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Evaluating the effects of rumen-protected glucose (RPG) on production, metabolism, and inflammation in transitioning dairy cows

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Evaluating the effects of rumen-protected glucose (RPG) on production, metabolism, and inflammation in transitioning dairy cows

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The student author, whose presentation of the scholarship herein was approved by the program of study committee, is solely responsible for the content of this thesis. The Graduate College will ensure this thesis is globally accessible and will not permit alterations after a degree is conferred.

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DEDICATION

This thesis is written in dedication to my grandparents, Larry and Suzanne Rhinesmith and Sharon Shouse. Thank you all for your support throughout my college career. I am so thankful to have you here to keep pushing me. I love you!

Carrie

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ABSTRACT

The transition period is an energetically demanding time for the dairy cow. Successfully converting from a pregnant, non-lactating state to a non-pregnant, lactating state requires exquisite metabolic adaptations to ensure adequate glucose sparing for lactation onset. The inability to effectively partition nutrients towards lactation is associated with metabolic disorders. Dysfunctional glucose trafficking may not only predispose cows to maladapt to lactogenesis, but also limit milk synthesis and reproductive function during established lactation. Therefore, we hypothesized that glucose availability may limit milk yield (**MY**) during the transition period. Consequently, objectives were to determine the effects of feeding a rumen-bypass glucose product during the periparturient period on milk production, energetic metabolism, and inflammatory response in dairy cows.

CHAPTER 1: LITERATURE REVIEW

The Transition Period

In dairy production, the time frame encompassing 3 weeks prepartum through 3 weeks postpartum is loosely defined as the transition period (Grummer, 1995; Zapata, 2015). The transition period is named as such because the cow undergoes a physiological transition from a late gestation, non-lactating state to a non-pregnant, lactating state. Prior to parturition, the dairy cow faces a substantial metabolic strain to support a growing fetus followed by the large energetic demand of lactation. Concomitant with these metabolic adaptations, dry matter intake (**DMI**) typically decreases prior to parturition (Weber et al., 2013; Song et al., 2016). Fetal growth, parturition and the onset of lactation require an orchestrated partitioning of nutrients, chiefly energy and protein, into different metabolic pools such as fetal tissue and membranes, mammary gland, and the gastrointestinal tract (**GIT**; Goff and Horst, 1997). Maladaptation to these metabolic shifts leads to inefficient utilization of nutrients; as a result, transition dairy cows are at a higher risk of health disorders. In fact, it is estimated that up to 50% of transitioning dairy cows experience at least one negative health outcome (Drackley, 1999). In addition, it has been reported dairy cows, regardless of health status, experience some degree of systemic inflammation throughout the transition period (Bradford et al., 2015). Inflammatory responses ostensibly have detrimental effects on the cow's well-being and productivity. Thus, it is important to implement nutritional and managerial strategies to help cows successfully navigate the transition period.

Nutrient Partitioning During the Transition Period

The transition period is a nutritionally challenging time; during pregnancy, the gravid uterus, fetus, and mammary gland increase their demand up to five-fold for fatty acids, up to

three-fold for glucose, and two-fold for amino acids (**AA**; Bell, 1995). Furthermore, lactation onset requires many tissues to undergo biological adaptations to metabolize all nutrient classes to meet the physiological demands of the mammary gland and other tissues (Baumgard et al., 2017). Major metabolic shifts include increased rate of adipose lipolysis, hepatic gluconeogenesis and skeletal muscle proteolysis (Bauman and Currie, 1980; Table 1). Beginning 3 weeks prior to parturition, DMI gradually decreases by 30 to 40% (Coppock et al., 1972; Grummer, 1995; Hayirli et al., 2002). Although not entirely understood, reduced DMI prior to parturition is associated with gut-fill due to a growing fetus (Ingvartsen et al., 1992) or physiological shifts mediated by endocrine mechanisms, namely increased circulating leptin and growth hormone (**GH**; Smith et al., 1976). Given the complexity of the various physiological processes during the transition period, it is vital for cows to partition nutrients accordingly to allow for copious milk production while maintaining a robust health status.

Working with sheep, Villalba et al. (2009) discovered that DMI decreased upon rumen distention, and subsequently increased when distention was relieved. Mimicking gut fill may have stimulated rumen stretch receptors triggering a satiety response (Grovum, 1979). Because a fetus is housed in the uterus, the stretch receptor theory may not be applicable to the transition cow scenario. However, it is reasonable to think that the volume of the enlarged uterus occupies more space in the abdomen, thus reducing the rumen's physical expansion capacity. In accordance with this, Forbes (1968) reported that the growing fetus compromised feed intake at week 5 prior to parturition in ewes. Therefore, a similar situation may be envisioned in dairy cows such that a reduction in rumen expansion capacity and the growing fetus may partly explain the reduction in DMI prior to calving.

Endocrine regulation of feed intake may also be associated with metabolic shifts in the periparturient period. Concentration of circulating leptin, a hormone regulating appetite (Campfield et al., 1995; Halaas et al., 1995; Pelleymounter et al., 1995), has been reported to be highly correlated with percent body fat in rodents and humans (Considine et al., 1996; Weigle et al., 1997). At parturition, plasma leptin concentration has been reported to decrease by up to 50%, inducing greater DMI during lactation (Block et al., 2001). Alternately, GH increases after calving (Bell and Bauman, 1997) and it can induce lipolytic activity (Lee et al., 1974) when cows are in negative energy balance (**NEBAL**; Bell and Bauman, 1997), thus mobilization of adipose tissue for a systemic energy source. At the same this period is characterized by decreased insulin sensitivity, demonstrated in monogastrics (McGowan et al., 1992; Barb et al., 1998; Larsson et al., 1998) and ruminants (Morrison et al., 2001). This phenomenon is associated with the initiation of catabolic activities such as adipose tissue lipolysis (Chilliard, 1993). These metabolic alterations may, in part, cause metabolic ailments (i.e. ketosis), but also allow for increased energy supply for lactation.

In addition to the endocrine changes influencing nutrient partitioning, glucose homeostasis is important for cow health and performance (Bell, 1995; Beever et al., 1999). Glucose is an important fuel for ATP production and synthesis of proteins, lipids, and nucleotides (Scott et al., 1976; Threadgold and Kuhn, 1979). Although complex, regulation of circulating glucose is chiefly governed by insulin and glucagon; through their antagonistic effects, these hormones ensure plasma glucose is “spared” for milk synthesis (Baumgard et al., 2017). This is due to the role of glucose as the precursor for lactose synthesis and reducing equivalents for milk fat synthesis (Malpress and Morrison, 1950; Neville et al., 1983; Cant et al., 2002). Lactogenesis requires large quantities of glucose; the mammary gland utilizes

approximately 72 g of glucose to produce 1 kg of milk (Kronfeld et al., 1982). For high-producing dairy cows (milk yield > 45 kg milk/d), the overall glucose turnover can exceed 3 kg/d with a large proportion being partitioned towards lactogenesis alone (Baumgard et al., 2017).

Furthermore, successfully managing body energy reserves is critical for navigating the transition period (McNamara, 1991, 2015). In order to spare glucose for milk synthesis, dairy cows increase their bioenergetic reliance on non-esterified fatty acids (**NEFA**; derived from adipose tissue mobilization) rather than glucose (Dunshea et al., 1990). In other words, the increase in circulating NEFA represents an alternative energy source that allows peripheral tissues to depend less on glucose and increase their dependence on lipids as a fuel source, thus sparing glucose for milk synthesis (Bauman and Currie, 1980; Drackley et al., 1991a, b). Blood NEFA concentrations are proportionate to the extent of NEBAL (Dunshea et al., 1990), in turn, hepatic NEFA uptake is proportionate to circulating levels (Drackley, 1999). There are 3 fates for NEFA entering the liver: they can be completely oxidized to carbon dioxide, re-esterified into a triglyceride (**TG**), or they can be partially oxidized to ketone bodies (Figure 1; Drackley, 1999; Nafikov et al., 2006). Non-esterified fatty acid-derived ketones can be used as a fuel source and precursors for milk fat synthesis for cows in NEBAL (Baumgard et al., 2017), therefore sparing glucose for production.

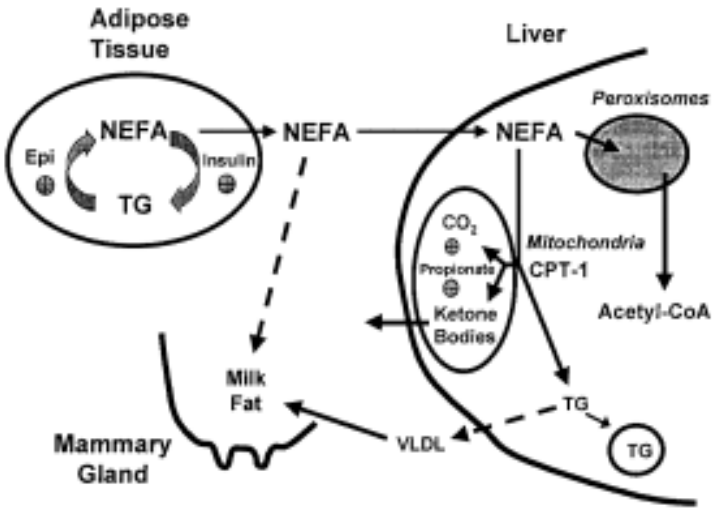

Figure 1. Fates of mobilized fat from adipose tissue in the lactating dairy cow. Adapted from Drackley (1999)

Table 1. A partial list of metabolic changes associated with the transition period in ruminants

Physiological Function	Metabolic Change	Tissues Involved
Milk Synthesis	Increased use of nutrients	Mammary
Lipid Metabolism	Increased lipolysis	Adipose Tissue
	Decreased lipogenesis	
Glucose Metabolism	Increased gluconeogenesis	Liver
	Increased glycogenolysis	
	Decreased use of glucose and increased use of lipid as energy source	Muscle and other body tissues
Protein Metabolism	Mobilization of protein reserves	Muscle and other body tissues

Adapted from Bauman and Currie, 1980.

Role of Hormones in the Transition Period

The orchestration of nutrient partitioning at lactation onset is mostly governed by modified endocrine signaling. These shifts involve alterations in tissue set-points and tissue sensitivities to allow for glucose sparing and uptake by the mammary gland (Table 2; Baumgard et al., 2017). Collectively, this is a strategy the dam utilizes to decrease systemic utilization of glucose and switch fuel preference in peripheral tissues toward utilization of NEFA and ketones as energy substrates.

Growth hormone

Many of the homeorhetic adaptations to support lactation stem from the somatotropic axis (Bauman and Currie, 1980). For example, adipose tissue lipolysis and muscle proteolysis occur to accommodate for the energy deficiency that is commonly encountered during the transition period. This adaptation is mediated by increased GH concentration in the prepartum dairy cow, coupled with a decrease in systemic insulin sensitivity. Combined, these metabolic shifts result in a scenario that triggers adipose tissue lipolysis and decreased glucose oxidation in peripheral tissues (Bell and Bauman, 1997).

In 1937, Asimov and Krouze pioneered the work to discover the effects of a pituitary gland substance to increase MY in dairy cows by subcutaneously injecting crude pituitary gland extracts. Subsequent work by Young (1947) further refined the crude pituitary gland substance to discover that GH was the contributing factor to the galactopoietic effect. At that time, studies of long-term exposure to GH injections were hard to conduct because 6,000 mg of GH were needed to elicit greater milk synthesis, but the amount of GH recovered from an anterior pituitary is only 5 to 15 mg (Young, 1947). Therefore, research stalled until the advent of recombinant technology allowed for the in vitro production of bovine somatotropin (**rbST**)

with similar effects to its native form (Peel et al., 1983). The mechanisms of action of rbST involve increased hepatic gluconeogenesis and suppression of the inhibitory effect of insulin on gluconeogenesis (Peel and Bauman, 1987). In addition, rbST increases complete fatty acid oxidation in the liver (Pocius and Herbein, 1986). Thus, if glucose is limiting MY in early lactation, rbST may increase the amount of glucose readily available for milk production. There are numerous studies on dairy cows determining the effect of rbST on MY (Peel et al., 1983; Richard et al., 1985). Although there is variation in stage of lactation and the magnitude of the response, all rbST-treated cows have shown increased MY ranging from 2 to 5 kg/d more milk compared with non-treated cows (Peel and Bauman, 1987). Later work by Bauman (1999), demonstrated that rbST was more effective at increasing MY once cows were in a positive energy balance (**EBAL**).

Insulin

Due to its importance in glucose homeostasis, it is not surprising that insulin plays a major role in sparing glucose for the mammary gland. Glucose-stimulated insulin secretion from the pancreas is blunted at lactation onset (Rhoads et al., 2004) and this explains why early lactating cows are hypoinsulinemic (Kunz et al., 1985). There is also a decreased sensitivity and responsiveness to insulin in muscle and other tissues (Vernon, 1986; Petterson et al., 1993; Bell, 1995; Bell and Bauman, 1997). The liver has a reduced response to insulin, decreasing the ability to inhibit hepatic gluconeogenesis at the onset of lactation (Bauman and Elliot, 1983; Bauman, 2000). Furthermore, insulin has anti-lipolytic properties (Chakrabarti et al., 2013; Morigny et al., 2016), so the decreased insulin concentration prior to calving allows for increased lipolysis and ultimately increased circulating NEFA as energy substrate. The mammary gland does not have appreciable amounts of glucose transporter type 4 (**GLUT4**;

Zhao et al., 1996, Zhao and Keating, 2007), therefore, it is not affected by insulinemic state. In addition, adipose glucose transporter type 1 (**GLUT1**) expression is decreased in early lactation, with a gradual increase as the lactation progresses (Komatsu et al., 2005). Collectively, the decreased sensitivity to insulin by peripheral tissues and reduced glucose uptake by adipose tissue allow for increased availability of glucose for the mammary gland.

Glucagon

Glucagon is a hormone produced by the pancreas which has the capacity to increase concentration of circulating glucose through glycogenolysis, AA uptake, and increasing gene expression of phosphoenolpyruvate carboxykinase and pyruvate kinase (Brockman et al., 1975; Flakoll et al., 1994; Hippen et al., 1999; Bobe et al., 2003, 2009; Nafikov et al., 2006). By stimulating lipolysis, glucagon increases glycerol availability (Brockman et al., 1975) for hepatic metabolism through gluconeogenesis and glycolysis (Remond et al., 1993; Goff and Horst, 2001). Osman and colleagues (2010) reported increased circulating glucose with a concomitant decrease in blood NEFA and BHB concentrations when glucagon was subcutaneously administered to early-lactation dairy cows.

Table 2. A partial list of alterations in the response to homeostatic responses that occur in different tissues and processes during lactogenesis and early lactation in ruminants[1]

Process or tissue	Homeostatic control	Response to altered set-points
Feed intake	Multiple controls	Appetite and satiety set-point
Adipose tissue	Insulin	Lipogenesis Uptake of preformed fatty acids
	Catecholamines	Stimulation of lipolysis
	Adenosine	Inhibition of lipolysis
Skeletal muscle	Insulin	Glucose uptake Protein synthesis Amino acid uptake Protein degradation
Liver	Insulin	Gluconeogenesis
Pancreas	Insulinotropic agents	Insulin release
Whole animal	Insulin	Glucose oxidation Glucose utilization by nonmammary tissues

[1]Adapted from Bauman and Elliot (1983), Bauman (2000), and Baumgard et al. (2017).

Energy Balance

Energy balance is calculated as the difference between energy consumed and energy expenditure in maintenance, growth, and milk synthesis. The EBAL for dairy cows is expressed as: EBAL = net energy intake – (net energy of maintenance + net energy of lactation; NRC, 2001). Beginning immediately prior to calving through up to 10 to 12 weeks in lactation, dairy cows are typically in NEBAL (Bertics et al., 1992; Grummer, 1995).

The net energy intake is determined by measuring the amount of feed consumed and its energetic content. The net energy for maintenance is calculated as: 0.08 × body weight (**BW**)$^{0.75}$. Although this equation is thought to be precise, especially in times of thermoneutral conditions (Moore et al., 2005), the net energy of maintenance may be difficult to measure in transitioning dairy cows, due to marked changes in body composition and increasing splanchnic mass (McDonald et al., 1995). Genetic selection for high producing animals has been accompanied by an increase in fasting heat production, therefore, the NEm is thought to have increased concomitantly (Veerkamp and Emmans, 1995; Agnew and Yan, 2000; Morases et al., 2015). In addition to energy requirements for maintenance, the onset of lactation triggers further energy partitioning towards synthesis of milk and milk components. In dairy cows, the equation to determine net energy of lactation (**NE$_L$**) is defined as: NE_L (Mcal/kg) = [(0.0929 × milk fat %) + (0.0547 × milk crude protein %) + (0.0395 × milk lactose %)] × milk production (kg) in the NRC (2001). The NE_L equation is thought to be relatively precise, given the concentrations of fat, protein, and lactose are known (Weiss, 2002). However, one caveat of this equation is the use of the crude protein (**CP**) concentration in the milk instead of true protein percent. Therefore, requirement for NE_L may be overestimated (NRC, 2001).

Metabolic Disorders

The profitability of a dairy farm is influenced, in large part, by cow health and productivity (Dijkhuizen and Morris, 1997; Galligan, 2006). The transition period, specifically the onset of lactation, is accompanied by social, dietary, and metabolic pressures, ultimately leading to metabolic ailments (Carpenter et al., 2018). Metabolic problems are broadly defined as disorders related to the vast metabolic shifts typically occurring in the periparturient period (i.e. ketosis and hypocalcemia; Carpenter et al., 2018). These disorders may be due to energetic deficits in the peri-partum period, stressors associated with calving, and the need to partition nutrients to support colostrum and milk synthesis. Therefore, implementing dietary strategies to help alleviate the myriad of metabolic disorders occurring after parturition will likely improve animal production and well-being.

Inflammation in the Transition Period

Inflammation is a response to infection and tissue injury, and it involves many physiological and pathological processes triggered by components of the innate and adaptive immune systems (Medzhitov, 2008; Bradford et al., 2015). It has been reported that nearly all dairy cows endure some degree of inflammation (Bradford et al., 2015) through the transition period. During this period, there are multiple pools where bacteria and associated endotoxins may infiltrate into systemic circulation; these pools include the mammary gland (Hogan and Smith, 2003), uterus (Sheldon et al., 2002), and GIT (Eckel and Ametaj, 2016). When lipopolysaccharide (**LPS**), a glycolipid present on the outer membrane of gram-negative bacteria (Mani et al., 2012), infiltrates into systemic inflammation, leukocytes and immune-active tissues (i.e. hepatocytes) produce an inflammatory milieu including cytokines and acute phase proteins (i.e. LPS-binding protein [**LBP**], serum amyloid A [**SAA**], and haptoglobin

[**Hp**]) which mediate leukocyte trafficking, promote vasodilation, and clear pathogens. Additionally, in response to these processes, cows exhibit mild hyperthermia, anorexia, and decrease milk production (Dantzer and Kelley, 2007; Bradford et al., 2015). Acute phase proteins are reliable and widely accepted markers of overall health and inflammatory status of the dairy cow (Ceciliani et al., 2012), however understanding the role of inflammation in the transition period requires further investigation.

Inflammation has been noted postpartum in cattle, pigs, mice, and humans (DiSilvestro, 1986; Humblet et al., 2006; Rosenbaum et al., 2012a, b; Gregor et al., 2013). Qu and colleagues (2014) discovered increased circulating Hp after calving, even in the absence of apparent disease; cows with clinical disease had even greater circulating Hp concentrations compared to the healthy cows. This response may be due to inflammation originating in the uterus (Yuan et al., 2015), adipose tissue (Sadri et al., 2010), liver, (Loor et al., 2005), intestine (Gott et al., 2015; Abuajamieh et al., 2016), or potentially from psychosocial (Silva et al., 2013) or heat stress (Tao et al., 2013; Zhang et al., 2014). Although inflammation ideally helps the body overcome and adapt to an antigen, it may also contribute to the development of metabolic disorders throughout the transition period (Bradford et al., 2015).

Rumen Acidosis

Prior to parturition, dairy cows are typically fed a high forage diet (Humer et al., 2018), and the rumen papillae have a reduction in height and a reduced ability to absorb volatile fatty acids (**VFA**) compared to cows on a lactating diet (Goff and Horst, 1997; Kleen et al., 2003). After calving, dairy cow diets are then modified to include more concentrates in an effort to increase dietary energy density to support milk synthesis (Humer et al., 2018). Because the rumen has been adapted to a dry cow diet, the rumen papillae are unable to meet the absorptive

capacity required to accommodate the large influx of VFA produced from a readily fermentable diet (Goff and Horst, 1997; Kleen et al., 2003). When the increase in VFA and lactic acid concentrations exceed the rate of VFA removal, rumen pH decreases (Owens et al., 1998). This disorder is termed rumen acidosis and is classified as subacute ruminal acidosis (**SARA**) when ruminal pH is < 5.5 (Garrett et al., 1999), or acute rumen acidosis, in which the rumen pH is < 5.2 (Owens et al., 1998; Nagaraja and Lechtenberg, 2007). Reduced DMI, liver abscesses, diarrhea, laminitis, and milk fat depression can result from ruminal acidosis (Kleen et al., 2003).

Although the incidence of SARA may be difficult to quantify, some studies in the United States have estimated that it is between 19 and 26% in early and mid-lactation cows, respectively (Garrett et al., 1997; Plaizier et al., 2008). In beef cattle, treatment costs alone amount to approximately $4.6 million annually (USDA, 2016); Stone (1999) estimated economic losses due to SARA in a dairy herd to be $1.12 per cow per day. The source of losses can be attributed to reductions in MY, milk fat production, milk production efficiency, and increased lameness which may also lead to an increase in culling rate (Nocek, 1997; Stone, 1999). Due to the large economic impact of SARA, many feeding strategies have been researched to reduce incidences of rumen acidosis, including manipulation of fiber particle size, feeding buffers and alkalizers, and the use of direct fed microbials (**DFM**).

Physically effective fiber (**peNDF**) is based on physical characteristics of a feedstuff which influences chewing activity and the biphasic nature of contents in the rumen (Mertens, 1997), and is essential in dairy cow diets, especially in early and mid-lactation. Generally, peNDF is calculated by multiplying the NDF concentration by the proportion of feed maintained on a 1.18-mm sieve (Mertens, 1997). Chewing activity, salivary buffer secretion,

rumen motility and mixing are maintained by peNDF (Allen, 1997). Saliva entering the reticulorumen contains buffers to help regulate pH. To ensure proper rumination and saliva production for rumen buffering, it has been recommended that at least 40% of feed particles be longer than 8 mm (Heinrichs and Kononoff, 2002).

Direct fed microbials have been defined as "a source of live, naturally occurring microorganisms" (Yoon and Stern, 1995) which benefit the host animal by improving the intestinal microbial balance (Fuller, 1989). Because consumers have become increasingly concerned with antibiotic use in animal production, research with DFM on health and performance of food-producing animals has become increasingly popular. Although DFM are administered for a variety of reasons, their effect on ruminal fermentation is of importance for transitioning dairy cows. Krehbiel and colleagues (2003) stated DFM may prevent rumen acidosis. For example, lactic acid utilizing bacteria, such as *Megasphaera elsdenii*, have been reported to decrease lactate accumulation when cows transition from a low- to high-concentrate diet (Greening, et al., 1991). *Propionibacterium*, also a lactic acid utilizing bacteria, does not decrease the amount of lactate in the rumen, but is a very efficient propionate producer (Krehbiel et al., 2003). Interestingly, lactic acid producing bacteria (**LAB**) such as *Lactobacillus* and *Enterococcus* have been shown to prevent rumen acidosis in transitioning dairy cows (Nocek et al., 2002). One theory as to how LAB decrease the risk for rumen acidosis is that other ruminal microorganisms adapt to the lactate being produced, thus "priming" the rumen microbiome to adapt to a greater VFA load with a higher concentrate diet (Yoon and Stern, 1995).

Fatty Liver

The transition period is characterized by increased adipose tissue lipolysis, this increases circulating NEFA that can be incorporated by the mammary gland to produce milk fat; oxidized by peripheral tissues (mainly skeletal muscle) for energy or taken up by the liver (Reynolds et al., 2003). The liver can then oxidize the NEFA or secrete it, but the ruminant liver has a low capacity to export lipids (Reid et al., 1979; Kleppe et al., 1988; Drackley, 1999). Thus, triacylglycerol (**TAG**) accumulation is likely when the esterification rate exceeds the rate of oxidation plus export of very low-density lipoprotein (**VLDL**; Grummer, 1993). This condition leads to a metabolic disorder known as hepatic lipidosis, otherwise commonly referred to as "fatty liver". This condition is a major welfare concern for high-producing dairy cows. Although the incidence of fatty liver is hard to determine because the diagnosis requires a liver biopsy, it is estimated that up to 50% of early lactation cows experience it to some extent (Reid, 1980; Grummer, 1993; Jorritsma et al., 2000, 2003). The estimated annual cost of fatty liver in the United States is over $60 million (Bobe et al., 2004) due to its detrimental effects on production (Littledike et al., 1981).

Even though hepatic lipidosis is hard to diagnose; mitigation and prevention are important because the disorder often leads to an increase in the incidence and severity of other diseases (Veenhuizen et al., 1991). Decreasing fatty acid mobilization, reducing liver fatty acid esterification, and increasing hepatic VLDL export would theoretically decrease the risk for fatty liver (Grummer, 1993). Unfortunately, research data on this subject is inconclusive and some strategies have been unsuccessful. For example, feeding prepartum dairy cows low- or high-energy diets (Grum et al., 1996; Douglas et al., 2004), or decreasing milk fat content to decrease the energy expense of lactation (Castañeda-Gutiérrez et al., 2005) did not diminish

rates of hepatic lipidosis. Similarly, administrating glucogenic precursors such as propylene glycol, propionate salts, and AA to alleviate lipolysis have resulted in inconsistent responses (Hoedemaker et al., 2004).

Ketogenesis and Ketosis

Post-parturition, circulating glucose and subsequently insulin, are decreased in the dairy cow, as a result, adipose tissue lipolysis occurs at an increased rate. In addition, an energetic deficit is likely to occur in early lactation due to the increased metabolic demands for milk production coupled with decreased DMI. In this scenario, NEFA are then mobilized and metabolized in 4 possible pathways: they can be directly used as an energetic substrate by peripheral tissues, such as skeletal muscle, incorporated into milk fat, esterified to a glycerol backbone in the liver, or intraconverted into a ketone body via incomplete hepatic fatty acid oxidation (Bell, 1979). The increase in circulating ketone bodies, namely acetoacetate, acetone, and β-hydroxybutyrate (**BHB**), is termed ketosis. This disorder can be classified as subclinical when blood BHB ≥ 1.2 mmol/L (Bobe et al., 2004), and clinical ketosis when circulating BHB ≥3.0 mmol/L (Oetzel, 2004; McArt et al., 2015).

Ketosis is the most prevalent post-partum metabolic disorder (Oetzel, 2004), and occurs more frequently in high producing cows. It is estimated ≥ 40% of dairy cows experience subclinical ketosis (McArt et al., 2015), whereas clinical ketosis affects about 20% of early lactation cows (Gillund et al., 2001). Estimated costs of ketosis are variable with no palpable consensus in published research. Nonetheless, it has been reported that each case of ketosis costs $77 ± 24 per case for primiparous cows and $181.91 ± 63.74 per case for multiparous cows; both amounting to much less than the previously estimated cost at $232.00 per case (Guard, 2008; Liang et al., 2017). Money loss due to decreased milk production is estimated

to be $1.00 ± 0.65 and $6.67 ± 1.69 per case for primiparous and multiparous cows, respectively (Liang et al., 2017). In addition, veterinary treatment costs were approximated at $52.44 ± 21.29 and labor costs were $11.76 ± 5.59 per case (Liang et al., 2017). The culling and death costs for primiparous cows were calculated to be $4.72 ± 1.06 and $5.42 ± 0.84, respectively, whereas the cost of culling and death for multiparous cows were estimated to be $6.87 ± 7.30 and $5.80 ± 0.85, respectively (Liang et al., 2017).

At the herd level, ketosis is associated with higher incidence of displaced abomasum (LeBlanc et al., 2005), reduced MY, increased culling rate, and increased death loss (Littledike et al., 1981; Duffield et al., 2009). Therefore, nutritional strategies that can decrease the incidence of ketosis on a commercial level would benefit multiple profitability metrics.

Endotoxemia

Although the most obvious and likely sources of endotoxemia are metritis and mastitis (Sheldon et al., 2002, Hogan and Smith, 2003), feeding regime may also predispose cows to face a surge in luminal endotoxin concentration as a result of lysis of certain rumen bacteria (Mao et al., 2013). The GIT serves a dual purpose: to digest and absorb dietary nutrients, and to prevent infiltration of unwanted compounds from the lumen into the bloodstream (Mani et al., 2012). The barrier function of the GIT is so critical to protect the cow from endotoxin infiltration that the majority of the immune system is harbored within the splanchnic bed (van der Heijden et al., 1987). Stressors that disrupt the epithelial barrier function, such as feed restriction or SARA, may lead to endotoxin translocation and cause systemic inflammation (Gozho et al., 2005; Emmanuel et al., 2007; Minuti et al., 2014). For example, Gozho et al. (2005) observed that concentration of circulating endotoxins increased by abruptly changing steers from a high fiber to a high-concentrate diet. The subsequent decrease in ruminal pH

occurring after ingesting a high concentrate-based diet may cause death and lysis of gram-negative bacteria, thus increasing concentration of LPS in the GIT (Nagaraja et al., 1987; Andersen et al., 1994; Mao et al., 2013).

Another stress factor that has been associated with increased endotoxemia is feed restriction. Recent work by Kvidera et al. (2017b) has shown that feed restriction of dairy cows compromises gut integrity. The response to increasing feed restriction resulted in altered morphology of the intestinal epithelium so that animals that were feed-restricted had decreased villus height and width as well as reduced crypt depth. These alterations in gut histology not only imply altered digestion and absorption processes but can also lead to loss of effective barrier function. In the same study, the authors also report increased concentration of circulating biomarkers of inflammation which may be a direct result of increased permeability due to loss of architectural integrity in the gut.

Endotoxemia causes decreased rumen motility by inhibiting smooth muscle contractions, ultimately causing decreased abomasal emptying (Eades, 1993; Wittek et al., 2004). Other metabolic diseases such as SARA and fatty liver are commonly associated with endotoxemia (Andersen, 2003; Eckel and Ametaj, 2016). Because endotoxin translocation has been shown to be caused by SARA (Nagaraja et al., 1987; Emmanuel et al., 2008; Khafipour et al., 2009) and feed restriction (Kvidera et al., 2017b), nutritional and managerial strategies that reduce these risks of may mitigate the negative impacts of endotoxemia.

Nutritional and Management Strategies to Mitigate Effects of NEBAL

Propylene Glycol

Propylene glycol (**PG**) is commonly administered as a drench (Grummer et al., 1994) or in dry form in feed (Chung et al., 2009) for the treatment of ketosis. The latter authors

reported that feeding dry PG to early lactating dairy cows decreased the incidence of subclinical ketosis. When drenched, a spike in plasma glucose occurs within 30 min after administration (Struder et al., 1993; Grummer et al., 1994) due to an increase in the absorption of propionate by the rumen (Manns and Boda, 1967). Regardless of the delivery method, most PG leaves the rumen intact, while some is metabolized to propionate (Emery et al., 1964). Overall, PG has been shown to be an effective precursor for hepatic gluconeogenesis that results in decreasing circulating NEFA and BHB (Fisher et al., 1971; Struder et al., 1993; Hoedemaker et al., 2004), likely due to an increase in circulating glucose (Struder et al., 1993) and insulin (Sauer et al., 1973; Patton et al., 2004).

Ionophores

Ionophores, such as monensin, are broad-spectrum antibiotics predominantly affecting Gram-positive bacteria by interfering with ion transport across cellular membranes causing bacterial cell death (Duffield et al., 2008). This mode of action results in a shift in rumen microbial populations (Duffield et al., 2008) accompanied by a shift in VFA production favoring propionate (Richardson et al., 1976; Armentano and Young, 1983; McGuffey et al., 2001). This is important because propionate is the primary glucogenic precursor in well-fed ruminants. However, hepatic propionate uptake and metabolism can either increase (McCarthy et al., 2015) or have no effect on gluconeogenesis (Larsen and Kristensen, 2009 a, b). The ionophore-induced shift in microbial populations in dairy cows impacts rumen metabolism by: increasing the efficiency of energy metabolism, improving nitrogen metabolism, and reducing bloat and lactic acidosis (Schelling, 1984).

In addition to increasing gluconeogenic precursors, ionophores have been shown to decrease circulating BHB concentrations especially during early-lactation (Abe et al., 1994;

Duffield et al., 1998, 2008). Similarly, circulating NEFA concentrations have been shown to decrease with supplementation of monensin (Duffield et al., 2008); however, results have differed among studies. Although not consistent, plasma glucose concentrations have been shown to increase with supplementation of monensin (Melendez et al., 2004; Kennerman et al., 2006; Duffield et al., 2008). Because of the aforementioned reasons, feeding ionophores may be a practical way to reduce the incidence or severity of metabolic diseases associated with the transition period.

Fat Supplementation

Because DMI generally decreases prior to calving, increasing the energy density of the feed may be a solution to increase energy intake throughout the transition period. Supplying additional dietary fat to dairy cows would be one potential strategy. Andersen et al. (2008) speculated that by increasing the lipid content in the diet of prepartum dairy cows, their bodies become "primed" to improve lipid metabolism upon calving. As such, hepatic capacity for β-oxidation of long-chain fatty acids would increase, leading to prevention of fatty liver via decrease TAG concentration and an increase in hepatic glycogen (Grum et al., 1996; Petit et al., 2007).

Feeding dry cows supplementary dietary fat has shown inconsistent responses by either decreasing (Grum et al., 1996; Moallem et al., 2007; Karimian et al., 2015; Zapata et al., 2015) or not affecting (Afzalzadeh et al., 2010) prepartum DMI. However, these contradictions may be due to the different amounts and forms of fat that were supplemented, fatty acid profile, palatability, or possibly inhibition of fiber digestion (Reidelberger, 1994). When feeding moderate (2% fat supplement, 1.68 Mcal NE_L/kg DM) or high (4% fat supplement, 1.74 Mcal NE_L/kg DM) levels of rumen-inert free fatty acids to dry cows 4 weeks prior to parturition,

Afzalzadeh and colleagues (2010) reported the additional dietary fat did not affect prepartum DMI. They also discovered no differences due to fat supplementation on circulating NEFA concentrations or prepartum blood glucose, however, circulating glucose concentration was increased in the fat-supplemented groups (Afzalzadeh et al., 2010). Supplying additional fat to lactating cows has been researched to determine if additional energy improves MY, DMI (Onetti et al., 2001; Duske et al., 2009), or reproductive performance (Rodney et al., 2015). Although there was no difference in DMI prior to calving, Afzalzadeh and colleagues (2010) found that MY is increased with supplementation of high amounts (540 g/kg, 1.74 Mcal NE_L/kg DM) of dietary fat prior to calving. This is contradicted by Hayirli et al. (2011), who found no differences in MY due to prepartum diets. Consequently, the effects of dietary fat on production variables in the transitioning dairy cow appears complex and dependent upon multiple parameters that are not clearly defined (Hayirli et al., 2011).

Protein Supplementation

In early lactation, the abrupt demand for synthesis of milk lactose and protein, increase the requirements for glucose and AA, respectively (Bertics et al., 1992; Reynolds et al., 2003). Consequently, there is a negative protein balance, estimated to be greatest at 7 days in milk (**DIM**; Grummer, 1995; Bell et al., 2000) in which AA are being mobilized from skeletal muscle and uterine smooth muscle (Gibb et al., 1992; Andrew et al., 1994). Drackley and colleagues (2001) hypothesized that feeding glucogenic AA can increase gluconeogenesis rates; interestingly, supplementing dairy cows with protein prior to calving provides inconclusive evidence to support this theory. For example, Putnam and Varga (1998) reported that, by increasing protein content in the dry-period diet, plasma glucose concentration increased, whereas VandeHaar et al. (1999) determined that supplying additional protein

prepartum had no effect on circulating glucose. The increase in circulating glucose concentration reported by Putnam and Varga (1998) may be attributed to the preferential role of gluconeogenic AA to support glucose supply in late lactation, due to the increasing levels of protein being fed. Contrary to this, Larsen and Kristensen (2013) reported that essential amino acids (**EAA**) did not produce more liver glucose release post-partum.

It is difficult to assess exactly how many dietary AA are taken up by the liver and what portion of absorbed AA are being utilized for gluconeogenesis. Additionally, protein appears to be partitioned toward protein synthesis over gluconeogenesis in early lactation. It is evident that further research is needed to elucidate the roles of specific AA on glucose metabolism. Utilization of labeled AA to aid in this research is warranted.

Carbohydrate Supplementation

Increasing carbohydrate balance in dairy cows to improve milk production has been of interest because of glucose's role in lactose synthesis. Dietary carbohydrate supplementation can take the form of neutral detergent fiber (**NDF**; Rabelo et al., 2003) or non-fiber carbohydrates (**NFC**) such as starches or sugars (Harmon, 1992). Evidence of carbohydrate supplementation affecting milk production responses are based on experiments assessing the effects of infusing exogenous glucose intravenously (Fisher and Elliot, 1966; Amaral et al., 1990; Brown and Allen, 2013), ruminally (Knowlton et al., 1998), abomasally (Clark et al., 1977; Huntington and Reynolds, 1986; Knowlton et al., 1998; Reynolds et al., 2001; Relling and Reynolds, 2008; Larsen et al., 2010), and duodenally (Lemosquet et al., 1997; Reynolds et al., 2001; Hurtaud et al., 1998). The premise of these experiments is that exogenous glucose supplementation would decrease reliance on alternative gluconeogenic precursors (Larsen and

Kristensen, 2009 a, b); however, results vary greatly upon supplementing exogenous glucose to dairy cows (Table 3 and Table 4).

Many studies have evaluated the effects of increased plasma glucose concentrations during lactogenesis. Fisher and Elliot (1966) infused glucose into the jugular vein of dairy cows between 1 and 10 months into their lactation. They did not observe any differences in DMI; however, they did note a 1.2 kg/d increase in MY. Interestingly, other experiments have reported no differences in DMI or MY upon intravenous glucose infusion in early lactation dairy cows (Amaral et al., 1990; Brown and Allen, 2013). The variability in responses to intravenously infusing glucose may be dependent on stage of lactation and overall EBAL of the cow. Intravenous infusion of glucose failed to increase MY in early-lactating dairy cows (Chelikani et al., 2003; Brown and Allen, 2013); whereas intravenous exogenous glucose infusion in mid- and late-lactation cows report varying results (Al-Trad et al., 2009; Curtis et al., 2018; Leane et al., 2018).

Because some studies have found an increase in MY with intravenously supplying exogenous glucose to lactating dairy cows (Fisher and Elliot, 1966; Curtis et al., 2018), it has been of interest to determine if supplying glucose post-ruminally will have the same effect on MY. Abomasally or duodenally infusing glucose in early- and mid-lactation cows has had inconclusive effects on MY (Clark et al., 1977; Dhiman et al., 1993; Knowlton et al., 1998). Increased MY reported by Knowlton et al. (1998) may be attributed to infusing 500 g/d of glucose more than Dhiman and colleagues (1993). This may have supplied additional glucose to increase milk production.

Since intravenous or post-ruminal administration of glucose to dairy cows is not a practical procedure for routine on-farm management, finding a way to supply carbohydrates to

the small intestine (**SI**) may be beneficial to improve animal performance. Increasing the dietary carbohydrate concentration has been shown to increase MY (Emmanuel et al., 2007); this response is likely due to a greater amount of propionate being made available for hepatic gluconeogenesis. However, if VFA production exceeds the rate of removal of VFA from the rumen, acidosis may occur (Plaizier et al., 2008). Abaker and colleagues (2017) observed that cows being fed a high-grain diet had a transient increase in MY, and subsequently, declined after 9 weeks to levels even lower than that of the control cows. They concluded the cows fed the high-grain diet had increased LPS translocation from the GIT to the blood, and likely caused an innate immune response (Abaker et al., 2017). This would trigger glucose partitioning towards activated immune cells, which become obligate glucose utilizers (Calder et al., 2007), diverting energy away from milk production. In a similar study infusing starch into the rumen of early lactation dairy cows, MY tended to increase (Knowlton et al., 1998) with no detrimental effects on animal health. The discrepancy in animal health outcome between these studies may be related to the fact that a short duration may not have had the long-term consequences of a sustained regime. Therefore, it is plausible that disruption of gut barrier for LPS translocation is the result of either an abrupt change in diet or a sustained insult to the intestinal epithelium.

Because of the possible negative consequences of introducing additional carbohydrates into the rumen, considering the development and supplementation with a rumen-bypass glucose product may be beneficial, assuming the cow will be able to absorb and utilize the glucose that reaches the SI.

The Small Intestine's Ability to Absorb Glucose

Carbohydrates are unique due to their dual role in digestion and physiology (Hall and Mertens, 2017). Compared to monogastric animals, the ability of the SI to digest and absorb starches and sugars typically is less important, due to carbohydrates being rapidly fermented in the rumen (Aschenbach et al., 2010). This limitation is controversial (Harmon and McLeod, 2001) because up to 50% of starch in a diet fed to ruminants may escape ruminal fermentation and reach the lower gut (Johnson and Bergen, 1982). Even though very little glucose is absorbed by the SI (Reynolds, 2006), many tissues rely on glucose as an energy source. Therefore, gluconeogenesis in the liver and kidneys and marginal glucose absorption by the SI are vital processes for the adult ruminant (Mayes, 1996).

Although only a proportion of dietary glucose enters the SI of ruminants, it has been recognized the metabolizable energy (**ME**) utilization from starch digested in the SI is more efficient than when the starch is fermented in the rumen (Armstrong et al., 1960). Even though the site of starch digestion remains ambiguous (Nocek and Tamminga, 1991; Reynolds et al., 1997), it has been reported that starch digestion in the SI is approximately 95% (Reynolds et al., 1997). The utilization of starch in the SI is influenced by many factors: 1) carbohydrase and pancreatic amylase activities in the intestine, 2) visceral metabolism of glucose, and 3) endogenous glucose regulation (Nocek and Tamminga, 1991).

Adequate access to starch granules by enzymes and the limited enzymatic activity for starch hydrolysis in ruminants may be limiting factors for intestinal starch digestion (Owens et al., 1986). In order for starch to be digested in the SI, enzymes capable of cleaving α-1-4 and α-1-6 glycolytic bonds are required. Pancreatic amylase cleaves off maltriose and maltose amylose in the SI (Nocek and Tamminga, 1991), and subsequently maltase activity yields

glucose (Coombe and Siddons, 1973). Because pancreatic amylase cannot hydrolyze α-1-6 bonds, hydrolysis of amylopectin is completed by isomaltase (Siddons, 1968). Additional protein in the diet has been shown to improve starch digestion in the SI (Rust et al., 1979; Veira et al., 1980) by sparing cholecystokinin-releasing peptide from trypsin digestion and allowing the release of cholecystokinin from the intestinal mucosa, thus, increasing amylase release (Fuskiki and Iwai, 1989). However, Remillard and Johnson (1984) reported that the infusion of amylase did not increase starch digestion in steers, implying amylase activity is not limiting starch digestion in the SI.

It is difficult to study how much glucose introduced to the SI is absorbed. Studies looking at nutrient absorption generally utilize catheters in the portal vein; however, these studies do not take into account the metabolism of glucose by intestinal epithelium (Kronfeld, 1976; Reynolds et al., 1991). Numerous studies (Lomax and Baird, 1983; Reynolds and Huntington, 1988) have found no net glucose absorption in the portal vein when fed a normal lactating diet, suggesting glucose absorption is limited or the glucose is being utilized by the gut. However, when infusing glucose, starch, or dextrin in the abomasum of steers, Kreikemeier et al. (1991) found a linear increase in portal vein glucose with increasing glucose infusion. From this study, the maximum rate of glucose in the portal vein was at 20 g/h when starch was infused, suggesting that hydrolysis, and not absorption, is a limiting factor. If glucose is absorbed, it has 5 fates: 1) oxidation, resulting in the production of CO_2 and H_2O, 2) partial glycolysis, producing either lactate or alanine from pyruvate, 3) stored as glycogen, 4) stored as fat, or 5) the carbons can be utilized for AA synthesis.

Because glucose is essential for lactose production, an increase in glucose concentration may seem a plausible way to increase MY. Many studies demonstrate increased

plasma glucose concentration when glucose is infused abomasally (Nichols et al., 2016; Gualdrón-Duarte and Allen, 2018); however, this is not practical in a commercial farm setting. Therefore, it may be beneficial to find a way to feed dairy cows in a way that delivers glucose to the SI to be absorbed and utilized for milk production.

Table 3. Milk yield responses to glucose infusion

Route	Lactation Stage	MY Response	Reference
IV	Early	=	3, 4, 19
	Mid	↑ / =	7 / 1, 2, 6
	Late	=	17
	Varied	↑	9
Abomasal	Early	↑ / =	15 / 8, 11, 16, 21
	Mid	↑ / =	10, 20 / 5, 22
	Late	=	23
	Not specified	=	26
Duodenal	Early	↑ / not measured	14, 23, 24 / 12
	Mid	↑ / =	25 / 13, 18

[1] Al-Trad et al., 2009
[2] Amaral et al., 1990
[3] Brown and Allen, 2013
[4] Chelikani et al., 2003
[5] Clark et al., 1977
[6] Curtis et al., 2014
[7] Curtis et al., 2018
[8] Dhiman et al., 1993
[9] Fisher and Elliot, 1966
[10] Frobish and Davis, 1977
[11] Gualdrón-Duarte and Allen, 2018
[12] Hurtaud et al., 1998a
[13] Hurtaud et al., 1998b
[14] Hurtaud et al., 2000
[15] Knowlton et al., 1998
[16] Larsen and Kristensen, 2009a
[17] Leane et al., 2018
[18] Lemosquet et al., 1997
[19] Léonard and Block, 1997
[20] Nichols et al., 2016
[21] Ørskov et al., 1977
[22] Relling and Reynolds, 2008
[23] Reynolds et al., 2001
[24] Rigout et al., 2002
[25] Rigout et al., 2003
[26] Vik-Mo et al., 1974

Table 4. Dry matter intake responses to glucose infusion

Route	Lactation Stage	MY Response	Reference
IV	Early	=	3, 4, 19
	Mid	↑ / =	6 / 1, 2, 7
	Late	=	17
	Varied	=	9
Abomasal	Early	= / ↓	11, 21 / 8, 15, 16
	Mid	= / ↓	5, 10, 22 / 20
	Late	=	23
	Not specified	=	26
Duodenal	Early	= / ↓	23, 12 / 14, 24
	Mid	= / ↑	13, 18 / 25

[1] Al-Trad et al., 2009
[2] Amaral et al., 1990
[3] Brown and Allen, 2013
[4] Chelikani et al., 2003
[5] Clark et al., 1977
[6] Curtis et al., 2014
[7] Curtis et al., 2018
[8] Dhiman et al., 1993
[9] Fisher and Elliot, 1966
[10] Frobish and Davis, 1977
[11] Gualdrón-Duarte and Allen, 2018
[12] Hurtaud et al., 1998a
[13] Hurtaud et al., 1998b
[14] Hurtaud et al., 2000
[15] Knowlton et al., 1998
[16] Larsen and Kristensen, 2009a
[17] Leane et al., 2018
[18] Lemosquet et al., 1997
[19] Léonard and Block, 1997
[20] Nichols et al., 2016
[21] Ørskov et al., 1977
[22] Relling and Reynolds, 2008
[23] Reynolds et al., 2001
[24] Rigout et al., 2002
[25] Rigout et al., 2003
[26] Vik-Mo et al., 1974

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CHAPTER 2: EFFECTS OF FEEDING RUMEN-PROTECTED GLUCOSE ON LACTATION PERFORMANCE, ENERGETIC METABOLISM AND INFLAMMATION IN TRANSITIONING DAIRY COWS

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Abstract

Objectives were to evaluate the effects of rumen-protected glucose supplementation on milk production, post-absorptive metabolism, and inflammatory biomarkers in transition dairy cows. Fifty-two multiparous cows were blocked by previous 305 mature equivalent milk yield and randomly assigned to one of two iso-energetic and iso-nitrogenous treatments: 1) control diet (CON; n = 26) or 2) a diet containing RPG (RPG, pre-fresh 8.4% RPG DM basis, post-fresh 9.5% RPG DM basis; n = 26). Cows received their respective dietary treatments from d -21 to 28 relative to calving. Weekly body weight (BW), milk composition, and fecal pH were recorded until 28 d in milk (DIM), whereas milk yield was recorded through 105 DIM. Blood

samples were collected on d -7, 3, 7, 14, and 28 relative to calving. Data were analyzed using repeated measures in the MIXED procedure of SAS. Previous 305 mature equivalent milk yield served as a covariate. Fecal pH was similar between treatments and decreased notably post-partum. Dry matter intake pre- and post-partum were unaffected by treatment. Consequently, MY did not differ throughout the experimental period. Milk fat, protein, and lactose were similar amongst treatments. Blood urea nitrogen and plasma glucose concentrations did not differ due to treatment; however, circulating β-hydroxybutyrate post-partum tended to be decreased with a concomitant reduction in concentration of non-esterified fatty acid (NEFA) in the RPG-fed cows compared to CON cows (630 vs. 456 ± 68 μEq/L). Circulating insulin tended to be increased in RPG compared with CON-fed cows. Overall, circulating lipopolysaccharide-binding protein (LBP) and haptoglobin (Hp) did not differ due to treatment, but at 7 DIM, RPG cows had decreased LBP and Hp concentrations by 31% and 27%, respectively compared to CON cows. Supplemental RPG improved some key metrics of post-absorptive bioenergetics and inflammation during the periparturient period, changes primarily characterized by increased insulin and decreased NEFA concentrations and reduced acute phase proteins.

Key words: bypass carbohydrate, ketosis, transition period

Introduction

There is a variety of situations during lactation in which insufficient glucose availability may limit productivity. Some of these situations include, the periparturient period, immunoactivation (Kvidera, 2017a), and heat stress (Baumgard and Rhoads, 2013). The transition period is commonly defined as three weeks prior to parturition, through three weeks post-parturition (Grummer, 1995) and is characterized by reduced dry matter intake (DMI) and

dramatic changes in metabolism (Drackley, 1999). During this time energy expenditure/output exceeds dietary energy intake and cows subsequently enter negative energy balance (NEBAL). The extent of NEBAL may predispose cows to be more susceptible to ketosis, fatty liver, displaced abomasum, mastitis, and infertility; in fact, only about 50% of cows in North America complete the transition period without experiencing at least one negative health outcome (Drackley, 1999).

Glucose is the precursor to lactose synthesis, and lactose is the primary osmoregulator of milk yield (Neville et al., 1983; Cant et al., 2002). The mammary gland requires 72 g of glucose to produce 1 kg of milk (Kronfeld et al., 1982; Bell and Bauman, 1997). During established lactation hepatic glucose output is exquisitely orchestrated to precisely meet peripheral tissue glucose requirements (mammary, muscle, adipose, central nervous system, etc.) and thus circulating glucose is homeostatically maintained within a narrow range (Baumgard et al., 2017). However, in early lactation, multiple tissues metabolically coordinate efforts to assist in the partitioning of glucose towards the mammary gland, and this is primarily accomplished through reduced insulin sensitivity. Reduced activity of insulin on adipose tissue allows for increased lipolysis, and the resulting NEFA are both directly oxidized by capable tissues and contribute to whole-body energetics by interconverting into ketone bodies (Bauman and Currie, 1980). Consequently, "glucose sparing" for mammary utilization occurs due to reduced glucose uptake by skeletal muscle and adipose tissue.

In addition to lactogenesis, mounting an immune response is a glucose-demanding process (Kvidera et al., 2017a). Bradford et al. (2015) indicated that practically all transitioning dairy cows experience some degree of inflammation, regardless of their clinical health status. The inflammation origin is not always clear; however, probable sources during the transition

period are the uterus and mammary gland (Bradford et al., 2015), as well as the gastrointestinal tract (Khafipour et al., 2009). Hence, glucose availability is critically important to promote maximal productivity and health; however, optimizing the post-absorptive "carbohydrate status" in ruminants is difficult as adding more dietary soluble carbohydrates may compromise rumen and post-absorptive health (Goff and Horst, 1997; Kleen et al., 2003). Therefore, providing a dietary source of glucose that is minimally digested in the rumen, but readily available in the small intestine (SI) may provide a safe nutritional strategy to increase the glucose availability in early lactating cows.

We hypothesized that early lactation hepatic glucose output and glucose-sparing mechanisms may be insufficient to sustain peripartum inflammation and optimum milk yield in dairy cows. Therefore, our objectives were to determine if feeding rumen-protected glucose (RPG) throughout the transition period would improve productivity, bioenergetic metabolism, and inflammation.

Materials and Methods

Animals, Diets, and Experimental Design

All procedures were approved by the Iowa State University Institutional Animal Care and Use Committee. Multiparous Holstein cows (n = 52), calving between March and October 2017, were blocked by their previous 305 mature equivalent milk yield, and assigned to 1 of 2 dietary treatments: 1) control diet (**CON**; n = 26) or 2) a diet containing RPG (pre-fresh 8.4% RPG dry matter [DM] basis, post-fresh 9.5% RPG DM basis; n = 26). Reduced pressure Maillard reaction with soybean meal and glucose was used to obtain the RPG product (US Patent United States Patent 8,507,025. Rupca LLC, Merced, CA). Treatments began 21 ± 5 d prior to expected parturition date and continued until 28 days in

milk (DIM). Immediately postpartum, cows were milked and processed according to standard operating procedures implemented at the Iowa State University Dairy Farm and moved into a lactation pen.

Pre- and post-parturition, cows were individually fed 110% of their ad libitum consumption using the Calan Broadbent feeding system (American Calan, Northwood, NH). For both groups, respective feed was delivered once daily, and orts were recorded prior to feeding. Diets were iso-nitrogenous, iso-energetic, and balanced to meet or exceed predicted requirements (NRC, 2001) of energy, protein, minerals and vitamins for each stage of production (Table 5). Cows were milked thrice daily at 0700, 1500, and 2300 h, and yield was automatically recorded at each milking.

Sampling and Data Collection

Feed sampling. Total mixed ration (TMR) samples were collected weekly on two consecutive days, composited to obtain one sample per week, and were stored at -20°C until the completion of the trial. The TMR samples were placed in a forced-air oven at 60°C for 48 h to determine the DM content. Diet composition was analyzed by an external laboratory (Cumberland Valley Analytical Services, Waynesboro, PA) and included DM (DM; method 930.15; AOAC International, 2000), nitrogen (N; method 990.03; Leco FP-528 Nitrogen Combustion Analyzer, Leco Corp., St. Joseph, MI), neutral detergent fiber (NDF; Van Soest et al., 1991), starch (Hall, 2009), ether extract using diethyl ether as the solvent (method 2003.05; AOAC International, 2006), ash (method 942.05; AOAC International, 2000), and phosphorus by inductively coupled plasma (method 985.01; AOAC International, 2000).

Milk production. Daily milk yield was condensed into weekly averages prior to statistical analysis. Individual milk samples were obtained at each of six consecutive milkings

on two consecutive days. Samples were stored at 4°C with a bronopol pellet as preservative (D & F Control System, San Ramon, CA) until analysis by Dairy Lab Services (Dubuque, IA) using AOAC approved analysis equipment and procedures. Milk samples were analyzed for milk fat, protein, lactose, and milk urea nitrogen (MUN) using Fourier transform infrared spectroscopy (MilkoScan FT+, FOSS Analytical, Eden Prairie, MN). Yield of protein, fat, lactose, were estimated using the corresponding milk yields for each sampling.

Blood metabolites, hormones, and acute phase proteins. Blood samples were obtained via coccygeal venipuncture once (1400 h) on d -14 (± 4 d) and -7 (± 4 d) relative to parturition and on d 3, 7, 14, 21, and 28 relative to actual parturition date. All samples were collected into 10 mL vacutainers (K_2EDTA; BD Franklin Lakes, NJ). Plasma samples were harvested following centrifugation at 1500 × *g* for 15 min at 4°C and were subsequently frozen at -20°C until analyses. Plasma samples from a subset of cows (n = 26) were retrospectively selected for blood analyses of non-esterified fatty acids (NEFA), β-hydroxybutyrate (BHB), blood urea nitrogen (BUN) and glucose. This dataset included samples from CON (n = 13) and RPG (n = 13) fed cows obtained on d -14, -7, 3, 7, 14, and 28 ± 1 relative to parturition. Samples for lipopolysaccharide-binding protein (LBP), haptoglobin (Hp), and insulin analyses were from d -7, 7, 14, and 28 ± 1 relative to parturition. Plasma insulin, NEFA, BHB, LBP, Hp, BUN, and glucose concentrations were determined using commercially available kits according to manufacturers' instructions (insulin, Mercodia AB, Uppsala, Sweden; NEFA, Wako Chemicals USA, Richmond, VA; BHB, Pointe Scientific Inc., Canton, MI; LBP, Hycult Biotech, Uden, The Netherlands; Hp: Immunology Consultants Laboratory Inc., Portland, OR; BUN, Teco Diagnostics Anaheim, CA; glucose, Wako Chemicals USA Inc., Richmond, VA).

Animal measurements. Body weight (BW) and body condition score (BCS) were determined twice weekly on consecutive days throughout the experimental period and condensed into weekly averages. Body condition scores were measured by two trained individuals utilizing Wildman and colleagues' (1982) scoring system but reported in 0.25 increments.

Fecal pH. Fecal samples were collected weekly from d -21 to 28 relative to parturition. Fresh samples were collected upon defecation or obtained via rectal palpation (~200 g wet basis). Samples were allowed to equilibrate to room temperature and pH was determined according to the method described by Branstad et al. (2017). Briefly, a subsample of 25 g was used in a 1:1 dilution with distilled water and homogenized for 1 min in a blender (Stomacher 80, Seward Ltd, West Sussex, UK). The homogenate was strained through one layer of cheesecloth. Fecal pH was then measured on the liquid using a portable pH meter (Oakton Instruments, Vernon Hills, IL). The remaining intact fecal sample was stored at -20°C.

Statistical Analysis

Data were analyzed as a completely randomized design with repeated measures using the mixed procedure of SAS (SAS Institute Inc., Cary, NC). Fixed effects included treatment, parity, time, and the interaction of treatment and time. Cow was included as a random effect. Each cow's previous 305 mature equivalent milk yield served as a covariate. The Akaike information criterion was used to select the most appropriate covariance structure. Autoregressive 1 structure was used to analyze BW, MY, milk components, energy corrected milk (ECM), 3.5% fat corrected milk (FCM), and feed efficiency. Spatial power structure was used to analyze DMI and plasma metabolites, hormones, and acute phase proteins. Data are

reported as least squares means and considered significant if $P \leq 0.05$ and a tendency if $0.05 < P \leq 0.10$.

Results

Dry Matter Intake, Fecal pH, and BW

Diets were formulated to be iso-energetic and iso-nitrogenic in both the pre- and post-partum period (Table 5). The NE_L for the pre-fresh CON and RPG TMR was calculated from actual laboratory analyses and was 97.1% of the targeted formulated value, whereas the analyzed CP for the pre-fresh CON and RPG TMR were 100.1 and 99.7% of the formulated CP content, respectively. Similarly, the NE_L values for the lactating CON and RPG TMR were 101.2% of the formulated diets, whereas the analyzed CP for the lactating CON and RPG TMR were 100.2 and 99.7% of the formulated CP content, respectively. Overall DMI was similar between treatments ($P = 0.64$; Figure 2A). However, a time effect was observed for both treatments as DMI initially decreased 10% prepartum ($P < 0.01$), followed by progressively greater DMI throughout 4 weeks postpartum ($P < 0.01$). Although no treatment differences were detected, fecal pH decreased 0.44 units from week -1 to 1 ($P < 0.01$; Figure 2B) and a further 0.18 unit decrease in fecal pH was observed from week 1 to 4 postpartum ($P = 0.03$). Cows on both treatments lost 34 kg throughout 28 DIM ($P < 0.01$) with no apparent difference in BW between treatments ($P = 0.16$; Figure 3A). Likewise, BCS was similar between treatments ($P = 0.41$; Figure 3B); cows lost 0.26 BCS points from weeks -1 to 1 ($P < 0.01$).

Milk Yield and Composition

Supplemental RPG had no effect on MY during the first 4 weeks of lactation (43.1 ± 1.2 kg/d; $P = 0.39$; Table 6). Similarly, there was no treatment effect ($P = 0.92$) on MY through

week 15 in lactation (47.2 ± 1.2 kg/d; Table 6). Milk composition for the first four weeks of lactation was similar for both treatments ($P \geq 0.40$; 4.4 ± 0.01%, 3.7 ± 0.1%, 4.8 ± 0.1%; for fat, protein, and lactose, respectively). Supplementing RPG tended ($P = 0.10$) to increase MUN compared to CON cows (12.6 vs. 11.7 mg/dL). No treatment differences were detected ($P = 0.63$; Table 6) in SCC; however, SCC decreased as lactation progressed for both treatment groups ($P < 0.01$). Energy and fat corrected milk were similar between treatments (49.2 ± 1.4 kg/d, $P = 0.64$ and 50.1 ± 1.7 kg/d, $P = 0.68$, respectively), but weekly average of ECM and FCM increased by 8.08 and 5.24 kg, respectively, when comparing week 1 with week 4 ($P < 0.01$; Table 6). Post-partum feed efficiency increased similarly in both treatments with time ($P < 0.01$; Table 6).

Blood Metabolites, Hormones, and Acute Phase Proteins

Overall, circulating glucose did not differ by treatment throughout the transition period ($P = 0.52$; 60.1 ± 1.9 mg/dL; Figure 4A); however, the distinctive and progressive hypoglycemic response associated with parturition was clear ($P \leq 0.01$). The temporal periparturient pattern in circulating insulin mirrored ($P < 0.01$) that of glucose but tended to be increased in RPG-fed cows relative to CON cows (27%; $P = 0.10$; Figure 4B); this response was largely driven by increased concentrations post-partum ($P = 0.09$). Overall, feeding RPG decreased circulating NEFA throughout the transition period ($P < 0.01$; Figure 4C). The reduction was most marked in the post-partum period, when cows consuming RPG had reduced circulating NEFA (28%; $P < 0.01$). Circulating BHB was similar ($P = 0.21$; Figure 4D) for both treatments throughout the transition period but tended to be decreased (24%; $P = 0.13$) in the post-partum period when cows received RPG. Concentrations of BUN

did not differ ($P = 0.53$; Figure 4E) between treatments; but progressively increased ($P = 0.05$) as lactation progressed.

Circulating LBP concentrations were not affected by treatment ($P = 0.18$; Figure 5A), however, both treatments peaked at 7 DIM and decreased with time ($P < 0.01$). Similarly, Hp concentrations did not differ due to treatment throughout the transition period (Figure 5B). Interestingly, circulating LBP and Hp from RPG-fed cows were decreased at 7 DIM relative to CON (30.7 and 27.4%, respectively; $P \leq 0.05$).

Discussion

This experiment aimed at determining the effect of supplemental rumen-undegradable carbohydrates under the premise that glucose availability may be limiting milk synthesis during early lactation (Amaral-Phillips et al., 1993). Since all cows seemingly experience some degree of inflammation after parturition (Bradford et al., 2015), and because mounting an immune response requires copious amounts of glucose (Kvidera et al., 2017a), it is important to consider glucose dynamics during both metabolically and immunogenic challenging periods. Because of this, we hypothesized that supplying RPG in the periparturient diet would increase milk yield and improve inflammatory biomarkers in dairy cows.

Starch reaching the rumen undergoes extensive microbial fermentation; degradation capacity varies from 30 to 90% (Allen, 2000), which implies that minimal glucose actually reaches the SI (Singleton, 1972). However, in some instances, up to 50% of dietary starch can reach the SI (Johnson and Bergen, 1982). Although the efficiency of the ruminants' SI to absorb glucose remains unclear (Harmon and McLeod, 2001), our results suggest that glucose from the tested supplement appeared to have been delivered to, and absorbed by, the SI primarily based upon increased insulin concentration in RPG-fed cows. Decreased circulating

insulin and insulin insensitivity of peripheral tissues after parturition play a key role in the partitioning of nutrients towards milk synthesis (Vernon, 1989; Baumgard et al., 2017). Despite difference in circulating insulin, blood glucose concentrations were not altered by RPG which is not entirely surprising considering that glucose is homeostatically controlled (Bauman and Currie, 1980). Similarly, Amaral et al. (1990) reported that insulin tended to increase when exogenous glucose was delivered intravenously to lactating cows with no change in circulating glucose concentration. It is likely RPG-fed cows had an increase in glucose turnover, which was responsible for the increased insulin concentrations. Since insulin is an anabolic hormone with potent antilipolytic properties (Brockman and Laarveld, 1986); it stands to reason that this mechanism was responsible for RPG-fed cows having decreased circulating NEFA concentrations and thus a numerical decrease in BHB concentration post-parturition. Knowlton et al. (1998) discovered similar results in blood bioenergetics when infusing starch into the abomasum of early-lactation cows.

Addition of a Maillard-derived protein-glucose supplement did not affect diet palatability as evidenced by similar DMI in both groups. No effect on DMI upon glucose supplementation is contrary to previous studies that reported decreased feed intake when lactating cows were infused with glucose ruminally (Knowlton et al., 1998) or abomasally (Larsen et al., 2010). However, our DMI results are consistent with several other studies which provided glucose either intravenously (Fisher and Elliot, 1966; Amaral et al., 1990), or with post-ruminal starch infusion (Clark et al., 1977; Reynolds et al., 2001; Relling and Reynolds, 2008). Reasons for the aforesaid discrepancies are not clear, but it is important to note that our treatments were iso-energetic and thus this may have contributed to the similarities in nutrient consumption.

In addition to the bioenergetics mentioned above, our data also suggest that RPG was digested and absorbed prior to reaching the large intestine based on the lack of treatment difference in fecal pH. It is noteworthy that fecal pH was markedly and consistently reduced for both treatments after calving. This observation highlights the possibility of compromised hind-gut barrier function when animals are transitioned to highly fermentable diets (Bissell and Hall, 2010). Though scarce and contrasting, literature on fecal pH in dairy cattle has centered on measurements during a high carbohydrate dietary challenge or abomasal infusion of carbohydrates. Results from these studies vary; for example, Gakhar and colleagues (2008) discovered that fecal pH was not altered by inducing SARA, whereas Morgante et al. (2009) reported that fecal pH decreased for cows with high risk of SARA. Therefore, it seems that starch or readily digestible carbohydrates that escape rumen fermentation may represent a risk factor for hind-gut acidosis. In accordance to this, fecal pH decreased upon abomasal infusion of oligofructose or starch (Reynolds et al., 2001; Bissell and Hall, 2010); however, this response is not highly repeatable across experiments (Gressley et al., 2011).

The onset of lactation and the sustained increase in milk production imposes a strong energy demand on high producing dairy cows but nutrient consumption is inadequate and does not equilibrate with energy output until much later in lactation (Bell, 1995). We hypothesized that dietary supplementation with RPG would provide more glucose in the intestinal lumen, hence, more precursors for milk production. This response has been reported with ruminal or abomasal glucose infusion (Knowlton et al., 1998), and intravenous glucose infusion (Brown and Allen, 2013); however, MY was not increased when cows consumed RPG in the current study. The lack of a MY response is consistent with previous studies infusing glucose in lactating dairy cows intravenously (Fisher and Elliot, 1966; Amaral et al., 1990), abomasally

(Clark et al., 1977; Reynolds et al., 2001; Relling and Reynolds, 2008), and duodenally (Lemosquet et al., 1997; Hurtaud et al., 1998; Reynolds et al., 2001). Reasons for this are not clear, but the mammary gland's capacity to acquire the necessary glucose appears fully adequate even during periparturient-induced hypoglycemia. There are a variety of different mammary glucose transporters and some of them have a Km for glucose as low as 2.4 mM (Zhao and Keating, 2007) and glucose concentrations in the current experiment were about 3.0 mM. Thus, data from our experiment and others suggest that increasing milk yield immediately post-partum is not entirely dependent on increased glucose availability. It is of practical and biological relevance to elucidate reasons for discrepancies within the literature to better characterize when and how supplemental glucose may increase milk production.

Similar to previous reports (Abuajamieh et al., 2016; Kaur et al., 2018, Zincola et al., 2018), inflammatory biomarkers, namely Hp and LBP, peaked during the first week of lactation and then gradually decreased with time. Since these proteins are synthesized as part of immunoactivation (Uchida et al., 1993), our data agree with previous literature indicating that even seemingly healthy cows experience inflammation and immunoactivation after parturition (Bradford et al., 2015). When activated, most leukocytes initiate a metabolic shift and rely primarily on aerobic glycolysis for energy production (Kelly and O'Neill, 2015), leading to a substantial increase in glucose consumption (Kvidera et al., 2017a). Thus, the immune system can put an additional strain on glucose homeostasis in early lactation. Interestingly, compared to CON cows, RPG-fed cows tended to have reduced Hp and LBP on d 7 postpartum when the peak of inflammation occurred. The reasons for the reduced "inflammatory state" with no positive translation into improved milk production are not clearly known, but certainly worthy of future investigation. Further evaluation of our data showed that

there was a strong correlation (R = 0.56; *P* = 0.06) for the CON cows to have increased Hp concentrations as DMI decreased to a greater extent prior to calving. On the other hand, RPG-fed cows had a strong negative correlation (R = -0.73; *P* = 0.01) such that blood Hp concentrations decreased with a more substantial decrease in DMI. Similarly, there was a strong correlation (R = 0.57; *P* = 0.07) for LBP concentrations to increase when fecal pH decreased to a greater extent after parturition for the CON cows. Although not significant (*P* = 0.45), the correlation shifted to being slightly negative (R = -0.24) such that blood LBP concentrations decreased with a more substantial decrease in fecal pH upon calving. While the mechanisms responsible for this are not clear, the changes are ostensibly a benefit to cow health.

Conclusion

Our experiment suggests that glucose availability does not limit milk yield immediately post-calving. Although difficult to determine, our data suggests that RPG improved bioenergetics of transition cows by delivering glucose to the small intestine based on the increased circulating insulin and decreased blood NEFA concentrations. Although the mechanisms are not completely understood, RPG supplementation appears to have benefited the immune system due to decreased inflammatory response on day 7 after calving, when the greatest immune insult appeared to have occurred. The mechanisms responsible for improving energetic status and ameliorating inflammation warrant future investigation.

Table 5. Ingredient and chemical composition of pre-fresh and lactating diets (average ± SD)

	Treatment[1]			
	Pre-fresh		Lactating	
Ingredient, % of DM	CON	RPG	CON	RPG
Corn silage	61.6	61.6	61.3	61.3
Wheat straw	8.5	8.5	1.6	1.6
Alfalfa hay	4.6	4.6	6.4	6.4
Cottonseed	-	-	2.4	2.4
Vitamin and mineral mix	13.9	13.8	16.3	16.3
Corn gluten feed	5.8	4.4	4.2	2.4
Expeller soybean meal	3.9	0.4	2.4	0.9
Molasses	1.7	1.6	-	-
Rumen-protected glucose	-	5.3	-	6.0
Nutrient analysis, % DM				
Dry matter	53.23 (3.87)	53.35 (3.48)	52.95 (2.11)	52.71 (1.68)
CP	13.52 (1.45)	13.66 (1.34)	16.33 (1.17)	15.86 (1.17)
NE_L (Mcal/kg)	1.38 (0.04)	1.38 (0.07)	1.63 (0.03)	1.62 (0.06)
NDF	46.87 (4.69)	47.15 (5.63)	34.82 (2.94)	36.13 (3.89)
ADF	31.93 (2.72)	32.20 (4.89)	23.38 (2.35)	24.57 (3.17)
Lignin	5.47 (0.72)	5.38 (1.08)	4.36 (0.62)	4.65 (0.79)
Starch	13.00 (2.09)	12.16 (2.63)	21.91 (2.50)	19.31 (2.69)
Ethanol soluble carbohydrate	2.88 (0.86)	5.15 (0.96)	2.88 (1.06)	5.11 (0.68)
NFC	29.58 (2.82)	30.07 (4.18)	37.63 (2.69)	37.91 (3.13)
Ether extract	2.71 (0.19)	2.41 (0.20)	4.79 (0.43)	4.54 (0.47)
Ash	8.77 (0.82)	8.29 (0.61)	7.81 (0.56)	7.32 (0.40)
Ca	1.07 (0.20)	0.99 (0.15)	0.82 (0.15)	0.73 (0.09)
P	0.36 (0.04)	0.32 (0.05)	0.44 (0.02)	0.40 (0.02)
Mg	0.37 (0.04)	0.35 (0.05)	0.34 (0.02)	0.33 (0.02)
K	1.45 (0.12)	1.39 (0.13)	1.39 (0.06)	1.35 (0.04)
S	0.42 (0.07)	0.40 (0.08)	0.25 (0.03)	0.24 (0.02)
Na	0.19 (0.03)	0.23 (0.03)	0.58 (0.08)	0.60 (0.06)
Cl	0.71 (0.07)	0.69 (0.11)	0.64 (0.05)	0.59 (0.04)
Fe (mg/kg)	336.50 (67.03)	312.09 (62.52)	342.58 (49.37)	301.71 (51.38)
Mn (mg/kg)	71.00 (7.09)	71.45 (12.50)	85.58 (9.02)	79.36 (7.88)
Zn (mg/kg)	70.50 (10.89)	67.36 (11.52)	116.58 (11.26)	107.14 (10.12)
Cu (mg/kg)	16.90 (3.25)	16.55 (2.91)	30.25 (3.98)	27.50 (4.05)

1CON = control; RPG = rumen-protected glucose diet.

Table 6. Production performance of post-partum dairy cows fed a control (CON) diet or a diet supplemented with rumen-protected glucose (RPG)

	Treatment[1]			*P*		
Item	CON	RPG	SEM	Trt	Time	Trt × Time
Dry matter intake, kg/d	21.7	21.3	0.7	0.65	< 0.01	0.42
Milk yield, wk 1-4, kg/d	43.7	42.5	1.2	0.39	< 0.01	0.62
Milk yield, wk 1-15, kg/d	47.1	47.3	1.2	0.92	< 0.01	0.90
Milk Components (wk 1-4)						
Fat, %	4.3	4.4	0.1	0.74	< 0.01	0.39
Protein, %	3.6	3.7	0.1	0.53	< 0.01	0.07
Lactose, %	4.8	4.7	0.1	0.40	< 0.01	0.06
MUN, mg/dL	11.7	12.5	0.1	0.10	0.74	0.07
SCC[2]	1.8	1.9	0.1	0.63	< 0.01	0.59
Energy corrected milk yield, kg/d	49.6	48.9	1.4	0.64	< 0.01	0.36
Fat corrected milk yield, kg/d	50.5	49.7	1.7	0.68	< 0.01	0.24
Feed Efficiency[3]	2.0	2.0	0.1	0.55	< 0.01	0.56

[1]CON = control; RPG = rumen-protected glucose diet.
[2]Somatic cell count, natural log transformed.
[3]Efficiency of milk production (kg of MY / kg of DMI).

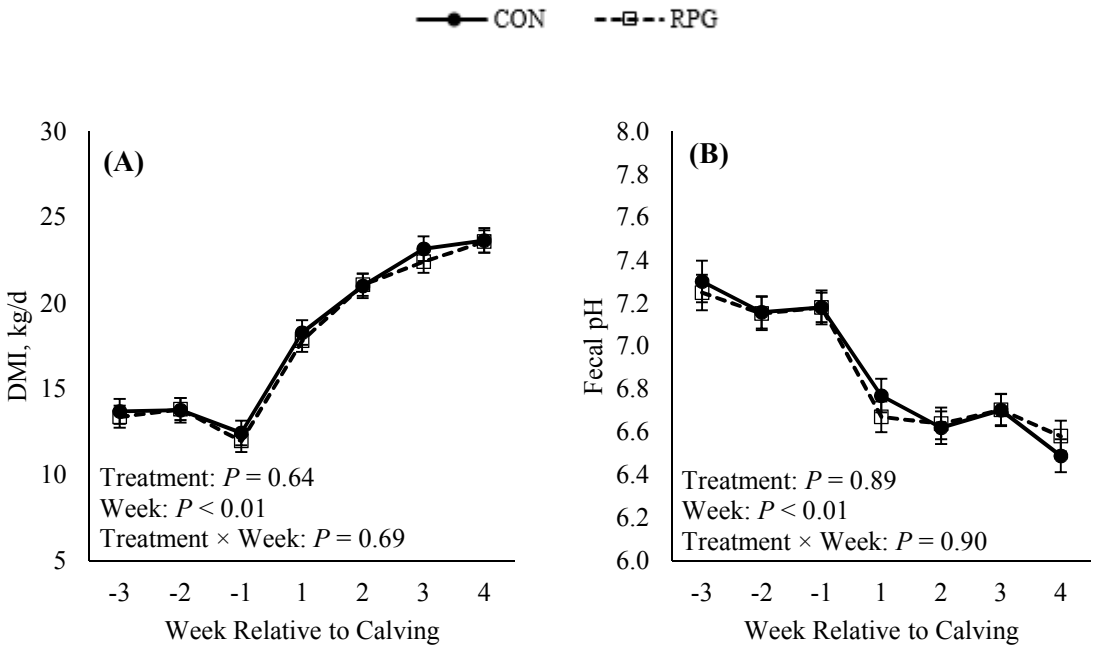


Figure 2. Effects of rumen-protected glucose on (A) DMI and (B) fecal pH. Treatments: CON = control diet and RPG = rumen-protected glucose diet.

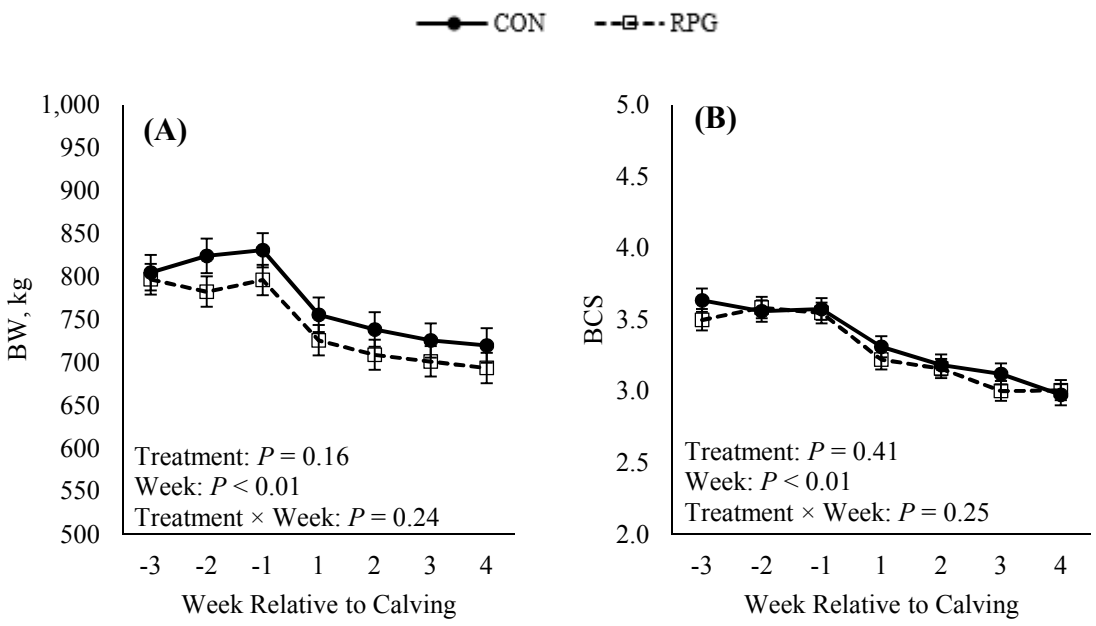
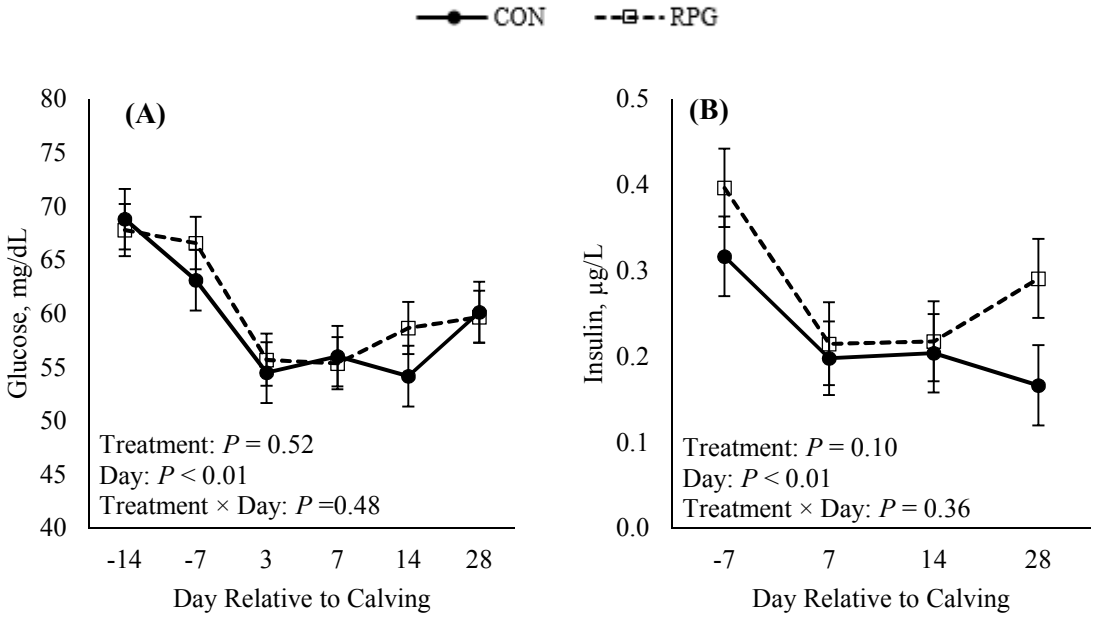


Figure 3. Effects of rumen-protected glucose on (A) BW and (B) BCS. Treatments: CON = control diet and RPG = rumen-protected glucose diet.



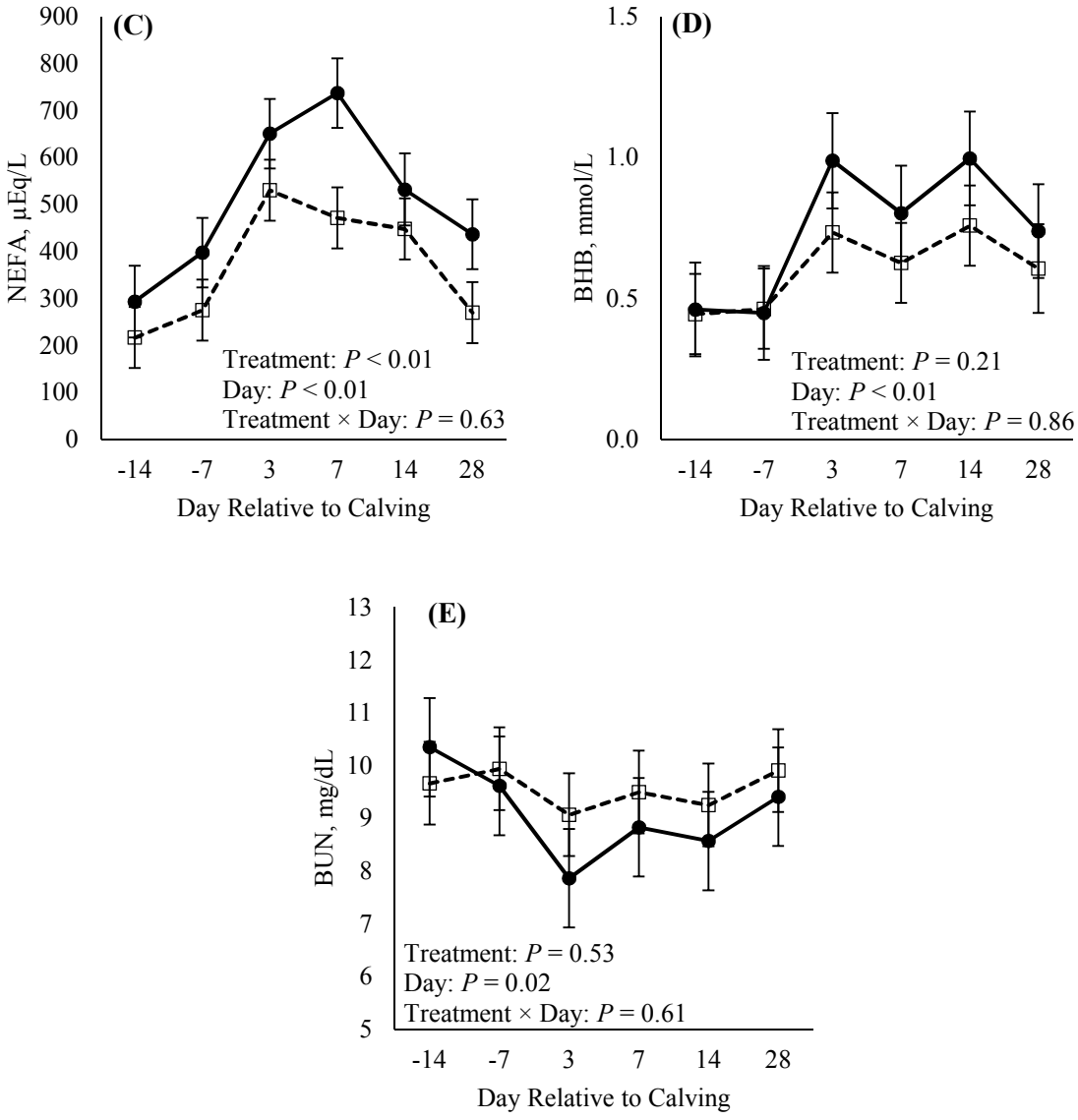


Figure 4. Effects of rumen-protected glucose on circulating (A) glucose, (B) insulin, (C) NEFA, (D) BHB, and (E) BUN. Treatments: CON = control diet and RPG = rumen-protected glucose diet.

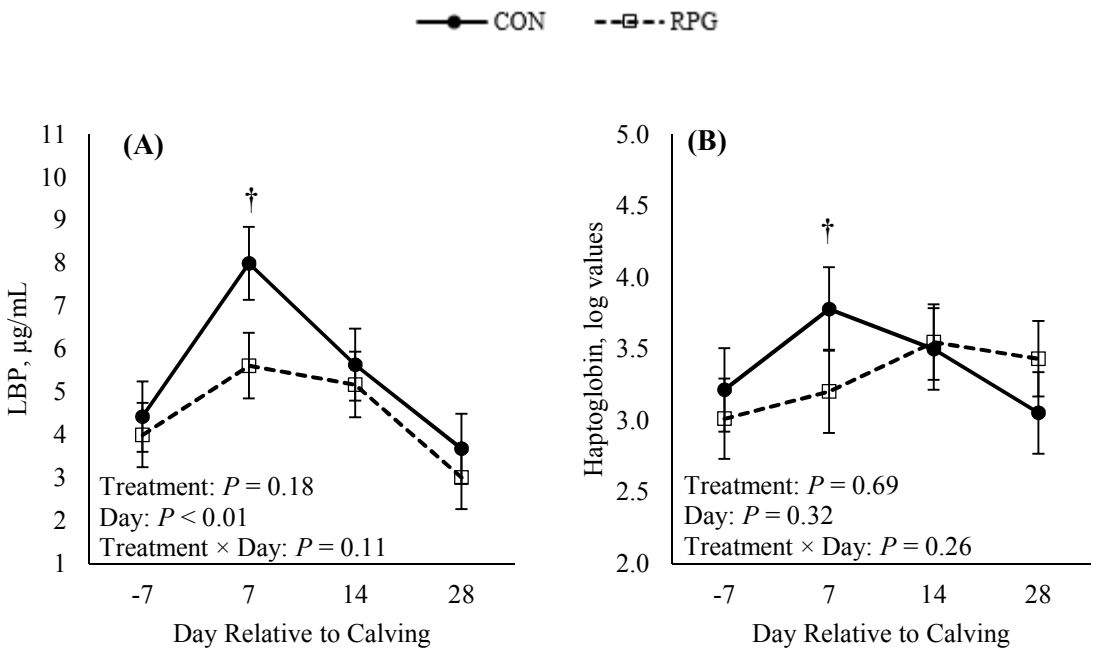


Figure 5. Effects of rumen-protected glucose on circulating (A) LBP and (B) Hp. Treatments: CON = control diet and RPG = rumen-protected glucose diet.

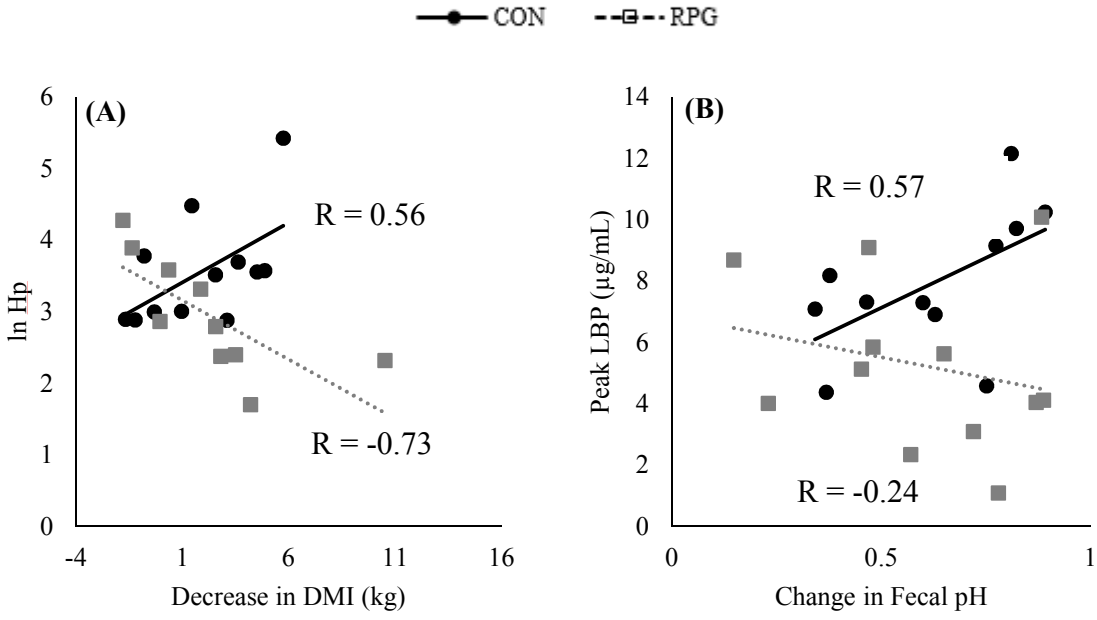


Figure 6. Correlations of the (A) decrease in DMI and Hp concentrations and (B) change in fecal pH and peak LBP concentrations.

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CHAPTER 3: SUMMARY AND IMPLICATIONS

There are a multitude of situations in which glucose availability may limit milk yield during lactation including the transition period, immunoactivation (Kvidera, 2017a), and heat stress (Baumgard and Rhoads, 2013). The transition period is characterized by reduced DMI prior to parturition, coupled with many metabolic changes (Drackley, 1999). Energy output exceeds energy intake in early-lactation, causing cows to enter NEBAL. Cows in NEBAL are predisposed to a myriad of negative health effects, including ketosis, fatty liver, displaced abomasum, mastitis, and infertility; up to 50% of transition dairy cows are affected by at least one of these negative outcomes (Drackley, 1999). Economic losses due to a poor transition into lactation include: decreased fecundity, cost of treatment, reduced life expectancy, and decreased milk production (Hailemariam et al., 2014). The greatest economic loss at the onset of lactation can be attributed to metritis, mastitis, laminitis, and infertility, accounting for $200 million lost per year (Ametaj et al., 2012). Additionally, these disorders mount an immune response, and the activated immune system requires copious amounts of glucose (Kvidera et al., 2017a). Furthermore, dairy cows in NEBAL mobilize body energy stores to spare glucose for milk production. The importance of glucose is its role as the precursor to lactose synthesis, and lactose is the osmoregulator of milk yield (Neville et al., 1983; Cant et al., 2002). However, carbohydrates are rapidly fermented in the rumen, requiring dairy cows to rely on hepatic gluconeogenesis for their glucose supply (Aschenbach et al., 2010). Therefore, supplying additional glucose during the transition period may improve the immune response to the health disorders associated with the periparturient period, and increase production performance.

Objectives of Chapter 2 were to determine the effects of dietary RPG on milk production, post-absorptive metabolism, and inflammatory biomarkers in transitioning dairy cows. Glucose is

important for milk production, and it is estimated that 72 g of glucose are required to produce 1 kg of milk (Kronfeld et al., 1982). Therefore, we hypothesized that RPG would allow for increased milk synthesis, improve circulatory energetics and reduce inflammatory biomarkers in transitioning dairy cows. Our results demonstrated that RPG did not increase MY in early-lactation or alter DMI. Interestingly, RPG supplementation had no effect on fecal pH, but both treatment groups saw a marked reduction in pH after calving. Although literature is scarce on fecal pH during the transition period, we conclude that RPG supplementation did not cause rapid fermentation in the hind-gut. Although there were no differences for circulating glucose concentration, RPG decreased circulating NEFA and BHB concentrations post-parturition. Additionally, RPG tended to increase blood insulin concentration. Because we did not see a difference in MY, we can speculate that RPG allowed less reliance on additional energy sources (NEFA and BHB) for milk production. Furthermore, the greatest immune insult occurred on day 7 post-parturition (characterized by peak APP concentrations), but RPG decreased LBP and Hp on day 7 of lactation. Although the mechanisms are not clear, the changes in APP are a benefit to cow health and warrants further investigation.

In conclusion, our study demonstrated supplying glucose to the SI of transitioning dairy cows benefited the health of the animals by decreasing the APP concentrations during the time of the greatest immune insult. Additionally, circulating NEFA and BHB concentrations were decreased by RPG supplementation. However, MY does not appear to be limited by glucose availability in the transition period.

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APPENDIX: EVALUATION OF THE METHOD OF SAMPLE PREPARATION FOR THE DETERMINATION OF FECAL PH IN DAIRY COWS

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Abstract

A standard procedure for measurement of fecal pH in dairy cows does not currently exist. Consequently, sample preparation may influence the precision of this measurement; thus, limiting comparisons across literature reports. The objectives of this study were to determine if differences exist based on preparation method, and to determine variation across methods. Thirty fresh fecal samples were collected from lactating Holstein cows housed in the same pen and consuming the same diet. Five samples were collected at a time and prepared according to the following methods: 1) direct measurement (DIR) in which the pH probe was directly inserted into the fecal sample; 2) strained fecal fluid (STR) obtained by squeezing the fecal sample through four layers of cheesecloth. Three dilution rates (distilled water:feces) were also tested: 3) 0.5:1 dilution (D1), 4) 1:1 dilution (D2), and 5) 2:1 dilution (D3). Each sample was prepared using all methods, resulting in a total of 150 pH measurements. The UNIVARIATE and GLM procedures of SAS were used to test normality and homogeneity of variance, respectively. The Shapiro-Wilk test confirmed that data was normally distributed ($P = 0.08$). The Levene's test showed heterogeneity of variance ($P = 0.02$), thus the SATTERTHWAITE approximation of degrees of freedom for denominator was used for the analysis of variance via the GLIMMIX procedure. Sample preparation method affected ($P < 0.01$) pH values, resulting in D3 having the highest pH of 6.91 ± 0.04, followed by

D2 with a value of 6.79 ± 0.04. Measurements of pH by D1 and DIR were similar, and averaged 6.67 ± 0.04 (P = 0.17); whereas, STR had the lowest value of 6.60 ± 0.04. Descriptive statistics showed the standard deviation for the STR method was 0.173 and 0.174 for D2, while that of D1, D3 and DIR was 0.224, 0.226 and 0.296, respectively. These results demonstrate that pH measurements in strained fecal fluid or a 1:1 dilution rate have reduced variability when compared to direct measurements and other dilution rates.