMP and 10g live yeast prepartum followed by 200g YMP and 10g live yeast postpartum) or control (0 g YMP or live yeast) group. In both studies, DMI and milk yield were recorded daily in the postpartum period. Milk samples were obtained twice weekly while BCS evaluation and blood collections were performed on d   -21, -14, -7, -3 and -1 relative to expected calving dates and d 1, 3, 7, 14, 21 and 28 postpartum.

In Experiment 1, there was no effect of YMP on DMI, BCS milk yield or milk composition. However, YMP supplementation significantly reduced serum non-esterified fatty acids (**NEFA**) concentration from d 3 postpartum until the end of the study. Serum concentrations of β-hydroxybutyrate (**BHBA**) were also reduced in YMP-supplemented cows on d 3 and 7 postpartum. Moreover, YMP supplementation significantly increased serum glucose levels on d 3 and 7 postpartum, and reduced serum aspartate transaminase (**AST**) levels on d 14 and 21 postpartum.

In Experiment 2, YMP and live yeast supplementation did not affect DMI, BCS or milk performance. Serum concentration of BHBA was not affected by YMPL supplementation. However, YMPL cows had higher serum NEFA concentrations on d 3 and 14 postpartum whereas serum AST concentrations were higher on d 1, 3 and 7 postpartum. This was accompanied by higher serum glutamate dehydrogenase (**GLDH**) and lower haptoglobin levels in YMPL cows on d 7 and 14 postpartum.

From Experiment 1, it was concluded that YMP supplementation to transition cows may significantly reduce metabolic stress leading to less adipose tissue mobilization and improved hepatic health without any negative impacts on milk performance. In Experiment 2, however, YMPL supplementation appeared to increase metabolic stress in transition cows as indicated by higher serum NEFA levels postpartum. However, serum NEFA levels decreased rapidly in YMPL cows as lactation progressed, suggesting that fat mobilization was not excessive. These conflicting results suggests that the mode of YMP supplementation (pellet or powder) may have a direct effect on its efficacy and that feeding YMP in combination with live yeast during the transition period merits further investigation.

ABRÉGÉ

Cette étude a pour but d'évaluer les effets d'une protéine microbienne extraite de la levure (YMP) et la levure vive pour la santé et la performance lactionnelle des vaches Holstein pendant leur période de transition. Durant la première étude, nous avons étudié les effets de suppléments YMP et durant la deuxième étude, nous avons examiné la consommation de YMP avec la levure vive (*Saccharomyces cerevisiae*). Les deux études ont débuté 21 jours avant la date de vêlage et se sont terminés 28 jours après le vêlage. Les vaches ont été bloquées selon leurs dates prévues de vêlage et parité. Les deux études ont évalué la composition ainsi que la production de lait, la composition de matières sèches (CMS), l'état de chair (BCS), les indicateurs métaboliques sériques, macro-mineraux, et protéines. Durant la première étude, vingt-sept vaches Holstein primipare and multipare ont été assignées de recevoir soit une granule contrôle (0g YMP) ou avec YMP (50g YMP avant le vêlage suivi de 200g YMP après le vêlage). Durant la deuxième étude, les vaches ont été assignées de recevoir la diète de YMP avec la levure vive (YMPL; 100g YMP et 10g levure vive avant le vêlage ainsi que 200g YMP et 10g levure vive après le vêlage) ou dans le groupe de contrôle (0g YMP ou la levure vive). Durant les deux études le rendement de lait et le CMS ont été enregistrés à chaque jour pendant la période après velage. Les échantillons de lait ont été obtenus deux fois par semaine, tandis que les BCS ont été évalués et les échantillons de sérum sanguin ont été obtenus les jours -21, -14, -7, -3 et -1 relatifs aux jours de vêlage prévues et aux jours 1, 3, 7, 14, 21 et 28 après le vêlage.

Les résultats de la première étude démontrent que l'alimentation de YMP n'a eu aucun effet sur CMS non plus le rendement de lait, la composition de lait ou le BCS. Toutefois, le YMP a eu des effets très positifs sur le métabolisme des vaches. En effet, le YMP a considérablement réduit ($P < 0.05$) les concentrations d'acides gras non estérifiés (NEFA) du sang à partir de 3 jours après le vêlage jusqu'à la fin de l'étude. De plus, le YMP a significativement réduit ($P < 0.05$) les niveaux de β-hydroxybutyrate (βHBA) à 3 et 7 post-partum. Les concentrations de glucose été plus élevées à 3 et 7 post-partum tandis que les niveaux d'aspartate transaminase été plus bas à 14 et 21 jours après le vêlage chez les vaches ayant reçu le YMP. Au finale, nos résultats de la première étude démontrent que le YMP peut améliorer de manière très significative le métabolisme (c.-à-d. moins de

mobilisation de réserves corporelles) des vaches en transition surtout pendant la période post-partum, sans effet négatif sur la production de lait.

Les résultats de la deuxième étude démontrent que l'alimentation de YMPL n'a eu aucun effet sur CMS non plus le rendement de lait, la composition de lait ou le BCS. Le YMPL à considérablement augmenté ($P < 0.05$) les concentrations de NEFA du sang à partir des jours 3 et 14 après le vêlage. Mais, le YMPL n'a pas eu d'effets ($P < 0.05$) les niveaux de BHBA ou de glucose. Les niveaux d'aspartate transaminase était plus haut à 3, 7 et 14 jours après le vêlage chez les vaches ayant reçu le YMPL et le glutamate déshydrogénase (GLDH) ont été plus haut les jour 7 et 14 après le vêlage. Mais, les niveaux de GLDH ont resté plus pas que 50 U/L, indiquant que les vaches sont restés dans le niveau normal pour les vaches après le vêlage. Les résultats de la deuxième étude démontrent que le YMPL augmentent le stress métabolique après le vêlage. Mais, les niveaux de NEFA et BHBA ont baisé très rapidement après le vêlage donc, cela indique que le montant de mobilisation des réserves corporelles n'a pas été trop extrême.

Finalement, les résultats de nos deux études démontrent que le YMP seule ou en avec de la levure vive n'a pas d'effets sur le CMS, la performance lactationelle ou le BCS. Le YMP réduit le stress métabolique après le vêlage et peut améliorer la sante du foie. Mais, le YMP avec la levure vive augmentent la mobilisation des réserves corporelles. Les résultats conflictuels suggèrent que le mode de supplémentation de YMP (en granules ou en poudres) peut jouer un rôle clé dans son efficacité et que la supplémentation de YMP avec la levure vive durant la période de transition mérite plus d'étude.

"Let your dreams stay big and your worries stay small." – Rascal Flatts

Acknowledgments

CONTRIBUTION OF AUTHORS

Authors of the presented manuscript

Valerie Higginson, Dr. Bushansingh Baurhoo, Yasmin Schuermann, Dr. Raj Duggavathi and Dr. Arif Mustafa

Dr. Arif Mustafa and Dr. Bushansingh Baurhoo designed experiments, reviewed the manuscript and supervised the primary author. Valerie Higginson designed and conducted all experiments, analyzed data and wrote the manuscript. Yasmin Schuermann assisted with sample collection and data analysis. Dr. Raj Duggavathi assisted with data analysis and reviewed the manuscript.

Chapter 1. GENERAL INTRODUCTION

The Canadian dairy industry is operated under supply management (**CDC**, 2016). Dairy producers are rewarded for superior milk yield and it is in their best interest to maintain high production while reducing expenses. As a result, high producing Holstein cows have become the preferred breed among Canadian producers. Holsteins contribute to about 93% of the total dairy cow population in 2015 (CDC, 2016). Since 1994, daily milk yield per cow has increased by approximately 375%. For this reason, Canadian dairy farms consist of less than half of the cow population than it did over 50 years ago whereas yearly milk production has nearly doubled (Capper et al., 2009). The dairy industry is held to a high standard and therefore considerable research is devoted to the Canadian dairy sector in order to further progress (CDC, 2016).

Such a major increase in cow productivity over the past years has been accompanied by a significant increase in health, reproduction and nutritional problems for the cow. For example, today, metabolic health disorders are of major concern to the industry, compromising cow productivity. Metabolic disorders such as displaced abomasum, ketosis, hepatic lipidosis and metritis mainly occur around time of calving. In transition cows, these disorders occur as a result of a severe negative nutrient balance (i.e. energy and protein), especially postpartum. At this time, cows experience a sudden drop in dry matter intake (**DMI**), whereas energy and protein demands, which are required for parturition and onset of milk synthesis, are extremely high. Up to 50% of cows may suffer from a metabolic health disorder during their transition period (Leblanc, 2010), leading to significant financial burden to the producer and compromised welfare of the animal. The transition period of a cow is usually defined as 3 weeks prepartum to 3 weeks postpartum.

Extensive research on nutritional management of transition cows has attempted to minimize metabolic health disorders (Hayirli et al., 2002; Mullins et al., 2012; Zaworski et al., 2014). Nevertheless, incidence of metabolic disorders among transition cows remains high, and therefore merits further investigation. Most studies have focused on negative energy balance whereas little scientific attention has been given to dietary supplementation with protein and amino acids (AA) as a dietary strategy to mitigate metabolic disorders in transition cows. Both negative energy and protein balance is, however, of equal importance which brings forth the subject of the current study.

1

Chapter 2. LITERATURE REVIEW

2.1 OVERVIEW OF THE TRANSITION COW

The transition period is the most critical and demanding time during the dairy cow's life cycle. The transition period is often defined as 21 days prepartum until 21 days postpartum (Grummer, 1995). According to Drackley (1999), success of a cow during this period subsequently determines her productivity during the rest of that lactation. The transition cow, however, is presented with many challenges that could compromise this success. Environmental stressors as well as the inability to consume sufficient quantities of dry matter (**DM**) to meet cow's nutritional requirements can lead to compromised health (Grummer et al., 2004; Contreras and Sordillo, 2011;).

A decline in dry matter intake (DMI) during the transition period is well documented (Van Suan and Sniffen 1996; Drackley, 1999; Grummer et al., 2004). Marquardt et al. (1979) reported that DMI can drop by as much as 72% for multiparous cows at 1 day postpartum when compared to 14 days prepartum. According to a more recent report, multiparous cows may decrease DMI by 30% at 2 days before calving. Healthy cows usually take 1 to 2 days postpartum until DMI begins to increase (Goff and Horst, 1997). This characteristic DMI drop around parturition is mainly attributed to nutritional, environmental and hormonal stress. Best outcomes occur when stress to transition cows is minimized around time of parturition and consumption of normal DM levels is encouraged (Grummer et al., 2004). However, because of high variability among individual cows and between farms, defining an efficient management or nutritional strategy specific for the transition period is somewhat difficult (Sundrum, 2015).

2.2 NEGATIVE NUTRIENT BALANCE

Low DMI of periparturient cows is coupled with exceptionally high nutrient demands during this stage (Drackley, 1999). Colostragenesis, fetal growth, mammary tissue synthesis and lactogenesis require considerable nutrient supply from the animal while endocrinological changes facilitate the repartitioning of nutrients away from maternal needs to that of the calf and milk production (Figure 2.1) (Bell 1995; Grummer et

2

al., 2004). The gradual DMI increase postpartum occurs too slowly to match such high nutrient demands (Grummer et al., 2004; Gordon et al., 2013). Grummer et al. (2004) stated that it seemed self-contradictory to have such low DMI at a time when nutrient requirements are so high. Consequently, transition cows experience a negative nutrient balance (NNB) in both energy and protein (Drackley, 1999). Figure 2.2 illustrates that both net energy and metabolizable protein requirements are greater than what the cow is capable of consuming at 4 days in milk (**DIM**). Much of what she does consume is used by the mammary gland.
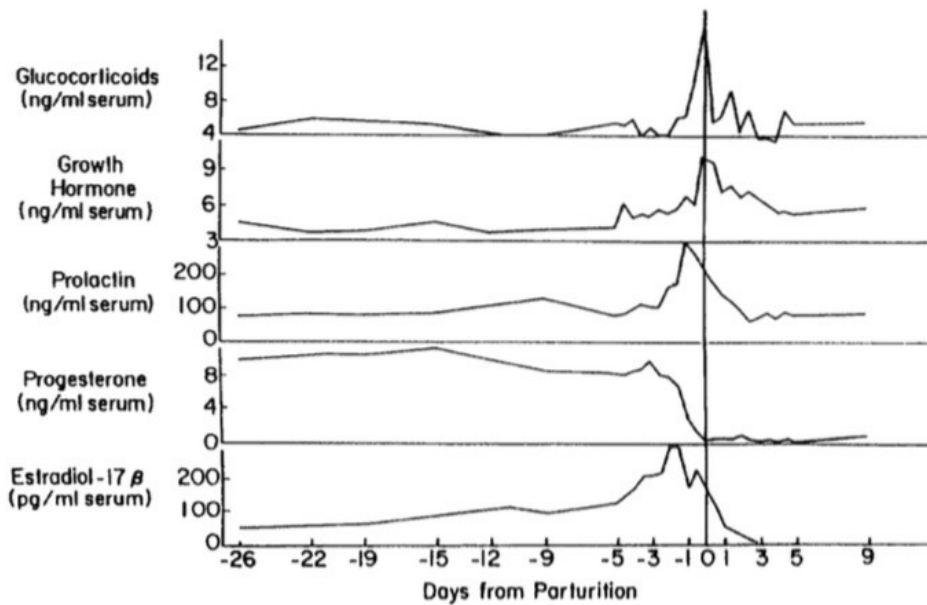
Figure 2.1 Hormonal changes in serum around parturition (Bell 1995)
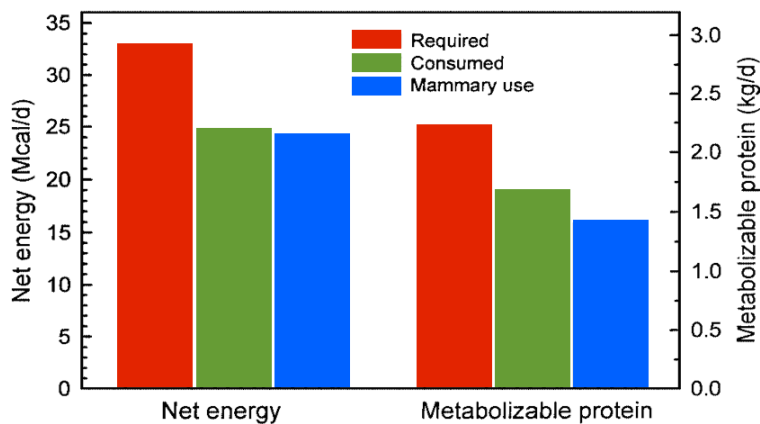
Figure 2.2 Net energy and metabolizable required (red), consumed (green) and used by the mammary gland (blue) at 4 DIM (Adapted from Drackley 1999).

3

2.3 ENERGY

2.3.1 *Requirements*

According to the NRC (2001), energy requirements for dairy cows are expressed as net energy for lactation (**NE$_L$**). The NE$_L$ for a Holstein cow in late gestation is estimated to be 14.4 Mcal/day and increases to 27.9 Mcal/day for fresh cows (NRC, 2001). A fresh cow with low DMI cannot meet such energy requirements which leads to a negative energy balance (NEB) (Figure 2.3).
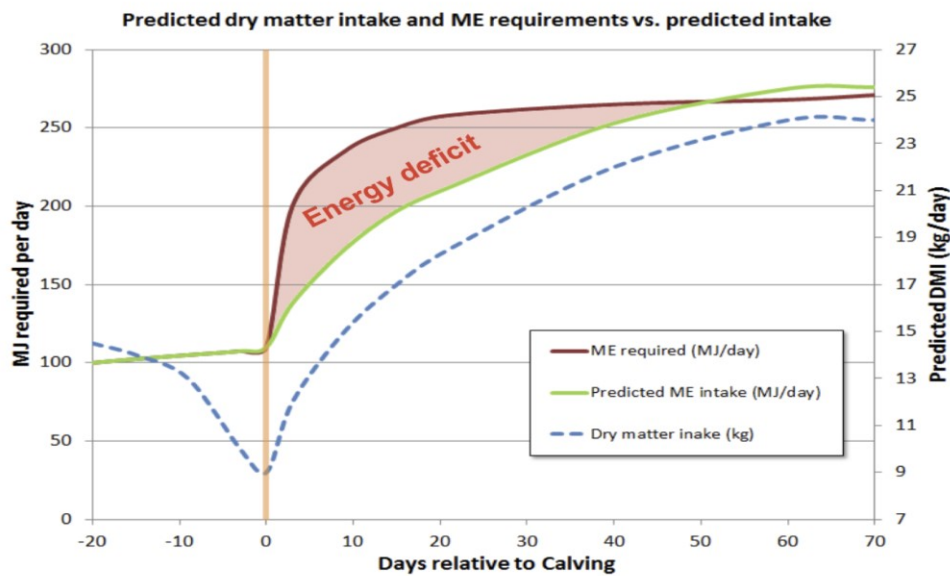
Figure 2.3 Mega joules (MJ) required per day, predicted dry matter intake and the energy deficit during the transition period (Adapted from Manning and Husband, 2014)

2.3.2 *Negative Energy Balance*

Negative energy balance is a primary challenge for the transition cow. Although NEB is a normal occurrence for all postpartum cows, it may result in lower production throughout that lactation if DMI does not begin to increase within 4-5 days postpartum. Negative energy balance results in triglyceride mobilization from adipose tissue and release of non-esterified fatty acids (**NEFA**) into circulation (Drackley, 1999). On average, cows may mobilize 54kg of body fat within 5 weeks after calving (Komaragiri and Erdman, 1997).

During periods of low DMI and severe NEB, blood NEFA levels can increase significantly because of fat mobilization (Drackley, 1999). A small concentration of NEFA is directed to the mammary gland as a milk precursor (Herdt and Gerloff, 2009) whereas the majority is taken up by the liver (Figure 2.4; Drackley, 1999). In the liver, NEFA may

4

undergo re-esterification into triacylglycerides (**TAG**) (Herdt and Gerloff, 2009). In the ruminant liver, TAG are exported at a low rate as very-low density lipoproteins (**VLDL**). However, TAG that cannot be exported promptly may accumulate in the liver during periods of high hepatic NEFA uptake (Bauchart et al., 1996). A second fate of NEFA in the liver is to undergo oxidation. Complete NEFA oxidation requires propionate (fed state) and will result in CO_2 production while partial oxidation in the absence of propionate during conditions of NEB produces ketone bodies (i.e. BHBA). Ketone bodies can enter the blood to be used as an energy source by many tissues (Bauchart et al., 1996; Drackley, 1999). However, these pathways of NEFA metabolism in the liver support conditions that facilitate development of metabolic disorders such hepatic lipidosis and ketosis (Herdt and Gerloff, 2009). These are discussed further below.
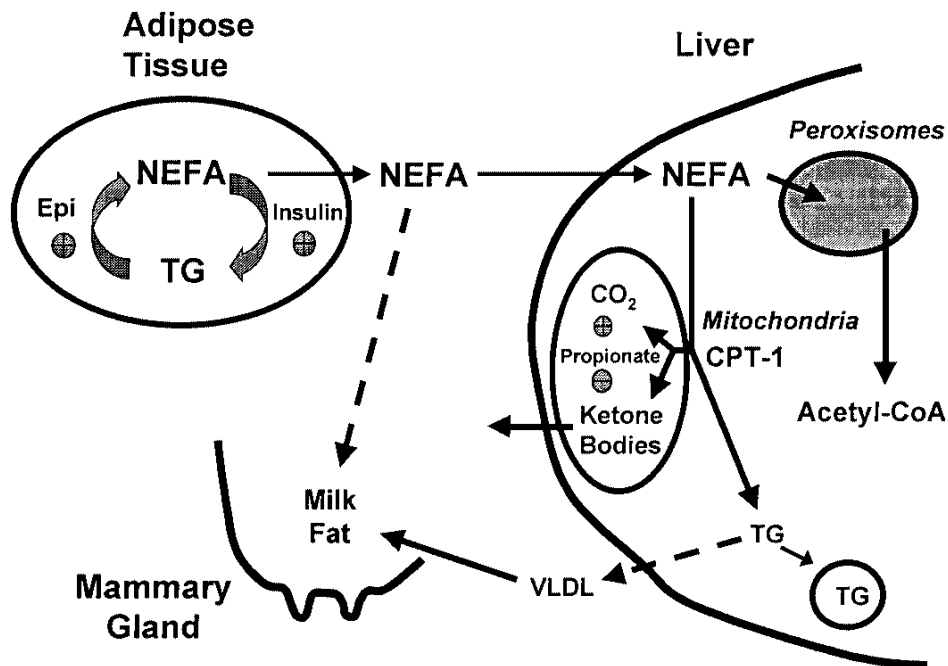
Figure 2.4 Fate of NEFA mobilized from adipose tissue during negative energy balance (Adapted from Drackley 1999)

2.4. PROTEIN

Protein is as essential a nutrient for cow maintenance and production as energy (Van Suan et al., 1993). Periparturient negative protein balance has historically been overshadowed by NEB, however negative protein balance has recently received more attention (Larsen et al., 2014). In a study by Komaragiri and Erdman (1997), cows lost 21kg of body protein within the first 5 weeks postpartum as a direct result of skeletal muscle mobilization to support lactogenesis and gluconeogenesis. In mature cows, this occurrence should be nearly non-existent (Overton and Burhans, 2013). Protein catabolism during the transition period can worsen the metabolic problems experienced by transition cows and may further lead to ketosis. Poor lactational and reproductive performance may be a direct result of protein deficiency or a secondary result of metabolic disorders (Van Suan and Sniffen, 1996). However, protein requirements for transition cows are currently not well defined (Van Suan and Sniffen, 1996).

2.4.1. *Protein Requirements*

Protein can be divided into two main categories: rumen undegradable protein (**RUP**) and rumen degradable protein (**RDP**) (Bach et al., 2005). Sufficient RDP is essential for microbial growth and MCP synthesis while RUP should be supplemented to optimize post-ruminal AA absorption (Van Soest, 1994; NRC, 2001; Sabbia et al., 2012).

The NRC (2001) recommends that prepartum diets should contain 12% crude protein (**CP**) (2.8% RUP). Feeding CP levels below this recommendation reduced DMI and milk yield relative to feeding 100% of the recommendation (Chew et al., 1984). However, increasing CP levels above NRC (2001) recommendation had no significant effect on cow performance (Van Suan and Sniffen, 1995; Huyler et al., 1999). Van Suan et al. (1993) fed two levels of CP (12.4 and 15.3%) with different levels of RUP (27 and 39% of CP, respectively) to prepartum cows. The authors found that cows fed high CP and RUP had greater body condition score at calving and milk protein percentage during the first five weeks postpartum. In contrast, Komaragiri and Erdman (1997) reported feeding a 19% CP diet to transition cows two weeks prepartum reduced milk yield and DMI during the first seven weeks postpartum compared with feeding a 16% CP diet. Similarly, Greenfield et al. (2000) found that multiparous cows receiving 12% CP (3.12% RUP) had increased milk yield and DMI until 56 days in lactation when compared to cows receiving 16% CP prepartum. High RUP prepartum (6.2% versus 4%) may also decrease DMI and milk yield postpartum (Hartwell et al., 2000). Lower lactational performance with increased %CP may be caused by higher ammonia levels, which requires energy to be metabolized into urea (Canfield et al., 1990; Santos et al., 2001). It is clear that much debate remains over total %CP and RDP:RUP for prepartum cows which is likely affected by untested dietary interactions.

For primiparous cows, the NRC (2001) recommends 15% CP with no specificity for RDP and RUP. Van Suan et al. (1993) found that prepartum heifers fed a diet containing either 15.3% CP (2.55% RUP) or 12.4% CP (2.18% RUP) had no effect on postpartum DMI or BCS. However, Santos et al. (2001) found that feeding a 14.7% CP diet versus a 12.7% CP diet to primiparous cows increased milk yield postpartum, suggesting that the NRC recommendation may overestimate requirements for primiparous cows. Multiparous cows in this study had lower 3.5% FCM and milk yield over the 305-d lactation period when fed the high CP diet prepartum. Primiparous cows generally have lower milk yield and therefore a lower energy deficit postpartum which may allow them to efficiently metabolize higher RDP levels than multiparous cows (Canfield, 1990).

For postpartum diets, the NRC (2001) recommends CP levels between 17.5% (10.5% RDP and 7.5% RUP) and 19.5% (10.5% RDP and 9.5% RUP). However, protein metabolized from tissue postpartum complicates postpartum dietary CP requirement (Weich et al., 2016). Research on dietary CP level in the immediate postpartum period is scarce. Few studies have reported that high producing cows in early lactation have greater yield when fed increasing amount of RUP with constant CP percentage (Cunningham, 1996; Baker et al., 1999; Kalscheur et al., 1999). When increasing RUP in isonitrogenous diets of early lactation cows, Hoffman et al. (1991) reported no significant differences on lactation performance. Larsen et al. (2014) abomasally infusion casein into postpartum cows and found that the treated group had significantly increased milk yield. Similar results were found by Ørskov et al. (1977). It is evident from the vast variation in results of feeding different CP and RDP:RUP levels, the source of protein and other nutrient content of the diet will largely affect the results.

2.4.2 *Amino acid requirements & importance*

The current NRC for dairy cattle does not define amino acid requirements. However, supplying a quality protein that contains a proper amino acid profile for the dairy cow is essential (Schwab, 2012). Lysine and methionine are known to be the first 2 limiting amino acids (**AA**) for dairy cows (NRC, 2001). When RDP is sufficient, AA absorbed post-ruminally will support milk production (Sabbia et al., 2012). According to Clark et al. (1992), AA supplemented in the diet should complement the MCP profile (Table 2.1).

Amino acids are one of the major gluconeogenic precursors for fresh cows and can be converted into fatty acids or immediate energy sources (Herdt, 1988; NRC, 2001). While propionate is the major gluconeogenic precursor during the fed state, amino acids contribute to approximately 20% of glucose synthesis during the first 30 days postpartum as cows do not consume sufficient quantities of DM (Herdt, 1988; Lean and DeGaris, 2010). Moreover, AA play a key role in maintaining proper immune functions, especially in postpartum animals. Transportation of fatty acids, macrophage regulation, signaling molecules and killing pathogens is a small list of AA contributions to an immune response (Li et al., 2007). Methionine, also has a key role in VLDL assembly which can be of great importance for the transition cow to reduce the severity and incidence of hepatic lipidosis (Hartwell et al., 2000; Osorio et al., 2013)

Table 2.1. Amino Acid profile of MCP. Adapted from Sabbia et al. (2012)

Amino Acid	MCP, % of total essential AA
Arg	10.2
His	4.0
Ile	11.5
Leu	16.3
Lys	15.8
Met	5.2
Phe	10.2
Thr	11.7
Typ	2.7
Val	12.5

2.4.3 *Protein metabolism in the transition cow*

The rumen contains a large and diverse microbiome consisting mostly of bacteria that aid in digestion (Indugu et al., 2016). Rumen health is important to dairy cows as changes in the microbial population can result in improper absorption of nutrients that will negatively impact cow performance (Zebeli et al., 2015).

Bach et al. (2005) classifies N metabolism in the rumen as two main events: protein degradation to provide N to microbes and MCP synthesis. In the rumen, RDP from concentrate and forages are broken down by microbes (Ørskov, 1992). Initially bacteria adhere to protein and extracellular breakdown occurs, resulting in peptides and AA (NRC, 2001; Bach et al., 2005). Non-protein N from the diet are used for microbial growth in the rumen (Bach et al., 2005).

Protein degradation rate depends on the rumen microflora population (Bach et al., 2005). Rumen passage rate has an inverse relationship with degradation and solubility, and therefore dietary composition also largely affects digestibility of protein and other nutrients (Van Soest, 1994; Bach et al., 2005). When the amount of N required by rumen microbes is exceeded, excess RDP from the diet is excreted (Bach et al., 2005).

When energy is available in the rumen, ammonia, peptides and AA as the primary breakdown products are used to synthesize microbial protein that leaves the rumen and is transported to the small intestine (Tamminga, 1979). Up to 80% of the total protein

supplied to the small intestine is derived from MCP synthesis (Bach, 2005). Moreover, MCP provides an ideal AA profile compared to dietary proteins (Kalscheur et al., 2006).

2.4.4 *Protein metabolism during NEB*

When the cow is in NEB, microbes may not have sufficient energy for MCP synthesis (Clark et al., 1992). Low MCP synthesis and low RDP intake causes the cow to have an inadequate AA supply (Kalscheur et al, 2006). During NEB, dietary supplementation of RUP with a proper AA profile will help to reduce the deficiency, by supplying AA directly into the intestine without requiring energy for MCP synthesis (NRC, 2001; Sabbia et al., 2012). Therefore, for cows in NEB, a supply of protein that can escape rumen degradation is beneficial (Van Soest, 1994).

2.5. METABOLIC DISORERS IN TRANSITION COWS

When nutrient intake from the diet does not meet body requirements, the transition cow falls into a NNB as previously discussed. Under such conditions, the transition cow is at great risk for several periparturient metabolic disorders (Suthar et al., 2013). Up to 50% of cows will suffer from a metabolic disorder in the transition period (Leblanc, 2010). The complex interrelationships of transition cow disorders are illustrated in Figure 2.5. It is known that metabolic disorders in transition cows are commonly linked to decreased DMI (Leblanc, 2010).
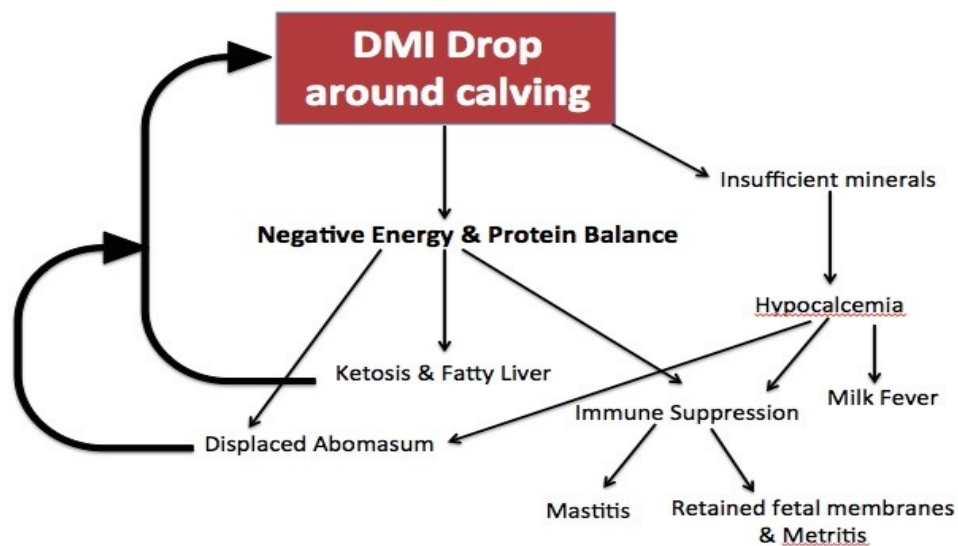
Figure 2.5. Interrelationships of transition cow metabolic disorders. Adapted from Goff (2006)

Among all metabolic disorders, displaced abomasum (DA) may be the most common for fresh cows. Up to 90% of DA cases occur within the first month after calving (Shaver, 1997). During the transition period when DMI is low, the risk for DA is greater. Low forage consumption, especially in herds fed by partial mixed ration, also increases the risk of a DA (Shaver, 1997; Goff, 2006).

The pathways for NEFA metabolism in the liver as previously described support conditions that facilitate occurrence of fatty liver and ketosis (Bauchart et al., 1996). These are two intricately linked postpartum disorders caused directly by a NNB. Oxidization of NEFA in the liver can result in high blood BHBA levels (Herdt and Gerloff, 2009). When BHBA levels become high, this can cause subclinical (SCK, 1.2- 2.9mmol/L BHBA) or clinical ketosis (>2.9mmol/L BHBA), further decreasing DMI (Oetzel, 2004; McArt et al., 2013). McArt et al. (2013) reported that prevalence of SCK is highest by 5 DIM. Cows diagnosed with SCK before 7 DIM are also more likely to develop a DA, produce less milk and had higher incidence of being removed from the herd (McArt et al., 2013).

Reesterification of NEFAs in the liver leads to TAG formation. Because of low VLDL export rate from the liver and high levels of adipose mobilization that occurs around the time of calving, TAG accumulation in the liver can occur rapidly. Within 48 hours of calving, TAG accumulation may increase from 5% of the total liver weight to 25% (Herdt

and Gerloff, 2009). Fatty liver can impair liver function including gluconeogenesis and ammonia detoxification (Herdt and Gerloff, 2009).

When the liver undergoes TAG accumulation this causes hepatocyte damage. Subsequently, a rise in gene expression for enzymes associated with liver damage will be observed, namely aspartate amino transferase (**AST**) and glutamate dehydrogenase (**GLDH**)(Gonzalez et al., 2010). The AST is a hepatocyte leakage enzyme that is not specific to the liver and can also be seen with skeletal or cardiac muscle damage (Gonzalez et al., 2010; Ok et al., 2013). However, when high AST is accompanied with high GLDH levels, it is often assumed to be reflective of hepatocyte damage. The GLDH is a liver-specific enzyme expressed when there is total hepatocyte death and therefore the levels of GLDH will not increase to the same extent as AST (Ok et al., 2013). Mostafavi et al. (2013) reported that cows with blood AST >120 U/L had a 2.9-fold increased risk of incurring fatty liver. Ok et al. (2013) and Mostafavi et al. (2013) claimed that high blood NEFA, AST and GLDH levels are indicative of fatty liver. Cows with fatty liver are also likely to have lower serum cholesterol levels, reflecting low TAG export from the liver (Mostafavi et al., 2013).

Postpartum immunosuppression can increase the risks for infections such as mastitis and metritis (Esposito et al., 2013). It is known that nutrition plays a vital role in immunological soundness. According to a study by Huzzey et al. (2007), cows that contracted metritis postpartum had decreased DMI during the first 2 weeks prepartum compared to their healthy counterparts. Nutritional management is a factor contributing to mastitis infections as cows in NEB have impaired udder defense mechanisms (Suriyasathaporn et al., 2000; Heinrichs et al., 2009).

2.5.1 Consequences of metabolic disorders in transition cows

For dairy producers, transition cows with one or more metabolic disorders present a financial cost (Mulligan and Doherty, 2008). Average costs of the most common metabolic disorders in transition cow are summarized in Table 2.2. These disorders have direct treatment costs and are also associated with lower milk production, cow replacement and decreased reproductive success in many cases (Geishauser et al., 1995; Leblanc et al. 2005). Eicker (1997) reported that cows diagnosed with a DA produced 350kg less of milk in the month following treatment than those without a DA.

Considerable improvements in nutrition and production practices are required in order to address early lactation disorders in cows. Dairy producers have recognized that these health issues also decrease their animals' welfare (Sundrum, 2015). Inflammatory responses leading to chronic issues and disorders that may require surgery and drug administration causes stress and physical discomfort to the animal. Optimizing cow welfare will not only improve animal welfare but to maintain positive public perception of the agricultural industry (Mulligan and Doherty, 2008).

Table 2.2. Costs of metabolic disorders considering milk discards, veterinary treatment, reproductive and culling losses.

Disorder	Occurrence	Cost per cow ($)	Cost per herd (100 cows, USD)
Milk fever	4	275	1100
Displaced Abomasum	4	494	1976
Ketosis	14	232	3248
Retained Placenta	15	315	4725
Mastitis	20	224	8960

Adapted from Guard (2008).

2.6 TRANSITION PERIOD MANAGEMENT

Many strategies have been tested and applied in the dairy industry to manage and prevent metabolic disorders in transition cows.

2.6.1 *Body Condition Score*

On commercial dairy farms, producers can use BCS assessment as a management tool (Vasseur, 2013). A widely used BCS system has been developed by Wildman et al. (1982) and includes a 5-point scale with 0.25 increments. The NRC (2001) recommends that dry cows should not gain BW except for fetal development. Cows losing significant BCS during transition (>1) or for being over-conditioned (≥BCS 4) have greater risks for incidences of ketosis and fatty liver postpartum, likely due to lower DMI (Wildman, 1982; Shaver, 1997; Hayirili et al., 2002; Lean and DeGaris, 2010; Shin et al., 2015). Over-conditioned cows

have lower blood leptin levels. Leptin induces voluntary feed intake (Esposito et al., 2013). Hayrili et al. (2002) observed that obese cows (BCS>4) had the lowest DMI prepartum when compared to thin (<3) or medium (3 ≤ BCS ≤ 4) cows.

2.6.2 *Current nutritional supplements used on commercial farms*

With increased milk production demands, the dairy industry is constantly evolving to augment cow performance through the use of feed additives and dietary manipulation.

2.6.2.1 *Ionophore antibiotics*

Monensin is a commonly used ionophore in dairy nutrition. It is supplemented to increase propionate production in the rumen which can lead to increased gluconeogenesis (Van Maanen et al., 1978; Mullins et al., 2012). Duffield et al. (1998) administered a controlled release monensin capsule to cows 3 weeks prepartum and observed significant reductions in BHBA levels compared to control cows during the first 3 weeks postpartum. The authors also found lower BCS losses and higher blood glucose in treated than control cows. When Mullins et al. (2012) fed monensin as a top dress 21 days before and after expected calving date, blood BHBA level and liver TAG accumulation were significantly reduced whereas meal frequency was increased in treated than control cows. However, recently, because of consumer demands, the use of ionophore antibiotics in dairy industry has been considerably reduced in North America and banned in Europe.

2.6.2.2 *Rumen protected AA*

The mammary extracts lysine and methionine greater than other AA (Bremmer et al., 1997). When feeds contain low levels of lysine and methionine, dietary supplementation with these amino acids may positively impact the cow (Schwab, 2012). Osorio et al. (2013) supplemented cows with a rumen protected methionine product from 21 days prepartum to 30 DIM, and observed significant increase in DMI from day 7 to 21 postpartum. In midlactation cows, Bremmer et al. (1997) supplemented a similar methionine and lysine product and observed increased milk fat percentage.

2.7 YEAST SUPPLEMENTATION TO DAIRY COWS

Various forms of yeast have been supplemented into the diets of dairy cows to enhance performance.

2.7.1 *Yeast fermentation products*

Yeast products have been used as a rumen buffer and to possibly alter ruminal fermentation, improving milk yield and DMI (Jouany et al., 2006). Zaworski et al. (2014) supplemented either 0g, 56g or 112g per day of a *Saccharomyces cerevisiae* fermentation product (SCFP) to multiparous cows from 4 weeks prepartum until 4 weeks postpartum and observed lower SCC and higher milk production, lactose and milk protein when compared to control cows. The authors also reported higher serum BHBA in cows fed SCFP than control ones at day 3 postpartum.

2.7.2 *Live Yeast*

Live yeast is supplemented into the diets of dairy cows to enhance feed digestion in the rumen. Because aerobic yeast requires oxygen, these will attach to newly ingested feed particles and consume the oxygen surrounding the particles. This creates an enhanced environment for the indigenous anaerobic bacteria in the rumen (Jouany et al., 2006). In mid-lactation cows, Dehghan-Banadaky et al. (2017) reported higher milk fat percentage and serum glucose in cows supplemented with 4g live yeast for 35 days. Similarly, Moallem et al. (2009) reported increased DMI, 4% FCM and milk fat percentage in midlactation cows supplemented with 1g of live yeast /4kg of DMI. Dann et al. (2000) supplemented a group of periparturient cows with *Saccharomyces cerevisiae* culture (SC) 30 days prepartum and observed improved DMI from 7 days prepartum until 42 days postpartum but milk yield was not affected. Similar results have been found by others (Robinson and Garret, 1999; Ramsing et al., 2009; Schingoethe et al., 2004). In transition cows, greater DMI during the first week of lactation was reported by Nocek et al (2003) when these were fed live yeast from 21 days prepartum until 10 weeks postpartum. In a similar study, Nocek et al. (2006) observed greater milk yield and DMI postpartum when cows were supplemented with live yeast in contrast to those not supplemented.

2.7.3 *Yeast Derived Microbial Protein*

Yeast derived microbial protein (YMP) is considered a rumen-escape protein, associated with the liquid phase in the rumen due to its small particle size (Sabbia et al., 2012). Interestingly, YMP has an ideal AA profile for the dairy cow that closely resembles that of MCP (Table 2.3; Sabbia et al., 2012). As previously discussed, unlike MCP synthesis which is dependent on energy availability in the rumen, a protein source that can reach the intestines during a time of NNB may be beneficial to the transition cow. Therefore, YMP may have a double advantage considering its physical by-pass mechanism and ideal amino acid profile.

In a study conducted by Sabbia et al. (2012), 16 midlactation, multi- and primiparous cows were fed a high-forage TMR and SBM was partially replaced with YMP at 0, 1.14, 2.28 and 3.41% DM. This study reported increased plasma BHBA concentration and a tendency for increased plasma glucose concentration in YMP-fed cows. The study also showed that SBM replacement with YMP at 1.14 and 3.41% DM increased DMI and that cows had the highest BW change. There was also a tendency for greater milk fat percentage in cows fed 1.14% of YMP. In another study with multiparous cows in early lactation, Neal et al. (2014) examined YMP supplementation either alone or in combination with slow release urea. YMP was supplemented at 1.14% DM in order to partially replace SBM and canola meal so that all diets were isonitrogenous. The authors found that YMP supplementation decreased DM but increased overall feed efficiency. Manthey et al. (2016) conducted a study with 16 midlactation cows (multiparous and primiparous). Dietary treatments included 4 low fiber and high fiber diets where YMP partially replaced SBM, soybean hulls and mechanically extracted SBM to maintain isonitrogenous and isoenergetic diets. Manthey et al. reported that YMP supplementation to mid-lactation cows decreased feed efficiency and did not affect milk performance.

However, until today, there is no reported study that has investigated YMP in transition cows. As discussed above, YMP may supply AA directly into the cow's intestine due to its theoretical mechanism of escaping ruminal degradation. Therefore, YMP may possibly reduce protein deficiency in postpartum cows as well as provide additional substrates for gluconeogenesis (Lean and DeGaris, 2010). For the transition cow, this may reduce the negative nutrient balance, increase performance and reduce the incidence of metabolic disorders postpartum. For the first time, the current study will investigate the effects of YMP and live yeast on the performance of periparturient cows.

Table 2.3. Amino Acid composition of yeast microbial protein (YMP), soybean meal (SBM and microbial crude protein (MCP). Adapted from: Sabbia et al. (2012)

AA, % of total essential AA	Yeast microbial protein	Soybean Meal	Microbial Crude Protein
Lys	16.0	13.9	15.8
Met	3.6	3.2	5.2
Arg	10.9	16.2	10.2
His	5.1	6.1	4.0
Ile	11.1	10.1	11.5
Leu	17.6	17.2	16.3

Chapter 3. EFFECTS OF YEAST-DERIVED MICROBIAL PROTEIN ON LACTATIONAL PERFORMANCE AND METABOLIC STATUS OF TRANSITION DAIRY COWS[1]

V. Higginson*, B. Baurhoo*†, Y. Schuermann*, R. Duggavathi* and A. F. Mustafa*

*Department of Animal Science, Macdonald Campus, McGill University , Ste-Anne-de-Bellevue, QC, H9X 3V9, Canada.

†Bélisle Solution Nutrition Inc., St-Mathias sur Richelieu, QC, J3L 6A7, Canada

[1] Submitted to *Journal of Dairy Science*

3.1 ABSTRACT

The objective of this study was to evaluate the effects of yeast-derived microbial protein (YMP) on performance and blood biochemical parameters in transition cows. Twenty-seven primiparous and multiparous Holstein cows were blocked according to expected calving date and parity, and randomly assigned to receive either a control (0g YMP) or treated (YMP; 50 g YMP prepartum followed by 200 g YMP postpartum) pellet containing beet pulp and molasses. The study commenced 21 d prior to expected calving date and ended 28 d postpartum. Individual dry matter intake (DMI) and milk yields were recorded daily. Milk samples were collected twice weekly on two non-consecutive days and analyzed for fat, protein, lactose, somatic cell count and urea. Blood samples collected on d -21, -14, -7, -3, -1, 1, 3, 7, 14, 21 and 28 relative to calving were analyzed for metabolites, macrominerals and proteins. There was no significant effect of YMP treatment on DMI, milk yield and milk composition. However, YMP supplementation significantly reduced serum concentrations of non-esterified fatty acids from d 3 postpartum until the end of the study. Serum concentrations of β-hydroxybutyrate were also reduced in YMP-supplemented cows on d 3 and 7 postpartum. Moreover, YMP supplementation significantly increased serum glucose levels on d 3 and 7 postpartum, and reduced serum aspartate transaminase levels on d 14 and 21 postpartum. Overall, our results suggest that YMP may significantly reduce metabolic stress and fat mobilization from the adipose tissue in transition cows especially during the postpartum period without significant adverse effects on milk production.

Key words: yeast-derived microbial protein, metabolism, negative nutrient balance, transition dairy cow

3.2 INTRODUCTION

The transition period, defined as 3 wk prepartum to 3 wk postpartum, is generally characterized by reduced DMI, but increased energy and protein demands for parturition and onset of milk synthesis, leading to severe negative nutrient balance and metabolic disorders such as ketosis, displaced abomasum and mastitis (Grummer, 1995; Ingvartsen et al., 2000; Drackley et al., 2001). This period is often marked by increased fatty acid and amino acid mobilization from the adipose tissue and muscles, respectively (Bell, 1995; Komaragiri and Erdman, 1997; LeBlanc, 2010). Metabolic disorders in transition cows are usually accompanied by a major financial burden to the producer due to treatment costs, lower reproductive success, decreased milk production and increased cow culling (LeBlanc et al., 2005). The consequences of negative energy balance have well been studied (Grummer, 1995; Ingvartsen et al., 2003; Mann et al., 2015). However, the impacts of negative protein balance on cow's metabolism and production have received little scientific investigation (Grummer, 1995; Bell et al., 2000).

In general, dairy cow diets should be formulated to meet the MP requirements for production and high N efficiency by feeding a diet balanced for RDP and RUP (Agle et al., 2010). Microbial CP (**MCP**) is the most important and reliable source of MP for dairy cows considering its ideal AA profile and that it can provide 50 to 80% of total MP requirements of high-producing cows for milk synthesis (NRC, 2001). The quantity of MCP synthesis in the rumen is dependent on microbial N uptake (NH_3-N, AA or peptides) from dietary RDP and energy obtained from fermentable carbohydrates (i.e. fiber, starch, sugars and organic acids) (Sniffen et al., 1992). The AA requirements of high-producing cows cannot be met by MCP alone (Brito and Broderick, 2007). In transition cows, low voluntary intake of RDP and fermentable carbohydrates significantly reduced MCP synthesis (Clark et al., 1992). Therefore, dietary RUP that escapes ruminal degradation may be a more reliable source of MP for transition cows. For decades, soybean meal, fishmeal and animal protein have been utilized as protein supplements in dairy ration formulations. Today, there is a need to develop novel dietary strategies that can improve both the AA profile of MP and their transfer into the small intestine of dairy cows, and most importantly for transition cows considering their negative protein balance and very high nutrient demands postpartum.

Yeast-derived microbial protein (YMP) is a by-product of yeast fermentation which, in contrast to other dietary CP sources such as soybean meal, contains an ideal amino acid

profile that closely matches that of MCP (Sabbia et al., 2012). Moreover, based on its small particle size, a large proportion of YMP presumably flows with the liquid phase of the rumen at a high passage rate escaping ruminal degradation by microbes, and allowing for increased AA absorption in the small intestine (Sabbia et al., 2012). In lactating cows, replacing soybean meal with YMP up to 3.41% of the diet DM had no adverse effects on milk, 4% FCM and ECM yields (Sabbia et al., 2012). Similar results were obtained when YMP (2.25% and 1.15% of diet DM, respectively) partially replaced soybean meal (Manthey et al., 2016) or a mixture of soybean meal and canola meal (Neal et al., 2014) in the diets of lactating cows. Therefore, YMP may provide an excellent source of high-quality and absorbable AA to support milk synthesis in transition cows. However, to the best of our knowledge, the effects of YMP in transition cows have not been investigated yet.

We hypothesize that YMP supplementation may significantly alleviate the metabolic stress of negative nutrient balance and mobilization of body reserves in transition cows by increasing AA delivery and utilization in the small intestine. The objectives of the current study were to evaluate the effects of YMP on DMI, milk yield, milk composition, blood biochemical parameters and BCS of transition dairy cows.

3.3 MATERIALS AND METHODS

All animal procedures were approved by the Animal Care Committee of the Faculty of Agricultural and Environmental Sciences of McGill University. This study was conducted on a commercial dairy farm close to McGill University between November 2015 and May 2016.

3.3.1 *Animals and Diets*

Twenty one multiparous and six primiparous Holstein transition cows were used in the study. Cows were enrolled in the study 21 d prior to their expected calving date until 28 d postcalving. Cows were blocked into 13 blocks according to parity and expected calving dates. Within blocks, transition cows (n = 13 or 14 per treatment) were randomly assigned to either a control or treatment (**YMP**; FOSTO; Alltech Inc., Nicholasville, KY) group. All YMP cows received 125 g of YMP pellets (equivalent to 50 g of YMP, 0.42% of DMI) prepartum and 500 g of YMP pellets (equivalent to 200 g of YMP, 1.25% of DMI) postpartum while control cows received the same amounts of pellets void of YMP. Pellets

were fed to cows as a top dress 30 min prior to morning (0600 h) feeding. Cows were housed in tie-stalls and had free access to water. All prepartum cows were fed the same dry cow diet as a TMR twice daily at 0600 and 1500 h for an individual DMI of 12 kg per day and ad libitum dry hay between the diets whereas all postpartum cows were fed the same diet as a TMR twice daily for ad libitum intake (5% orts, on an as-fed basis) (Table 3.1). Orts were weighed once daily to determine daily feed intake for individual cows.

3.3.2 *Chemical Analysis*

Samples of the TMR were collected once weekly, dried in a forced air oven at 65°C for 48 h, and then stored at -20°C until later analysis. Samples were composited on a monthly basis and ground through a 1-mm screen using a Wiley mill (A.H Thomas Philadelphia, PA). Subsamples were then analyzed for DM, ash and ether extract according to AOAC (1990). Neutral (Van Soest et al., 1991) and acid (AOAC, 1990) detergent fiber were determined using an Ankom Fiber Analyzer (Ankom Technology Corp., Macedon, NY). Crude protein (N x 6.25) was analyzed using a Leco Nitrogen Analyzer (Truspec Nitrogen Determinator System, Leco Corp., St. Joseph, MI). Neutral and acid detergent insoluble protein were estimated by analyzing NDF and ADF residues, respectively, for total N (Licitra et al., 1996). A composite sample of YMP was dried for 48 h at 65°C and then analyzed for AA composition using an AA analyzer (Sykam, Eresing, Germany) according to the method of AOAC (1990; method 982.30) and as described by Woyengo et al. (2010).

3.3.3 *Milk Production and Analysis*

Cows were milked twice daily at 0630 and 1730 h, and individual milk yields were recorded at each milking. Milk samples were collected twice daily on d 4, 6, 11, 13, 18, 20, 25, and 27 postpartum at each milking and composited by cow according to volume. Milk samples were analyzed for fat, protein, lactose, and MUN using an infrared analyzer whereas SCC was analyzed using a SCC analyzer (Valacta, Sainte-Anne-de-Bellevue, Canada). Milk TS were determined according to AOAC (1990).

3.3.4 *Serum Collection and Analysis*

Blood samples were collected from individual cow on d -21, -14, -7, -3, and -1 relative to expected calving date and on d 1, 3, 7, 14, 21, and 28 postpartum. Blood samples were obtained by venipuncture of the coccygeal vein or artery at least 30 min prior to

morning feeding. Blood was drawn into a 10-mL serum Monoject blood collection tubes (Monoject Blood Collection Tube, Mansfield, MA) using 20G vacutainer needles (BD Vacutainer PrecisionGlide Multiple Sample Needle, Plymouth, UK). Blood samples were allowed to clot at room temperature for approximately 30 min, immediately placed on ice, and centrifuged within 2 h of collection at 2,500 x g for 15 min at room temperature to separate the serum layer. Serum samples were then stored at -80°C until later analysis. All serum samples were analyzed at the Animal Health Laboratory (University of Guelph, ON, Canada) for BHBA, NEFA, glucose, protein, albumin, globulin, urea, cholesterol, total Ca, P, Na, K, Mg, Cl, GLDH, AST, GGT and haptoglobin using a Roche Cobas 6000 c501 automated chemistry analyzer (Roche, Mississauga, Canada). All test reagents were supplied by Roche diagnostics (Indianapolis, IN) except for BHBA and NEFA which were supplied by Randox Laboratories (Crumlin, UK). Analytical procedures were performed according to the manufacturer's guidelines. Serum haptoglobin was determined using a modified protocol (Makimura and Suzuki, 1982; Skinner et al., 1991).

Serum fatty acids were analyzed by gas chromatography (Varian 3900 with 8400 auto sampler with flame ionization detector at 260°C, Varian Analytical Instruments, Walnut Creek, CA) as described previously (Huang, 2016).

3.3.5 *BCS*

Body condition scores of each experimental cow were recorded prior to morning feeding on d -21, -14, -7, and -1 relative to expected calving and d 1, 7, 14, 21, and 28 postpartum. Body condition scoring was conducted by 2 independent and trained evaluators using a 5-point scale (Wildman et al., 1982).

3.3.6 *Statistical Analysis*

Data were analyzed as repeated measures using an analysis of variance (ANOVA) with PROC MIXED of SAS (SAS Institute, 2010) and the following model:

$$Y_{ijk} = \mu + trt_i + cow_{ij} + block_j + time_k + trt_i{*}time_k + e_{ijk}$$

where Y_{ijk} represents the parameter being measured, μ is the overall mean, trt_i is the fixed effect of the ith treatment (i = 1 or 2), cow_{ij} is the random effect of cow from the jth block receiving the ith treatment, $block_j$ is the fixed effect of the jth block (j = 1, 2,...or 13), $time_k$ represents the fixed effect of the kth time point (k= 1, 2...or 11 for blood biochemical

parameters) or (k = 1, 2,...or 8 for BCS) or (k = 1, 2, 3 or 4 for DMI, milk yield and milk composition), trt_i*time_k is the fixed effect of interaction of ith treatment and k^{th} time point, and e_{ijk} represents the residual error [$e_{ijk} \sim N (0, \sigma^2_e)$]. Treatment differences were declared at $P < 0.05$.

3.4 RESULTS

3.4.1 *Amino Acid profile of YMP*

The essential AA profile of YMP was similar to that of milk and MCP for many AA (Table 3.2). Interestingly, in contrast to soybean meal, YMP had higher concentrations for Met and Lys which are the most limiting AA for milk production (Schwab et al., 1995).

3.4.2 *Dry Matter Intake and BCS*

Treatment had no significant effect on DMI and BCS. Cows in both treatment groups consumed all of the prepartum diet. Postpartum DMI was similar between treatment groups and, as expected, DMI increased ($P < 0.01$) as lactation progressed (Figure 3.1A). There was a significant ($P < 0.01$) effect of time on BCS, which dramatically decreased in both treatment groups immediately after calving and until 14 d postpartum (Figure 3.1B). However, no treatment x time interactions were detected for DMI or BCS. In contrast to YMP-supplemented cows, BCS tended ($P = 0.93$) to be higher among control cows at 7 d post-calving.

3.4.3 *Milk Production and Composition*

There were no treatment x time interactions for milk production or composition ($P = 0.16$ to 0.96); therefore, only main effects are reported (Table 3.1). Treatment had no effect on milk, fat, protein, SNF, SCM and ECM yields. However, as expected, there was a significant effect of time ($P < 0.01$) on milk yield, which increased for all cows as lactation progressed. Cows fed the control diet tended ($P = 0.08$) to produce more milk at wk 4 postpartum than cows fed the YMP-supplemented diet (Figure 3.1C). However, 4% FCM yield was greater ($P < 0.05$) at 3 wk postpartum in control- than YMP-fed cows.

Milk fat, protein, lactose and TS percentages were similar between treatments (Table 3). Similar results were observed for MUN and SCC levels in milk. Milk protein,

lactose and TS percentages decreased ($P < 0.01$) for all cows as lactation progressed, probably due to higher milk yield.

3.4.4 *Blood Biochemical Parameters*

Treatment had no significant effect on serum biochemical parameters; except for lower ($P < 0.05$) serum NEFA and higher ($P < 0.05$) serum urea concentrations in YMP-supplemented cows (data not shown). Time effects were significant ($P < 0.01$) for all blood biochemical parameters. Significant treatment x time interactions were observed for serum NEFA concentration which was reduced ($P < 0.05$) in YMP-supplemented cows from d 3 to d 28 postpartum (Figure 3.2A). Similar findings were observed for BHBA at d 3 and 7 postpartum (Figure 3.2B). For both treatment groups, serum NEFA and BHBA concentrations peaked after calving and decreased as lactation progressed, which follows the normal physiological changes in dairy cows. Serum glucose concentrations were higher ($P < 0.05$) in YMP-supplemented cows at d 3 and 7 postpartum when compared with control cows (Figure 3.2C).

Serum concentrations of AST were lower ($P < 0.05$) in YMP-supplemented cows at d 14 and 21 postpartum when compared with control cows (Figure 3.3A). Similar observations were observed for serum GLDH concentration at d 21 postpartum (Figure 533B). In contrast, serum cholesterol concentrations were greater ($P < 0.05$) in YMP-supplemented cows than control cows at d 14, 21, and 28 postpartum (Figure 3.3C). Serum haptoglobin concentration was also greater ($P < 0.05$) in YMP-supplemented cows at d 7 postpartum (Figure 3.3D).

3.4.5 *Serum Fatty Acid Composition*

Treatment had no significant effect on serum concentrations of C14:0, C16:0, C16:1, C18:0, C18:1, C18:2 (data not shown). Significant treatment x time interaction effects were observed for C18:1 and C18:3 fatty acids (Figure 3.4). Serum C18:1 (d 3 prepartum and d 14 postpartum) and C18:3 (d 7 prepartum) concentrations were lower ($P < 0.05$) in YMP-supplemented cows than in control cows. However, serum C18:3 fatty acid concentration was higher ($P < 0.05$) in YMP-supplemented cows than in control cows at d 3 postpartum.

3.4.6 *Blood Macrominerals*

Serum Ca concentration was higher ($P < 0.05$) in YMP-supplemented cows than in control cows at d -1 prepartum (Figure 3.5A). Similar results were observed for serum P concentrations at d 7, 14 and 21 postpartum (Figure 5.5B). However, serum Ca:P was lower ($P < 0.05$) in YMP-supplemented cows at d 7 postpartum (Figure 3.5C). There were no treatment effects on serum Mg, Na, K, Cl and Na:K concentrations (data not shown).

3.4.7 *Metabolic Disorders*

None of the cows used in this trial experienced clinical metabolic disorders that would have otherwise required veterinary assistance.

3.5 DISCUSSION

In dairy cows, the transition period is extremely stressful and is frequently associated with incidences of metabolic health disorders, leading to production and economic losses. The principal objectives of this study were to determine the effects of supplementing YMP, a rumen-escape microbial protein source, on the lactational performance and nutrient metabolism of transition cows. The effects of YMP in lactating cows have previously been demonstrated (Sabbia et al., 2012; Neal et al., 2014; Manthey et al., 2016). However, the effects of YMP in transition cows have not been reported yet.

Dry matter intake was not influenced by YMP supplementation during early lactation. Data pertaining to the effects of YMP supplementation on DMI in cows are inconsistent. Feeding YMP to lactating cows had no effect (Manthey et al., 2016), increased (Sabbia et al., 2012) or decreased (Neal et al. 2014) DMI. However, in contrast to the current study, the previous studies involved the use of lactating cows and YMP partially replaced soybean meal or canola meal in iso-nitrogenous diets. The impact of YMP supplementation on DMI of transition cows is still unclear and warrant further investigations.

The supplementation of YMP to transition cows had no effect on BCS, probably due to the lack of differences in DMI. Other studies have also reported no effects of YMP supplementation on BCS (Sabbia et al., 2012; Neal et al., 2014; Manthey et al., 2016). According to Bernabucci et al. (2008), BCS decreases during late pregnancy and continues to decline until about 30 DIM as a result of mobilized fat. However, BCS loss is more significant in cows with high BCS prepartum (>3) than for cows with medium (2.6 to 3.0)

or low (<2.5) BCS (Bernabucci et al., 2008; Schulz et al., 2013). In our study, cows in both groups had a high BCS (>3.25) which significantly declined throughout the study. However, our similar results of BCS between YMP and control groups indicate that changes adipose tissue storage did not differ greatly between the two groups during the trial period.

Milk yield and milk composition were not significantly different between YMP and control cows. Similar findings are reported when YMP partially replaced soybean meal or a mixture of soybean meal and canola meal in the diets of lactating cows (Sabbia et al., 2012; Neal et al., 2014; Manthey et al., 2016). In these studies, dietary nutrients including CP, RDP, RUP and NE_L were balanced across diets. Manthey et al. (2016) explained similarity in milk yield by the lack of difference in AA concentrations between YMP and control diets. In the study by Neal et al. (2014), DMI was reduced in YMP-supplemented cows but the increased supply of AA may have increased energy partitioning towards milk production and milk composition. However, in this study, YMP was supplemented to transition cows in addition to the rations during the close-up and early lactation periods. Considering the additional intake of AA due to YMP supplementation, significant increase in milk yield and milk composition were expected. However, none of these parameters were altered by YMP. Our findings suggest that additional AA intake as a result of YMP supplementation was partitioned towards metabolic pathways and tissues rather than the mammary gland.

Approximately 50% of milk fat is synthesized de novo in the mammary gland from precursors such as acetate, BHBA, NEFA and triacylglycerides (mostly very-low-density lipoproteins) (Akers, 2002). Milk fat percentage was expected to be higher in control cows because of higher concentrations of serum BHBA and NEFA postpartum. However, milk composition was not altered by treatments in the current study. The lack of significant differences in milk fat percentages is difficult to explain at this time. According to the NRC (2001), milk protein content and yield may be influenced by the AA profile of MP, and the quantity of surplus protein and fermentable carbohydrates in the diet. However, in agreement to our findings, Bequette et al. (1998) reported lack of differences in milk yield and milk protein when protein, AA or energy was supplemented into the diets. Therefore, our findings suggest that dietary energy and protein were not limiting factors for milk production and composition (Brun-Lafleur et al., 2010).

Serum BHBA and NEFA concentrations were lower in YMP-supplemented cows than control cows during most of the postpartum period. However, in a study with mid-lactation cows, plasma BHBA concentrations were increased whereas NEFA levels were not affected

as a result of YMP supplementation (Sabbia et al., 2012). These conflicting results may be explained by differences in lactation stage (transition vs mid-lactation) and nutritional requirements of cows. The relationship between serum BHBA and NEFA concentrations and body tissue mobilization is well-documented. For instance, high levels of NEFA and BHBA are indicative of adipose tissue mobilization (Drackley, 1999). In response to a negative energy balance, as in transition cows for example, large amounts of triglycerides are mobilized from adipose tissue and released as NEFA. High blood NEFA (> 0.7 mmol/L) in cows is indicative of severe negative nutrient balance and greater risks for additional metabolic disorders such as displaced abomasum and clinical ketosis (McArt et al., 2013). Blood NEFA concentrations were < 0.7 mmol/L in YMP-supplemented cows only.

In general, high NEFA is consistent with increased serum BHBA levels. Indeed, partial oxidation of NEFA in the liver is the main source of ketone bodies (BHBA) which is also an energy substrate (Drackley et al., 1992). Sub clinical ketosis (1.2 to 2.9 mmol/L BHBA) or clinical ketosis (> 2.9 mmol/L BHBA) (Oetzel, 2004) increases the risk for displaced abomasum, lower conception rates and herd removal (McArt et al., 2013). In this study, none of the YMP and control cows exceeded 1.2 mmol/ L BHBA at any time during the transition period suggesting that cow's nutritional requirements were sufficiently met by the diets. However, cows supplemented with YMP may have mobilized less adipose tissue as indicated by their lower NEFA and BHBA levels (Drackley 1999), probably because these cows were in lower energy deficit than control cows. Indeed, serum glucose levels were higher in YMP-supplemented cows at 3 and 7 d postpartum, suggesting that higher AA supply into their intestine may have been utilized in gluconeogenesis (Herdt, 1988). Moreover, lower adipose tissue mobilization among YMP cows was evidenced by their lower serum concentration of C18:1 and numerically lower levels of C16:0, C16:1 and C18:0. These are the major fatty acids which are mobilized from the adipose tissue (Rukkwamsuk et al., 1999). On the other hand, greater mobilization of energy (adipose tissue) and/or AA (skeletal muscle) in control cows were probably utilized for BW gain (BCS) and milk production which tended to be higher at 7 and 28 d post-calving, respectively.

Cows fed YMP had lower serum concentrations of AST (d 7 and 14) and GLDH (d 21) postpartum when compared to control cows. Liver damage and fatty liver occur when NEFA uptake by the liver exceeds its capacity to oxidize fat and that NEFA are reesterified into triacylglycerol (TAG). At this time, AST, a hepatocyte leakage enzyme, is expressed and

reflects liver damage (Gonzalez et al., 2011; Ok et al., 2013). The, AST is not specific to the liver. However, when accompanied with high concentrations of GLDH, it may reflect hepatocyte and not skeletal muscle damage. Moreover, GLDH, which is more specific and sensitive to the liver, is expressed when there is complete cell death and therefore may not increase as much as AST (Ok et al., 2013). Mostafavi et al. (2013) reported that cows with AST >120 U/L had 2.9x greater risk for fatty liver whereas Herdt and Gerloff (2009) reported optimal threshold for AST to be 100 U/L. In our study, neither group of cows had AST levels above 120 U/L. However, AST concentration in YMP cows remained below 100 U/L at all times during the transition period, suggesting that these cows were at lower risks of hepatic lipidosis and damage than control cows. The lower serum AST and GLDH levels observed in YMP cows are in agreement with our other result about lower serum NEFA concentrations. Taken together, all of these findings indicate lower fat mobilization due to YMP supplementation. Furthermore, higher serum cholesterol in YMP cows are indicative of greater triglycerides export from the liver as VLDL, which suggest improved liver health and lower risk of lipidosis. Our hepatic results are consistent with previous findings that cows with hepatic lipidosis had lower serum cholesterol concentrations but higher NEFA, AST and GLDH levels (Ok et al., 2013; Mostafavi et al., 2013).

To the best of our knowledge, the effects of YMP supplementation on serum mineral concentration have not been investigated yet. Blood Ca level below 2 mmol/L is indicative of hypocalcaemia and this metabolic disease usually occurs between 12 and 24 h after calving when Ca requirement is extremely high (Goff, 2008). In the current study, blood Ca dropped below 2.1 mmol/L on the day after parturition in both YMP and control cows, but then reached normocalcaemia levels (2.1 to 2.5 mmol/L) by d 3 postpartum. However, YMP cows had higher serum Ca levels (2.1 vs 2.3 mmol/L; Figure 3.5B) than control cows one day before calving, and were presumably at lower risk for hypocalcaemia. Short-term casein supplementation to rats significantly increased intestinal Ca absorption (Gaffney-Stomberg et al., 2010). Phosphorus is a major component of phospholipids, phosphoproteins, nuclei acids, energy-transferring molecules such as ATP, acid-base buffer system and bone. Blood P should be maintained between 1.3 to 2.6 mmol/L in adult cows (Goff, 2000). Cows in both the YMP and control groups were within or slightly above this range during the entire transition period. However, YMP cows had greater serum P on d 1, 7 and 14 postpartum. Serum Ca:P were within acceptable range for both groups throughout the trial, but was lower in YMP cows postpartum probably due to higher serum

P levels. According to Goff (2000), Ca:P ratio is of little importance if it falls between 1 and 7, and that blood Ca and P levels are of greater importance. Our findings suggest that YMP may have increased intestinal absorption of Ca and P in transition cows, but further investigation is required to test this hypothesis.

3.6 CONCLUSIONS

In this study, YMP supplementation significantly reduced metabolic stress and mobilization of the adipose tissue as indicated by lower serum NEFA and BHBA concentrations. Whereas control cows required adipose tissue mobilization as energy source for parturition and milk synthesis, energy requirements in YMP-supplemented cows were principally met through gluconeogenesis of AA contained in YMP. Moreover, YMP supplementation significantly increased serum Ca concentration one day prior to calving, hence reducing the risks for hypocalcaemia. However, YMP supplementation to transition cows had no significant effect on DMI, milk production, and milk composition. Finally, YMP may significantly reduce periparturient metabolic stress without negatively impacting cow performance.

Table 3.1. Ingredients and chemical composition (mean ± SD) of pre- and post-partum diets

Item, % of DM	TMR Diet[1]	
	Prepartum	Postpartum
Ingredient		
Corn silage	19.50	17.37
Alfalfa silage	-	34.94
Alfalfa hay	50.17	5.11
Soybean meal	12.86	6.44
Roasted soybean	-	6.21
Dried ground corn	14.78	23.10
Dried rye grains	-	4.01
Mineral Premix – prepartum[2]	2.69	-
Mineral Premix – postpartum[3]	-	2.82
Chemical composition, % of DM (unless otherwise noted)		
DM	44.95 ± 6.15	43.084 ± 3.08
Ash	3.67 ± 0.50	3.078 ± 0.22
CP	14.39 ± 1.04	14.18 ± 0.93
Ether extract	3.02 ± 1.24	3.84 ± 0.34
NDF	32.84 ± 3.43	40.236 ± 4.52
ADF	17.68 ± 3.34	22.180 ± 1.99
ADL	4.21 ± 0.75	2.99 ± 0.16
Neutral detergent-insoluble CP, % of CP	7.78 ± 1.90	8.92 ± 1.90
Acid Detergent- insoluble CP, % of CP	3.23 ± 2.20	3.68 ± 0.75
NE_L,[4] Mcal/kg	1.82	1.89

[1]Cows were fed exactly 12 kg of the prepartum diet daily whereas the postpartum diet was provided for ad libitum intake.

[2]Contained 19.63% Ca, 1.29% P, 11.33% Mg, 1.20% K, 0.51% Na, 0.001% Cl, 2.42% S, 0.08% Mn, 0.03% Cu, 0.06% Fe, 1.00 Zn, 0.005% I, 0.008% Co, 0.004% Se, 467 KIU/kg of vitamin A, 113 KIU/kg of vitamin D, 7, 092 mg/kg of vitamin E, 19.2 mg/kg of Biotine. Minerals were mostly in organic form.

[3]Contained 14.00% Ca, 1.79% P, 2.87% Mg, 0.71% K, 0.29% Na, 0.0003% Cl, 1.42% S, 0.05% Mn, 0.02% Cu, 0.08% Fe, 0.06% Zn, 0.003% I, 0.005% Co, 0.002% Se, 262 KIU/kg of vitamin A, 61 KIU/kg of vitamin D, 1, 803 mg/kg of vitamin E, 19.2 mg/kg of Biotine. Minerals were mostly in organic form.

[4]Estimated according to NRC (2001).

Table 3.2. Essential AA composition of yeast-derived microbial protein (YMP), soybean meal, milk, and microbial CP (MCP)

AA, % of total essential AA	YMP	Soybean meal	Milk[1]	MCP[1]
Arg	9.1	17.0	7.2	10.2
His	6.6	6.9	5.5	4.0
Ile	10.4	9.4	11.4	11.5
Leu	16.6	16.7	19.5	16.3
Lys	16.6	14.7	16	15.8
Met	3.3	2.3	5.5	5.2
Phe	10.1	11.2	10.0	10.2
Thr	11.7	9.1	8.9	11.7
Trp	2.4	2.7	3.0	2.7
Val	13.0	10.0	13.0	12.5

[1] Table 5-10 (NRC, 2001).

Table 3.3. Effects of feeding yeast microbial protein (YMP) on milk performance in Holstein cows during the first 28 d postpartum

Parameter	Treatment[1]		SEM[2]	P-value[3]	
	Control	YMP		Trt	Time
Production, kg/d					
Milk	36.7	31.9	1.9	0.98	<0.0001
Fat	2.02	1.76	0.17	0.28	0.51
Protein	1.15	1.05	0.06	0.23	0.90
SNF	8.89	8.86	0.19	0.91	0.03
SCM	43.32	37.83	2.90	0.19	0.12
ECM	45.92	40.39	3.11	0.22	0.11
4% FCM	44.89	39.24	3.16	0.22	0.09
MUN, mg/dl	8.14	9.04	0.50	0.19	0.12
Composition, %					
Fat	5.64	5.46	0.33	0.70	0.20
Protein	3.20	3.27	0.06	0.44	<0.0001
Lactose	4.05	4.40	0.05	0.91	0.001
TS	14.52	14.31	0.34	0.67	0.0002
SCC, cells / μL	310	531	176	0.39	0.32

[1]Pellets without (control) and with yeast microbial protein (YMP: 50 g prepartum followed by 200 g postpartum) provided to cow 30 mins prior to morning feeding.

[2]Average SEM of the two treatment groups.

[3]P-value for treatment effects.
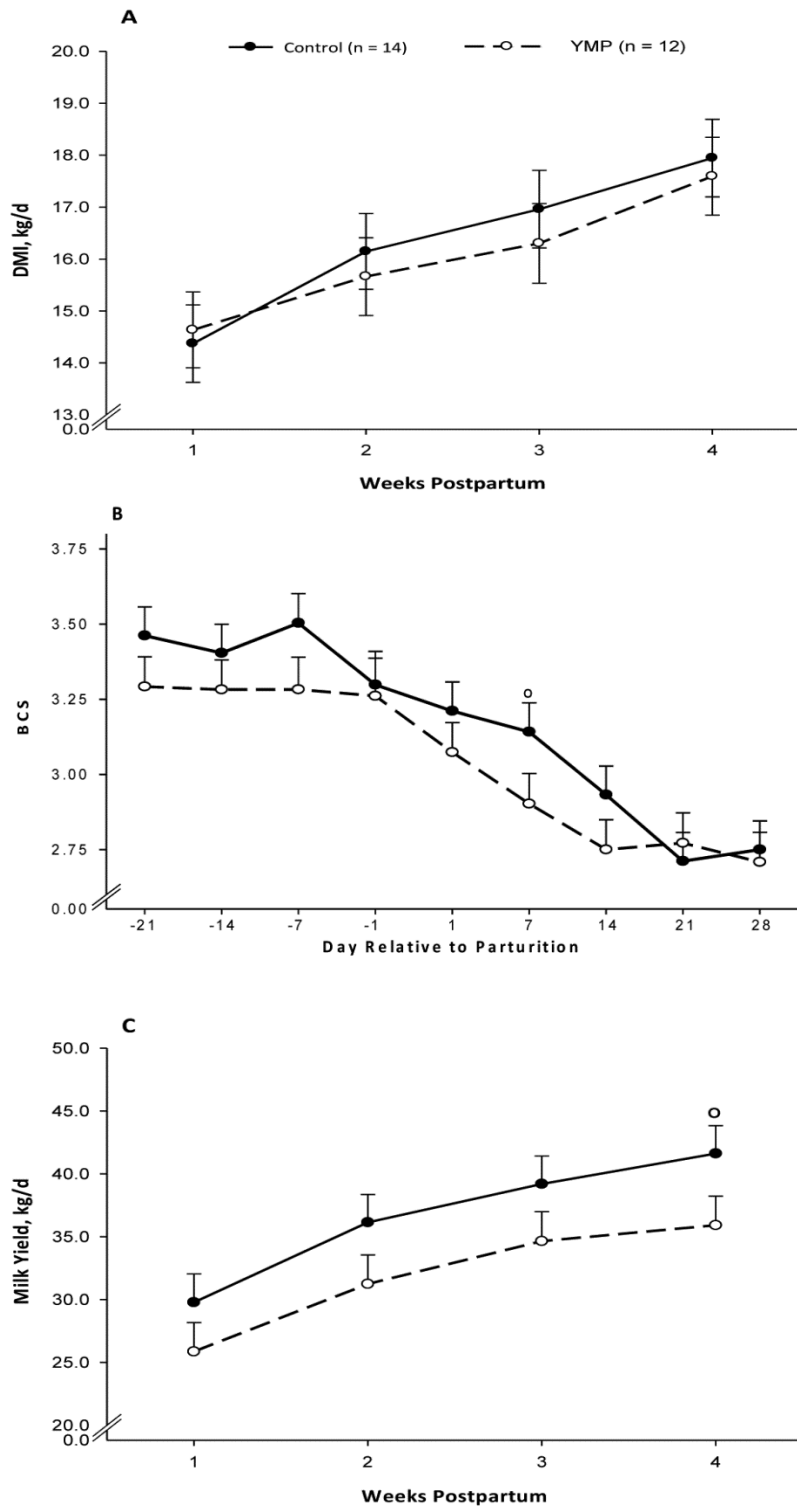
Figure 3.1. Effects of yeast microbial protein (YMP; 50 g prepartum followed by 200 g postpartum) supplementation on DMI (A), BCS (B) and milk yield (C) in transition cows. [0] = Tendency for statistical differences ($0.05 < P < 0.10$) between YMP-fed and control cows.
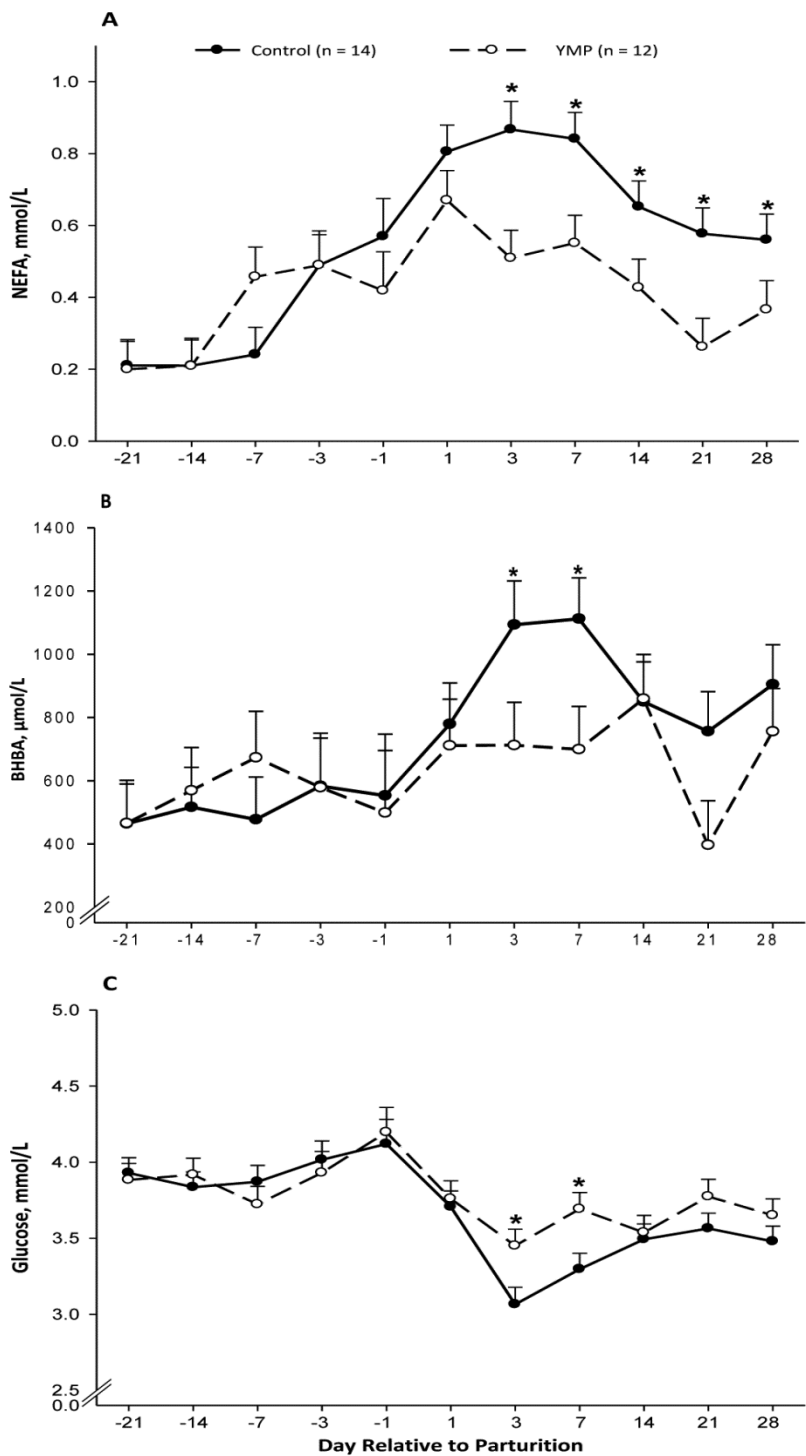
Figure 3.2. Effects of yeast microbial protein (YMP; 50 g prepartum followed by 200 g postpartum) supplementation on serum concentrations of NEFA (A), BHBA (B) and Glucose (C) in transition cows. * = Differences ($P < 0.05$) between YMP-fed and control cows.
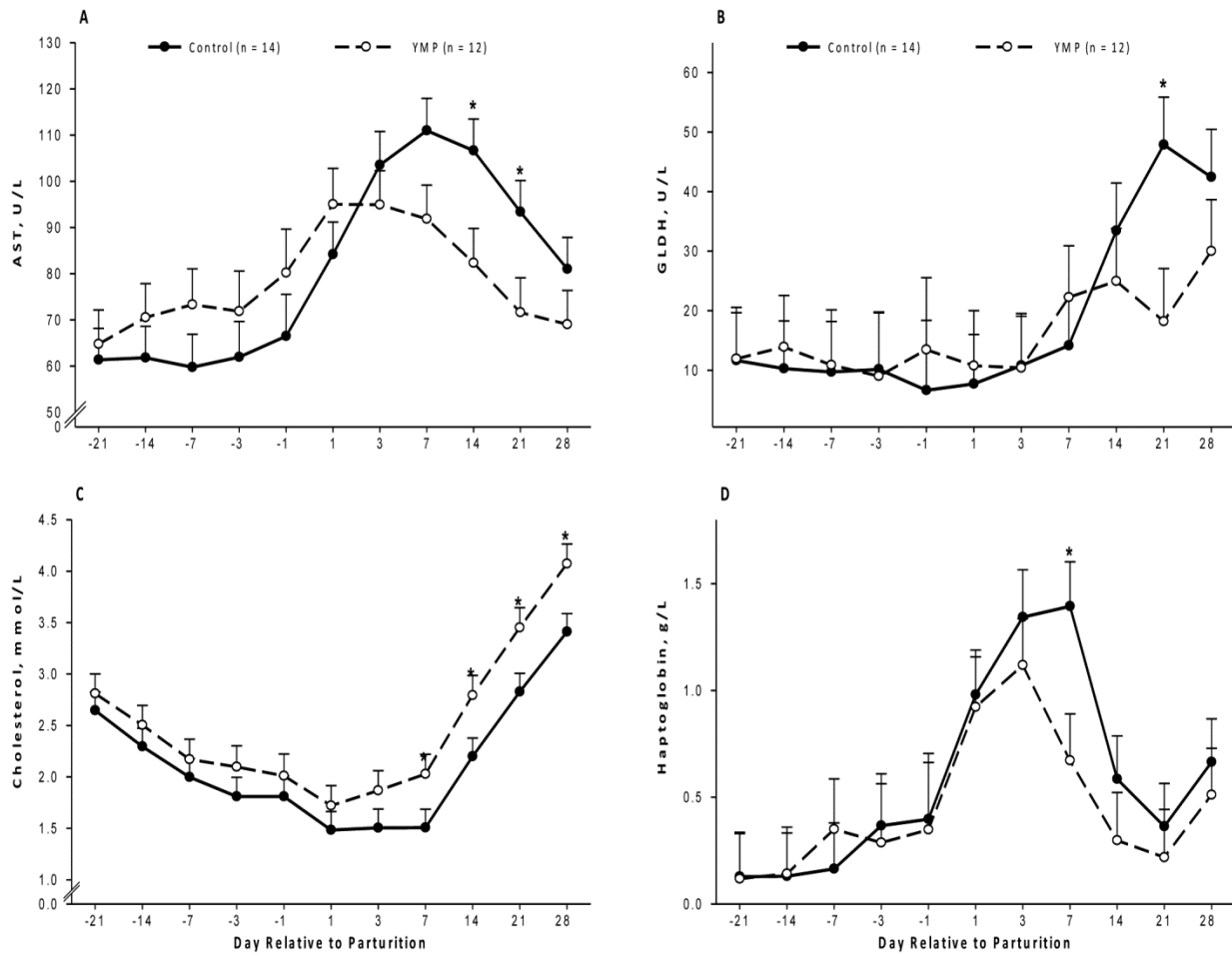
Figure 3.3. Effects of yeast microbial protein (YMP; 50 g prepartum followed by 200 g postpartum) supplementation on serum concentrations of AST (A), GLDH (B), cholesterol (C) and haptoglobin (D) in transition cows. * = Statistical differences (*P* < 0.05) between YMP-fed and control cows.
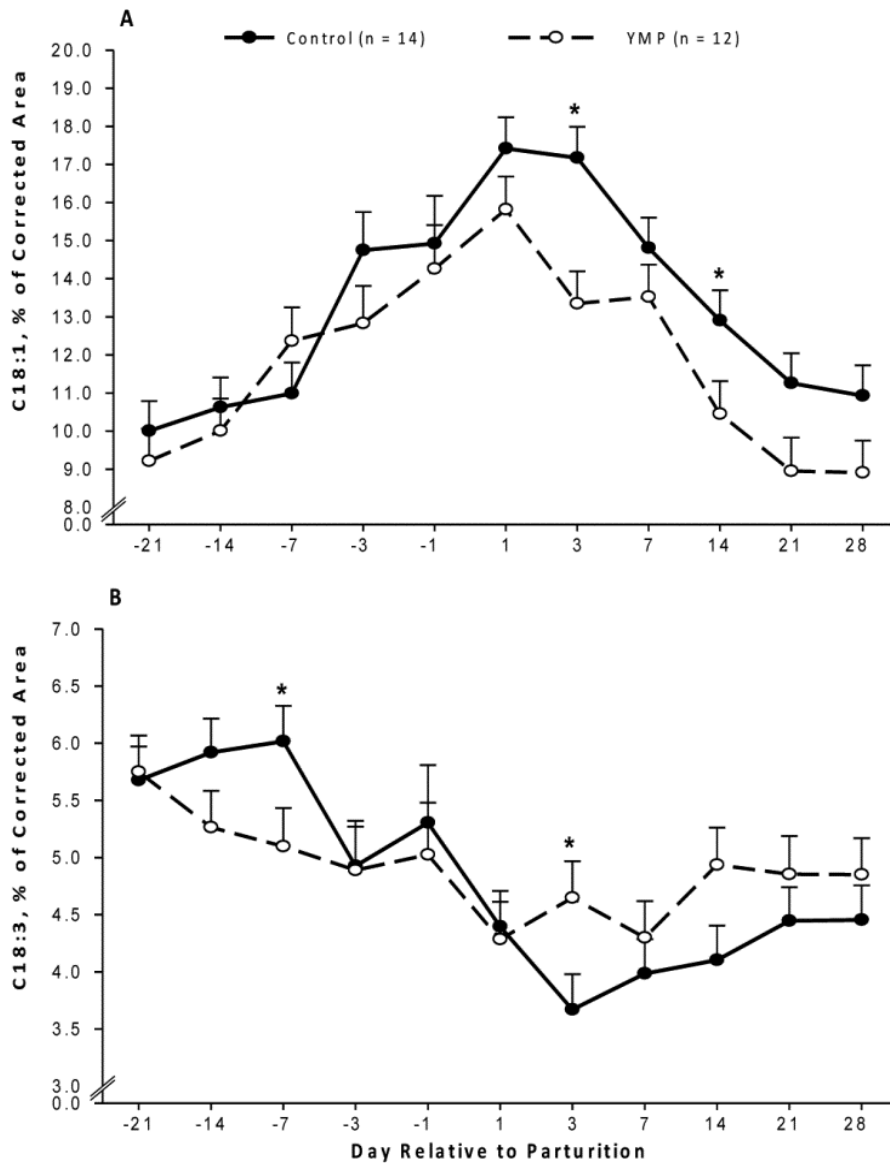
Figure 3.4. Effects of yeast microbial protein (YMP; 50 g prepartum followed by 200 g postpartum) supplementation on serum concentrations of C18:1 (A) and C18:3 (B) in transition cows. * = Statistical differences ($P < 0.05$) between YMP-fed and control cows.
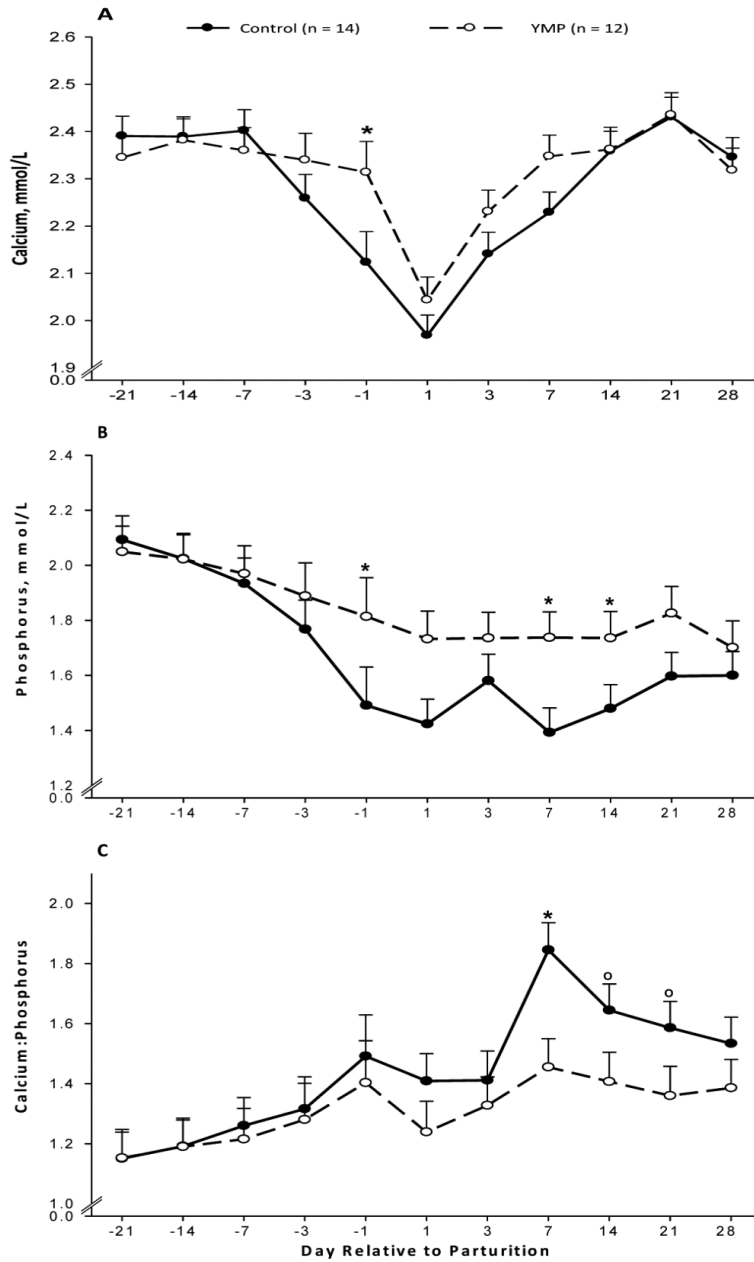
Figure 3.5. Effects of yeast microbial protein (YMP; 50 g prepartum followed by 200 g postpartum) supplementation on serum concentrations of calcium (A), phosphorus (B) and calcium:phosphorus (C) in transition cows. * = Statistical differences ($P < 0.05$) between YMP-fed and control cows. [0] = Tendency for statistical differences ($P < 0.10$) between YMP-fed and control cows.

CHAPTER 4. CONNECTING STATEMENT

In Experiment 1, we examined the effects of feeding YMP, a yeast-derived microbial protein that escapes ruminal degradation on Holstein transition cows fed a high forage ration. Feeding YMP reduced metabolic stress as reflected by lower serum NEFA, BHBA, AST and GLDH levels accompanied by higher serum glucose and cholesterol during part of the postpartum period. However, in this study, YMP had no effect on DMI or lactational performance. Considering the positive effects of live yeast in improving milk yield and DMI in transition cows (Wohlt et al., 1998), we were interested to investigate the combined effects of YMP and live yeast on the performance and metabolism of transition cows.

CHAPTER 5. EFFECTS OF YEAST-DERIVED MICROBIAL PROTEIN AND LIVE YEAST ON METABOLIC STATUS AND MILK PERFORMANCE OF HOLSTEIN TRANSITION COWS

V. Higginson*, B. Baurhoo*†, Y. Schuermann*, R. Duggavathi* and A. F. Mustafa*

*Department of Animal Science, Macdonald Campus, McGill University , Ste-Anne-

de-Bellevue, QC, H9X 3V9, Canada.

†Bélisle Solution Nutrition Inc., St-Mathias sur Richelieu, QC, J3L 6A7, Canada

5.1 ABSTRACT

Feeding yeast-derived microbial protein (YMP) may reduce metabolic stress in transition cows. The objective of this study was to investigate the effects of combining YMP with live *Saccharomyces cerevisiae* on blood biochemical parameters, dry matter intake and milk performance in transition cows. Twenty-three primiparous and multiparous Holstein cows were blocked according to expected calving date and parity, and randomly assigned to receive a total mixed ration without (control) or with (YMPL; 100 g YMP prepartum with 10g live yeast followed by 200 g YMP postpartum with 10g live yeast) supplementation. The supplementation began 21 d prior to expected calving date until 28 d postpartum. Individual dry matter intake (DMI) and milk yields were recorded daily. Milk samples were collected twice weekly and analyzed for fat, protein, lactose, somatic cell count and urea. Blood samples collected on d -21, -14, -7, -3, -1, 1, 3, 7, 14, 21 and 28 relative to calving were analyzed for metabolites, macrominerals and proteins. There was no effect of YMPL treatment on DMI, milk yield, milk composition or body condition scores (BCS). Moreover, YMPL had no effect on serum β-hydroxybutyrate concentrations. However, YMPL significantly increased serum concentrations of non-esterified fatty acid on d 3 and 14 postpartum whereas only a tendency was observed on d 7 postpartum. Feeding YMPL to cows significantly increased serum concentrations of aspartate aminotransferase (AST) on d 1, 3 and 7 postpartum and serum glutamate dehydrogenase (GLDH) levels on d 7 and 14 postpartum. Moreover, serum haptoglobin levels were significantly reduced due to YMPL supplementation on d 7 and 14 postpartumIn conclusion, feeding YMPL to transition cows may help reduce risks of liver inflammation without major effects on milk performance or BCS. However, the effects of YMPL on nutrient metabolism, particularly higher NEFA as an indication of adipose tissue mobilization, were unexpected and warrant further investigation.

5.2 INTRODUCTION

The transition period, generally defined as 3 wk prepartum until 3 wk postpartum, is critical for dairy cows because nutritional requirements for parturition and onset of milk synthesis exceed intake (Drackley, 1999). This period is often characterized by a negative nutrient balance and increased mobilization of the adipose tissue (fatty acids) and skeletal muscles (amino acids), which increase the risk of metabolic disorders such as ketosis, displaced abomasum and mastitis (Grummer, 1995; Drackley et al., 2001; Bell, 1995; Komaragiri and Erdman, 1997; Ingvartsen, 2006). Approximately, 30 to 50% of transition cows suffer from one of these disorders leading to compromised cow welfare and lower farm profitability due to treatment costs, cow culling and lower reproductive success (Leblanc et al., 2005). There is considerable research about the consequences of negative energy balance in transition cows (Grummer, 1995; Loor, 2010; Mullins et al., 2012). However, research pertaining to the impacts of negative protein balance in transition cows and potential dietary remedial strategies are scarce (Larsen et al., 2014; Amanlou et al., 2017).

Microbial CP (MCP) can supply 50 to 80% of total MP requirements of high-producing cows (NRC, 2001). Moreover, MCP has an ideal AA profile when compared with plant proteins such as soybean meal (Clark et al., 1992). Therefore, nutritional strategies that can maximize MCP synthesis are highly desirable. However, MCP synthesis in the rumen is considerably reduced in transition cows as a result of lower voluntary intake of RDP and fermentable carbohydrates (Clark et al., 1992). Indeed, microbial N uptake from dietary RDP and energy from fermentable carbohydrates are required for MCP synthesis (Sniffen et al., 1992). Therefore, feeding transition cows with high-quality RUP may be a more reliable strategy to increase AA delivery into the small intestine of transition cows.

Yeast-derived microbial protein (YMP) is a by-product of yeast fermentation with an amino acid profile that closely matches that of MCP and presumably flows with the liquid phase of the rumen at high passage rate allowing for increased AA absorption in the small intestines (Sabbia et al., 2012). In lactating cows, partial

43

replacement of dietary vegetable proteins (soybean and canola meals) with YMP did not affect milk, 4% FCM and ECM yields (Sabbia et al., 2012; Neal et al., 2014; Manthey et al., 2016). In our previously study, YMP supplementation to transition cows did not only increase blood glucose (possibly through gluconeogenesis of higher amino acids), but considerably reduced metabolic stress (lower NEFA and BHBA) of cows without compromising milk production (Higginson et al., under review).

There is evidence that live yeast supplementation to dairy cows may improve feed efficiency, cow performance and reduce health disorders. For instance, live yeast significantly improved rumen function, dry matter intake and milk yields by increasing the growth and activity of cellulolytic microorganisms for greater fibre digestibility, stabilizing the rumen pH, and reducing the incidence of SARA (Al Ibrahim et al., 2012; Alzahal et al., 2014; Chaucheyras-Durand et al., 2015). It is thought that live yeast can modify the rumen environment by utilizing available oxygen and other components (Newbold et al., 1996). There is also evidence that live yeast may increase milk fat percentage (Deghan-Banadaky et al., 2017) and 4% FCM (Mollaem et al., 2009) in mid-lactation cows. In transition cows, live yeast supplementation increased DMI and milk yield (Wohlt et al., 1998). To our knowledge, the combined effect of YMP and live yeast on transition cows have not yet been examined.

We hypothesize that simultaneous supplementation of YMP and live yeast to transition cows may help reduce negative nutrient balance and metabolic stress in by increasing DMI and the quantity of high-quality AA reaching the small intestine. The objectives of this study were to investigate the effects of YMP and live yeast supplementation on DMI, blood biochemical parameters and lactational performance of transition dairy cows.

5.3 MATERIALS AND METHODS

5.3.1 *Animals and Experimental Design*

All animal procedures were approved by the Animal Care Committee of the Faculty of Agricultural and Environmental Sciences of McGill University. This study was conducted on a commercial dairy close to McGill University between October 2016 and March 2017. Twenty multiparous and three primiparous Holstein transition cows were enrolled in the study 21 d prior to their expected calving date until 28 d postpartum. All cows were housed in a tie stall facility with free access to water throughout the trial.

5.3.2 *Treatments and Dietary Management*

Cows were blocked into 13 blocks according to parity and expected calving dates. Cows (n = 10 or 13 per treatment) were blocked into 13 blocks according to parity and expected calving dates, and were randomly assigned to either a control (0 g YMP and 0 g live yeast) or treatment [**YMPL**, 100 g of YMP (FOSTO; Alltech Inc., Nicholasville, KY) prepartum plus 10 g of live yeast (YeaSacc; Alltech Inc.) from d 21 prepartum followed by 200 g YMP plus 10 g of live yeast from calving until 28 d postpartum] diet. Both YMP and live yeast were mixed in the morning (0600 h) TMR and fed to treated cows. Control cows received the same TMR void of YMP and live yeast. Each dry cow was fed 10kg of prepartum TMR twice daily at 0600 and 1500 h with ad libitum dry hay (Table 5.1). Postpartum cows were also fed by TMR twice daily for ad libitum intake (5% orts, on an as-fed basis; Table 5.1) and orts were weighed once daily to determine feed intake for individual cows.

5.3.3 *Chemical Analysis*

Representative samples of TMR were collected once weekly and dried in a forced air oven at 65°C for 48 h. The dried samples were then stored at -20°C until later analysis. Samples were composited on a monthly basis, ground through a 1-mm screen using a Wiley mill (A.H Thomas Philadelphia, PA) and then analyzed for DM, ash and ether extract according to AOAC (1990). Neutral (Van Soest et al., 1991) and acid (AOAC, 1990) detergent fiber were determined using an Ankom Fiber Analyzer (Ankom Technology Corp., Macedon, NY). Crude protein (N x 6.25) was

analyzed using a Leco Nitrogen Analyzer (Truspec Nitrogen Determinator System, Leco Corp., St. Joseph, MI). Acid and neutral detergent insoluble protein were estimated by analyzing total N of ADF and NDF residues, respectively (Licitra et al., 1996).

5.3.4 *Milk Production and Analysis*

Individual milk yields were recorded at each milking (0630 and 1730 h). Milk samples were collected twice daily on d 4, 6, 11, 13, 18, 20, 25, and 27 postpartum and composited by cow according to volume. Milk samples were analyzed for fat, protein, lactose, and MUN using an infrared analyzer whereas SCC was analyzed using a SCC analyzer (Valacta, Sainte-Anne-de-Bellevue, Canada). Total milk solids were determined according to AOAC (1990).

5.3.5 *Serum Collection and Analysis*

Blood samples were obtained by venipuncture of the coccygeal vein or artery at least 30 min prior to morning feeding on d -21, -14, -7, -3, and -1 relative to expected calving date and on d 1, 3, 7, 14, 21, and 28 postpartum. Blood samples were collected into 10-mL serum Monoject blood tubes (Monoject Blood Collection Tube, Mansfield, MA) using 20G vacutainer needles (BD Vacutainer PrecisionGlide Multiple Sample Needle, Plymouth, UK). Blood sample processing and serum extraction were as previously described (Higginson et al., under review). Serum samples were then stored at -80°C until later analysis. All serum samples were analyzed at the Animal Health Laboratory (University of Guelph, ON, Canada) for BHBA, NEFA, glucose, protein, albumin, globulin, haptoglobin, urea, cholesterol, total Ca, P, Na, K, Mg, Cl, GLDH, AST, and haptoglobin using a Roche Cobas 6000 c501 automated chemistry analyzer (Roche, Mississauga, Canada). Analytical protocols and test reagents are as described previously (Higginson et al., under review).

Serum fatty acids were analyzed by gas chromatography (Varian 3900 with 8400 auto sampler with flame ionization detector at 260°C, Varian Analytical Instruments, Walnut Creek, CA) as described previously (Neveu et al., 2013).

5.3.6 *BCS*

Body condition scores of each cow were assessed by 2 independent and trained evaluators using a 5-point scale (Wildman et al., 1982). Individual BCS were recorded prior to morning feeding on d -21, -14, -7, and -1 relative to expected calving and d 1, 7, 14, 21, and 28 postpartum.

5.3.7 *Statistical Analysis*

Data were analyzed as repeated measures using an analysis of variance (ANOVA) with PROC MIXED of SAS (SAS Institute, 2010) and the following model:

$$Y_{ijk}= \mu + trt_i + cow_{ij} + block_j + time_k + trt_i*time_k + e_{ijk}$$

where Y_{ijk} represents the parameter being measured, μ is the overall mean, trt_i is the fixed effect of the ith treatment (i = 1 or 2), cow_{ij} is the random effect of cow from the jth block receiving the ith treatment, $block_j$ is the fixed effect of the jth block (j = 1, 2,...or 13), $time_k$ represents the fixed effect of the kth time point (k= 1, 2...or 11 for blood metabolites) or (k = 1, 2,...or 8 for BCS) or (k = 1, 2, 3 or 4 for DMI, milk yield and milk composition), trt_i*time_k is the fixed effect of interaction of ith treatment and k^{th} time point, and e_{ijk} represents the residual error [$e_{ijk} \sim N (0, \sigma^2_e)$]. Treatment differences were declared at $P < 0.05$ while statistical tendencies were declared at $0.05 < P < 0.10$.

5.4 RESULTS

5.4.1 *Dry Matter Intake and BCS*

All cows consumed their allotted 10kg of prepartum TMR. Postpartum DMI was similar between control and YMPL cows and increased ($P < 0.05$) as lactation progressed (Figure 5.1A). Likewise, BCS was not influenced by treatments but there

was a significant time effect: BCS continued to decrease (P < 0.05) as lactation progressed (Figure 5.1B). There were no significant time x treatment interactions for DMI (P = 0.35) or BCS (P = 0.72).

5.4.2 *Milk Production and Composition*

No treatment x time interactions were observed for milk production or composition; therefore, only main effects are herein reported (Table 5.2). Dietary treatments had no effect on yields of milk, milk fat, milk protein, SNF, 4%FCM, SCM , and ECM. However, milk yield was affected by time and increased (P < 0.01) as lactation progressed (Figure 5.1C).

Feeding cows YMPL did not influence milk fat, lactose or TS percentages (Table 5.2). Milk levels of MUN and SCC were also not different between treatments. However, milk protein percentage was greater (P < 0.05) among control cows than YMPL-fed cows during wk 1 and 2 postpartum (Figure 5.1D). Overall, milk fat, protein and TS percentages decreased (P < 0.05) as lactation progressed, probably due to increased milk yield.

5.4.3 *Blood Biochemical Parameters*

The YMPL treatment had no significant effects on serum biochemical parameters (Table 5.3). However, time effects were significant (P < 0.02) for all parameters. Significant treatment x time interactions were observed for serum NEFA, AST and GLDH concentrations. For example, serum NEFA concentrations were lower (P < 0.05) in control cows on d 3 and 14 postpartum, and there was a tendency (P = 0.06) for lower serum NEFA concentration on d 7 postpartum (Figure 5.2A). However, despite higher NEFA in control cows, serum BHBA levels were not different between treatments (Figure 5.2B). Nevertheless, both NEFA and BHBA levels peaked immediately after parturition and gradually decreased as lactation progressed following the normal physiological changes in transition cows. Serum AST concentrations were greater (P < 0.05) in YMPL-supplemented cows on d 1, 3 and 7 postpartum when compared with control cows (Figure 5.2C). Similarly, serum GLDH concentrations were greater (P < 0.05) in YMPL-supplemented cows on d 7

and 14 postpartum (Figure 5.2D). Serum haptoglobin concentration was lower ($P <$ 0.05) in YMPL- supplemented cows than control cows on d 7 and 14 postpartum (Figure 5.2E). Blood urea nitrogen was greater ($P < 0.05$) in YMPL- supplemented cows than control cows on d 1 post-calving.

5.4.4 *Serum Fatty Acid Composition*

The YMPL treatment had no significant effect on serum concentrations of long chain fatty acids (C14:0 to C20:4; Table 5.4). All serum fatty acids were significantly affected by time, except for C14:0. Significant treatment x time interactions were observed for C16:1, C18:3 and C20:4. Serum C16:1 and C20:4 concentrations were lower ($P < 0.05$) in YMPL-supplemented cows when compared to control cows on d -7 prepartum whereas C18:3 level was greater ($P < 0.05$) in YMPL-supplemented cows on d 3 postpartum (Figure 5.3).

5.4.5 *Blood Macrominerals*

The YMPL treatment had no major effect on serum concentrations of macro-minerals (Table 5.5). There were significant treatment x time interactions for serum Ca and K concentrations. Serum Ca concentration was lower ($P < 0.05$) in YMPL-supplemented cows than control cows on d 1 postpartum (Figure 5.4). In contrast, serum K levels were greater ($P < 0.05$) in YMPL-supplemented cows when compared to control cows on d -1 prepartum. The Ca:P ratio was greater ($P < 0.05$) on d 21 postpartum while Na:K ratio was lower ($P < 0.05$) on d -1 prepartum in YMPL-supplemented cows compared to control cows. However, serum concentrations of P, Mg, Na and Cl were not influenced by treatments (data not shown).

5.5 DISCUSSION

This study attempted to overcome the major nutritional and metabolic challenges of transition cows. As a new nutritional strategy, transition cows were supplemented with YMP in combination with live yeast. Our findings indicate that

YMPL supplementation had little or no positive effects on the lactation performance or serum biochemical parameters of transition cows.

Dry matter intake of early lactation cows was unaffected by YMPL. In our previous study, similar findings were observed when transition cows were fed YMP (Higginson et al., under review). The effects of YMP supplementation on DMI have mostly been researched in lactating dairy cows. However, results are inconsistent. For example, feeding YMP to lactating cows increased (Sabbia et al., 2012), decreased (Neal et al., 2016) or had no effect (Manthey et al., 2016) on DMI. In agreement with our findings, Yuan et al. (2015) found no effect of live yeast supplementation on prepartum or postpartum DMI in transition cows. However, in other studies, feeding live yeast during the transition period increased prepartum and postpartum (Dann et al., 2000) or prepartum (Ramsing et al., 2009) DMI. Live yeast has been reported to increase DMI by increasing the growth and activity of cellulolytic microorganisms for greater fibre digestibility (Alzahal et al., 2014; Chaucheyras-Durand et al., 2008, 2015). However, this effect of live yeast was not evidenced in our study. Our DMI result suggests the possibility of unknown interactions between YMP and live yeast that are yet to be determined.

Body condition score was not influenced by the YMPL treatment, likely due to the lack of differences in DMI. Similar findings were obtained when transition cows were fed YMP alone (Higginson et al., under review). In lactating cows, BCS was also not affected as a result of YMP (Sabbia et al., 2012; Neal et al., 2014; Manthey et al., 2016) or live yeast (Yuan et al., 2015; Ambris-Vilchis et al., 2017) supplementation. However, BCS decreased for all cows during late pregnancy and as lactation progressed which is consistent with previous studies (Doepel et al, 2008; Yuan et al., 2015). This decline in BCS has been attributed to fat mobilization (Bernabucci et al., 2008).

Milk yield and milk composition were also similar between YMPL and control cows. Similar findings were observed in lactating cows when YMP partially replaced dietary proteins such as soybean or canola meal (Sabbia et al., 2012; Neal et al., 2014; Manthey et al., 2016). The lack of differences in milk performance was explained by the fact that YMP and control diets were balanced for AA, CP, RDP, RUP

and NE_L (Manthey et al., 2016). However, in this study, AA density was not balanced between treatments. Therefore, it is likely that the additional AA intake as a result of YMP supplementation was utilized by other body tissues than the mammary gland. In agreement with our findings, Yuan et al. (2015) also observed no effect on milk yield when transition cows were supplemented with different levels (0, 30, 60, and 90 g/d) of the yeast *Saccharomyces cerevisiae*. However, milk protein percentage was lower in YMPL cows during weeks 1 and 2 postpartum. The later finding was unexpected and difficult to explain at this time. Nevertheless, neither YMP (Sabbia et al., 2012) nor live yeast (Nocek and Kautz, 2006; Yuan et al., 2015) supplementation was able to increase milk protein percentage in lactating or transition cows, respectively. Therefore, it can be hypothesized that amino acids supplied by YMPL were unable to reach the intestines in sufficiently high concentrations. Cows fed YMPL had higher MUN during week 1 postpartum. Sabbia et al. (2012) also observed a quadratic increase in MUN with increasing levels of dietary YMP. Taken together, these finding may indicate deamination of additional amino acids supply from YMP by the rumen microbes and N recycling into the milk. Indeed, rumen degradation of dietary protein and MUN are positively correlated (Schepers and Meijer, 1998). The transition from prepartum to postpartum was marked by drastic changes in serum NEFA, BHBA, and glucose concentrations which are in agreement with previous reports (AlZahal et al., 2014; Zaworski et al., 2014; Yuan et al., 2015). The peak in serum NEFA concentration at parturition is typical for transition cows (Doepel et al., 2002; Janovick Guretzky et al., 2006; Zaworski et al., 2014). At parturition, transition cows mobilize large amounts of triglycerides from adipose tissue in the form of NEFA into the blood causing a rapid decline in body weight and BCS. However, serum NEFA concentrations were higher in YMPL-supplemented cows on d 3 and 14 postpartum and tended to be higher on d 7 postpartum. Moreover, on d 1, 3, 7 and 14 postpartum, NEFA concentrations exceeded 0.7 mmol/L, indicating severe energy deficits and rapid mobilization of the adipose tissue (Drackley, 1999; McArt et al., 2013). Mobilization of the adipose tissue in YMPL cows was evidenced by higher serum concentration of C18:1 on d 3 postpartum. At calving, C18:1 contributes extensively to serum NEFA concentration,

and is reported to be a major fatty acid present in and mobilized from the adipose tissue (Rukkwamsuk et al., 2000). The higher NEFA concentration from YMPL supplementation is difficult to explain at this time. In contrast, NEFA concentration in control cows remained below this threshold (0.7 mmol/L) throughout the study.

The effects of YMP or yeast supplementation on blood biochemical parameters of lactating and transition cows are inconsistent. Feeding YMP to transition cows significantly reduced serum concentrations of both NEFA and BHBA (Higginson et al., under review). In mid-lactation cows, however, plasma NEFA concentration was unaffected while BHBA concentration increased linearly as the level of dietary YMP increased (Sabbia et al., 2012). Live yeast supplementation to transition cows increased plasma BHBA concentration, but NEFA concentration was not affected (Nocek and Kautz, 2006; Yuan et al., 2015). Conversely, when transition cows were fed live yeast, plasma NEFA concentration was not altered from d 1 to 7 postpartum, but reduced from d 8 to 22 postpartum whereas BHBA concentration was not affected throughout the study (Nocek et al., 2003).

Despite higher serum NEFA concentration in YMPL cows, serum BHBA concentration was not different from control cows and remained below the threshold (1.2 mmol/L) for sub-clinical ketosis (McArt et al., 2013). Our NEFA and BHBA findings are somewhat atypical for transition cows with regards to negative energy balance and adipose tissue mobilization. In transition cows, higher NEFA is usually consistent with higher BHBA (Doepel et al., 2002; Zaworski et al., 2014; Yuan et al., 2015; Higginson et al., under review). Indeed, hepatic oxidation of NEFA is the main substrate for BHBA (Drackley et al., 2001; Grummer et al., 2004) which is used as energy substrate by other body tissues. Nevertheless, our BHBA results suggest that adipose tissue mobilization or NEFA transit into the liver of YMPL cows may not have been excessive. The YMPL cows had higher energy deficits from d 3 to 14 postpartum, but then recovered quickly.

Serum glucose concentration was not altered by YMPL supplementation. However, YMP (Higginson et al., under review) or live yeast (Nocek et al., 2003) supplementation have been shown to increase blood glucose concentrations in transition cows. Interestingly, in both studies, transition cows were less

metabolically stressed (lower NEFA and BHBA levels). We previously reported that higher glucose in YMP-fed transition cows was derived through gluconeogenesis of amino acids supplied by YMP, and that the synthesized glucose was preferentially utilized as energy source such that adipose tissue mobilization was not as necessary.

In transition cows, blood glucose concentration is usually lowest at time of calving due to very high glucose demand for lactose synthesis in milk. Ingvartsen et al. (2000) attributed the changes in blood metabolites (i.e. glucose and NEFA) in postpartum cows to greater demand for glucose by the mammary gland and the use of lipid (NEFA) as an energy source to support lactation. Consequent to a negative energy balance, transition cows in early lactation rely mostly on gluconeogenesis as principal source of glucose for milk synthesis. During early lactation, increased gluconeogenesis and NEFA mobilization from the adipose tissue are mediated by higher glucagon and lower insulin concentrations (Drackley et al., 2001). High insulin resistance affects glucose utilization by making glucose less available to insulin-dependent tissues (i.e. skeletal muscles and adipose tissue), but more available to insulin-independent tissues such as the mammary gland (Bell, 1995).

Cows fed the YMPL diet had higher serum concentrations of AST (d 1, 3 and 7) and GLDH (d 7 and 14) postpartum, probably due to higher hepatic NEFA uptake and lipid accumulation in the liver (Bobe et al., 2004). When NEFA uptake by the liver exceeds its capacity to oxidize fat, NEFA are reesterified into triacylglycerol (TAG) and an accumulation of TAG may cause fatty liver and liver damage. Under such conditions, AST, a hepatocyte leakage enzyme, is expressed and reflects liver damage (Gonzalez et al., 2011; Ok et al., 2013). However, AST is not specific to the liver while GLDH is more specific and sensitive to the liver, and is expressed when there is complete cell death (Ok et al., 2013). According to Mostafavi et al. (2013), cows with AST >120 U/L had 2.9x greater risk for fatty liver. An optimal threshold has been established for AST at 100 U/L (Herdt and Gerloff, 2009). In our study, AST concentrations in YMPL-supplemented cows was >100 U/L on d 1, 3 and 7 postpartum, and then declined <90 U/L. Serum concentrations of GLDH were higher in YMPL cows at d 7 and 14 postpartum when compared to control cows, but did not exceed the reference value of 50 U/L for healthy fresh cows as established by the

Animal Health Lab of the University of Guelph (Animal Health Lab, 2015). Nevertheless, the higher AST and GLDH levels in YMPL cows suggest that these cows may have greater risks for TAG accumulation in the liver than control cows. Reasons for such results are unknown.

Haptoglobin is an acute phase protein synthesized by hepatic cells in response to inflammation or stress (Murata et al., 2004; Bannikov et al., 2011). Elevated haptoglobin concentration is linked to various disorders such as mastitis, metritis and displaced abomasum (Huzzey et al., 2009; Duffield, 2015). A positive correlation has been reported between plasma haptoglobin and incidence of fatty liver (Ametaj et al., 2002). In our study, YMPL-supplemented cows had lower serum haptoglobin concentrations on d 7 and 14 postpartum suggesting that YMPL cows experienced less liver inflammation than control cows. Amino acids supplied by YMPL supplementation may have assisted in immunoregulation postpartum. According to Li et al. (2007), amino acids enhance immune functions such as macrophage activation, antioxidation and mitochondrial fatty acid synthesis

The YMPL treatment had no major effect on serum macrominerals, except that Ca concentrations was lower on d 1 postpartum whereas K level was higher on d -1 prepartum. Serum Ca concentration is generally maintained between 2.1 to 2.5 mmol/L (Goff, 2008). However, in both groups of cows, serum Ca concentrations were below 2.0 mmol/L on d 1 postpartum, suggesting that fresh cows were at risk for hypocalcemia. Hypocalcaemia usually occurs between 12 and 24 h after calving when Ca requirement is extremely high (Goff, 2008). It is well documented that hypocalcaemia reduces DMI and milk yield, compromises immune functions and increases the risk for metabolic disorders (Kimura et al., 2006; Goff, 2008). By d 3 postpartum, cows regained Ca homeostasis and were above the hypocalcemic threshold (i.e. >2.0 mmol/L) (NRC 2001). For both groups of cows, serum P levels (1.33 – 1.98 mmol/L) were within the acceptable range of 1.3- 2.6 mmol/L throughout the study (Goff 2000).

5.6 CONCLUSIONS

The supplementation of YMPL to transition cows had no significant effect on milk performance, DMI or BCS. Cows fed YMPL had higher serum NEFA concentration around time of calving (d 3 to 14), but were able to recover quickly. As BHBA and glucose levels were not affected by YMPL, it is feasible to believe that adipose tissue mobilization was not excessive. Interestingly, YMPL significantly reduced serum haptoglobin levels, indicating lower risk of liver inflammation. With reference to our previous study (Higginson et al., under review), our current findings regarding higher NEFA reflecting adipose tissue mobilization was unexpected, and warrants further investigation. Finally, when compared to our previous study, results from the current study indicate that mode of supplementing YMP (pellet or powder form) may possibly affect its efficacy.

Table 5.1. Ingredients and chemical composition (mean ± SD) of pre- and post-partum diets

Item, % of DM	TMR Diet[1]	
	Prepartum	Postpartum
Ingredient		
Corn silage	31.50	29.17
Alfalfa hay	56.93	52.67
Soybean meal	8.37	7.75
Dried ground corn	-	7.58
Mineral Premix – prepartum[2]	3.07	-
Mineral Premix – postpartum[3]	-	2.83
Chemical composition, % of DM (unless otherwise noted)		
DM	43.65 ± 4.12	42.93± 4.08
Ash	3.08 ± 0.87	3.09 ± 0.79
CP	14.21 ± 1.38	16.03 ± 0.71
NDF	38.65 ± 4.93	39.60± 5.01
ADF	23.51 ± 2.98	24.89± 3.54
ADL	5.71 ± 0.85	5.53 ± 0.76
Neutral detergent-insoluble CP, % of CP	6.39 ± 1.31	7.23 ± 0.96
Acid Detergent- insoluble CP, % of CP	4.30 ± 0.98	5.01 ± 0.69
NE_L,[4] Mcal/kg	1.78	1.91

[1]Cows were fed exactly 10 kg of the prepartum diet daily whereas the postpartum diet was provided for 5% orts.

[2]Contained 19.63% Ca, 1.29% P, 11.33% Mg, 1.20% K, 0.51% Na, 0.001% Cl, 2.42% S, 0.08% Mn, 0.03% Cu, 0.06% Fe, 1.00 Zn, 0.005% I, 0.008% Co, 0.004% Se, 467 KIU/kg of vitamin A, 113 KIU/kg of vitamin D, 7, 092 mg/kg of vitamin E, 19.2 mg/kg of Biotine. Minerals were mostly in organic form.

[3]Contained 14.00% Ca, 1.79% P, 2.87% Mg, 0.71% K, 0.29% Na, 0.0003% Cl, 1.42% S, 0.05% Mn, 0.02% Cu, 0.08% Fe, 0.06% Zn, 0.003% I, 0.005% Co, 0.002% Se, 262 KIU/kg of vitamin A, 61 KIU/kg of vitamin D, 1, 803 mg/kg of vitamin E, 19.2 mg/kg of Biotine. Minerals were mostly in organic form.

[4]Estimated according to NRC (2001).

Table 5.2. Effects of feeding yeast microbial protein and live yeast (YMPL) on milk performance in Holstein cows during the first 28 d postpartum

| | Treatment[1] | | | P-value[2] | |
	Control	YMPL	SE[3]	Trt	Time
Parameter					
Production, kg/d					
Milk	34.16	36.18	2.52	0.579	<0.0001
Fat	2.22	2.36	0.18	0.572	0.005
Protein	1.12	1.12	0.07	0.986	0.034
SNF	8.33	8.11	0.216	0.463	0.009
SCM	43.30	45.15	3.00	0.67	<0.0001
ECM	47.54	49.96	3.50	0.631	<0.0001
4% FCM	46.98	49.84	3.56	0.58	<0.0001
MUN, mg/dl	7.35	8.98	0.63	0.08	<0.0001
Composition, %					
Fat	6.65	6.68	0.32	0.97	0.005
Protein	3.34	3.16	0.06	0.033	<0.0001
Lactose	4.35	4.31	0.45	0.54	0.0005
TS	14.97	14.73	0.29	0.56	<0.0001
SCC, cells / μL	414.080	1371.830	522.06	0.21	0.22

[1]Total mixed ration without (control) or with yeast microbial protein and live yeast (YMPL; 100 g YMP prepartum with 10g live yeast followed by 200 g YMP with 10g live yeast postpartum) supplementation to cow.

[2]Average SEM of the two treatment groups.

[3] *P*-value for treatment effects

Table 5.3. Pooled effects of feeding yeast microbial protein and live yeast (YMPL) on serum biochemical parameters in Holstein cows from the last 21 days in gestation to the first 28 d postpartum

| Parameter | Treatment[1] | | | P-value [2] | | |
	Control	YMPL	SE[3]	Trt	Time	Trt * Time
BHBA, umol/L	668.13	613.00	108.18	0.72	0.03	0.81
NEFA, mmol/ L	0.38	0.50	0.06	0.18	<0.0001	0.21
Glucose, mmol/ L	3.66	3.65	0.09	0.90	<0.0001	0.97
Total protein, g/L	68.09	69.47	1.35	0.48	<0.0001	0.57
Urea, mmol/L	2.85	3.32	0.18	0.07	0.0001	0.80
Cholesterol, mmol/L	2.17	2.25	0.17	0.76	<0.0001	0.99
AST, U/L	68.15	73.99	6.19	0.51	<0.0001	0.60
GLDH, U/L	11.80	18.97	4.43	0.26	0.02	0.84
GGT, U/L	17.97	21.98	1.90	0.16	0.001	0.73
Albumin, g/L	33.10	34.64	0.66	0.11	0.007	0.25
A:G	1.00	1.05	0.57	0.51	<0.0001	0.83
Haptoglobin, g/L	0.53	0.34	0.10	0.17	0.0002	0.80

[1]Total mixed ration without (control) or with yeast microbial protein and live yeast (YMPL; 100 g YMP prepartum with 10g live yeast followed by 200 g YMP with 10g live yeast postpartum) supplementation to cow.

[2]Average SEM of the two treatment groups.

[3] P-value for treatment effects

Table 5.4. Pooled effects of feeding YMP and live yeast (YMPL) serum fatty acids in Holstein cows from the last 21 days in gestation to the first 28 d postpartum

| Parameter | Treatment[1] | | SE[3] | P-value [2] | | |
	Control	YMPL		Trt	Time	Trt * Time
Fatty acid, % of corrected area						
C14:0	2.13	2.19	2.13	2.19	2.132	2.19
C16:0	17.46	18.06	0.51	0.40	0.000	0.84
C16:1	1.77	1.54	0.19	0.38	0.0036	0.003
C18:0	17.14	17.95	0.52	0.28	<0.0001	0.99
C18:1	12.43	13.51	0.68	0.27	<0.0001	0.35
C18:3	5.92	5.26	0.48	0.34	0.01	0.07
C20:4	3.11	3.04	0.19	0.78	<0.0001	0.16

[1]Total mixed ration without (control) or with yeast microbial protein and live yeast (YMPL; 100 g YMP prepartum with 10g live yeast followed by 200 g YMP with 10g live yeast postpartum) supplementation to cow.

[2]Average SEM of the two treatment groups.

[3] P-value for treatment effects

Table 5.5. Effects of feeding YMP and live yeast (YMPL) serum macrominerals in Holstein cows during the last 21 days in gestation and first 28 d postpartum

| Parameter | Treatment[1] | | SE[3] | P-value [2] | |
	Control	YMPL		Trt	Time
Ca, mmol/ L	2.27	2.29	0.03	0.56	<0.0001
P, mmol/L	1.57	1.65	0.05	0.24	<0.0001
Ca: P	1.49	1.46	0.05	0.58	0.001
K, mmol/ L	4.66	4.87	0.09	0.10	0.0002
Na, mmol/ L	139.71	140.40	0.58	0.41	<0.0001
Na: K	30.11	29.09	0.52	0.17	<0.0001
Mg, mmol/L	0.90	0.86	0.03	0.44	<0.0001
Cl, mmol/ L	98.53	99.01	0.53	0.52	<0.0001

[1]Total mixed ration without (control) or with yeast microbial protein and live yeast (YMPL; 100 g YMP prepartum with 10g live yeast followed by 200 g YMP with 10g live yeast postpartum) supplementation to cow.

[2]Average SEM of the two treatment groups.
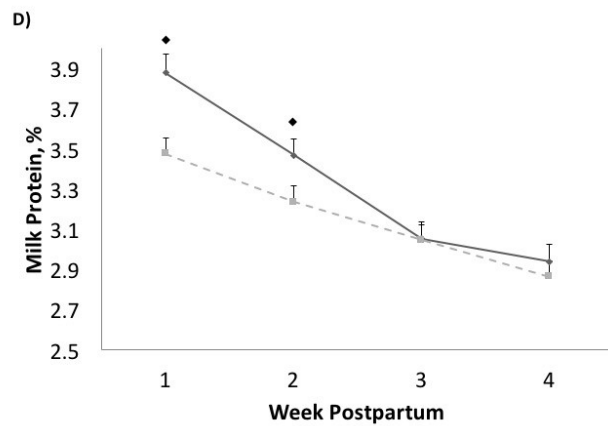
[3] P-value for treatment effects

Figure 5.1 Effects of yeast microbial protein and live yeast (YMPL; 100 g YMP prepartum with 10g live yeast followed by 200 g YMP with 10g live yeast postpartum) supplementation on DMI (A), BCS (B), milk yield (C) and milk protein percentage (D).

A)

B)

C)
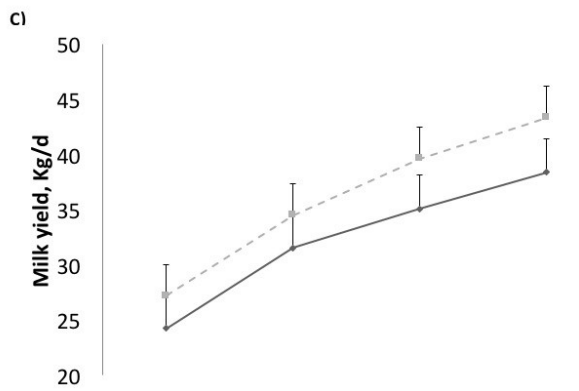
D)

Day Relative to Parturition

64

Figure 5.2. Effects of yeast microbial protein and live yeast (YMPL; 100 g YMP prepartum with 10g live yeast followed by 200 g YMP with 10g live yeast postpartum) supplementation on serum NEFA (A), BHBA (B), AST(C), GLDH (C) and haptoglobin (D) concentrations ♦ = statistical difference ($P < 0.05$) between YMPL and control cows

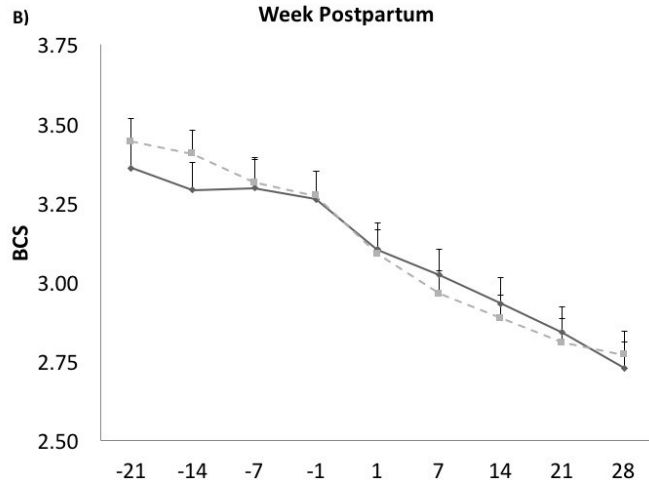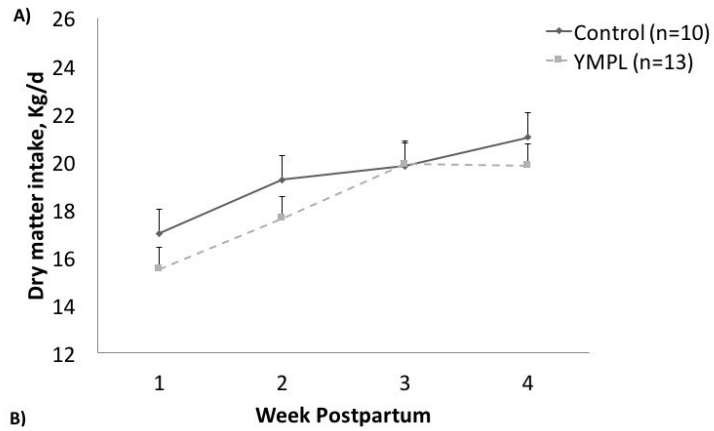◉ = statistical tendency ($P < 0.1$) between YMPL and control cows

Figure 5.3. Effects of yeast microbial protein and live yeast (YMPL; 100 g YMP prepartum with 10g live yeast followed by 200 g YMP with 10g live yeast postpartum) supplementation on serum C16:1 (A), C18:3 (B) and C20:4 (C) levels ♦= statistical difference ($P < 0.05$) between YMPL and control cows
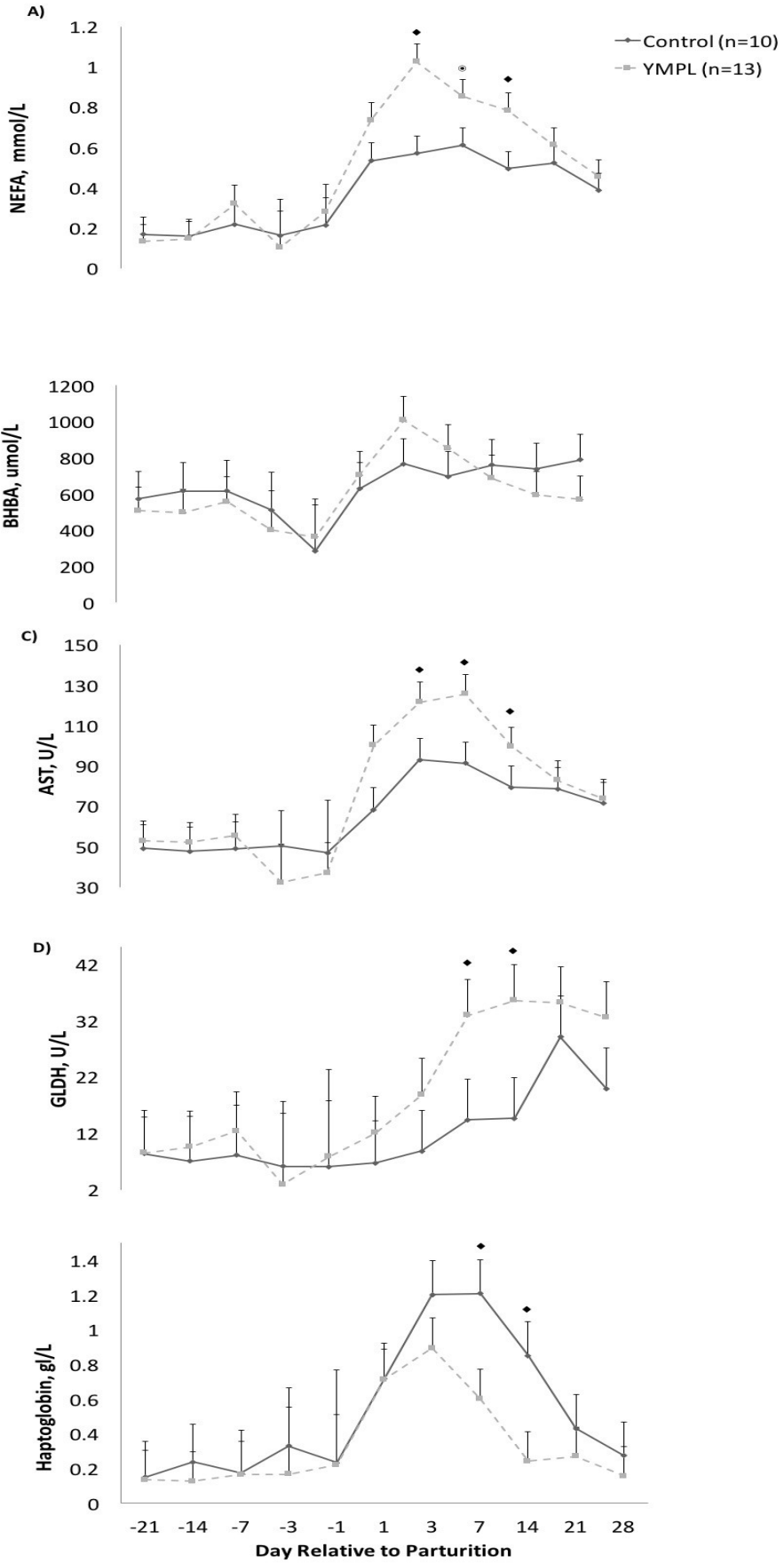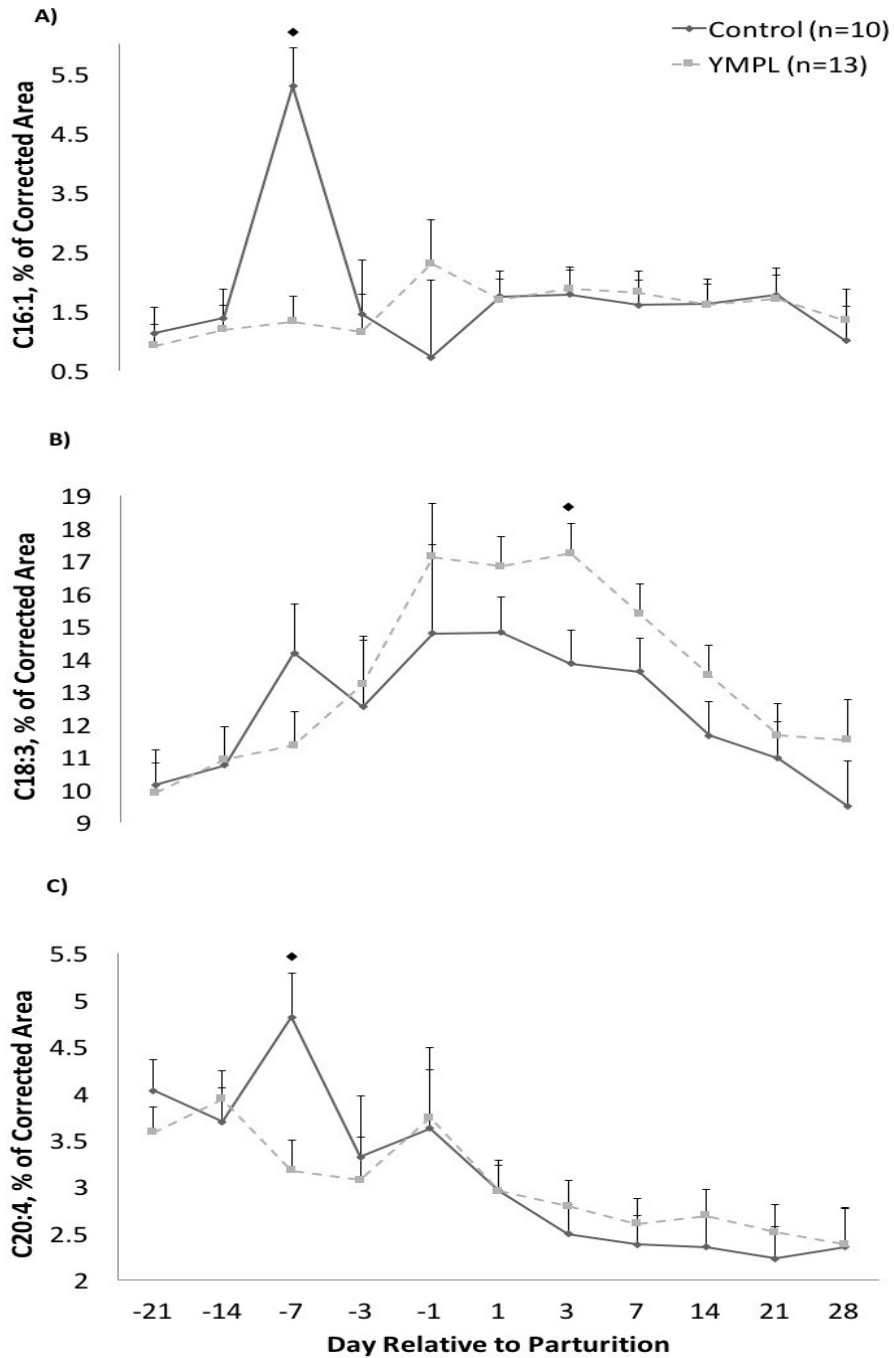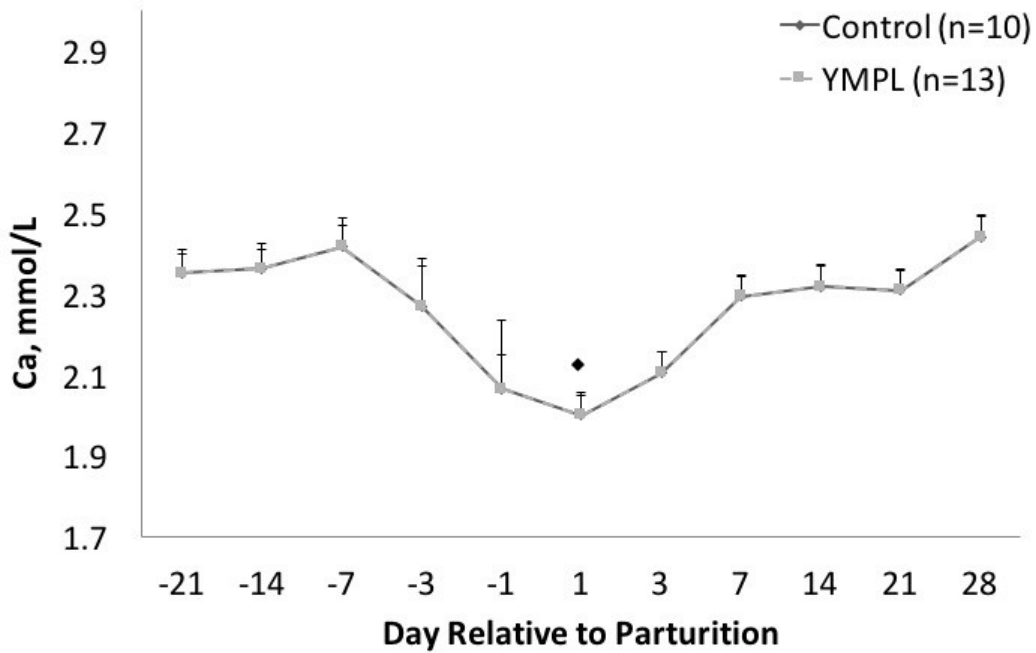
Figure 5.4. Effects of yeast microbial protein and live yeast (YMPL; 100 g YMP prepartum with 10g live yeast followed by 200 g YMP with 10g live yeast postpartum) supplementation on serum calcium levels ♦= statistical difference ($P < 0.05$) between YMPL and control cows

CHAPTER 6. GENERAL DISCUSSION AND CONCLUSION

Two experiments were conducted to address the postpartum negative energy balance (NNB) that is associated with metabolic disorders (i.e. ketosis, displaced abomasum and mastitis) in transition cows. YMP has an AA profile similar to that of MCP (Sabbia et al., 2012). Most importantly, because of its small particle size, YMP is presumed to escape ruminal degradation by the rumen bacteria to provide AA directly into the duodenum. We hypothesize that YMP may help address the NNB of transition cows, especially under conditions of low DMI. In Experiment 1, we examined the effects of YMP on DMI, milk production, milk composition, BCS, serum biochemical parameters and serum macrominerals. In Experiment 2, we examined the effects of YMP fed in combination with the live yeast (*Saccharomyces cerevisiae*; **YMPL**) on the same measured parameters as in Experiment 1.

In both studies, milk production, milk composition, BCS and DMI were not influenced by YMP or YMPL supplementations. Our findings were similar to previous results reported for early and midlactation cows in which YMP was supplemented at different dosages (Sabbia et al., 2012; Neal et al., 2014; Manthey et al., 2016). However, live yeast supplementation during the transition period of dairy cows has been reported to increase DMI (Wohlt et al., 1998; Dann et al., 2000). We believe that some unknown interactions between YMP and live yeast may have hindered any positive effects on DMI.

In Experiment 1, we found that metabolic stress was significantly reduced by YMP supplementation. Treated cows had lower serum NEFA and BHBA concentrations during most of the postpartum period. In contrast, YMP supplementation to mid-lactation cows has been reported to increase plasma BHBA concentration without affecting NEFA levels (Sabbia et al., 2012). These inconsistent results may possibly be explained by differences in stage of lactation of cows (transition vs lactating). High blood NEFA and BHBA are associated with postpartum metabolic disorders (McArt et al., 2013) that negatively impact animal health and welfare as well as farm profitability. Interestingly, YMP-supplemented cows had higher serum glucose levels which was most like derived through

gluconeogenesis of AA contained in YMP. The energy requirements for parturition and especially milk synthesis of YMP cows were principally met through the higher blood glucose whereas control cows had to rely on adipose tissue mobilization. Serum AST and GLDH were also lower at few time-points of the postpartum period which, together with higher blood cholesterol, suggest that YMP supplemented cows had improved hepatic health and possibly lower risk for hepatic lipidosis postpartum. Previous studies have reported that cows with hepatic lipidosis had lower serum cholesterol accompanied by higher NEFA, AST and GLDH concentrations (Mostafavi et al., 2013; Ok et al., 2013).

In Experiment 2, cows fed the YMPL diet had greater serum NEFA concentration on d 3 and 14 postpartum with a tendency for NEFA to be higher on d 7 postpartum. However, serum BHBA concentrations were unaffected by YMPL supplementation. Serum BHBA levels remained below SCK threshold (1200 umol/L) throughout the trial, suggesting that fat mobilization was not excessive and that cows were at low risk for metabolic disorders (McArt et al., 2012). Feeding transition cows with YMPL also increased serum AST on d 3, 7 and 14 postpartum and serum GLDH on d 7 and 14 postpartum. However, serum GLDH level remained within normal range (< 50 U/L) for postpartum cows (Animal Health Lab, University of Guelph). Considering the lack of significant differences in serum cholesterol or glucose between both groups, YMPL cows likely had proper liver functions and were not at higher risks of fatty liver, despite our findings about higher AST and GLDH concentrations. We believe that YMPL-supplemented cows may have been subjected to more metabolic stress (higher NEFA) around time of calving; however, these cows recovered quickly as indicated by rapid drop in serum NEFA levels to within normal range (<100 U/L). Moreover, cows did not experience any metabolic health disorders that would otherwise have required veterinary assistance. In Experiment 2, YMPL-supplemented cows had lower serum haptoglobin levels. Haptoglobin is an acute phase protein that is released in response to immunological challenge or stress. Free haptoglobin has been reported to increase liver inflammation. Therefore, our finding suggests that YMPL-supplemented cows may have experienced less liver inflammation postpartum.

Based on findings of Experiment 1, we may conclude that feeding YMP alone to transition cows may be an effective dietary strategy to reduce metabolic stress and possibly reduce risk of metabolic disorders without negatively impacting cow performance. However, when fed in powdered form or in combination with live yeast as in Experiment 2, YMPL increased specific serum biochemical parameters (NEFA, AST and GLDH) as an indication of fat mobilization and fat accumulation in the liver. These inconsistent results merit further investigation into the mode of YMP supplementation (pellet in Experiment 1 versus powdered form in Experiment 2) and the possibility of any negative interaction with live yeast which may have hindered the activity of both supplements during the transition period.

BIBLIOGRAPHY

Agle, M., A. N. Hristov, S. Zaman, C. Schneider, P. Ndegwa, and V. K. Vaddella. 2010. The effects of ruminally degraded protein on rumen fermentation and ammonia losses from manure in dairy cows. J. Dairy Sci. 93:1625–1637.

Akers, R. M. 2002. Lactation and the mammary gland. 1st ed. Iowa State Press, Ames, IO.

Al Ibrahim, R. M., A. K. Kelly, L. O'Grady, V. P. Gath, C. McCarney, and F. J. Mulligan. 2010. The effect of body condition score at calving and supplementation with Saccharomyces cerevisiae on milk production, metabolic status, and rumen fermentation of dairy cows in early lactation. J. Dairy. Sci. 93:5318-5328.

AlZahal, O., H. McGill, A. Kleinberg, J. I. Holliday, I. K. Hindrichsen, T. F. Duffield and B. W. McBride. 2014. Use of a direct-fed microbial product as a supplement during the transition period in dairy cattle. J. Dairy Sci. 97:7102-7114.

Amanlou, H., T. Amirabadi Farahani, and N. Eslamian Farsuni. 2017. Effects of rumen undegradable protein supplementation on productive performance and indicators of protein and energy metabolism in Holstein fresh cows. J. Dairy Sci. 100:3628-3640.

Ambriz-Vilchis, V., N. S. Jessop, R. H. Fawcett, M. Webster, D. J. Shaw, N. Walker, and A. I Macrae. 2017. Effect of yeast supplementation on performance, rumination time, and rumen pH of dairy cows in commercial farm environments. J. Dairy Sci. 100:5449-5461.

Ametaj, B. N., Bradford, B. J., Bobe, G., and Beitz, D. C. 2002. Acute phase response indicates inflammatory conditions may play a role in the pathogenesis of fatty liver in dairy cows. J. Dairy Sci. 85:189 (Suppl. 1).

71

AOAC. 1990. Official Methods of Analysis. 15th ed. Association of Official
Analytical Chemistry, Arlington, VA.

Aschenbach, J. R., N. B. Kristensen, S. S. Donkin, H. M. Hammon and G. B. Penner.
2010. Gluconeogenesis in dairy cows: the secret of making sweet milk from
sour dough. Life. 62:869-877.

Bach, A., S. Calsamiglia and M. D. Stern. 2005. Nitrogen metabolism in the
rumen. J. Dairy Sci. 88: E9-E21 (Suppl. 1).

Baker., M. J., H. E Amos, A. Nelson, C. C. Williams, and M. A Froetschel. 1996.
Undegraded intake protein: effects on milk production and amino acid
utilization of cows fed wheat silage. Can. J. Anim. Sci. 76:367-376

Bauchart, D. D., D. Gruffat and D. Durand. 1996. Lipid absorption of hepatic
metabolism in ruminants. Proc. Nutr. Soc. 55:39-47.

Bell A. W. 1995. Regulation of organic nutrient metabolism during transition
from late pregnancy to early lactation. J. Anim. Sci. 73:2804-2819

Bell, A. W., W. S. Burhans, and T. R. Overton. 2000. Protein nutrition in late
pregnancy, maternal protein reserves and lactation performance in dairy
cows. Proc. Nutr. Soc. 59:119–126.

Bernabucci, U., B. Ronchi, N. Lacetera, and A. Nardone. 2005. Influence of body
condition score on relationships between metabolic status and oxidative
stress in periparturient dairy cows. J. Dairy Sci. 88:2017–2026.

Bobe, G., J. W. Young, and D. C. Beitz. 2004. Invited Review: Pathology, etiology,
prevention, and treatment of fatty liver in dairy cows. J. Dairy Sci. 87:3105-
3124.

Bremmer, D. R., T. R. Overton and J. H. Clark, 1997. Production and composition
of milk from Jersey cows administered bovine somatotropin and fed
ruminally protected amino acids. J. Dairy Sci. 80:1374-1380.

Brito, A. F., and G. A. Broderick. 2007. Effects of different protein supplements on milk production and nutrient utilization in lactating dairy cows. J. Dairy Sci. 90:1816–1827.

Canadian Dairy Commission. 2016. The Industry. Accessed Nov, 10, 2016. http://www.cdc-ccl.gc.ca/CDC/index-eng.php?id=3796

Canfield, R. W., C. J. Sniffen and W. R. Butler. 1990. Effects of Excess Degradable Protein on Postpartum Reproduction and Energy Balance in Dairy Cattle. J. Dairy Sci. 73:2342-2349.

Capper, J. L., R. A Cady, and D. E Bauman. 2009. The environmental impact of dairy production: 1944 compared with 2007. J Anim. Sci. 87: 2160-2167.

Chuaucheyras-Durand, F. and G. Fonty. 2001. Establishment of celluloytic bacteria and development of fermentative activities in the rumen of gnotobiotically-reared lambs receiving microbial additive Saccharomyces cerevisiae CNCM I-1077. Reprod. Nutr. Dev. 41:57-68.

Chuaucheyras-Durand, F., N. D. Walker and A. Bach. 2008. Effects of active dry yeasts on the rumen microbial ecosystem: Past, present and future. Anim. Feed Sci. Technol. 145:5-26.

Chew, B. P., F. R. Murdock, R. E. Riley, and J. K. Hillers. 1984. Influence of prepartum dietary crude protein on growth hormone, insulin, reproduction, and lactation of dairy cows. J. Dairy Sci. 67:270-275.

Clark, J. H., T .H. Klusmeyer, and M. R. Cameron. 1992. Microbial protein synthesis and flows of nitrogen fractions to the duodenum of dairy cows. J. Dairy Sci. 75:2304–2323.

Clark, J. H., T. H Klusmeyer, and M. R. Cameron. 1992. Symposium: Nitrogen metabolism and amino acid nutrition in dairy cattle. J. Dairy Sci. 75: 2304-2323.

Contreras, G. A. and L. M Sordillo, 2011. Lipid mobilization and inflammatory responses during the transition period of dairy cows. Comp. Immunol . Microbiol. Infect. Dis. 34:281-289.

Cunningham, K. D., M.J.Cecava, T. R. Johnson and P. A. Ludden. 1996. Influence of source and amount of dietary protein on milk yield by cows in early lactation. J. Dairy Sci. 79:620-630.

Dann, H. M., J. K. Drackley, G. C. McCoy, M. F. Hutjens and J. E. Garrett. 2000. Effects of yeast culture (Saccharomyces cerevisiae) on prepartum Intake and postpartum intake and milk Production of Jersey cows. J. Dairy Sci. 83:123-127

Deghan- Banadaky, M., M. Ebrahimi, R. Motameny, and S. R. Heidari. 2012. Effects of live yeast supplementation on mid-lactation dairy cow performances, milk composition, rumen digestion and plasma metabolites during hot season. J. Appl. Anim. Res. 41:137-142.

Doepel L., H. Lapierre and J. J. Kennelly. 2002. Prepartum performance and metabolism of dairy cows in response to prepartum energy and protein intake. J. Dairy Sci. 85:2315-2334.

Drackley, J. K. 1999. Biology of dairy cows during the transition period: The final frontier? J. Dairy Sci. 82:2259–2273.

Drackley, J. K., M. J. Richard, D. C. Beitz, and J. W. Young. 1992. Metabolic changes in dairy cows with ketonemia in response to feed restriction and dietary 1,3-butanediol. J. Dairy Sci. 75:1622–1634.

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. J. Dairy Sci. 84: E100–E112 (E. Suppl.).

Duffield, T. F., D. Sandals, K. E. Leslie, K. Lissemore, B. W. McBride, J. H. Lumsden, P. Dick and R. Bagg. 1998. Efficacy of Monensin for the prevention of subclinical ketosis in lactating dairy cows. J. Dairy Sci. 81:2866-2873

Duffield, T. F. 2015. Update on management of transition cows. Adv. Dairy
    Technol. 27: 319- 330.

Eicker SW. 1995 Milk production loss after displaced abomasum disease in New
    York Holsteins. ADSA:169 (Suppl. 1).

Esposito, G., P. C..Irons, E. C.Webb and A. Chapwanya. 2014. Interactions between
    negative energy balance, metabolic diseases, uterine health and immune
    response in transition dairy cows. Anim. Rep. Sci. 144:60-71.

Gaffney-Stomberg, E., B. Sun, C.E. Cucchi, C. a Simpson, C. Gundberg, J.E.
    Kerstetter, and K.L. Insogna. 2010. The effect of dietary protein on intestinal
    calcium absorption in rats. Endocrinol. 151:1071–1078.

Geishauser, T., K. Leslie and T. Duffield. 2000. Metabolic aspects in the etiology of
    displaced abomasum. Vet. Clinc. Nort. Am. Food Anim. Pract. 16:255-265.

Goff, J. P. 2006. Major Advances in Our Understanding of Nutritional Influences
    on Bovine Health. J. Dairy Sci. 89:1292-1301.

Goff, J. P. 2008. The monitoring, prevention, and treatment of milk fever and
    subclinical hypocalcemia in dairy cows. Vet. J. 176:50–57.

Goff, J. P. and R. L. Horst. 1997. Physiological changes at parturition and their
    relationship to metabolic disorders. J. Dairy Sci. 80:1260-1268.

González, F. D., R. Muiño, V. Pereira, R. Campos, and J. L Benedito. 2010.
    Relationship among blood indicators of lipomobilization and hepatic function
    during early lactation in high-yielding dairy cows. J. Vet. Sci. 12: 251-255.

Gonzalez, F. D., R. Muino, V. Pereira, R. Campos, and J. L. Benedito. 2011.
     Relationship among blood indicators of lipomobilization and hepatic
    function during early lactation in high-yielding dairy cows. J. Vet. Sci. 12:251–
    255.

Gordon, J. L., S. LeBlanc, and T. F. Duffield. 2013. Ketosis treatment in lactating
    dairy cattle. Vet. Clin. Food Anim. 29: 433- 445.

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 cattle. J. Dairy Sci. 83:703-710.

Grummer, R. R. 1995. Impact of changes in organic nutrient metabolism on feeding the transition dairy cow. J. Anim. Sci. 73:2820–2833.

Grummer, R. R. 1995. Impact of changes in organic nutrient metabolism of dairy cows in response to prepartum energy and protein intake. J. Dairy Sci. 73:2820-2833.

Grummer, R. R. G. Doug, D. G. Mashek and A Hayirli. 2004 Dry matter intake and energy balance in the transition period. Vet. Clin. Food Anim. 20:447-470.

Guard, C. 2008. The costs of common diseases of dairy cattle. Proc. Continuing Veterinary Education. San Diego, Ca.

Hartwell, J. R., M. J. Cecava and S. S. Donkin. 2000. Impact of dietary rumen undegraded   protein and rumen-protected choline on intake, peripartum liver triglyceride, plasma metabolites and milk production in transition dairy cows. J. Dairy Sci. 83:2907-2917.

Hayirli, A., R. R. Grummer, E. V. Nordheim, and P. M. Crump. 2002. Animal and dietary factors affecting feed intake during the prefresh transition period in Holsteins. J. Dairy Sci. 85: 3430-3443.

Heinrichs. A. J., .S. S. Costello and C. M. Jones. 2009. Control of heifer mastitis by nutrition. Vet. Microbiol. 134:172-176

Herdt, T. H. 1988. Fuel homeostasis in the ruminant. Vet. Clin. North Am. Food Anim. Pract. 4:213–231.

Herdt, T. H. and B. J. Gerloff. 2009. Ketosis. Anim. Food Practice. 5th ed. Elsevier. pp 141-144.

Herdt, T. H., and B. J. Gerloff. 2009. Fatty liver in dairy cattle. In: Food Animal Practice. Elsevier, St. Louis, MO.

Herdt, T.H. 1988. Fuel homeostasis in the ruminant. Vet Clin North Am Food
Anim Pract. 4:213-231.

Higginson, V., B. Baurhoo, Y. Schuermann, R. Duggavathi, and A. Mustafa. Effects
of yeast-derived microbial protein on lactational performance and
metabolic status of transition cows. J. Dairy Sci: under review.

Hoffman, P. C., R. R. Gruymer, R. D. Shaver, A. Broderick and T. R. Drendel. 1991.
Feeding early supplemental fat and undegraded intake protein lactation
to dairy cows. J. Dairy Sci. 74:3468-3474.

Huang, S. 2016. Effects of feeding extruded flaxseed on layer performance.
Thesis. McGill University.

Huyler. M. T., R. L. Kincaid and D. F. Dostal. 1999. Metabolic and yield responses
of multiparous Holstein cows to prepartum rumen-undegradable
protein. J. Dairy Sci. 82:527-536.

Huzzey JM, D. M. Veira, D. M. Weary and M. A. von Keyserlingk. 2007. Prepartum
behavior and dry matter intake identify dairy cows at risk for metritis. J Dairy
Sci. 90:3220-3233.

Indugu, N., K. Bittinger, S. Kumar, B. Vecchiarelli, and D. Pitta. 2016. A
comparison of rumen microbial profiles in dairy cows as retrieved by 454
Roche and Ion Torrent (PGM) sequencing platforms. PeerJ 4:e1599.

Ingvartsen, K. L., and J. B. Andersen. 2000. Integration of metabolism and intake
regulation: A review focusing on periparturient animals. J. Dairy Sci.
83:1573–1597.

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.
Livest. Prod. Sci. 83:277–308.

Janovick Guretzky, N. A., N. B. Litherland, K. M. Moyes, and J. K. Drackley. 2006. Prepartum energy intake effects on health and lactational performance in primiparous and multiparous Holstein cows. J. Dairy Sci. 89(Suppl. 1) (Abstr,).

Jensen, G. S., K. M. Patterson and I. Yoon. 2008. Yeast culture has anti-inflammatory effects and specifically activates NK cells. Comp. Immun. Microbiol. Infect. Disease. 31:487-500.

Jouany J. P 2006. Optimizing rumen functions in the close-up transition period and early lactation to drive dry matter intake and energy balance in cows. Anim. Reprod. Sci. 96:250–264.

Kalscheur, K. F., J. H. Vandersall, R. A. Erdman, R. A. Kohn and E. Russek-Cohen. 1999. Effects of dietary crude protein concentration and degradability on milk production responses of early, mid, and late lactation dairy cows. J. Dairy Sci. 82:545-554.

Kalscheur, K. F., R. L. Baldwin, B. P Glenn, and R. A. Kohn. 2006. Milk production of dairy cows fed differing concentrations of rumen-degraded protein. J. Dairy Sci. 89: 249-259.

Kimura, K., T.A. Reinhardt, and J.P. Goff. 2006. Parturition and hypocalcemia blunts calcium signals in immune cells of dairy cattle. J. Dairy Sci. 89:2588-2595.

Komaragiri, M. V. S. and R. A. Erdman. 1997. Factors affecting body tissue mobilization in early lactation dairy cows. 1. Effect of dietary protein on mobilization of body fat and protein. J. Dairy Sci. 83:2907-2917.

Larsen, M., H. Lapierre and N. B. Kristensen. 2014. Abomasal protein infusion in postpartum transition dairy cows: Effect on performance and mammary metabolism. J. Dairy Sci. 97:5608-5622.

Lean, I., and P. DeGaris. 2010. Transition cow management: A review for nutritional professionals, veterinarians and farm advisers. Dairy Australia. Southbank, Victoria.

LeBlanc, S. 2010. Monitoring metabolic health of dairy cattle in the transition period. J. Reprod. Dev. 56:29–35.

LeBlanc, S. J., K. E. Leslie and T. F. Duffield. 2005. Metabolic predictors of displaced abomasum in dairy cattle. J. Dairy Sci. 88:159-170.

Li, P., Y-L. Yin, D. Lin, S. W. Kim, and G. Wu. 2007. Amino acids and immune function. Br. J. Nutr. 98: 237- 252.

Licitra, G., T. M. Hernandez, and P.J. Van Soest. 1996. Standardization of procedures for nitrogen fraction of ruminant feeds. Anim. Feed Sci. Technol. 57:347–358.

Loor, J. J. 2010. Genomics of metabolic adaptations in the peripartal cow. Anim. 4:1110–1139.

Makimura, S., and N. Suzuki. 1982. Quantitative determination of bovine serum haptoglobin and its elevation in some inflammatory diseases. Japanese J. Vet. Sci. 44:15–21.

Mann, S, F. A. Leal Yepes, T. R. Overton, J. J Wakshlag, A. L. Lock, C. M. Ryan, and D. V. Nydam. 2015. Dry period plan of energy: Effects on feed intake, energy balance, milk production and composition in transition dairy cows. J. Dairy Sci. 98: 3366- 3382.

Manning, J., and J. Husband. 2014. Transition cows and ketosis. Accessed: Sept 21, 2016. http://beefandlamb.ahdb.org.uk/wp content/uploads/2014/07/Transition-cows-100714.pdf

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. J. Dairy Sci. 99:2775–2787.

McArt, J. A., D. V Nydam, G. R. Oetzel, T. R. Overton, and P. A. Ospina. 2013. Elevated non‑esterified fatty acids and beta-hydroxybutyrate and their association with transition dairy cow performance. Vet. J. 198:560–70.

Moallem, U., H.Lehrer, M. .Zachut and S.Yakoby. 2009. The effects of live yeast supplementation to dairy cows during the hot season on production, feed efficiency, and digestibility J. Dairy Sci. 92: 343-351.

Mostafavi, M., H. A. Seifi, M. Mohri, and A. Jamshidi. 2013. Optimal thresholds of metabolic indicators of hepatic lipidosis in dairy cows. Rev. Med. Vet. 164:564–571.

Mulligan, F. J., and M. L Doherty. 2008. Production diseases of the transition cow. Vet J. 176:3-9.

Mullins, C. R., L. K. Mamedova, M. J. Brouk, C. E. Moore, H. B. Green, K. L. Perfield, J. F. Smith, J. P. Harner and B. J. Bradford. 2012. Effects of monensin on metabolic parameters, feeding behavior, and productivity of transition dairy cows. J. Dairy Sci. 95:1323-1336.

Murata, H., N. Shimada, and M. Yoshioka. 2004. Current research on acute phase proteins in veterinary diagnosis: An overview. Vet. J. 168:28–40.

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 cows. J. Dairy Sci. 97:7716–7728.

Newbold, C. J., R. J. Wallace, and F. M. McIntosh. 1996. Mode of action of the yeast Saccharomyces cerevisiae as a feed additive for ruminants. Br. J. Nutr. 76: 249- 261.

Nocek, J. E., W. P. Kautz, A. Z. Leedle, and E. Block. 2003. Direct-fed microbial supplementation on the performance of dairy cattle during the transition period. J Dairy Sci. 86: 331- 335.

Nocek, J. E., and W. P Kautz. 2006. Direct-fed microbial supplementation on ruminal digestion, health and performance of pre- and postpartum dairy cattle. J. Dairy Sci. 89: 260-266.

NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Press, Washington, D.C.

Oetzel, G. R. 2004. Monitoring and testing dairy herds for metabolic disease. Vet Clinc. Food Anim. 20:651-674.

Oetzel, G.R. 2004. Monitoring and testing dairy herds for metabolic disease. Vet. Clin. North Am. - Food Anim. Pract. 20:651–674.

Ok, M., İ. Şen, H. Güzelbekteş, M. Boydak, C. Er, U. Aydoğdu, and R. Yildiz. 2013. The importance of concentrations of sorbitol dehydrogenase and glutamate dehydrogenase and b-mode ultrasonographic examination in The Diagnosis of Hepatic Lipidosis in Dairy Cows. Kafkas Univ. Vet. Fak. Derg. 19:117–123.

Ørskov, E. R. 1992. Protein nutrition in ruminants. Academic Press. Madison, WN.

Ørskov, E. R., D. A Grubb, and R. N. B. Kay. 1977. Effect of postruminal glucose or protein supplementation on milk yield and composition in Freisian cows in early lactation and negative energy balance. Br. J. Nutr. 38: 397.

Osorio, J. S., P. Ji, J. K. Drackley, D. Luchini and J. J. Loor. 2013. Supplemental smartamine M or metasmart during the transition period benefits postpartal cow performance and blood neutrophil function. J. Dairy Sci. 96:6248-6263.

Overton, T.E. and W.S. Burhans. 2013. Protein metabolism of the transition cow. Proc. Cornell Nutr. Conf., Syracuse, NY, pp. 91-100.

Ramsing, E. M., J. A. Davidson, P. D. French, I Yoon, M. Keller and H. Peters-Flockenstein. 2009. Effects of yeast culture on peripartum intake and milk

production of primiparous and multiparous Holstein cows. J. Dairy Sci. 93:4239-4251.

Rukkwamsuk, T., M. J. H. Geelen, T. A. M. Kruip, and T. Wensing. 2000. Interrelation of fatty acid composition in adipose tissue, serum, and liver of dairy cows during the development of fatty liver postpartum. J. Dairy Sci. 83:52–59.

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. J. Dairy Sci. 95:5888–5900.

Santos, J. E., 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. J. Dairy Sci. 84:213-224.

SAS Institute. 2010. SAS/ STAT 9.3 User's Guide: Statistics. SAS Inst. Inc. Cary, NC.

Schepers, A. J. and R.G. M. Meijer. 1998. Evaluation of the utilization of dietary nitrogen by dairy cows based on urea concentration in milk. J. Dairy Sci. 81: 579- 584.

Schulz, K., J. Frahm, U. Meyer, S. Kersten, D. Reiche, J. Rehage, and S. Dänicke. 2014. Effects of prepartal body condition score and peripartal energy supply of dairy cows on postpartal lipolysis, energy balance and ketogenesis: an animal model to investigate subclinical ketosis. J. Dairy Res. 81:257–266.

Schwab, C. G. 1995. Protected proteins and amino acids for ruminants (Chapter 7), R. J. Chesson In: Biotechnology in Animal Feed and Animal Feeding. VCH, Germany.

Schwab, C. G. 2012. The principles of balancing diets for amino acids and their impact on N utilization efficiency. Accessed on Sept 19, 2016. http://dairy.ifas.ufl.edu/rns/2012/1SchwabRNS2012.pdf

Schwab, C. G., C. K. Bozak, N. L. Whitehouse, and M. M. A. Mesbah. 1992. Amino acid limitation and flow to duodenum at four stages of lactation. 1. Sequence of lysine and methionine limitation. J. Dairy Sci. 75:3486–3502.

Shaver, R. D. 1997. Nutritional risk factors in the etiology of left displaced abomasum in dairy cows: A review. J. Dairy Sci. 80:2449-2453.

Skinner, J., R. Brown, and L. Roberts. 1991. Bovine haptoglobin response in clinically defined field conditions. Vet. Rec. 128:147–149.

Sniffen, C. J., J. D. O'Connor, P. J. Van Soest, D. G. Fox, and J. B. Russell. 1992. A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. J. Anim. 70:3562-3577.

Sundrum, A. 2015. Metabolic disorders in the transition period indicate that the dairy cows' ability to adapt is overstressed. Animals. 5:978-1020.

Suriyasathaporn, W., H. Schukken, M. Nielen and A. Bran. 2000. Low somatic cell count: a risk factor for subsequent clinical mastitis in a dairy herd. J. Dairy Sci. 83:1248- 1255.

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. J. Dairy Sci. 96: 2925- 2938.

Tamminga, S. 1979. Protein degradation in the forestomachs of ruminants. J. Anim. Sci. 49: 1615- 1630.

Van Maanen, R. W., J. H. Herbein, A. D. McGilliard and J. W. Young. 1978. Effect of monensin on in vivo rumen propionate production and blood glucose kinetics in cattle. J. Nutr. 108:1002-1007.

Van Saun, R. J. and C. J. Sniffen. 1995. Comparison of pre- and postpartum performance in dairy cattle having either a single or twin pregnancy. J Dairy Sci 78 (Suppl. 1):266, Abstr.

Van Saun, R. J. and C. J. Sniffen. 1996. Nutritional management of the pregnant dairy cow to optimize health, lactation and reproductive performance. Anim. Feed Sci. Technol. 59:13-26.

Van Saun, R.J., S.C. Idleman, and C.J. Sniffen. 1993. Effect of undegradable protein amount fed prepartum on postpartum production in first lactation Holstein cows. J. Dairy Sci. 76:236-244.

Van Soest, P. 1994. Nutritional Ecology of the Ruminant. 2nd ed. Cornell University Press, Ithaca. pp. 476.

van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583–3597.

Vasseur, E., J. Gibbons and J., Rushen and A. .M.de Passillé. 2013. Development and implementation of a training program to ensure high repeatability of body condition scoring of dairy cows. J. Dairy Sci. 96:4725-4737.

Weich, W. D., K. F. Kalscheur, and D. P Casper. 2016. Postpartum protein nutrition for   dairy cows. Abstr. ASAS-ADSA Midwest Meeting. Demoines, IA.

Wildman, E. E., G. M. Jones, P. E. Wagner and R. L. Boman. 1982. A Dairy cow body   condition scoring system and Its relationship to selected production characteristics. J. Dairy Sci. 65:495-501.

Wohlt, J. E., T. T. Corcicone, and P. K. Zajac. 1999. Effect of yeast on feed intake and performance of cows fed diets based on corn silage during early lactation. J. Dairy Sci. 81: 1345- 1352.

Woyengo, T. A., E. Kiarie, and C. M. Nyachoti. 2010. Metabolizable energy and standardized ileal digestible amino acid contents of expeller-extracted canola meal fed to broiler chicks. Poult. Sci. 89:1182–1189.

Yuan. K., T. Liang, M. B. Muckey, L. G. D. Mendonça, L. E. Hulbert, C. C. Elrod and B. J. Bradford. 2015. Yeast product supplementation modulated feeding behavior and metabolism in transition dairy cows. J. Dairy Sci.98:532.540.

Zaworski, E. M., C. M. Shriver-Munsch, N. A. Fadden, W. K. Sanchez, I. Yoon, and G. Bobe. 2014. Effects of feeding various dosages of Saccharomyces cerevisiae fermentation product in transition dairy cows. J Dairy Sci. 97: 3081- 3098.

Zebeli, Q., K. Ghareeb, E. Humer, B.U. Metzler-Zebeli, and U. Besenfelder. 2015. Nutrition, rumen health and inflammation in the transition period and their role on overall health and fertility in dairy cows. Res. Vet. Sci. 103: 126- 136.