Effects of Rumen-Protected Glutamate Supplementation during the Periparturient Period on Digestibility, Inflammation, Metabolic Responses and Performance in Dairy Cows

by

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A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

in

Animal Science

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Abstract

Dairy cows often experience negative energy balance, fat mobilization and inflammation after calving, which increases health problems. Glutamate (Glu) is a primary energy source for the small intestine and a glucogenic amino acid in the liver, and feeding Glu enhances gastrointestinal growth, barrier function, and digestive and absorptive capacity in monogastric animals. However, the effects of feeding Glu in dairy cows during the calving transition period have not been studied. The objective of this thesis was to evaluate the effects of feeding rumen-protected Glu during the periparturient period (d -21 ± 3 to d 21 ± 3 relative to calving) on apparent total-tract digestibility (ATTD), inflammation, metabolic responses, and production performance of dairy cows. Fifty-two multiparous Holstein cows were blocked by parity, body condition score, and expected calving date, and randomly assigned to one of the experimental diets with rumen-protected monosodium Glu (RP-Glu; intestinally available Glu = 8.8%) or without RP-Glu (control) at d -21 ± 3 relative to expected calving date. The RP-Glu was fed at 4% and 3% of dietary dry matter, before and after calving, respectively. Prepartum diets contained 17.1% and 16.5% crude protein, and 13.1% and 13.3% starch, and postpartum diets contained 18.8% and 18.3% crude protein, and 22.5% and 22.7% starch on a dry matter basis, respectively for RP-Glu and CON treatments. A subset of nineteen cows was used to measure ATTD. Cows fed the RP-Glu had greater ATTD of dry matter (70.6 vs. 69.1 %; $P = 0.05$), crude protein (75.1 vs. 72.6 %; $P = 0.03$), and ether extract (66.0 vs. 61.2 %; $P = 0.05$) on d 5 ± 1 after calving. Cows fed the RP-Glu also had greater dry matter intake (15.7 vs. 13.7 kg/d; $P = 0.03$) on d 1 after calving. Cows fed the RP-Glu had greater plasma concentrations of Glu (4.60 vs. 3.89 μmol/dL; $P < 0.01$) and insulin-like growth factor-1 (44.2 vs. 30.1 ng/mL; $P < 0.01$), lower serum concentrations of free fatty acids (670 vs. 981 μEq/L; $P < 0.01$) and total bilirubin (0.22

vs. 0.34 mg/dL; $P < 0.01$), and lower plasma 3-methylhistidine concentration (1.28 vs. 1.50 μmol/dL; $P = 0.03$) on d 4 after calving. However, these treatment effects observed between d 1 and d 5 ± 1 immediately after calving did not continue until d 21 after calving. Concentrations of serum amyloid A, serum haptoglobin, and plasma lipopolysaccharide binding protein were not affected by the treatment. In addition, no differences were observed for serum β-hydroxybutyrate concentration and milk yield during the postpartum period between the two groups, and cows fed the RP-Glu decreased lactose yield. These results indicate that feeding RP-Glu during the periparturient period can increase digestive capacity and feed intake, and decrease body fat and protein mobilization immediately after calving without increasing milk production. Dietary Glu requirement is not established for dairy cows, however, our findings suggest that cows may not be able to synthesize sufficient Glu in the body immediately after calving.

Preface

The research in this thesis received research ethics approval from the University of Alberta Animal Care and Use Committee for Livestock (AUP#3193) and conducted according to the guidelines of the Canadian Council on Animal Care (2009).

Chapter 2 of this thesis "Effects of Rumen-Protected Glutamate Supplementation during the Periparturient Period on Digestibility, Inflammation, Metabolic Responses and Performance in Dairy Cows" was accepted by the Journal of Dairy Science. I was responsible for the data and sample collection and analysis, and manuscript composition. A. Haruno and T. Fujieda contributed to sample analysis and provided financial support and the rumen-protected monosodium Glu prototype product. T. Sugino contributed to sample analysis. A. Haruno and T. Sugino contributed to develop experimental design, data analysis and manuscript edits. M. Oba was the corresponding author and was involved with experimental design, data and sample collection and analysis, and manuscript composition.

Acknowledgements

I thank Dr. Masahito Oba for accepting me as his student, and for his patient guidance with the broad knowledge throughout my program.

I thank my supervisory committee members, Dr. Leluo Guan and especially Dr. Anne Laarman for their warm support and helpful comments.

I thank the staff at the Dairy Research and Technology Centre for their contribution in my research.

I thank Lauren E. Engelking and Matthew Burdick for their advice, support, and friendship.

I thank my friends at the University of Alberta for their support, encouragement, and friendship: Yajing Ban, Manjot M. J. Singh, Maddi Degenshein, Iswarya Rajesh, Courtnee Hainstock, and Stephanie Chang.

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List of Abbreviations

AA	Amino acids
Ala	Alanine
Arg	Arginine
Asn	Asparagine
Asp	Aspartate
ATTD	Apparent total-tract digestibility
BCAA	Branched-chain amino acids
BCS	Body condition score
BHB	β-hydroxybutyrate
BW	Body weight
CCK	Cholecystokinin
CON	Control
CP	Crude protein
Cys	Cysteine
DCAD	Dietary cation-anion difference
DM	Dry matter
DMI	Dry matter intake
EAA	Essential amino acids
ECM	Energy-corrected milk
FFA	Free fatty acids
Gln	Glutamine
GLP-2	Glucagon-like peptide-2

Glu	Glutamate
GLUT	Glucose transporter
His	Histidine
IGF-1	Insulin-like growth factor-1
Ile	Isoleucine
iNDF	Indigestible NDF
LBP	Lipopolysaccharide binding protein
Leu	Leucine
LPS	Lipopolysaccharide
Lys	Lysine
ME	Metabolizable energy
Met	Methionine
MP	Metabolizable protein
MUN	Milk urea nitrogen
NDF	Neutral detergent fiber
NEAA	Nonessential amino acids
NFC	Nonfiber carbohydrates
OM	Organic matter
peNDF	Physically effective neutral detergent fiber
Phe	Phenylalanine
Pro	Proline
RP-Glu	Rumen-protected monosodium glutamate
SARA	Sub-acute rumen acidosis

SCC	Somatic cell count
Ser	Serine
Thr	Threonine
TMR	Total mixed ration
Trp	Tryptophane
Tyr	Tyrosine
Val	Valine
VLDL	Very-low-density lipoprotein

Chapter 1: Literature Review

1.1 Introduction

Annual milk yield per cow in 2020 was 10,784 kg, which increased by 40% over the last 50 years (United States Department of Agriculture, 2021). The increase in milk production of dairy cows is partly due to genetic improvement and advances in nutritional management (Baumgard et al., 2017). Nutritional management of the calving transition is critical to health of dairy cows and profitability because approximately 75% of disease of dairy cows happens in the first month after calving (LeBlanc et al., 2006). In the last 50 years, the understanding of dairy cow nutrition has progressed, and nutritional formulation has become more precise. For example, protein nutrition in dairy cows formulated to meet crude protein (CP) requirement previously, however, protein nutrition now formulates for metabolizable protein (MP), which is a true protein that is digested post-ruminally and amino acids (AA) absorbed in the small intestine (NRC, 2001). Amino acids are categorized as essential AA (EAA) or nonessential AA (NEAA). Cows do not have dietary requirement for NEAA (NRC, 2001), however, the number of studies about dietary NEAA in dairy cows are increasing gradually due to the increase of understandings of the role of dietary NEAA in monogastric animals.

In this literature review, I will discuss the physiology and nutritional management during the calving transition, AA research in ruminants, and the effects of dietary Glu, which is one of the NEAA, in monogastric animals and ruminants.

1.2 Calving Transition in Dairy Cows

1.2.1 Physiology of Calving Transition

The calving transition period is defined as 3 weeks before to 3 weeks after calving (Drackley, 1999). Metabolism and nutrient requirements of dairy cows change significantly from late gestation through the lactation period (NRC, 2001). The regulatory function for metabolic adaptation to support the physiological state, such as lactation, during the transition period is called homeorhesis (Bauman and Currie, 1980). The adaptations for lactation occur in the mammary gland as increased use of nutrients, liver as increased gluconeogenesis and glycogenolysis, adipose as increased lipolysis and decreased lipogenesis, gastrointestinal tract as increased its mass, and muscle as mobilization of protein reserves (Bauman and Currie, 1980).

The gluconeogenesis in the liver greatly increases after calving in dairy cows because glucose requirement for lactose synthesis in the mammary gland increases (Baumgard et al., 2017). Propionate is a major precursor of gluconeogenesis mainly derived from starch fermentation in the rumen. However, propionate synthesis is often insufficient for the greater metabolic demand associated with lactation due to low feed intake after calving (Aschenbach et al., 2010; Larsen and Kristensen, 2013). Therefore, cows mobilize body fat which elevates circulating free fatty acids (FFA) concentration (Reece et al., 2015; Baumgard et al., 2017). Body fat mobilization occurs in most lactating mammals, and it is regulated by the endocrine system (NRC, 2001; Mulligan and Doherty, 2008). The mobilized body fat is oxidized to acetyl coenzyme A in the liver and generates energy in the tricarboxylic acid cycle. Excessive acetyl coenzyme A following excessive FFA mobilization to the liver is converted to ketone bodies, and circulating β-hydroxybutyrate (BHB) concentration increases in dairy cows (Aschenbach et al., 2010; Baumgard et al., 2017).

A previous study (Bell et al., 2000) reported that metabolizable protein (MP) balance in postpartum dairy cows decreased to nadir in 1 week after calving, and increased gradually to zero balance until approximately 3 weeks after calving. Plasma AA concentration in transition dairy cows reaches nadir immediately after calving and increases gradually until day 28 after calving (Zhou et al., 2016). Decreased plasma AA concentration immediately after calving may be associated with increased AA utilization for gluconeogenesis or milk protein synthesis. Lactating cows can mobilize 25% of body muscle protein (McCabe and Boerman, 2020) to meet a great requirement for AA (Bell et al., 2000). However, there is likely a large variation among individual dairy cows about the amount of body muscle protein mobilization and milk production (McCabe and Boerman, 2020).

Metabolic disorders such as fatty liver, ketosis, and rumen acidosis, and infections are more likely to occur for dairy cows during the transition period (Drackley, 1999; Bell et al., 2000; Mulligan and Doherty, 2008). These transition diseases can have negative impacts on health and productivity of dairy cows (Drackley, 1999; Mulligan and Doherty, 2008; Bertoni and Trevisi, 2013), and decrease the income of dairy producers. Improved transition cow nutrition and management can increase milk yield per lactation by 500 to 1000 kg (Lean et al., 2013). Therefore, many studies focused on nutrition and management during the calving transition period because it is extremely important for production and profitability.

1.2.1.1 Fetal Growth

During the late gestation period from three weeks before calving, the nutritional requirements of dams increase for placental and fetal growth (Bell et al., 2000; NRC, 2001). Fetal tissue accounts for almost 80% of uterine dry weight at day 270 of pregnancy in dairy

cows, and MP requirement for conceptus growth is 300 g/d (Bell et al., 1995). The fetus cannot take lipid substrate mobilized by dam directly, therefore, most of the energy and nitrogen required for fetal growth and metabolism are supplied by glucose and AA from the maternal circulation and taken up by the placenta (Bell, 1995). The placental glucose transport depends on the maternal and fetal glucose concentrations gradient. Therefore, circulating fetal glucose concentration responds to the changes in maternal circulating glucose concentration (Bell, 1995). Maternal glucose deficiency is compensated for by increased AA catabolism, however, fetal growth is reduced (Bell, 1995). It is essential to supply adequate nutrition to dams for fetal growth.

1.2.1.2 Mammary Development

Massive mammary cell proliferation occurs just before and after calving which is stimulated primarily by hormones such as prolactin and growth hormone (Akers, 2017), and the colostrum is produced in the last week of gestation (McCabe and Boerman, 2020). Therefore, the nutritional requirement for late gestation dairy cows is greater due to the proliferation of mammary gland and colostrum synthesis (Bell et al., 2000). The requirement of MP for mammary growth in late gestation is estimated to be approximately 120 g/d (Bell et al., 2000) to 130 g/d (NRC, 2001), however, this depends on the MP required for milk protein and the quality and quantity of colostrum synthesis (McCabe and Boerman, 2020). Mammary development completes within several days postpartum with the onset of copious milk secretion (Bell, 1995; Akers, 2006). The requirements for glucose, AA, and fatty acids in the mammary gland within a few days of calving due to milk secretion increases significantly, by approximately 2.7, 2.0, and 4.5 times, respectively, which is greater than the requirements of glucose, AA, and fatty acids in

the gravid uterus during late gestation (Bell, 1995). Glucose, AA and fatty acids are required in the mammary gland after calving mostly for lactose synthesis, lactose and milk protein synthesis, and milk fat synthesis, respectively (Bell, 1995).

1.2.1.3 Gut Development

Gastrointestinal mass growth occurs in transition dairy cows. Reynolds et al. (2004) measured the visceral mass of a total of 36 Holstein cows by slaughtering at 21 or 7 days before the expected calving date, or 10 or 22 days after calving. The weights of the reticulo-rumen, small and large intestines 3 weeks after calving gained approximately 2.0 kg, 0.6 kg, and 1.0 kg, respectively, compared with 3 weeks before calving in dairy cows (Reynolds et al., 2004). They concluded that the changes in the mass of gastrointestinal tracts were due to increased feed intake and not the onset of lactation. Increased gastrointestinal mass and absorptive capacity typically benefit cows (Ingvartsen and Anderson, 2000; Steele et al., 2016) although gastrointestinal mass growth and maintenance increase nutrient requirement (Johnson et al., 1990).

1.2.1.4 Feed Intake

Feed intake decreases by approximately 30% during the last week before calving (Lean et al., 2013). After calving, feed intake increases gradually (Drackley, 1999) and reaches the maximum at 8 to 22 weeks after calving (Ingvartsen and Andersen, 2000). The greater feed intake could result in the less lipid accumulation in the liver and higher milk production after calving (Lean et al., 2013). Feed intake is regulated by metabolism, endocrine changes, and fatty acid oxidation, and immune system may also affect feed intake during infection (Ingvartsen and

Andersen, 2000). In addition, reduced feed intake decreases nutrient flow to the gastrointestinal epithelium which causes gastrointestinal barrier dysfunction (Pearce et al., 2013; Kvidera et al., 2017) and induces gut-derived inflammation (Pearce et al., 2013; Bradford et al., 2015).

1.2.1.5 Insulin Resistance

When feed intake increases and the circulating propionate concentration elevates in animal body in dairy cows, insulin is secreted from the pancreas (Allen et al., 2009). Specifically, insulin suppresses circulating glucose concentration by reducing gluconeogenesis in the liver and glycogenolysis, and increasing glucose uptake, protein synthesis in the muscle, and lipogenesis (Baumgard et al., 2017; De Koster and Opsomer, 2013). Circulating glucose is taken up via glucose transporter (GLUT). The expression of GLUT1, which is insulin-independent, increases in the mammary gland after calving (Komatsu et al., 2005; De Koster and Opsomer, 2013). In addition, GLUT 4, which is insulin-dependent, is not present in the mammary gland (Komatsu et al., 2005; De Koster and Opsomer, 2013). Therefore, the mammary gland can take up glucose to synthesize lactose without being affected by insulin.

Cows generally experience a state of insulin resistance in late gestation and early lactation (De Koster and Opsomer, 2013). Under insulin resistance, gluconeogenesis or body fat and muscle protein mobilization are not suppressed (Baumgard et al., 2017), and glucose is conserved for lactose synthesis in the mammary gland (Lucy, 2008; De Koster and Opsomer, 2013; Baumgard et al., 2017), therefore, insulin resistance can promote greater milk production.

1.2.1.6 Energy Metabolism in the Liver

Greater circulating FFA and BHB concentrations due to excess body fat mobilization can increase the incidences of fatty liver, ketosis, and other metabolic disorders (Drackley, 1999; Mulligan and Doherty, 2008; Baumgard et al., 2017). Ketosis is a metabolic disorder that occurs in early lactation in a state of negative energy balance in dairy cows. When cows are in a state of negative energy balance, cows break down triglycerides in body fat and utilize FFA as an energy source (Reece et al., 2015). Excess FFA is converted to ketone bodies which elevate circulating BHB concentration (Reece et al., 2015). Cows have a limited ability to metabolize ketone bodies although ketone bodies are an energy source for dairy cows, therefore, ketone bodies are accumulated and ketosis occurs in cows (Reece et al., 2015). When body fat mobilization is excessed, triglycerides are reformed in the liver (Drackley, 1999). The synthesis of very-low-density lipoprotein (VLDL) which transports triglycerides from the liver requires phosphatidylcholine, and the synthesis of phosphatidylcholine requires choline (NRC, 2001; Mulligan and Doherty, 2008). Methionine (Met) works as a methyl donor for the synthesis of choline (NRC, 2001; Reece et al., 2015). However, the ability of ruminants to synthesis VLDL is low in the liver, therefore, decreased transportation of triglycerides from the liver results in fatty liver in dairy cows (Drackley, 1999; Mulligan and Doherty, 2008).

1.2.2 Nutritional Management during the Calving Transition

1.2.2.1 Energy

Carbohydrates, fat, and AA are dietary energy sources for dairy cows. Carbohydrates include fiber and non-fiber carbohydrates (NFC; organic acids, sugar, starch, and soluble fiber) which are fermented by rumen microbes to produce volatile fatty acids (i.e. acetate, propionate,

and butyrate) as energy sources. Acetate and butyrate are substrates for fatty acid synthesis in mammary gland and adipose tissue (Reece et al., 2015), and propionate is a primary glucogenic precursor (Aschenbach et al., 2010; Larsen and Kristensen, 2013; Reece et al., 2015) in dairy cows. Feeding fat in transition diets can provide energy, however, feeding fat does not suppress body fat mobilization which may result in decreased feed intake (Drackley, 1999). Glucogenic AA can be converted to glucose. Drackley (1999) suggested increasing the supply of AA and glucogenic precursors, and minimizing the use of supplemental fat during the early postpartum period.

1.2.2.1.1 Prepartum

Prepartum diets can affect the incidence of metabolic disorders during the periparturient period in dairy cows. Dairy NRC (2001) suggested increasing energy density by increasing NFC in close-up cow diet, such as grains. By increasing grains, it allows rumen microbes to adapt to a highly fermentable postpartum cow diet, and growth of rumen papillae is increased which increases the capacity to absorb more acid (NRC, 2001). However, a recent study (Shi et al., 2020) reported that feeding a high-starch close-up cow diet (26.1% starch on a dry matter (DM) basis) did not have any advantages because cows fed the high-starch close-up cow diet did not mitigate prepartum and postpartum rumen pH depression compared with cows fed a low-starch close-up diet (14.0% starch on a DM basis). In addition, feeding the high-energy close-up cow diet increases energy reserve and body condition score (BCS). An optimum calving BCS of 3.0 to 3.25 (5-point scale; Wildman et al., 1982) is recommended (Roche et al., 2009). Lower BCS at calving can reduce milk production, whereas greater BCS at calving (≥ 3.5; 5-point scale)

reduces feed intake in early lactation and milk production, and increase the risk of metabolic disorders (Roche et al., 2009) such as ketosis.

There are two types of ketosis: type I and type II (Oetzel, 2004). Type I ketosis often occurs in high-producing dairy cows in 2 to 3 weeks after calving, associated with feeding an insufficient gluconeogenic precursor diet (Reece et al., 2015). Cows with type I ketosis usually well respond to glucose intravenous injections and administering propylene glycol. In contrast, type II ketosis occurs within week 1 after calving, which appears to be associated with rapid fat accumulation in the liver (Reece et al., 2015). Over-conditioned dry cows compared with adequately conditioned dry cows have greater risks of type II ketosis because over-conditioned cows mobilize more body fat (Drackley, 1999; Ingvartsen and Andersen, 2000; Mulligan and Doherty, 2008). Excessive body weight (BW) loss immediately after calving should be avoided, especially to prevent type II ketosis and fatty liver.

To prevent ketosis, it is recommended to maintain optimum BCS before calving by feeding a controlled energy dry cow diet using chopped straw (Dann et al., 2006; Janovick and Drackley, 2010; Havekes et al., 2020). In addition, reducing pen moves, regrouping, or overstocking to decrease environmental stressors (Mulligan and Doherty, 2008; Van Saun and Sniffen, 2014) for late gestation dairy cows is important to minimize reducing their feed intake.

1.2.2.1.2 Postpartum

The requirement for net energy of lactation for fresh cows is three to four times greater than that of close-up cows depending on milk production (NRC, 2001). Postpartum dairy cows are often fed a high grain fresh cow diet to meet the increased nutritional requirement associated with increased milk production after calving. The results of recent studies evaluating animal

performance in transition dairy cows by feeding fresh cow diets differing in starch content are inconsistent. Shi et al. (2019) reported that cows fed a low-starch fresh cow diet (22.1% starch on a DM basis) had greater milk yield compared with a high-starch fresh cow diet (28.3% starch on a DM basis). Cows in both treatments were fed a close-up diet with approximately 14% starch on a DM basis. Cows fed the low-starch fresh cow diet may have experienced less sudden and drastic change in diet fermentability after calving compared with cows fed the high-starch fresh cow diet (Shi et al., 2019). Another study (Haisan et al., 2021) reported that greater milk yield was observed for cows fed a high-starch fresh cow diet (32.8% starch on a DM basis) than cows fed a low-starch fresh cow diet (25.1% starch on DM basis). The starch content of close-up cow diets in both studies were similar which was approximately 14% on a DM basis, however, the result of Haisan et al. (2021) contradicts the result of Shi et al. (2019). One explanation for the greater milk yield for cows fed the high-starch fresh cow diet may be due to free-choice hay supply only for the first 3 days after calving which could allow cows to change starch content of diets and diet fermentability at their own rate (Haisan et al., 2021).

The sudden and drastic change in diet fermentability after calving produces excessive volatile fatty acids, depress rumen pH, and increase the risk of sub-acute rumen acidosis (SARA; Krause and Oetzel., 2006; Steele et al., 2016). The thresholds of SARA are when the rumen pH remains below 5.6 for more than 3 consecutive hours per day (Gozho et al., 2005), or below 5.8 for more than 5.2 hours per day (Zebeli et al., 2010), and rumen pH can be measured by a continuous indwelling rumen pH system (Gozho et al., 2005). Grain-based SARA disturbs rumen microbial richness, diversity, and functionality, and results in reduced feed intake and productivity, induced health disorders, and decreased profitability (Plaizier et al., 2018) with losses in the income of US$ 400 to US$ 475 per cow per year (Krause and Oetzel., 2006). In

addition, grain-based SARA reduces rumen fermentation which can increase the amount of rumen-bypass starch. The large amount of rumen-bypass starch increases fermentation by the microbes in the hindgut which increases the risk of hindgut acidosis because of an accumulation of VFA (Gressley et al., 2011; Plaizier et al., 2018).

The rumen epithelium permeability increases due to lower rumen pH by SARA because the rumen epithelium is not protected by mucus (Steele et al., 2016; Plaizier et al., 2018). The intestinal epithelium is more fragile due to the single-layered structure compared with the rumen epithelium which has multi-layered structure although the intestinal epithelium is protected by mucus (Gressley et al., 2011; Li et al., 2012; Steele et al., 2016). When cows are in the state of hind-gut acidosis, mucin casts appear in the feces because of the damage to the large intestine epithelium (Gressley et al., 2011). In addition, the state of SARA increases concentration of lipopolysaccharide (LPS), also known as endotoxin, released by gram-negative bacteria (Ribeiro et al., 2010) in the rumen (Steele et al., 2016). Previous studies reported that LPS produced in the rumen can be detoxified by acidic pH (Ribeiro et al., 2010) in the abomasum (Khiaosa-ard and Zebeli, 2018) and by bile acids in the small intestine (Bertók, 1998). However, the concentration of LPS is also increased in the large intestine during the state of hind-gut acidosis (Gressley et al., 2011). The increased LPS in the rumen and hindgut translocate to the bloodstream due to increased permeability causes systemic inflammation and detrimental health effects in dairy cows such as laminitis (Plaizier et al., 2018).

Inflammatory responses normally help animals to recover from infection or injury (Bradford et al., 2015). Cows experience systemic inflammation after calving even when they do not display symptoms of disease (Bradford et al., 2015), and acute phase proteins such as serum amyloid A, haptoglobin and LPS binding protein (LBP) increase (Ceciliani et al., 2012). Damage

of the uterus during parturition, body fat mobilization, oxidative stress, gut barrier dysfunction, social stress, and infections (i.e. metritis or mastitis) are associated with systemic inflammation (Bradford et al., 2015). Increases in serum amyloid A concentration can be an indicator of inflammation in early stages because serum amyloid A has higher sensitivity to inflammation than haptoglobin (Khafipour et al., 2009a). Serum amyloid A and haptoglobin are systemic inflammation markers which respond to infections such as metritis or mastitis, but are not specific markers for gut-derived inflammation (Ceciliani et al., 2012; Plaizier et al., 2018). Circulating LBP concentration indicates the extent of the intestinal barrier dysfunction (Kvidera et al., 2017) because LBP binds with LPS to clear it from the circulation (Ceciliani et al., 2012). Amino acids concentrations are reduced during inflammation likely because AA is consumed by the liver to produce acute phase proteins (Zhou et al., 2016). Inflammation can reduce milk production because immune system activation consumes glucose to meet its energy demand and decreases available glucose for milk production (Bradford et al., 2015).

Previous studies reported that grain-based SARA challenge increased LPS in the rumen and inflammatory markers such as circulating concentrations of LPS binding protein (LBP; Emmanuel et al., 2008; Khafipour et al., 2009a; Li et al., 2012), serum amyloid A (Emmanuel et al., 2008; Khafipour et al., 2009a), and haptoglobin (Khafipour et al., 2009a). Interestingly, SARA induced by feeding low physically effective neutral detergent fiber (peNDF; fiber that stimulates chewing activity; Martens, 1997) by replacing alfalfa hay with alfalfa pellet in the diet with a low starch did not increase inflammatory markers (Khafipour et al., 2009b; Li et al., 2012) although LPS in the rumen was increased. The low peNDF in the diet with a low starch may not induce hind-gut acidosis, therefore, systemic inflammation may be associated with hind-gut acidosis.

1.2.2.2 Protein

Metabolizable protein for dairy cows consists of rumen microbial protein, rumen undegradable protein, and endogenous protein (NRC, 2001). Microbial protein is synthesized in the rumen from rumen degradable protein and organic matter (OM) such as carbohydrates by ruminal microbial fermentation. Microbial proteins are a highly efficient AA source for dairy cows because the AA composition of microbial proteins is similar to the compositions of milk protein and muscle protein (NRC, 2001). Therefore, it is necessary to feed diets that can optimize rumen microbial fermentation and maximize microbial protein synthesis. Also, rumen undegradable protein including rumen-protected products is necessary for high-producing cows. However, feeding an excess protein diet for dairy cows increases feed costs. In addition, an excessive AA results in increased N excretion (Reece et al., 2015) which increases environmental issues (Jonker et al., 1998). Therefore, optimizing feed protein is important for milk production with reducing disadvantages of cost and environmental issues.

1.2.2.2.1 Prepartum

Protein nutrition in the prepartum period in dairy cows is still controversial. When dietary protein is insufficient in the diet during the prepartum period, cows may start mobilizing body muscle protein and result in greater risk for impaired health and productive efficiency (Van Saun and Sniffen, 2014). Feeding a high MP diet during the prepartum period is likely to reduce body muscle protein mobilization (Carder and Weiss, 2017). However, a previous meta-analysis study (Lean et al., 2013) reported that feeding a high-CP diet to prepartum cows did not increase milk yield. A recent meta-analysis (Husnain and Santos, 2019) reported that cows fed a greater MP

diet during the prepartum period increased milk and milk protein yields in primiparous cows, and increased milk protein yield in multiparous cows which produced greater than 36 kg/d of milk yield. The requirement of MP for close-up cows with 680 kg of BW is 810 to 901 g/d (NRC, 2001), however, recent studies suggest that greater than 1,100 g/d of MP supply for close-up cows may be appropriate (Van Saun and Sniffen, 2014; Boerman, 2021). Further studies are needed to determine the protein and AA requirements (NRC, 2001).

1.2.2.2.2 Postpartum

The AA requirements increase significantly in postpartum dairy cows due to milk protein synthesis and gluconeogenesis (Bell et al., 2000; Reece et al., 2015; McCabe and Boerman, 2020). The requirement of MP for fresh cows with 680 kg of BW and 35 kg/d of milk yield is 2,157 g/d (NRC, 2001), which is significantly higher than that of close-up cows. Carder and Weiss (2017) reported that cows fed a high MP diets after calving had lower plasma 3-methylhistidine concentrations which indicates lower body muscle protein mobilization, and cows fed a high MP with lysine (Lys) and Met supplemented diet increased milk protein synthesis. In addition, cows fed a high MP with a better AA profile diet only during the first three weeks of lactation increased milk protein yield, and the effect on milk protein yield was continued during the following 40 days (Carder and Weiss, 2017). This positive response due to feeding a high MP with Lys and Met supplemented diet during early lactation in milk production may carry over after the treatment is completed.

1.3 Amino Acid Research for Dairy Cows

Amino acids are the building blocks of proteins and have important functions for enhancing animal health, maintenance, growth, lactation, and reproduction (Wu 2009). Amino acids are categorized as either essential or nonessential (NRC, 2001). Animals must obtain EAA through the diets because EAA cannot be synthesized in the animal body, or not in sufficient amounts. Histidine (His), isoleucine (Ile), leucine (Leu), Lys, Met, phenylalanine (Phe), threonine (Thr), tryptophan (Trp), and valine (Val), which are defined as EAA in dairy cows (NRC, 2001). Milk protein synthesis is generally dependent on AA supply (Doepel et al., 2004). Essential AA that is the shortest supply to the requirements of milk and milk protein synthesis is called limiting EAA (Wu et al., 2013). Lysine and Met are generally the first-limiting EAA, and His can be a limiting EAA after Lys and Met in dairy cows (NRC, 2001).

In contrast, NRC (2001) does not establish dietary requirement for NEAA for cows because NEAA can be synthesized in the animal body (NRC, 2001). Nonessential AA includes alanine (Ala), arginine (Arg), asparagine (Asn), aspartate (Asp), cysteine (Cys), Glu, glutamine (Gln), proline (Pro), serine (Ser), tyrosine (Tyr) for dairy cows (Doepel and Lapierre, 2010). Mostly in monogastric animals, it is known that NEAA has important roles and NEAA can be a conditional EAA under specific conditions (Wu, 2009). For example, NEAA become conditional EAA when the amount of NEAA demand exceeds the amount of NEAA intake and synthesis due to reduced feed intake. Animals need to take the conditional EAA from the diet to meet the requirement (Wu, 2009). Deficiency of conditional EAA impairs not only protein synthesis but also systemic homeostasis (Wu, 2009). However, there are limited studies about NEAA in dairy cows, therefore, I will discuss studies of Arg which is considered as a semi EAA, Gln which has a similar physiological role to Glu, and other NEAA in this review.

1.3.1 Methionine

Methionine can be a limiting AA for milk protein synthesis in dairy cows fed corn silage and soybean meal-based diets which are major feed sources in North America (NRC, 2001). A recommendation for intestinal available Met to maximize milk protein yield and content is 2.4 % of MP (NRC, 2001). Meta-analysis studies reported that cows fed rumen-protected Met increased both the concentration and yield of milk protein (Patton, 2010; Lean et al., 2018), and slightly increased milk yield (Patton, 2010). In postpartum cows, plasma Met concentration decreased after calving and did not recover until day 28 after calving which was probably due to the increased Met requirements for milk production and gluconeogenesis (Zhou et al., 2016). In addition, cows fed rumen-protected Met from 21 days before expected calving until 30 days after calving increased feed intake and yields of milk and milk protein, and decreased incidence of ketosis (Zhou et al., 2017). Increased Met supply can increase choline (NRC, 2001; Reece et al., 2015) and VLDL production (NRC, 2001; Reece et al., 2015) which could mitigate fatty liver (Reece et al., 2015). Moreover, Met is required for the synthesis of antioxidants such as glutathione and taurine (Schwab and Broderick, 2017). Therefore, Met plays important roles to increase milk and milk protein yield, enhance triglycerides transportation from the liver, and mitigate oxidative stress during the periparturient period in dairy cows.

1.3.2 Lysine

Lysine can be a limiting AA when feeding corn or corn byproduct-based diet because Lys content in corn is low (NRC, 2001). A recommendation for intestinal available Lys to maximize milk protein yield and content is 7.2 % of MP, and optimum ratio of Lys and Met is 3:1 (NRC,

2001). Feeding rumen-protected Lys in an MP deficient diet to mid-lactation cows increased milk protein yield (Giallongo et al., 2016). Contrarily, feeding rumen-protected Lys to mid-lactation multiparous Jersey cows did not affect yields of milk and contents, however, feeding rumen-protected Lys increased body muscle protein accretion (Morris and Kononoff, 2020). A meta-analysis study also reported that increased in Lys was not associated with increased milk yield and milk protein yield (Lean et al., 2018).

In contrast, several studies reported the positive effects of supplementing dietary Lys during the periparturient period. Cows fed rumen-protected Lys only during the close-up period increased milk protein and energy-corrected milk (ECM) yields in early lactation (Fehlberg et al., 2020). They indicated that increased intestinally available Lys during the close-up period may supply MP for body protein reserve and mammary gland growth (Fehlberg et al., 2020). In addition, feeding both Lys and Met in a diet for transition dairy cows increased milk and milk protein yields (Socha et al., 2005; Osorio et al., 2013; Zhou et al., 2017). Based on the aforementioned studies, feeding both rumen-protected Lys and Met especially during the periparturient period likely increases milk and milk protein yields in dairy cows.

1.3.3 Histidine

Histidine can be the third limiting AA when dairy cows are fed grass forage-based diets with low rumen microbial synthesis which is mostly seen in North Europe (Vanhatalo et al., 1999). Intestinal available His supply can be less than the other EAA because His content is low in rumen microbial protein (NRC, 2001). A meta-analysis study reported that His may play an important role in increasing milk production in dairy cows (Lean et al., 2018). Supplementing incremental amounts of rumen-protected His (48 to 58 g/d of available His; 2.2 to 2.7 % His/MP)

tended to increase milk yield in a dose-dependent manner, however, milk protein yield increased only when cows fed the largest dose of available His (Zang et al., 2019). Previous studies also reported that supplementing His increased milk yield (86 or 96 g/d of available His; 3.0% His/MP; Räisänen et al., 2021) or milk protein yield (67 g/d of available His; 2.4% His/MP; Giallongo et al., 2016). Both milk and milk protein yields were increased by supplementing His (59 g/d of available His; 2.7 % His/MP; Lee et al., 2012). In addition, His deficiency diet is likely to decrease feed intake (Lee et al., 2012; Giallongo et al., 2016, 2017), however, the exact mechanism is unclear. Moreover, His can be insufficient in the calving transition period because plasma His concentration in the postpartum dairy cows decreased after calving, and did not recover until d 28 after calving (Zhou et al., 2016).

Based on the results of the aforementioned studies, feeding additional His seems to have a potential of the positive effects such as increased milk and milk protein yields and feed intake in dairy cows, however, the amount of available His and His/MP ratio to increase the positive effects is not determined. The recommendation of His has a wide range from 2.4 % (Doepel et al., 2004) to 3.2 % (Lapierre et al., 2021) of MP. Feeding additional His may not show positive effects when Lys or Met or both supplies are insufficient because Lys and Met are generally the first-limiting AA in dairy cows.

1.3.4 Threonine

A meta-analysis study reported that metabolizable Thr (g/d) intake was correlated with milk yield (Lean et al., 2018). A previous study (Schwab et al., 1976) with abomasum AA infusion in dairy cows reported that Thr may be a limiting AA that comes after Lys and Met. However, Doepel et al. (2016) reported that the deficiency of Thr did not affect productivity and

mammary metabolism. Therefore, the response to milk production by Thr seems inconsistent. However, Thr has an important role to maintain the integrity and barrier function of the mucosa in the intestine (Mao et al., 2011) because Thr is a primary AA in the mucins, the main structural component of mucus (Kim and Khan, 2013) in swine. Similar effects of Thr may be observed in the intestine of dairy cows. Further studies are warranted to understand the effects of Thr on milk and mucin production in dairy cows.

1.3.5 Tryptophan

It is possible that Trp would be insufficient when dairy cows are fed corn-based diet because corn contains low Trp (NRC, 2001). A meta-analysis study (Lean et al., 2018) reported that 0.27 kg of milk yield increased with every 1g of metabolizable Trp increased without plateau. However, no additional responses in milk and milk protein yields were observed by post-ruminal Trp infusion (Schwab et al., 1976). In addition, milk protein yield was not affected by post-ruminal EAA infusion when Trp was removed. (Doelman et al., 2015; Doepel et al., 2016). Thus, the role of Trp in milk and milk protein production remains unclear. Tryptophan is also the substrate for the biosynthesis of serotonin and melatonin (Kollmann et al., 2008). Plasma Trp and melatonin concentrations were increased by feeding rumen-protected Trp in heifers (Kollmann et al., 2008). Feeding rumen-protected Trp increased plasma Trp concentration and milk yield slightly only at morning milking in dairy cows (Kollmann et al., 2008). However, studies to evaluate the effects of dietary Trp in dairy cows are very limited.

1.3.6 Phenylalanine

Phenylalanine can be a limiting AA after Lys and Met (Schwab et al., 1976; NRC, 2001), however, the effects of Phe have not been extensively studied in dairy cows. Lean et al. (2018) summarized that abomasal infusion of Phe had reduced rumen passage rates and increased ruminal NDF digestion. Feeding rumen-protected Phe with 7.5 g/d (Swanepoel et al., 2015) and 15 g/d (Swanepoel et al., 2016) of intestinally available Phe to early lactation cows did not increase milk production, however, feeding 15 g/d of intestinally available Phe (Swanepoel et al., 2016) increased total-tract NDF and acid detergent fiber digestibility.

1.3.7 Branched-Chain Amino Acids

Branched-chain AA (BCAA) are Leu, Ile, and Val, which account for more than 44% of EAA of milk protein (NRC, 2001). In addition, BCAA uptake in the mammary gland is greater than BCAA output in secreted milk protein (NRC, 2001; Lapierre et al., 2012). Excess BCAA uptake can be used for the synthesis of NEAA, or could be oxidized as an energy source in the mammary gland (Lapierre et al., 2012). Branched-chain AA in skeletal muscle is lower than BCAA in milk (35% and over 44% of the total essential AA, respectively), therefore, it is possible that skeletal muscle would be mobilized in excess to meet BCAA demand for milk protein synthesis in early lactation (McCabe and Boerman, 2020).

Leucine can be a limiting AA when cows are grazing in the pasture or fed a grass silage-based diet (Rulquin and Pisulewski, 2006). Another study (Larsen et al., 2014) reported that Leu can be a limiting AA for early lactating cows fed typical North European diets based on small grains combined with rapeseed meal. A meta-analysis study (Lean et al., 2018) reported that there was a positive correlation between the amount of dietary Leu and milk yield, and milk

protein yield was also associated with the amount of dietary Leu. In the previous study, post-ruminal Leu infusion in cows fed corn silage-based diet increased milk protein yield, however, milk protein yield did not increase in a dose-dependent manner (Rulquin and Pisulewski, 2006). Post-ruminal EAA infusion with deletion of Ile did not affect milk production, however, deletion of Val decreased both milk protein yield and content (Haque et al., 2013). Infusion of BCAA into the jugular vein (Appuhamy et al., 2011) and BCAA post-ruminal infusions (Korhonen et al., 2002; Yepes et al., 2009; Curtis et al., 2018) did not increase milk protein yield. Branched-chain AA deficiency may decrease milk production, however, the response of additional BCAA supply appears to be inconsistent in dairy cows although the BCAA is abundant in milk.

1.3.8 Arginine

Arginine is a NEAA but it is considered a semi-EAA because Arg synthesis in animal body is not sufficient in high-producing dairy cows (NRC, 2001). Arginine uptake in the mammary gland is greater than Arg output in secreted milk protein (NRC, 2001; Lapierre et al., 2012). Excess Arg uptake can be used to synthesize ornithine or other NEAA such as Pro, Glu, and Ser (Doepel and Lapierre, 2011; Lapierre et al., 2012). However, post-ruminal Arg infusion in dairy cows did not increase milk protein yield (Schwab et al., 1976; Doepel and Lapierre, 2011). In addition, post-ruminal infusion with deletion of Arg in EAA did not decrease milk and milk protein yield compared with the infusion of both EAA and Arg (Doepel and Lapierre, 2011; Haque et al., 2013). Doepel and Lapierre (2011) suggested that the post-ruminal infusion with deletion of Arg in EAA resulted in reduced Arg uptake in the mammary gland, however, there was minimal effect on milk and milk protein yields when the other EAA were supplied sufficiency.

1.3.9 Glutamine

Glutamine is a NEAA that is abundant in milk casein, and it is possible that Gln affects milk protein synthesis (Meijer et al., 1993). The NRC (2001) mentioned that Gln may be a limiting AA for early postpartum cows having malnutrition due to metabolic disorders. Post-ruminal Glu infusion of 300 g/d in lactating cows (Plaizier et al., 2001) and early lactation cows (Doepel et al., 2006, 2007) increased plasma Gln concentration, however, neither treatment increased yields of milk and milk protein. Another study reported that cows intravenously infused with Gln (106 or 212 g/d) for one week after calving did not increase yields of milk and milk protein as well (Jafari et al., 2006). In addition, post-ruminal Gln infusion in cows over the first 3 weeks post-calving had limited effects on metabolism and immune status (Doepel et al., 2006). The effects of Gln in dairy cows are not consistent and warranted further research into the mechanisms.

1.3.10 Other Nonessential Amino Acid

Bahloul et al. (2021) evaluated the effect of additional supply of both EAA and NEAA, or only EAA by post-ruminal infusion during the postpartum period in dairy cows. Cows with post-ruminal infusions of both EAA and NEAA had greater yields of milk and milk protein and lactose compared with cows with only EAA infusion which indicates that some or all NEAA are as important as EAA in the postpartum dairy cows (Bahloul et al., 2021). In contrast, Doepel and Lapierre (2010) evaluated the effects of post-ruminal infusion of only EAA, only NEAA, and both EAA and NEAA when feeding protein deficient diets in lactating dairy cows. Cows with post-ruminal EAA infusion increased milk and milk protein yields, however, cows with post-

ruminal NEAA infusion did not which suggests that NEAA supply in the diet and NEAA synthesized were sufficient in lactating cows (Doepel and Lapierre, 2010). Cows can synthesize NEAA, however, under specific conditions such as in the early postpartum period, the amount of NEAA dietary intake and synthesis can be less than that required (Doepel et al., 2006).

1.4 Glutamate Research in Livestock Animals

Glutamate is a NEAA, a glucogenic AA (Wu, 2013; Heitmann and Bergman, 1981), a primary energy source in the small intestine (Reeds et al., 2000; Burrin and Stoll, 2009), and one of the most abundant AA in diet and animal tissues (Hou and Wu, 2018). In addition, Gln is converted to Glu, and Glu and Gln plays similar physiological roles in the gut tissues (Heitmann and Bergman, 1981). Furthermore, other AA such as BCAA, Ala, Asp are catabolized to Glu (Hou and Wu, 2018) and Glu is also a precursor of glutathione as an antioxidant (Newsholme et al., 2003).

1.4.1 Glutamate in Monogastric Animals

Many studies have been conducted to evaluate the effects of Glu on the gastrointestinal function in swine. Glutamate is a primary energy source in the small intestine (Reeds et al., 2000; Burrin and Stoll, 2009), and approximately 95% of the dietary Glu in the diet is metabolized in the small intestine (Reeds et al., 2000). Glutamate is a key neurotransmitter, however, most of the absorbed Glu is catabolized in the small intestine, therefore, the possibility of neurotoxicity is very low (Burrin and Stoll, 2009).

Previous studies evaluated the capacity of the intestine to metabolize Glu with doses above the normal intake. Excessive Glu was metabolized in the small intestine without any toxic

effects when infant pigs were infused with a 4-folds of excess Glu relative to a normal intake (Janeczko et al., 2007), and post-weaning pigs were fed 4-fold greater monosodium Glu with a basal diet (Rezaei et al., 2013). In addition, the excessive Glu was metabolized to Asp, Gln, and ornithine in infant pigs (Janeczko et al., 2007), and ornithine, citrulline, Pro, and Ala in post-weaning pigs (Rezaei et al., 2013). Hou and Wu (2018) summarized several studies with supplementing varying amounts of Glu or monosodium Glu during 14 to 120 days in weaning, finishing, pregnant, and lactating pigs, and they suggested that pigs in all stages of production can be fed 2% of Glu in diets with any adverse effects on growth or health.

Glutamate is the second highest AA in the milk of sows to support the growth of neonate piglets, however, Glu intake decreases during weaning in pigs due to decreased feed intake by transition from milk to solid feed (Hou and Wu, 2018). In addition, post-weaning piglets experience intestinal atrophy along with intestinal oxidative stress due to decreased feed intake, including Glu (Hou and Wu, 2018). Glutamate plays an important role in mucosal barrier function by enhancing cell growth and maintaining membrane integrity in response to oxidative stress (Jiao et al., 2015). Supplementing 1% of Glu in the post-weaning diet in pigs increased the small intestinal villus height and mucosal thickness (Wu et al., 2012). In addition, supplementing 1 to 4% of monosodium Glu in the post-weaning diet in pigs decreased an incidence of diarrhea in a dose-dependent manner during the first week after weaning, increased intestinal absorptive capacity, and enhanced weight gain and feed efficiency (Rezaei et al., 2013). Based on the aforementioned studies, Glu synthesis in the animal body may be insufficient under weaning or other stress conditions such as reduced feed intake, and Glu supply from the diet may be necessary.

Supplementation of a product containing Glu and Gln from late gestation to early lactation period in pigs increased milk Glu and Gln concentration (Manso et al., 2012; Santos de Aquino et al., 2014) which may provide more dietary Glu and Gln for piglets by suckling from the sows. Glutamate is abundant in fetal pig allantois fluid which suggests that Glu may also have an important role in fetal growth and development (Hou and Wu, 2018).

1.4.2 Glutamate in Ruminants

The NRC (2001) defined Glu as a NEAA and NEAA do not need to be fed from the diets of dairy cows. The effects which were observed in monogastric animals by supplementing Glu may also be observed in ruminants. However, research on dietary Glu supplementation in ruminants is limited because Glu is utilized by rumen microbes (Chalupa et al., 1976), therefore, abomasum or duodenum infusion via cannula or rumen-protection is necessary. In fact, most of the recently published studies on Glu supplementation in ruminants were conducted with post-ruminal infusion (Ansia et al., 2017; Elsabagh et al., 2018).

Dietary Glu requirement may be increased under specific conditions such as reduced feed intake during the periparturient period in dairy cows because plasma Glu concentration decreased after calving and did not recover to the concentration of plasma Glu in the prepartum period until day 60 after calving (Maeda et al., 2012). In addition, Glu is a glucogenic AA and Glu contributed up to 4% of the total glucose produced in sheep (Heitmann and Bergman, 1981). Abomasal Glu infusion (42 g/d of available Glu) in feed restricted mid-lactation multiparous Holstein cows (Ansia et al., 2017), and duodenal Glu infusion (10% of MP requirements for maintenance) in fasted sheep (Elsabagh et al., 2018) increased plasma Glu and glucose concentrations. In addition, duodenal Glu infusion in fasted sheep decreased plasma BHB

concentration. These results indicate that the post-ruminal Glu supply affected its plasma concentration and Glu may contribute to gluconeogenesis when feed intake was restricted. Furthermore, a duodenal Glu infusion in fasted sheep increased concentration of plasma ghrelin (Elsabagh et al., 2018) which is a hormone increases feed intake (Kojima et al., 1999).

The period of Glu supplementation may affect starch digestibility in the small intestine in ruminants. Glutamate duodenal infusion in steers for less than a 6-day period (Brake et al., 2014) or a 12 day period (Blom et al., 2015) enhanced the small intestinal starch digestion, however, a 58 day period did not increase the small intestinal starch digestion (Trotta et al., 2020). The specific mechanism for the limitation with prolonged Glu infusion was unknown (Trotta et al., 2020). More research is needed to determine the effective Glu dose and period in ruminants.

1.5 Knowledge Gap

There are limited studies to evaluate the effects of supplementing NEAA in dairy cows because dietary NEAA requirement is not established for dairy cows although many positive animal responses are reported by supplementing NEAA in monogastric animals. In monogastric animals, NEAA, such as Glu, can be insufficient when feed intake of animals is low. It is possible that Glu is insufficient in transition dairy cows due to decreased feed intake around the parturition, increased nutritional demand with the onset of lactating, and decreased plasma Glu concentration after calving. However, the effect of supplementing Glu on plasma Glu concentration in transition dairy cows has not been studied. As Glu is a glucogenic AA and supplementing Glu increased circulating glucose concentration in feed restricted ruminants, supplementing Glu in transition dairy cows may increase circulating glucose concentration and increase milk yield. However, the effects of supplementing Glu on metabolites and milk

production in transition dairy cows have not been evaluated. Reduced feed intake around the calving causes gastrointestinal barrier dysfunction. As Glu is a primary energy source in the small intestine, supplementing Glu in transition cows may also enhance barrier function by increasing cell growth and maintaining membrane integrity in the gastrointestinal tract as previously seen in monogastric animals, however, the effects of supplementing Glu on barrier function and inflammatory markers in transition dairy cows have not been explored. In addition, supplementing Glu in transition dairy cows may enhance gastrointestinal mass growth and increase digestive and absorptive capacity, as supplementing Glu increased villus height and absorptive capacity in monogastric animals. The gastrointestinal mass growth increases nutrient requirement, however, increased gastrointestinal mass and digestive and absorptive capacity provide cows with more benefits. Therefore, evaluating the effects of supplementing Glu on the mass of gastrointestinal tract and digestibility in transition dairy cows is needed. Some studies evaluated the effects of supplementing Glu with post-ruminal infusion in ruminants, however, the effects of feeding Glu in transition dairy cows have not been studied because the rumen-protection process is needed.

The overall hypothesis of this thesis is feeding rumen-protected Glu during the calving transition period in dairy cows would increase apparent total-tract digestibility, decrease inflammation, and increase milk production. The objective of my thesis study is to evaluate the effects of rumen-protected Glu supplementation during the calving transition period on ATTD, inflammation, blood metabolites, and productivity of dairy cows.

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Chapter 2: Effects of Rumen-Protected Glutamate Supplementation during the Periparturient Period on Digestibility, Inflammation, Metabolic Responses and Performance in Dairy Cows.

2.1 Introduction

Nutrient requirements of dairy cows increase with the onset of lactation (NRC, 2001). The nutrients are required for not only lactation but also gastrointestinal growth (Johnson et al., 1990). Mass of the gastrointestinal tract tissues increases after calving, which is likely to be promoted by increased feed intake (Reynolds et al., 2004). In addition, dairy cows can experience gastrointestinal barrier dysfunction which can be caused by reduced feed intake (Pearce et al., 2013; Kvidera et al., 2017) around the calving. Compromised gastrointestinal integrity and barrier dysfunction can induce gut-derived inflammation (Pearce et al., 2013; Bradford et al., 2015). Previous studies reported inflammation related with sub-acute rumen acidosis (SARA; Emmanuel et al., 2008; Khafipour et al., 2009a, b) occurred during the calving transition period (Knoblock et al., 2019), but nutritional management to mitigate inflammation during the calving transition period has not been extensively studied.

In addition, dairy cows often experience negative energy balance as the glucose requirement for lactose synthesis in the mammary gland increases with the onset of lactation (Baumgard et al., 2017) while feed intake increases gradually. Transition dairy cows mobilize body fat to cover the negative energy balance, which would elevate circulating free fatty acids (FFA) and β-hydroxybutyrate (BHB) concentrations (Reece et al., 2015). However, excess circulating FFA and BHB increase the incidence of metabolic disorders which results in reduced milk production (Drackley, 1999). Supplying glucogenic precursors could reduce the synthesis of ketones and increase milk production (Aschenbach et al., 2010).

Nonessential amino acids (NEAA) such as glutamate (Glu) can be synthesized in the animal body (Burrin and Stoll., 2009; Wu, 2013), and their dietary requirements are not established for dairy cows (NRC, 2001). However, Glu is recognized as a primary energy source in the small intestine in swine (Reeds et al., 2000; Burrin and Stoll, 2009) and cows (El-Kadi et al., 2009). Supplementing Glu in the diet of weaning piglets increased small intestinal villus height (Wu et al., 2012) and absorptive capacity of the small intestine (Rezaei et al., 2013). In addition, Glu contributes to the intestinal mucosal barrier function by enhancing cell growth and maintaining membrane integrity (Jiao et al., 2015), and increased antioxidant function (Yin et al., 2015) in swine. Furthermore, Glu contributed up to 4% of the total glucose produced in sheep (Heitmann and Bergman, 1981). Other studies also reported that post-ruminal Glu infusion increased plasma glucose concentration in sheep (Elsabagh et al., 2018) and lactating dairy cows (Ansia et al., 2017). Glutamine and Glu play similar physiological roles in the gut tissues (Heitmann and Bergman, 1981), and effects of post-ruminal Gln infusion were evaluated for transition dairy cows (Doepel et al., 2006). However, dietary Glu is extensively degraded in the rumen, and the effects of supplementing Glu in the diet of dairy cows during the calving transition have not been studied.

Therefore, we hypothesized that feeding rumen-protected Glu during the calving transition period would increase apparent total-tract digestibility (ATTD), decrease inflammation, and increase milk production. The objective of our study is to evaluate the effects of rumen-protected Glu supplementation during the calving transition period on ATTD, inflammation, blood metabolites, and productivity of dairy cows.

2.2 Material and Methods

All experimental procedures were pre-approved by the University of Alberta Animal Care and Use Committee for Livestock (AUP#3193) and conducted according to the guidelines of the Canadian Council on Animal Care (2009).

2.2.1 Animals, Diets, and Experimental Design

Fifty-two multiparous Holstein dairy cows (Table 2.7.1) were blocked by parity, expected calving date, and body condition score (BCS), and randomly assigned to one of the two treatments. Cows were fed diets with rumen-protected monosodium Glu monohydrate prototype supplement (RP-Glu; Ajinomoto Co. Inc., Tokyo, Japan; n = 26) as a part of TMR or without RP-Glu (CON; n = 26) from d 21 ± 3 before the expected calving date till d 21 ± 3 after calving. The RP-Glu contained 50.2% of hydrogenated soy oil to coat Glu to protect from ruminal digestion, 38.4% of Glu, 6% of sodium, and 5.4% of water on a 55°C dry matter (DM) base. Its in situ ruminal degradability of Glu was 19%, and in vitro intestinal digestibility, determined in a three-step in vitro procedure (Miyazawa et al., 2014), was 23% (of the total Glu). The RP-Glu was expected to provide 8.8% of intestinally available Glu (38.4% × 23%). Prepartum and postpartum diets were formulated using AMTS Cattle Professional version 4.15.0 (Agricultural Modeling and Training Systems LLC; Groton, NY) to meet or exceed nutrient requirements for a 680-kg BW cow through the peripartum period and producing 31 kg/d of milk with 4% milk fat and 3.3% milk CP for the postpartum period (Table 2.7.2). Cows were fed RP-Glu at 4% of dietary DM during the prepartum period and at 3% of dietary DM during the postpartum period. We intended to supply RP-Glu at 0.48 kg/d on a DM basis assuming DMI was 12 and 16 kg/d for prepartum and postpartum periods, respectively, to provide 42 g/d of intestinally available

Glu, which was evaluated in a previous study (0.4 mmol/kg BW; Ansia et al., 2017). In the literature, Glu absorption in the small intestine ranges from 191 to 262 g/d in lactating dairy cows (Stern et al., 1985; Waltz et al., 1989; Berthiaume et al., 2001), as such, the 42 g/d of intestinally available Glu is expected to increase Glu absorption by approximately 20%. The hydrogenated soy oil and sodium bicarbonate were added to the CON diet to match the hydrogenated soy oil and available sodium content of RP-Glu. Anionic salts were added to both prepartum diets, but we could not add sufficient enough to make the DCAD below zero due to palatability-related concerns. Cows were housed in individual tie-stalls at the Dairy Research and Technology Centre and had free access to water. All cows were individually fed experimental diets as TMR in mangers once daily at 0800 h to allow for 5 to 10% refusals (as-fed basis) based on actual feed intake of the previous day. Cows were allowed to exercise in a paddock for 3 h 3 times a week. After calving, cows were milked twice daily at 0600 h and 1700 h.

2.2.2 Data and Sample Collection

Individual DMI was recorded daily throughout the experimental period. Forage and concentrate samples were collected once weekly. The DM concentrations of forage and concentrate samples were determined in a forced-air oven at 55°C for 48 h. Experimental diets were adjusted with the determined DM as necessary to feed the same experimental diets on a DM basis.

Milk yield was recorded at each milking from d 1 to 21 after calving. Milk samples were collected from two consecutive milkings (p.m. and a.m.) weekly. The body weight (BW) and BCS were measured on d -21 ± 3 relative to the expected calving date, immediately after calving, and on d 21 ± 3 after calving. After calving, BW was measured after milking and before feeding

in the morning on two consecutive days, and BCS was taken by 2 individuals using a 5-point scale (Wildman et al., 1982). Both BW and BCS were averaged before statistical analysis, and BW changes after calving and BCS changes before and after calving were calculated.

Blood samples were collected before feeding on d -21 ± 3, and twice weekly from d -10 before the expected calving date. The samples on d -3 ± 2 relative to the actual calving date were selected for analyses. After calving, blood samples were collected after morning milking but before feeding on d 4, 7, 10, and 21. Blood samples were collected via the coccygeal vein into evacuated tubes containing no anticoagulant (Fisher Scientific Company, Nepean, ON Canada) for serum collection and evacuated tubes containing sodium heparin (Fisher Scientific Company) or K_2EDTA (Fisher Scientific Company) for plasma collection. Evacuated tubes without anticoagulant were kept at room temperature for at least 30 min to harvesting serum. A protease inhibitor, aprotinin (5 μL; 0.01 mg/μL; A1153-25mg, Sigma-Aldrich Canada Co., Oakville, ON Canada), was added to 10-mL evacuated tubes containing sodium heparin immediately after sampling, and both evacuated tubes with sodium heparin or K_2EDTA were placed on ice after sampling. All evacuated tubes were centrifuged at 3000 × *g* (20 min, 4°C) to harvest serum or plasma. Serum and plasma samples were stored at -80°C freezer until analysis.

A subset of twenty cows with close calving dates (within a 2-month period) was used to measure ATTD of DM, organic matter (OM), CP, neutral detergent fiber (NDF), ether extract, and starch. Fecal samples were collected from the rectum, starting on d 5 ± 1 and 21 ± 3 after calving, every 9 h over a 72-h period to account for diurnal variation, composited for each cow, and stored at -20°C. Fecal samples were thawed at room temperature and dried in a forced-air oven at 55°C for 72 h.

2.2.3 Sample Analyses

Dried feed and fecal samples were ground with Wiley mill (Thomas Scientific, Swedesboro, NJ) with a 1-mm screen and analyzed by Cumberland Valley Analytical Services (Waynesboro, PA) for DM (AOAC International, 2002; method 930.15), OM (AOAC International, 2002; method 942.05), CP (AOAC International, 2000; method 990.03), NDF (Van Soest et al., 1991), ether extract (AOAC International, 2006; method 2003.05), and starch (Hall, 2009). Indigestible NDF (iNDF) was determined after 240 h of in vitro digestion (Goering and Van Soest., 1970), which was used as an internal marker to predict fecal output to calculate ATTD (Cochran et al., 1986). The ATTD was calculated with the following equation:

ATTD (% of nutrient intake) = 100 – [100 × (dietary iNDF content, %DM / fecal iNDF content, %DM) × (fecal nutrient content, %DM / dietary nutrient content, %DM)]

Milk samples were analyzed individually for concentrations of milk fat, milk CP, lactose, milk urea nitrogen (MUN), somatic cell count (SCC), and BHB by mid-infrared spectroscopy (ISO-IDF, 2013; ISO 9622|IDF 141; Foss System MilkoScan 7RM, Foss North America, Brampton, ON, Canada) at Lactanet Canada Central Milk Testing Laboratory (Edmonton, AB, Canada). The energy-corrected milk (ECM) was calculated as (0.3246 × milk yield, kg) + (12.86 × milk fat yield, kg) + (7.04 × milk CP yield, kg) according to the equation described by Bernard (1997). Feed efficiency was calculated as ECM divided by DMI.

Serum samples were analyzed using commercial kits for concentrations of serum amyloid A (Tridelta Development Limited, Kildare, Ireland) and haptoglobin (Tridelta Development Limited). In addition, serum samples were analyzed for concentrations of FFA (Eiken Chemical co, Ltd, Tokyo, Japan), BHB (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan), total

bilirubin (Alfresa Pharma Corporation, Osaka, Japan), and urea N (Denka Company Limited, Tokyo, Japan) using 7180 Clinical Analyzer (Hitachi High-Technologies Corporation, Tokyo, Japan).

Plasma samples treated with sodium heparin and aprotinin were analyzed for lipopolysaccharide binding protein (LBP) concentration using a commercial kit (Hycult Biotech, Frontstraat, PB Uden, Netherlands), and for concentrations of insulin-like growth factor 1 (IGF-1) and glucagon-like peptide-2 (GLP-2) with a time-resolved fluoroimmunoassay technique using a 2030 Multilabel Reader ARVO X4 (PerkinElmer Inc., Waltham, MA) as described by Engelking et al. (2020). Plasma samples treated with K_2EDTA were analyzed for plasma glucose concentration using a commercial kit (FUJIFILM Wako Diagnostics U.S.A. Corporation, Mountain View, CA), and for concentrations of AA and 3-methylhistidine using Amino Acid Analyzer L-8900 (Hitachi High-Technologies Corporation) as described by Zang et al. (2019).

2.2.4 Statistical Analysis

All data were analyzed using the Fit Model procedure of JMP Pro 14.3 (SAS Institute Inc., Cary, NC). Data were analyzed separately for d -3 ± 2 relative to calving and for the postpartum period using the following models [1] and [2], respectively:

$$Y_{ijkl} = \mu + T_i + B_k + C(B)_{l(k)} + e_{ijkl}, \qquad [1]$$

$$Y_{ijkl} = \mu + T_i + P_j + TP_{ij} + B_k + C(B)_{l(k)} + e_{ijkl}, \qquad [2]$$

where Y_{ijkl} = observations for dependent variables; μ = overall mean, T_i = fixed effect of treatment (CON or RP-Glu); P_j = fixed effect of time period (day or week) as a repeated measure; TP_{ij} = fixed effect of treatment and time period interaction; B_k = fixed effect of block;

$C(B)_{l(k)}$ = random effect of cow nested in block; and e_{ijkl} = residuals. As the pre-trial FFA concentration (d -21 ± 3) was different between treatments, it was used as a covariate for analysis of all FFA data. For repeated measures, a covariance structure with the smallest Akaike's information criterion was selected (Littell et al., 1996). The *t*-test was used when interactions between treatment by time period were detected. Significance was declared at $P \leq 0.05$ and tendency was at $0.05 < P \leq 0.10$.

2.3 Results

Postpartum data of two cows were not used for statistical analyses; one cow fed the RP-Glu was euthanized after milk fever, and the other cow fed the CON diet, to be used for ATTD measurements, had a severe teat injury. Following parturition, 20 cases of postpartum health disorders in 13 cows were treated (3 milk fever, 4 subclinical ketosis, 2 displaced abomasum, 2 mastitis, and 1 metritis for the RP-Glu treatment; 2 milk fever, 1 subclinical ketosis, 2 retain placenta, 1 edema, 1 pneumonia, and 1 teat injury for control). For cows treated with antibiotics, anti-inflammatory drugs, or propylene glycol, data collected immediately after treatment were excluded from statistical analyses.

2.3.1 DMI, BW and BCS, and Milk Production

No treatment effects were observed between cows fed the RP-Glu and CON in DMI (14.3 vs. 13.6 kg/d; $P = 0.23$) and BCS change (-0.16 vs. -0.09; $P = 0.27$) during the prepartum period (Table 2.7.3). However, an interaction between treatment and day was detected for DMI in the postpartum period ($P = 0.03$) and cows fed the RP-Glu had a greater DMI on d 1 after calving (15.7 vs. 13.7 kg/d; $P = 0.03$; Figure 2.7.1). There were no differences between the two

groups in postpartum changes in BW and BCS, yields of milk and milk fat, however, cows fed the RP-Glu had lower lactose yield (1.96 vs. 2.16 kg/d; $P = 0.05$), and tendencies for lower yields of milk CP (1.46 vs. 1.59 kg/d; $P = 0.06$), milk total solids (5.77 vs. 6.26 kg/d; $P = 0.06$), and ECM (48.2 vs. 51.9 kg/d; $P = 0.08$).

2.3.2 Circulating Metabolites, Hormone, Inflammatory Markers, and AA

No differences were observed between the two treatments in concentrations of circulating metabolites, plasma IGF-1, and serum and plasma inflammatory markers on d -3 relative to calving date (Table 2.7.4). During the postpartum period, no treatment effects were observed in concentrations of serum BHB and plasma glucose between RP-Glu and CON diets (Table 2.7.5). Interactions between treatment and day during postpartum period were observed in concentrations of serum FFA ($P < 0.01$), serum total bilirubin ($P < 0.01$), plasma 3-methylhistidine ($P = 0.03$), and plasma IGF-1 ($P = 0.02$). On d 4 after calving, feeding RP-Glu diet compared with CON decreased concentrations of serum FFA (670 vs. 981 μEq/L; $P < 0.01$; Figure 2.7.2A), total bilirubin (0.22 vs. 0.34 mg/dL; $P < 0.01$; Figure 2.7.2B), and plasma 3-methylhistidine (1.28 vs. 1.50 μmol/dL; $P = 0.03$; Figure 2.7.2C) and increased plasma IGF-1 concentrations (44.2 vs. 30.1 ng/mL; $P < 0.01$; Figure 2.7.2D). There were time effects for concentrations of serum amyloid A ($P < 0.01$), and haptoglobin ($P < 0.01$), and plasma LBP ($P < 0.01$) as inflammatory markers, however, no treatment effects or treatment and time interactions were detected during the postpartum period. Cows fed RP-Glu compared with CON had lower Gln (306 vs. 344 μ*M*; $P = 0.05$; Table 2.7.6) on d -3 ± 2 relative to calving. Lower plasma histidine (His; 47.3 vs. 52.9 μ*M*; $P = 0.04$; Table 2.7.7), and Thr (86.1 vs. 96.6 μ*M*; $P = 0.05$) concentrations were observed for cows fed RP-Glu during the postpartum period. There were

interactions between treatment and day in plasma concentrations of Trp ($P = 0.02$), Asn ($P = 0.02$), Asp ($P = 0.01$), Gln ($P < 0.01$), Glu ($P = 0.02$), and Tyr ($P = 0.02$) during the postpartum period. Lower plasma concentrations of Trp (29.6 vs. 33.7 μ*M*; $P = 0.04$; Figure 2.7.3A) on d 21 after calving and Asn on d 10 (47.6 vs. 55.0 μ*M*; $P = 0.03$; Figure 2.7.3B) and d 21 (49.0 vs. 55.7 μ*M*; $P = 0.04$) after calving were observed for cows fed RP-Glu compared with CON. Cows fed RP-Glu had greater plasma concentrations of Asp (4.1 vs. 3.3 μ*M*; $P < 0.01$; Figure 2.7.3C), Gln (357 vs. 315 μ*M*; $P = 0.02$; Figure 2.7.3D), Glu (46.0 vs. 38.9 μ*M*; $P < 0.01$; Figure 2.7.3E), and Tyr (43.8 vs. 37.3 μ*M*; $P = 0.05$; Figure 2.7.3F) compared with CON only on d 4 after calving.

2.3.3 Apparent Total-Tract Digestibility

Treatment by time interactions ($P < 0.05$; Table 2.7.8) were observed for ATTD of DM, OM, CP, and NDF. Cows fed RP-Glu had greater ATTD of DM (70.6 vs. 69.1 %; $P = 0.05$) and CP (75.1 vs. 72.6 %; $P = 0.03$) on d 5 ± 1 after calving, but lower ATTD of CP (73.9 vs. 74.7 %; $P = 0.01$) on d 21 ± 3 compared with CON.

2.4 Discussion

In the present study, cows fed the RP-Glu diet had greater plasma Glu concentration only on d 4 after calving, and positive responses to the RP-Glu diet, such as greater DMI on d 1 after calving, plasma IGF-1 concentration on d 4 after calving, and ATTD on d 5 ± 1 after calving, and lower concentrations of serum FFA, serum bilirubin, and plasma 3-methylhistidine on d 4 after calving were observed. However, once the RP-Glu supplementation did not affect plasma Glu concentration (i.e., after d 4), we did not observe any positive animal responses to the RP-

Glu treatment. These results indicate that animal responses to RP-Glu may be related to whether additional Glu supply from diet increases its plasma concentration.

Glutamate is considered as a NEAA, and NRC (2001) does not establish its dietary requirement for dairy cows. However, previous studies reported that post-ruminal Glu infusion increased plasma Glu concentration in 48-h fasted sheep (Elsabagh et al., 2018) and feed restricted mid-lactation cows (Ansia et al., 2017). Similarly, we observed that cows fed the RP-Glu diet had greater plasma Glu concentration only right after calving. These findings suggest that Glu supply from the gut lumen can affect its plasma concentration when intake is low. Although Glu can be synthesized from other AA or metabolites (Burrin and Stoll., 2009; Wu, 2013), the fact that RP-Glu supplementation increased plasma Glu concentration. Feeding the RP-Glu resulted in several positive animal responses at the same time indicates that Glu synthesis can be insufficient under certain conditions, such as reduced feed intake, or increased nutrient demands with the onset of lactation, and that animals can benefit from additional Glu supply from the diet.

Cows fed RP-Glu had greater ATTD of DM and CP on d 5 ± 1 after calving compared with CON although Glu was coated with hydrogenated soy oil and lower in its availability. These results suggest that additional Glu supply can enhance digestive capacity. Our findings are consistent with previous studies showing that feeding Glu to weaning piglets increased villus height (Wu et al., 2012) and absorptive capacity (Rezaei et al., 2013) of the small intestine. As Glu is a major metabolic fuel in the small intestine in swine (Reeds et al., 2000; Burrin and Stoll, 2009) and cows (El-Kadi et al., 2009), it is also possible that periparturient RP-Glu supplementation expedited the intestinal growth, leading to greater ATTD during the first week after calving. However, the RP-Glu treatment did not affect ATTD of DM at 3 weeks after

calving. Dietary Glu supply may not affect the intestinal growth or digestive capacity once the critical periparturient period has passed. In fact, cows fed the RP-Glu diet had lower ATTD of CP at 3 weeks after calving. The extent of nutrient digestion is determined by feed characteristics, such as processing method, as well as digestive capacity of the animal. As the total in situ / in vitro digestibility of Glu was only 42% (19% in the rumen and 23% post-rumen) for the RP-Glu prototype, if digestive capacity of the animal is similar, cows fed the RP-Glu would likely have lower CP digestibility.

The greater DMI observed for cows fed RP-Glu on d 1 after calving may be related to delayed satiety signals due to less hepatic oxidation (Allen et al., 2009). As discussed above, if the RP-Glu supplementation expedited intestinal growth, more metabolic fuels would be oxidized by the gut tissues, resulting in less oxidative fuels reaching the liver. Less hepatic oxidation can delay satiety and increase DMI (Allen et al., 2009). Another possible reason for greater DMI for cows fed the RP-Glu is hormonal control. In a previous study, duodenal Glu infusion in 48-h fasted sheep increased plasma ghrelin concentration (Elsabagh et al., 2018). As ghrelin increases feed intake (Kojima et al., 1999), the greater DMI for the RP-Glu treatment might be mediated by ghrelin. However, cows fed the RP-Glu did not have greater DMI beyond d 1 after calving, indicating that additional dietary Glu supply from the RP-Glu supplementation did not have sustained effects on hepatic oxidation, ghrelin secretion, or other unidentified mechanisms regulating feed intake.

Cows fed the RP-Glu had greater plasma IGF-1 concentration on d 4 after calving, which is likely due to greater nutritional intake (Clemmons and Underwood, 1991) from greater DMI immediately after calving for the RP-Glu treatment. Cows fed the RP-Glu also had lower plasma 3-methylhistidine concentration on d 4 after calving, which suggests less body muscle protein

mobilization (McCabe and Boerman, 2020). Greater metabolizable protein (MP) supply from the higher DMI immediately after calving for cows fed RP-Glu may have contributed to less muscle catabolism (Carder and Weiss, 2017). In addition, the greater DMI likely reduced serum FFA concentration on d 4 after calving for cows fed the RP-Glu, suggesting less body fat mobilization (Reece et al., 2015). The reduced body fat mobilization may subsequently contribute to enhanced liver functions, indicated by less serum total bilirubin concentration (West, 1990; Bertoni and Trevisi, 2013) on d 4 after calving for cows fed the RP-Glu. However, these responses in metabolites did not continue as the treatment effect on DMI disappeared.

Intestinal barrier dysfunction can be caused by reduced feed intake (Pearce et al., 2013; Kvidera et al., 2017), increasing gut-derived inflammation right after calving. We hypothesized that feeding the RP-Glu would reduce gut-derived inflammation because Glu contributes to the intestinal mucosal barrier function by enhancing cell growth and maintaining membrane integrity (Jiao et al., 2015), and increased antioxidant function (Yin et al., 2015) in swine. However, no treatment effects were observed for concentrations of circulating acute-phase proteins such as serum amyloid A, serum haptoglobin, and plasma LBP although they peaked on d 4 after calving in both groups, indicating that the RP-Glu supplementation did not decrease systemic inflammation. Plaizier et al. (2018) reported that SARA increased concentrations of circulating acute phase proteins in some studies, but not in others. In the current study, we did not observe treatment effects on these inflammation markers. Serum amyloid A and haptoglobin are not specific markers for gut-derived inflammation, but systemic inflammation markers that also respond to infections such as mastitis or metritis (Ceciliani et al., 2012). However, we did not see treatment effects on LBP concentrations, either. In the bloodstream, LBP binds with lipopolysaccharide (LPS) to clear it from the systemic circulation (Ceciliani et al., 2012), and

circulating LBP concentration indicates the extent of the intestinal damage and barrier function (Kvidera et al., 2017). As such, our findings suggest that gut-derived inflammation was not be mitigated by additional dietary Glu supply.

As Glu is a primary energy source in the small intestine (Reeds et al., 2000) and a glucogenic AA (Heitmann and Bergman, 1981), we had also expected that feeding RP-Glu would spare glucose utilization, reduce serum BHB concentration, and increase milk production. However, there were no differences in concentrations of plasma glucose and serum BHB, and milk yield between the RP-Glu treatment and CON. In fact, cows fed the RP-Glu had lower lactose yield and tended to have lower milk protein, total solids, and ECM yields. Cows fed the RP-Glu had lower plasma concentrations of His and Thr, and this may partly account for the tendency for lower milk component yields for the RP-Glu treatment. A positive correlation was observed between milk protein yield and plasma His concentration ($r = 0.34$; $P = 0.02$) in the present study. Previous studies reported that His can be a limiting AA when cows fed MP-deficient diet (Lee et al., 2012; Giallongo et al., 2015), and feeding His-deficient diets decreased milk and milk protein yield (Giallongo et al., 2016, 2017). Therefore, His may limit milk production for cows fed the RP-Glu in the current study, however, the exact reason for the lower plasma His concentration for the RP-Glu cows after calving is unknown.

The lower plasma Thr concentration may also account for the lower milk component yields for cows fed the RP-Glu diet. In the current study, plasma Thr concentration was positively correlated with milk protein yield ($r = 0.58$; $P < 0.01$) and ECM yield ($r = 0.42$; $P = 0.01$). Threonine is the primary AA in mucins, the main structural component of mucus in the small intestine (Kim and Khan, 2013). As the mucus thickness in the small intestine was increased by Glu perfusion in rats (Akiba et al., 2009) or by adding Glu to the diet in weaned

piglets (Wu et al., 2012), it is possible that cows fed the RP-Glu had greater mucin secretion, which increases Thr utilization (Van Der Schoor et al., 2002). If more Thr is consumed in the gut tissues, cows fed the RP-Glu would have decreased plasma concentration of Thr, decreasing its availability for milk protein production.

In the current study, feeding the RP-Glu did not increase milk production nor decrease inflammation, and all the positive responses to the RP-Glu treatment, such as greater ATTD and DMI, were observed transiently only between d 1 and 5 after calving. Overall, Glu may not be required in the diet of dairy cows, but our findings suggest that cows may not be able to synthesize sufficient Glu in the body immediately after calving, and that Glu supplementation during the periparturient period can contribute to greater ATTD and DMI, and to less mobilization of body fat and muscle protein immediately after calving. Dairy NRC (2001) does not establish NEAA requirements including Glu, but further research is warranted to evaluate de novo synthesis and whole body utilization NEAA under specific conditions when animals experience metabolic stresses.

2.5 Conclusion

Feeding the RP-Glu to dairy cows from the close-up period through the fresh period increased ATTD of DM and CP on d 5 ± 1 after calving. In addition, RP-Glu increased DMI on d 1 after calving, which is associated with greater plasma IGF-1 concentration, lower concentrations of serum FFA, serum total bilirubin, and plasma 3-methylhistidine on d 4 after calving. However, these positive responses were observed only between d 1 and 5 after calving and did not continue beyond that. In addition, RP-Glu did not decrease inflammation nor increase milk production, but decreased lactose yield. Increasing dietary Glu supply during the

calving transition period may not increase milk production, but its physiological roles immediately after calving should be considered in future research.

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2.7 Tables and Figures

Table 2.7.1 Parity, BCS, and BW of dairy cows at enrollment (d -21 ± 3 relative to expected calving date)

	Treatment[1]	
Variable	CON (± SD)	RP-Glu (± SD)
n	26	26
Parity	2.5 ± 1.4	2.3 ± 1.1
BCS	3.20 ± 0.35	3.21 ± 0.34
BW, kg	791 ± 90	792 ± 79
Subset[2]		
n	9	10
Parity	2.0 ± 1.0	1.9 ± 1.3
BCS	3.26 ± 0.23	3.13 ± 0.28
BW, kg	821 ± 69	791 ± 60

[1] CON = control diet, no rumen-protected monosodium glutamate (RP-Glu) supplementation; RP-Glu = RP-Glu supplemented diet.
[2] A subset of 19 cows was used for digestibility measurements.

Table 2.7.2 Ingredients and nutrient composition of experimental diets[1]

	Prepartum		Postpartum	
Item	CON	RP-Glu	CON	RP-Glu
Ingredients of basal TMR, % of DM				
Barley silage	60.5		40.9	
Alfalfa hay	-		7.2	
Corn grain, ground, dry	5.9		13.9	
Barley grain, ground	4.5		16.1	
Beet pulp	5.7		1.1	
Soybean hull	3.7		-	
Canola meal	6.4		9.5	
Corn distillers	2.0		-	
Corn gluten meal	0.9		1.5	
Bypass soybean meal[2]	5.1		7.4	
Mineral and vitamin mix[3]	2.6		2.4	
Rumen-protected choline[4]	0.4		-	
Anionic mineral[5]	2.3		-	
Ingredients of experimental diets, % of DM				
Basal TMR for prepartum	97.6	96.0	-	-
Basal TMR for postpartum	-	-	98.2	97.0
Hydrogenated soy oil[6]	2.0	-	1.5	-
Sodium Bicarbonate[7]	0.4	-	0.3	-
RP-Glu[8]	-	4.0	-	3.0
Nutrient composition, % of DM				
DM	42.0	42.4	50.5	50.8
CP	16.5	17.1	18.3	18.8
NDF	41.9	41.2	33.1	32.7
Forage NDF	31.8	31.3	24.3	24.0
NFC	29.4	28.9	37.8	37.3
Starch	13.3	13.1	22.7	22.5
Ether extract	5.3	5.2	4.6	4.6
ME, Mcal/kg for DM[8]	2.4	2.4	2.6	2.5
DCAD, mEq/kg	9.1	8.9	-	-
ME allowable milk[8], kg/d	-	-	31.2	31.0
MP allowable milk[8], kg/d	-	-	34.7	34.6

[1] CON = control diet, no rumen-protected monosodium glutamate (RP-Glu) supplementation; RP-Glu = RP-Glu-supplemented diet.
[2] Soyplus (Landus, Ames, IA)
[3] Prepartum mix contained 25.3% Ca, 5.5% Mg, 2.7% P, 1.7% S, 0.1% K, 0.1% Na, 2,515 mg/kg Zn, 2,160 mg/kg Mn, 1,427 mg/kg Fe, 370 mg/kg Cu, 43 mg/kg Co, 35 mg/kg I, 19 mg/kg Se, 1 mg/kg monensin sodium, 340 KIU vitamin A, 70 KIU vitamin D, and 5,754 IU vitamin E. Postpartum mix contained 19.1% Ca, 9.5% Na, 8.1% Cl, 3.0% Mg, 1.8% P, 1.0% S, 0.3% K,

2,168 mg/kg Zn, 1,870 mg/kg Mn, 976 mg/kg Fe, 345 mg/kg Cu, 37 mg/kg Co, 30 mg/kg I, 10 mg/kg Se, 0.88 mg/kg monensin sodium, 213 KIU of vitamin A, 50 KIU of vitamin D and 1,520 IU of vitamin E.

[4] ReaShure Choline (Balchem Corporation, New Hampton, NY).

[5] Animate (Phibro Animal Health Corporation, Teaneck, NJ).

[6] To provide the same amount of hydrogenated soy oil as RP-Glu.

[7] To provide the same amount of available sodium as RP-Glu.

[8] RP-Glu (Ajinomoto Co. Inc., Tokyo, Japan). Contained 50.2% hydrogenated soy oil, 38.4% glutamate, and 6.0% sodium, and 5.4% water on a 55 ℃ DM basis.

[9] Estimated from AMTS cattle professional version 4.15.0. The estimated DMI for postpartum was 22 kg/d.

Table 2.7.3 The effects of supplementation of rumen-protected monosodium glutamate (RP-Glu) on DMI and production performance[1]

				P-value		
Variable	CON	RP-Glu	SE	Trt	Time	Trt × Time
Prepartum						
DMI, kg/d	13.6	14.3	0.4	0.23	0.09	0.47
BCS change, /21 d	-0.09	-0.16	0.05	0.27	-	-
Postpartum						
DMI, kg/d[2]	19.5	19.7	0.6	0.80	< 0.01	0.03
BW change, kg/d	-2.34	-2.95	0.26	0.12	-	-
BCS change, /21 d	-0.43	-0.32	0.06	0.18	-	-
Yield, kg/d						
Milk	42.1	39.4	1.2	0.12	< 0.01	0.26
Fat	2.05	1.92	0.05	0.28	0.53	0.11
CP	1.59	1.46	0.05	0.06	0.04	0.54
Lactose	2.16	1.96	0.07	0.05	< 0.01	0.24
Total solids	6.26	5.77	0.17	0.06	0.05	0.64
ECM	51.9	48.2	1.4	0.08	0.03	0.24
ECM/DMI	2.62	2.37	0.09	0.08	< 0.01	0.04
Wk 1	3.01	2.50	0.17	< 0.01	-	-
Wk 2	2.59	2.45	0.17	0.38	-	-
Wk 3	2.28	2.18	0.17	0.54	-	-
Milk composition						
Fat, %	4.26	4.34	0.19	0.76	< 0.01	0.03
Wk 1	4.90	4.60	0.35	0.41	-	-
Wk 2	4.26	4.32	0.33	0.85	-	-
Wk 3	3.67	4.15	0.34	0.16	-	-
CP, %	3.29	3.32	0.04	0.65	< 0.01	0.75
Lactose, %	4.45	4.47	0.15	0.55	< 0.01	0.24
Total solids, %	13.0	13.1	0.2	0.68	< 0.01	0.04
Wk 1	14.0	13.8	0.4	0.48	-	-
Wk 2	12.9	13.0	0.4	0.85	-	-
Wk 3	12.1	12.6	0.4	0.15	-	-
MUN, mg/dL	15.3	15.2	0.6	0.98	0.24	0.41
SCC, 10^3 cells/mL	212	139	80	0.53	0.43	0.03
Wk 1	378	87	160	0.07	-	-
Wk 2	171	57	151	0.45	-	-
Wk 3	87	275	153	0.22	-	-
BHB, mmol/L	0.12	0.12	0.01	0.67	0.48	0.61

[1] CON = control diet, no RP-Glu supplementation; RP-Glu = RP-Glu-supplemented diet.
[2] As the Trt × Time interaction is significant, daily DMI data are shown in Figure 2.7.1.

Table 2.7.4 The effects of supplementation with rumen-protected monosodium glutamate (RP-Glu) on circulating concentrations of metabolites, hormones, and inflammatory markers on d -3 relative to calving[1]

Variable	CON	RP-Glu	SE	*P*-value
Metabolite				
Serum				
Free fatty acids, μEq/L	375	335	36	0.44
BHB, μmol/L	329	356	27	0.50
Total bilirubin, mg/dL	0.15	0.13	0.01	0.24
Urea N, mg/dL	16.9	17.4	0.5	0.53
Plasma				
Glucose, mg/dL	71.4	70.3	0.8	0.38
3-methylhistidine, μmol/dL	1.33	1.36	0.10	0.82
Hormone				
Plasma				
IGF-1, ng/mL	82.0	83.8	6.4	0.85
Glucagon-like peptide-2, ng/mL	0.91	0.99	0.07	0.43
Inflammatory marker				
Serum				
Serum amyloid A, μg/mL	35	57	22	0.50
Haptoglobin, mg/mL	0.10	0.31	0.10	0.15
Plasma				
LPS binding protein, ng/mL	2795	2954	183	0.56

[1] CON = control diet, no RP-Glu supplementation; RP-Glu = RP-Glu-supplemented diet.

Table 2.7.5 The effects of supplementation with rumen-protected monosodium glutamate (RP-Glu) on concentrations of circulating metabolites, hormones, and inflammatory markers during the postpartum period[1][2]

				P-value		
Variable	CON	RP-Glu	SE	Trt	Time	Trt × Time
Metabolite						
Serum						
Free fatty acids, μEq/L[3]	885	843	50	0.58	< 0.01	< 0.01
BHB, μmol/L	669	737	81	0.56	0.08	0.62
Total bilirubin, mg/dL[3]	0.27	0.24	0.02	0.39	< 0.01	< 0.01
Urea N, mg/dL	16.3	16.8	0.5	0.49	< 0.01	0.44
Plasma						
Glucose, mg/dL	61.8	62.2	1.2	0.80	< 0.01	0.14
3-methylhistidine, μmol/dL[3]	1.09	0.97	0.06	0.15	< 0.01	0.03
Hormone						
Plasma						
IGF-1, ng/mL[3]	31.8	41.1	3.3	0.06	0.76	0.02
Glucagon-like peptide-2, ng/mL	1.01	1.01	0.06	0.97	0.98	0.80
Inflammatory markers						
Serum						
Serum amyloid A, μg/mL	177	208	42	0.61	< 0.01	0.69
Haptoglobin, mg/mL	0.46	0.37	0.10	0.54	< 0.01	0.95
Plasma						
LPS binding protein, ng/mL	3818	4037	152	0.33	< 0.01	0.42

[1] CON = control diet, no RP-Glu supplementation; RP-Glu = RP-Glu-supplemented diet.
[2] Metabolite concentrations were measured on d 4, 7, 10 and 21 after calving, and the others were measured on d 4, 7 and 10 after calving.
[3] As the Trt × Time interaction is significant, data for each time point are shown in Figure 2.7.2.

Table 2.7.6 The effects of supplementation with rumen-protected monosodium glutamate (RP-Glu) on plasma AA profile (μ*M*) on d -3 relative to calving [1]

Variable	CON	RP-Glu	SE	*P*-value
Arg	65.3	61.0	0.33	0.38
His	54.1	53.7	0.21	0.88
Ile	115	103	7	0.25
Leu	155	139	10	0.26
Lys	77.5	68.5	0.46	0.20
Met	25.7	23.4	1.5	0.29
Phe	54.7	54.7	3.3	1.00
Thr	90.1	76.8	5.1	0.09
Trp	24.5	23.0	1.4	0.48
Val	216	194	10	0.15
Ala	206	206	10	0.50
Asn	44.6	41.1	0.25	0.36
Asp	3.6	4.1	0.3	0.22
Cys	ND[2]	ND[2]	-	-
Gln	344	306	12	0.05
Glu	42.3	46.1	2.2	0.25
Gly	511	515	33	0.93
Pro	88.9	81.2	3.9	0.19
Ser	115	116	8	0.89
Tyr	39.4	38.3	2.3	0.76

[1] CON = control diet, no RP-Glu supplementation; RP-Glu = RP-Glu-supplemented diet.
[2] ND = not detected.

Table 2.7.7 The effects of supplementation with rumen-protected monosodium glutamate (RP-Glu) on plasma AA profile (μ*M*) on day 4, 7, 10, and 21 after calving[1]

Variable	CON	RP-Glu	SE	*P*-value		
				Trt	Time	Trt × Time
Arg	64.9	63.6	1.8	0.63	< 0.01	0.32
His	52.9	47.3	1.8	0.04	< 0.01	0.24
Ile	119	121	6	0.87	< 0.01	0.26
Leu	166	165	6	0.91	< 0.01	0.30
Lys	77.4	76.1	2.1	0.68	< 0.01	0.78
Met	25.3	24.8	0.6	0.60	< 0.01	0.38
Phe	55.5	55.7	1.1	0.91	0.06	0.75
Thr	96.6	86.1	3.5	0.05	< 0.01	0.24
Trp[3]	28.1	26.0	1.0	0.23	< 0.01	0.02
Val	231	232	8	0.93	< 0.01	0.18
Ala	221	213	7	0.45	< 0.01	0.18
Asn[3]	48.8	45.2	1.5	0.12	< 0.01	0.02
Asp[3]	3.4	3.6	0.2	0.45	< 0.01	0.01
Cys	ND[2]	ND[2]	-	-	-	-
Gln[3]	333	332	9	0.94	0.03	< 0.01
Glu[3]	40.5	43.4	1.3	0.14	0.04	0.02
Gly	605	580	21	0.41	< 0.01	0.86
Pro	105	102	4	0.60	< 0.01	0.25
Ser	120	115	4	0.45	0.23	0.07
Tyr[3]	41.7	41.9	1.7	0.93	< 0.01	0.02

[1] CON = control diet, no RP-Glu supplementation; RP-Glu = RP-Glu-supplemented diet.
[2] ND = not detected.
[3] As the Trt × Time interaction is significant, data for each time point are shown in Figure 2.7.3.

Table 2.7.8 The effects of supplementation of rumen-protected monosodium glutamate (RP-Glu) on apparent total-tract digestibility (%) of DM, OM, CP, NDF, and starch on day 5 ± 1 and 21 ± 3 after calving[1]

Variable	CON	RP-Glu	SE	*P*-value		
				Trt	Time	Trt × Time
DM	70.1	70.3	0.41	0.73	0.11	< 0.01
d 5 ± 1	69.1	70.6	0.70	0.05	-	-
d 21 ± 3	71.0	70.0	0.70	0.14	-	-
OM	71.5	71.6	0.38	0.85	0.12	< 0.01
d 5 ± 1	70.6	71.9	0.68	0.06	-	-
d 21 ± 3	72.5	71.4	0.68	0.12	-	-
CP	73.7	74.5	0.52	0.27	0.46	< 0.01
d 5 ± 1	72.6	75.1	0.65	0.03	-	-
d 21 ± 3	74.7	73.9	0.65	0.01	-	-
NDF	47.1	47.1	0.58	0.97	0.64	0.02
d 5 ± 1	45.8	48.0	1.18	0.08	-	-
d 21 ± 3	48.4	46.2	1.18	0.07	-	-
Starch	99.5	99.5	0.11	0.84	0.05	0.12

[1] CON = control diet, no RP-Glu supplementation; RP-Glu = RP-Glu-supplemented diet

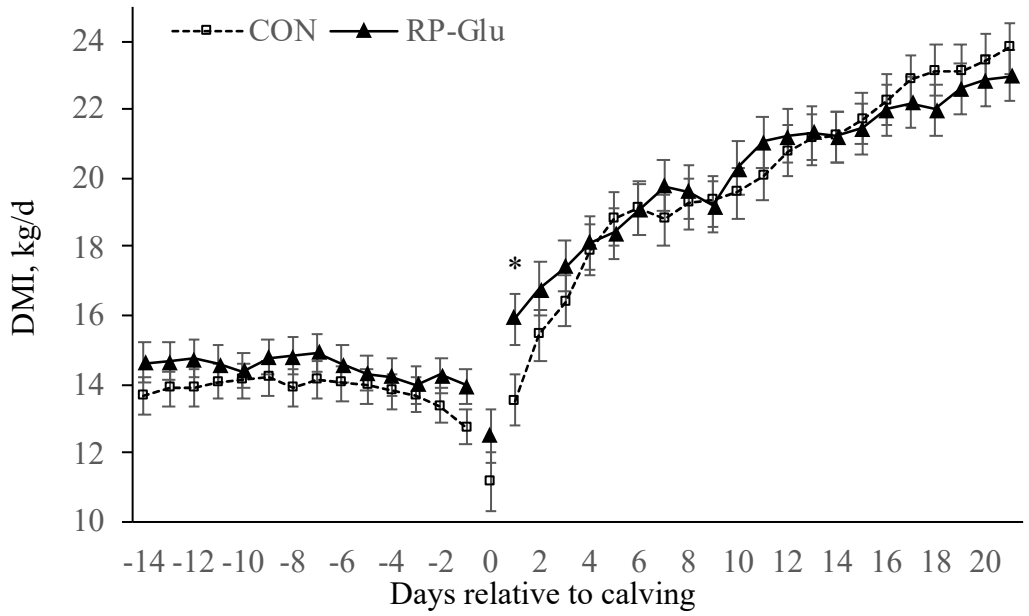

Figure 2.7.1 Daily DMI of cows supplemented with rumen-protected monosodium glutamate (RP-Glu) and without (CON) during the periparturient period. The *P*-values for treatment, day, and their interaction during the prepartum period were 0.23, 0.09, and 0.47, respectively. The *P*-value for treatment on d 0 relative to calving was 0.27. The *P*-values for treatment, day and their interaction during postpartum period were 0.80, < 0.01, and 0.03, respectively. Postpartum data were analyzed individually for each time point using *t*-test. *Effect of RP-Glu on DMI was significant ($P < 0.05$) on d 1 after calving. Data shown are LSM ± SE.

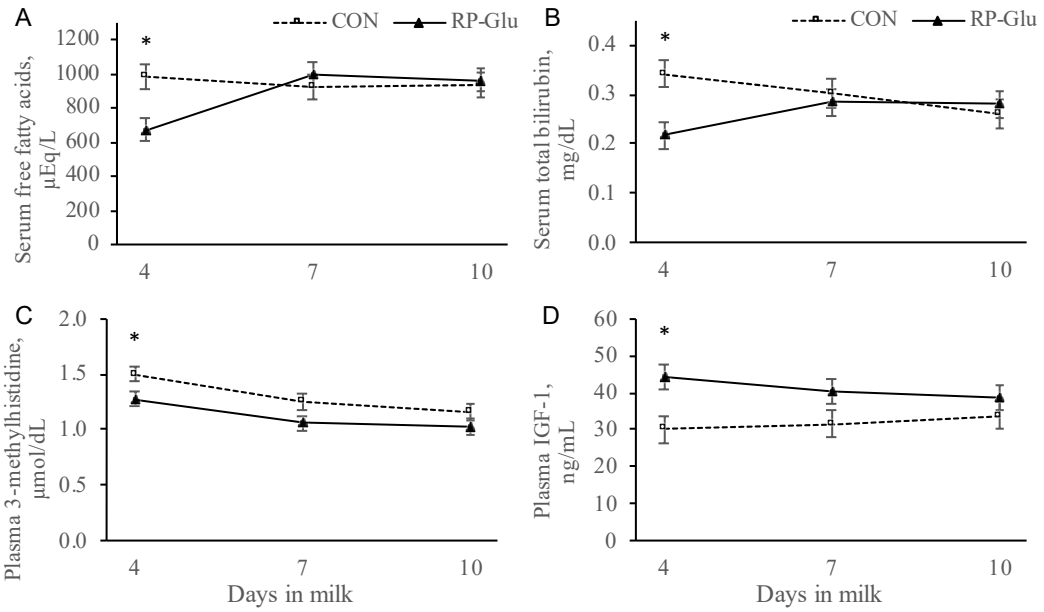

Figure 2.7.2 The effects of supplementation of rumen-protected monosodium glutamate (RP-Glu) on concentrations of serum free fatty acids (A), serum total bilirubin (B), plasma 3-methylhistidine (C), and plasma IGF-1 (D). As interactions between treatment and day were significant, data were analyzed individually for each time point using *t*-test ($P < 0.05$ shown as *). Data shown are LSM ± SE.

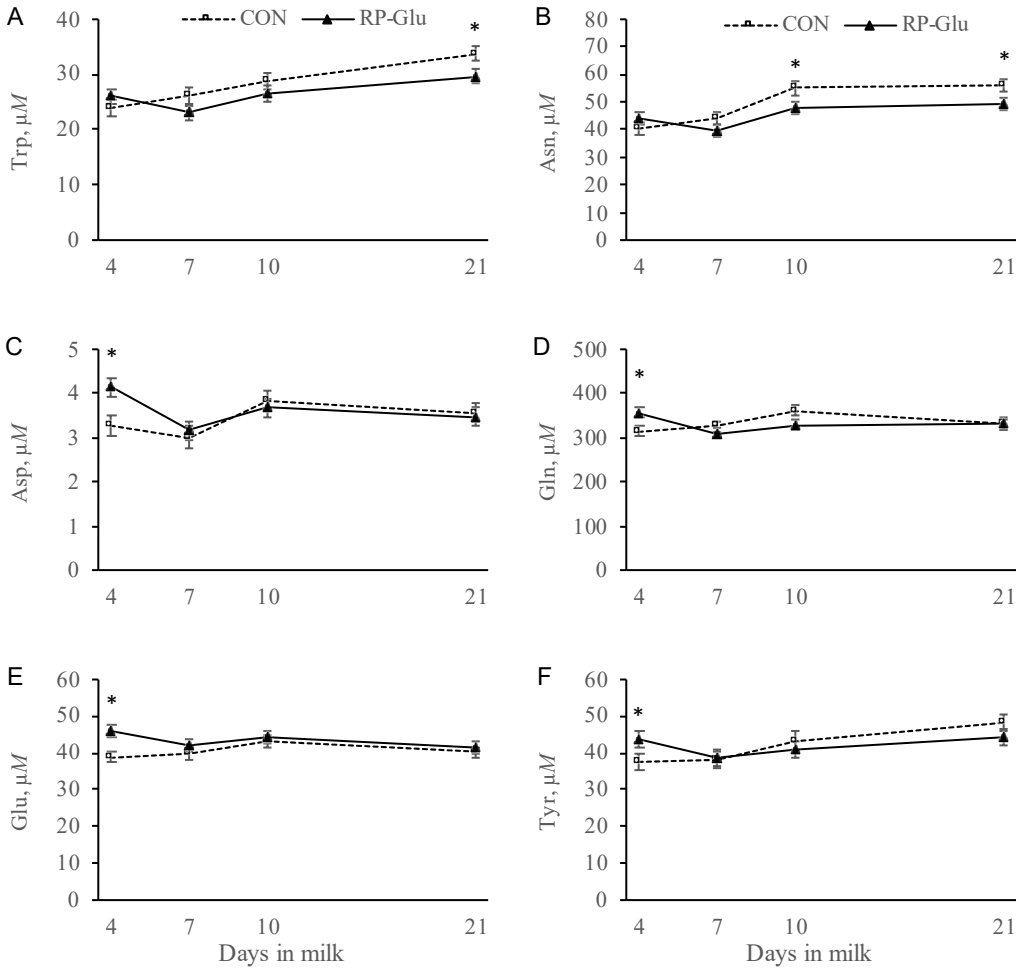

Figure 2.7.3 The effects of supplementation of rumen-protected monosodium glutamate (RP-Glu) on plasma concentrations of Trp (A), Asn (B), Asp (C), Gln (D), Glu (E), and Tyr (F). As interactions between treatment and day were significant, data were analyzed individually for each time point using *t*-test ($P < 0.05$ shown as *). Data shown are LSM ± SE.

Chapter 3. General discussion

3.1 Summary and Industry Implications

In the present study, feeding Glu to dairy cows did not reduce systemic inflammation nor increase milk production throughout the experimental period. However, cows fed Glu increased ATTD of DM and CP, DMI, and plasma IGF-1 concentration immediately after calving. These findings suggest Glu provided a greater nutrition supply to cows immediately after calving because of increased nutrient demand with the onset of lactation. In addition, cows fed Glu had lower postpartum serum FFA, and total bilirubin concentrations, and lower plasma 3-methylhistidine concentration on d 4 after calving. These findings suggest that cows fed Glu during the transition period decreased mobilization of body fat (Reece et al., 2015) and muscle protein (McCabe and Boerman, 2020) due to greater DMI on d 1 after calving, and reduced body fat mobilization may have contributed to enhanced liver functions (West, 1990; Bertoni and Trevisi, 2013).

Greater plasma Glu concentration was observed for cows fed Glu only on d 4 after calving, and greater ATTD and DMI were observed only immediately after calving, and did not continue until d 21 after calving which was consistent with the animal response in plasma Glu concentration. These findings indicate that Glu synthesis in the body may be insufficient around the calving in dairy cows when feed intake is low. Feeding Glu increases Glu available to cows, likely overcoming Glu deficiency immediately after calving when feed intake is low and resulted in the positive animal responses.

Management of dairy cows during the calving transition is important. It is no exaggeration to say that incidence of metabolic diseases, milk production, reproduction, and the cull rate on a dairy farm are determined by transition cow management. In the present study,

cows fed Glu did not have a reduced incidence of metabolic disorders nor increased milk production, therefore, I do not recommend feeding 4% and 3% of the RP-Glu during the prepartum and postpartum periods, respectively, at this point. However, we observed some positive responses immediately after calving likely due to feeding Glu diet during the periparturient period which suggests that Glu may be insufficient after calving, despite Glu being a NEAA. While I do not recommend feeding Glu based on the results of the present study, the positive responses immediately after calving from in cows fed Glu suggest there is potential for Glu to improve cow performance in some capacity, and this could be explored in future research.

3.2 Limitations

In the present study, sodium content in the prepartum RP-Glu diet (0.36% of dietary DM) was higher than the recommendation in NRC (2001; 0.14% of dietary DM) because of the sodium derived from monosodium Glu in the RP-Glu. Therefore, we added sodium bicarbonate in CON diets both in the prepartum and postpartum period to match the available sodium content of RP-Glu, and we had to add 2.3% of dietary DM of anionic mineral to decrease DCAD to prevent milk fever in the prepartum TMR. Supplementing anionic minerals decreases DMI in dairy cows and increases cost. In addition, cows have an increased risk of SARA after calving because of higher starch from fresh cow diets compared with close-up diets. Subacute ruminal acidosis is associated with reduced feed intake and milk production, and inflammation. In the CON diet, it is possible that sodium bicarbonate worked as a buffer to mitigate SARA with neutralization of the acids produced in the rumen to reduce ruminal pH depression. Therefore, the condition of the rumen between CON and RP-Glu treatments may not be fair to compare.

As the monosodium Glu was coated with hydrogenated soy oil to protect from ruminal degradation, hydrogenated soy oil was added to CON diet to match the hydrogenated soy oil of RP-Glu. Therefore, ether extract content increased in the experimental diets, and the increased ether extract supplementation in the prepartum diets increased energy supply. Over-conditioned prepartum cows have greater risks of excessive body fat mobilization, ketosis, and reduced feed intake.

Glutamate is a primary energy source in the small intestine (Reeds et al., 2000; Burrin and Stoll, 2009) and feeding Glu enhances gastrointestinal barrier function in swine (Jiao et al., 2015), however, we did not evaluate gastrointestinal barrier function in the present study. If I measured the intestinal permeability, I could have evaluated the effects of feeding Glu on gastrointestinal barrier function specifically.

Apparent total-tract digestibility of DM, CP were greater and that of NDF tended to be greater on d 5 ± 1 after calving for cows fed Glu compared with CON. In contrast, feeding Glu did not affect ATTD of DM, and cows fed Glu had lower ATTD of CP and tended to have lower NDF on d 21 ± 3 after calving compared with CON. However, we did not measure variables that explain animal responses in digestibility. We speculated that feeding Glu may enhance gut growth and digestive capacity on d 5 ± 1 after calving but may not affect the gut growth or digestive capacity beyond d 5 ± 1 after calving. However, we did not measure the gut growth and digestive capacity. In addition, when cows have greater gut capacity, digesta retention time becomes longer and digestibility can be greater, however, we did not measure digesta retention time. Furthermore, in the present study, cows fed Glu may have lower cholecystokinin (CCK) secretion. Cholecystokinin is a gut hormone secreted in the duodenum and inhibits gastric emptying (Allen et al., 2009), therefore, digesta retention time can be shorter and digestibility

becomes lower when CCK secretion is low. Plasma CCK concentration was elevated by increasing protein in the small intestinal lumen in lactating cows (Relling and Reynolds, 2008). In the present study, cows fed Glu may have lower cholecystokinin (CCK) secretion attributed to less protein exposure in the small intestine because the availability of Glu was low due to rumen protection. However, we did not measure plasma CCK concentration in the present study. If I evaluated overall gut growth, villus height, mucosal thickness, digesta retention time, and plasma CCK concentration, I could have explained the reasons for animal responses in digestibility.

Cows fed Glu had lower postpartum concentrations of serum FFA and plasma 3-metylhistidine on d 4 after calving, which suggest lower body fat and muscle protein mobilization, but we did not measure the amount of mobilized body fat and muscle.

We observed positive animal responses by feeding Glu only in the first week after calving. However, there were no treatment effect on milk components immediately after calving, which may be because sampling was not frequent enough. We collected milk samples for two consecutive milkings (p.m. and a.m.) weekly in the present study. However, if I collected milk samples daily during d 1 to d 7 after calving when I observed the positive responses, I may observe additional effects in milk components production by feeding Glu which could provide further evidence of the effects of feeding Glu to transition dairy cows.

Cows fed Glu had greater DMI immediately after calving. We speculated that the greater DMI may be because of ghrelin (Kojima et al., 1999) based on a previous study that reported that dietary Glu supplementation increased ghrelin concentration (Elsabagh et al., 2018), however, we did not measure plasma ghrelin concentration. If I measured plasma ghrelin concentration after calving, I could have determined if the feeding Glu stimulated ghrelin secretion in dairy cows.

3.3 Future Research

In the present study, cows fed Glu showed some positive responses such as greater ATTD and DMI only immediately after calving, which was likely due to feeding Glu during the prepartum period. However, cows fed Glu had lower ATTD of CP at three weeks after calving and yields of milk protein and ECM throughout the postpartum period which suggests that feeding Glu during the postpartum period can result in some negative responses. Feeding Glu to dairy cows only during the prepartum period may be effective to induce the positive responses such as greater ATTD and DMI immediately after calving without aforementioned negative responses. Therefore, it would be interesting to evaluate the effects of feeding Glu to transition dairy cows during the prepartum and postpartum periods independently: 1) a control with no Glu in both the prepartum and postpartum periods, 2) a treatment group with Glu fed only in the prepartum period, 3) a treatment group with Glu fed only in the postpartum period, or 4) a treatment group with Glu fed in both the prepartum and postpartum periods.

In the present study, we could not determine the optimal dosage of Glu in the diet because we fed only one dosage of Glu to transition dairy cows. Therefore, it would be interesting to evaluate the effects of feeding multiple dosages of Glu to transition dairy cows. We do not currently know the maximum dosage of feeding Glu in dairy cows although Glu is a key neurotransmitter. However, post-weaning pigs did not experience neurotoxicity by infusing a 4-folds greater amount of Glu with baseline diet (Janeczko et al., 2007), the possibility of neurotoxicity appears to be low (Burrin and Stoll, 2009). In the present study, the RP-Glu which we fed was expected to increase Glu absorption by approximately 20%. Future research should consider testing different dosages of intestinal available Glu, to determine an optimal dosage.

In the present study, we had hypothesized that feeding Glu would reduce inflammation. We fed Glu in low-starch fresh cow diet (22.5% and 22.7 % of starch on a DM basis, respectively for RP-Glu and CON treatments), and cows fed Glu did not have reduced inflammatory markers. In future research, it would be interesting to evaluate the effects of feeding Glu on inflammatory markers when feeding a high-starch fresh cow diet which is expected to induce greater gut-derived inflammation (Emmanuel et al., 2008; Khafipour et al., 2009; Li et al., 2012).

Cows fed Glu did not increase yields of milk protein and ECM in the present study, which may be partially attributed to lower plasma His concentration. The exact reason for the lower plasma His concentration is unknown, however, microbial protein synthesis may be lower for cows fed Glu due to the RP-Glu diet was slightly lower rumen fermentable carbohydrate. If there was more microbial synthesis from feeding a high-starch diet, there may be more His production. Cows fed a high-starch diet could synthesize greater microbial protein compared with the present study. In addition, starch may not be a limiting factor of the microbial protein synthesis by feeding a high-starch diet. Therefore, feeding Glu in high-starch diet may not decrease yields of milk protein and ECM. It would be interesting to measure microbial protein synthesis which may provide additional evidence to explain lower plasma His concentration.

3.4 Conclusion

In conclusion, cows fed the RP-Glu diet did not have increased milk production nor reduced inflammation. In addition, cows fed the RP-Glu diet during the periparturient period had greater ATTD, DMI, lower body fat and muscle protein mobilization immediately after calving, however, these responses did not continue beyond the first week of calving. Therefore, I do not

recommend dairy farmers to feed Glu to dairy cows during the calving transition period. However, cows may not be able to synthesize sufficient Glu immediately after calving when feed intake is low although Glu can be synthesized in the animal body. Further research for the role of dietary NEAA could clarify if limiting NEAA, such as Glu, impacts cow performance during the transition period.

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