Effect of dietary choline and diet fermentability on feeding behavior and performance of postpartum dairy cows

by

Kelsey Rose Pasch

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Major Professor
William E. Brown

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Abstract

Depressed intake and hepatic lipid accumulation are major contributors to metabolic stress in transition dairy cows. Highly fermentable diets have also been shown to depress dry matter intake (DMI), compounding the risk of metabolic stress. Choline is commonly supplemented to periparturient dairy cows to facilitate hepatic lipid metabolism and support postpartum health and performance. Our objective was to evaluate effects of diet fermentability (DF) and rumen-protected choline (RPC) on postpartum DMI and performance. Multiparous Holstein cows (n = 65) were enrolled on a rolling basis according to body condition score, expected calving date (ECD), and 305-d mature equivalent milk yield, and were randomly assigned to 1 of 4 postpartum treatments in a randomized complete block design with a 2x2 factorial arrangement of starch fermentability rate (low [dry-rolled corn; LFERM] vs. high [wheat; HFERM]) and RPC (no RPC [C-] vs. RPC [C+; 30 g/d]). Prepartum RPC supplementation began 21 d before ECD in a total mixed ration for C+ cows and continued postpartum, whereas C- cows received no RPC. Cows were fed with a Roughage Intake Control (RIC) computerized feeding system during the prepartum period (Hokofarm Group, Emmeloord, The Netherlands). Postpartum cows (n = 55) were fed for 21 d postpartum in a tie-stall with a computerized feeding behavior system. Feeding behaviors, DMI, and milk yield were collected daily, and milk components were collected 2 d/wk. Data were analyzed using linear mixed models for the fixed effects of treatment, time, and their interactions using PROC MIXED in SAS (v 9.4). Means are presented with 95% confidence interval. Prepartum, C+ decreased DMI compared with C- (14.8 [13.8, 15.9] vs. 17.1 kg [16.0, 18.2]). Postpartum, there was no evidence of treatment effect on DMI. Time and RPC interacted for meal size, with C- increasing meal size more rapidly than C+ through d 10 and after d 15. Time and DF interacted to influence milk

yield with HFERM increasing milk yield after d 3 compared with LFERM. Compared with LFERM, HFERM decreased milk fat % (4.4 [4.0, 4.8] vs 4.8% [4.4, 5.2]) but not fat yield. Time and DF interacted on protein % and yield; HFERM decreased protein yield from wk 1 to 3 (3.1 [2.8, 3.4] vs. 2.8 [2.6, 3.1]), but there was no difference across wk for LFERM. Compared to LFERM, HFERM decreased milk fatty acid concentrations and preformed fatty acids. There was no evidence of treatment effects on ECM or FCM. For blood metabolites, C+ decreased plasma BHB (1.0 mmol/L [0.9, 1.2] vs. 1.3 [1.1, 1.5]) and tended to increase glucose concentration (49.0 mg/dL [46.4, 51.5] vs. 46.0 [43.5, 48.5]) compared to C-. Time and DF interacted on BUN with HFERM increasing BUN concentration (13.5 mg/dL [12.4, 14.5] vs. 12.6 [11.5, 13.6]). In conclusion, supplementation of RPC, even at a low rate of inclusion, decreased plasma BHB concentrations suggesting improved metabolic status postpartum. Prepartum DMI was reduced in cows supplemented with RPC, which requires further work to elucidate potential mechanisms of action showing similar results in TMR-fed cows. Diet fermentability and RPC supplementation did not interact to affect feeding behavior, which may be reflective of relatively lesser differences in starch digestibility between treatments than anticipated. Formulated starch digestibility was 60.1% for HFERM and 44.6% for LFERM, whereas actual analyzed values were 53.8% and 46.7%, respectively, suggesting that modest differences in starch fermentability may have limited treatment responses.

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Dedication

To my father and gramps

The two men whose passion and dedication to the dairy industry have been my greatest inspiration. Thank you for teaching me the value of honest work and a life lived with purpose!

Chapter 1 - Review of Literature

Introduction

The transition period, defined as 3 weeks before and 3 weeks after calving, is one of the most critical timeframes for a dairy cow (Holcomb et al., 2001). During this period, the dairy cow is experiencing physiological, metabolic, and immunological changes, while also at a greater risk for metabolic disorders, such as hepatic lipidosis and ketosis (Drackley, 1999; Sundrum, 2015). In the immediate days leading up to calving, the dairy cow may experience a pronounced decline in dry matter intake (DMI) which coincides with greater energy demands for milk production (Drackley, 1999). This difference in energy intake and expenditure results in negative energy balance (NEB) during the onset of lactation as the cow is unable to consume the amount of dietary energy needed to match the increased demands to produce milk. In response, the dairy cow mobilizes body fat from adipose tissue as a metabolic energy source. Management and feeding strategies that promote feed intake and limit fat mobilization may help optimize cow health, increase lactation milk yield, improve reproductive efficiency, and maximize farm profitability. Indeed, numerous studies have evaluated appropriate nutritional and management strategies to optimize transition cow health and support feed intake. While DMI is often observed, relatively few studies have focused on the underlying mechanisms of intake and feeding behavior in relation to cow health during the transition period. This review will explore the management and nutritional factors influencing feed intake, feeding behavior, and performance of transition dairy cows.

Dry Matter Intake and Feeding Behavior During the Transition Period

Prepartum Feeding Behavior Patterns

During the early dry period, DMI tends to remain relatively constant (Hayirli et al., 2002, 2003). However, during the final weeks of gestation, energy requirements increase substantially due to rapid fetal growth and metabolic adaptations in preparation for lactation. Recent updates from NASEM (2021) redefined prepartum energy requirements, recommending a diet containing 1.28 Mcal/kg of net energy of lactation (NEL) to be fed from dry off to -21 d before parturition and 1.49 Mcal/kg of NEL during the remaining 3 weeks until parturition. Alongside this increase in energy demand preceding calving, DMI often declines, making it difficult to meet these needs through DMI and predisposes cows to postpartum metabolic stress (Drackley et al., 2005). Hayirli et al. (2003) designed and validated a model to predict changes in DMI during the weeks leading up to parturition, reporting a 32% decrease in DMI from -21 to -1 d relative to parturition, with the most pronounced decline occurring in the final week.

Previous studies have investigated the timing and extent of this notable decline in intake in the days surrounding parturition. Santos et al. (2024) investigated multiple factors and their association with changes in DMI during the prepartum period where they found a negative association between prepartum DMI and rumination time, likely as a response to reduced DMI in the week preceding parturition. Similarly, Schirmann et al. (2013) investigated changes in rumination and feeding behavior from 96 h before and 48 h after calving, reporting that a reduction in rumination within 24 h of calving is linked with a decrease in DMI and feeding time. Notably, the onset of decreased rumination and feeding time occurred at 8 h before calving, while DMI also declined (Schirmann et al., 2013). These reductions in intake and behavioral changes have been consistently observed across studies in different environments and dietary

compositions, suggesting a decline in DMI is a normal physiological response during late gestation (Santos et al., 2024).

Factors Affecting DMI

In the transition period, there are several animal, dietary, and management factors that contribute to a reduction in DMI. Traditionally, transition cow management has focused on maximizing feed intake during this timeframe (Holcomb et al., 2001). However, recent work suggests that minimizing variation in DMI during the transition period may mitigate hypophagia and reduce metabolic disorders postpartum. Santos et al. (2024) investigated changes in DMI during the prepartum period where they reported large reductions in DMI is linked with important adjustments of energy metabolism and increased risk for postpartum diseases. Nutritional and management strategies aimed at maintaining DMI through the dry period have become a key focus for improving transition cow success. Still, diet composition remains complex as variation in ingredients, feeding strategies, and delivery practices contribute to inconsistent outcomes. Several dietary and animal factors have been identified as primary influencers of DMI during the prepartum period including body condition score (BCS), parity, stage of gestation, and dietary concentration of neutral detergent fiber (NDF) and ether extract (EE; Hayirli et al., 2002; Grummer et al., 2004).

Achieving and maintaining an optimal BCS during the transition period is important for metabolic health and minimizing changes in DMI. Although industry guidelines recommend a BCS at time of dry-off of 3.25-3.5 for multiparous cows, excessive body condition has been associated with adverse health outcomes and reduced prepartum DMI (Hayirli et al., 2002; Daros et al., 2021). Hayirli et al. (2002) found that DMI is inversely related to BCS during the

transition period as cows with a BCS greater than 4 (on a 5-point scale) had a greater depression of DMI during the 3 weeks prior to calving compared to animals with a BCS less than 4. Similarly, Daros et al. (2021) defined animals with a BCS ≥ 3.5 as overconditioned and found that overconditioned animals had 0.6 kg less daily DMI and spent 28 fewer minutes eating per day compared to animals with a BCS < 3.5.

Several studies have evaluated BCS and changes in BCS during the transition period and their relationship to DMI and transition cow performance. Generally, slight gain or loss in body condition has been considered normal during the prepartum period; however, excessive changes of more than 1 BCS point have been associated with increased risk of metabolic disorders and suppressed DMI. Casaro et al. (2024) investigated the relationship of BCS at 21 d before calving with prepartum and postpartum DMI, energy balance, and milk yield of multiparous cows. DMI was lowest for overconditioned cows (BCS ≥ 4.00; 9.97 kg/d) compared to moderate (BCS = 3.25-3.75; 11.15 kg/d) and thin cows (BCS ≤ 3.00; 11.92 kg/d) during both the prepartum and postpartum period, while energy balance and milk yield were also lesser. Furthermore, overconditioned cows experienced greater losses in BCS and body weight during the prepartum period and were already in negative energy balance by 21 d before calving, indicating enhanced lipid mobilization relative to their contemporaries. This mobilization of adipose tissue may not be solely attributed to reduced DMI as near the time of calving, lipolysis increases and appears to be influenced by biological processes that prioritize nutrient partitioning toward milk production (Roche et al., 2009). Thus, overconditioned cows in the dry period may be predisposed to elevated lipid mobilization due to internal biological mechanisms, further exacerbating NEB and potentially suppressing intake through feedback on hepatic oxidation pathways.

Aside from animal-related factors, one of the most important dietary factors influencing DMI during the transition period is diet fermentability. Diet fermentability is influenced by the source and concentration of carbohydrates and NDF, which in turn influences net energy for lactation (NASEM, 2021). Several studies have investigated different fiber and forage sources with varying NDF concentrations and their effect on feed intake. Low quality forages such as straw and cottonseed hulls can restrict DMI (NASEM, 2021). In the prepartum cow, physical fill is a predominant factor due to reduced rumen capacity. Poorly digested, low quality forages remain in the rumen longer, thereby reducing DMI and energy intake and inducing satiety before energy requirements are fulfilled (Zebeli et al., 2012). In contrast, inclusion of more digestible fibrous sources, such as soyhulls (Holcomb et al., 2001), in prepartum diets increases DMI, presumably due to higher digestibility and lower starch concentration to support rumen fill without suppressing feed intake or disrupting the rumen environment (Zebeli et al., 2012). Additionally, starch source, processing method, and overall particle size can impact diet fermentability, rumen fermentation, and DMI. Fermentability of prepartum diets should be carefully considered to promote DMI without compromising the ruminal environment.

In addition to dietary composition, non-dietary factors including pen movements, overcrowding, social interactions, heat stress, and bunk management also may influence DMI and should be considered to improve transition cow success. The effects of these management and environmental stressors on feeding behavior and transition cow performance have been described in previous reviews (Grant and Albright, 2001; Cook and Nordlund, 2004; Huzzey et al., 2005; Giannone et al., 2023).

Physiological and Metabolic Challenges During the Transition Period

Energy Balance and Hepatic Lipid Mobilization

As previously discussed, dairy cows typically experience a timeframe of NEB during the transition period, characterized by lipid mobilization and elevated circulating concentrations of free fatty acids (FFA; Drackley, 1999; Grummer et al., 2004). The liver extracts circulating FFA to utilize as an energy source to meet the demands of early lactation. In the liver, the possible fate of FFA includes (i) complete oxidation to produce CO_2 and ATP, (ii) re-esterified to triglyceride (TAG) for storage or packaged into very low density lipoproteins (VLDL), or (iii) partially oxidized and exported as ketone bodies including acetoacetate, beta-hydroxybutyrate (BHB), and acetone (Bauchart, 1993; Drackley, 1999; Roche et al., 2009). Although this metabolic adaptation of the liver to take up FFA provides a method for redistributing needed energy sources, hepatic FFA influx may exceed the capacity for complete oxidation and VLDL secretion, resulting in hepatic lipidosis and increased risk of clinical or subclinical ketosis (Goff and Horst, 1997; Ospina et al., 2010). Several reviews have described in greater detail the biochemical process of ketogenesis (Drackley et al., 2005; Rico and Barrientos-Blanco, 2024).

Although mobilized lipids provide an energy source for milk synthesis during early lactation, hepatic oxidation of fatty acids can generate satiety signals that further suppress intake and alter concentrations of blood metabolites. At time of calving, plasma FFA concentrations are greater and remain elevated throughout the first weeks of lactation, accompanied by elevated BHB and reduced glucose concentrations (Douglas et al., 2007). Persistently high FFA and ketone concentrations are associated with greater prevalence of transition disorders such as ketosis, displaced abomasum and metritis (Drackley, 1999; Huzzey et al., 2007; Kerwin et al., 2022). These hepatic metabolic shifts contribute to the regulation of feed intake during the

transition period. The following section outlines how oxidation of fuels like fatty acids and propionate influence satiety signaling.

Regulation of Feed Intake

Feed intake regulation in ruminants is a complex physiological process strongly influenced by diet composition, metabolic state, and endocrine feedback. Traditional intake regulation models often fall short of predicting changes in intake and feeding behaviors, particularly during early lactation when animals are experiencing metabolic stress. Research has shown that hepatic metabolism regulates meal size and frequency through hepatic oxidation of fuels that generate satiety signals (Allen et al., 2009). This concept, known as the hepatic oxidation theory (HOT), proposes that hepatic fuel oxidation alters signals sent from the liver to the brain via the vagus nerve, thereby influencing feed intake (Niijima, 1981; Anil and Forbes, 1988; Allen et al., 2009). Fuel availability to the liver is dependent on absorption of fuels derived from the diet or extracted from the blood, including fatty acids, amino acids, glycerol, and lactate. In dairy cattle, feed intake is limited by physical factors such as gut distension and hepatic oxidation. Allen et al. (2009) extensively reviewed hepatic fuel oxidation patterns, discovering minute-to-minute oxidation influences feeding behavior. These effects on intake and feeding behavior vary with metabolic state, as they depend on acetyl-CoA influx and capacity of the tricarboxylic acid (TCA) cycle (Oba and Allen, 2003a; Allen and Bradford, 2012; Allen, 2020). Understanding these underlying metabolic signals is essential for developing feeding strategies that support optimal intake and performance, especially during the transition period when lipid mobilization and hepatic oxidation intensify.

Among the fuels oxidized in the liver, propionate is a significant contributor to signaling satiety due to its rapid uptake during meals and its roles in gluconeogenesis and acetyl-CoA oxidation. During early lactation when glucose demands rise and hepatic acetyl-CoA accumulates from fatty acid oxidation, hypophagic effects of propionate become more pronounced. This problem is exacerbated when cows are fed higher-energy diets in early lactation, which stimulate greater ruminal propionate production and further intensify satiety signaling. Indeed, DMI was reduced by 20% when propionate was infused in early lactation cows, and this hypophagic effect increased as the concentration of acetyl-CoA in the liver increased (Stocks and Allen, 2012). In cannulated mid-lactation dairy cows, intraruminal infusion of iso-osmotic mixtures containing increasing proportions of propionic acid resulted in linear declines in metabolizable energy (ME), DMI, and meal size, while intervals between meals increased (Oba and Allen, 2003b). These results suggest that propionate infusion decreased feed intake due to fuel-specific mechanisms that trigger satiety. In a companion study, hypophagic effects of propionate infusion were greater for early-lactation cows compared to mid-lactation cows and were associated with elevated FFA and reduced BHB concentrations as infusion of propionate decreased (Oba and Allen, 2003a).

Fatty acid oxidation has received attention among recent work on early lactation dairy cows, with evidence linking hepatic fatty acid oxidation to suppressed feed intake. As dairy cows mobilize body fat during the transition period, an influx of FFA enters the liver for oxidation. Increased fatty acid oxidation is associated with suppressed intake, potentially due to changes in hepatic metabolism that signal satiety. Indeed, Piantoni et al. (2015) demonstrated that hepatic acetyl-CoA concentrations post-feeding were inversely correlated to subsequent DMI over a 4-hour period in both early and late lactation cows. Likewise, increased duodenal flow of

unsaturated fatty acids suppressed DMI compared to control (24.1 vs. 27.3 kg/d) by reducing meal size (2.28 vs. 2.51 kg/meal), likely mediated through enhanced hepatic oxidation (Harvatine and Allen, 2006). Furthermore, when glucose precursors are limited and fatty acid oxidation increases, TCA cycle intermediates decline, which limits the liver's capacity to completely oxidize acetyl-CoA. This results in acetyl-CoA accumulation and increased hepatic export of ketone bodies, further reinforcing satiety signals (Roche et al., 2009). This situation is especially relevant during the transition period when cows experience NEB, extensive lipid mobilization, and reliance on hepatic oxidation for energy; however, extensive oxidation of fatty acids in the liver may lead to excessive storage of triglycerides in the liver, resulting in hepatic lipidosis (Bobe et al., 2004).

These nutrient-derived hepatic signals interact closely with hormonal and endocrine mechanisms to influence DMI. Hormones including insulin, glucagon, somatotropin, and leptin influence hepatic oxidation by altering supply of acetyl-CoA and pool of TCA cycle intermediates available for oxidation (Allen, 2020). Insulin, for instance, plays a multifaceted role in regulating DMI and overall metabolism during the transition period. Under normal circumstances, insulin stimulates lipogenesis and suppresses lipolysis; however, during the transition period, dairy cows often exhibit a state of insulin resistance, diminishing peripheral tissue responsiveness to insulin and thereby increasing lipolysis and elevating concentrations of circulating FFA (De Koster and Opsomer, 2013; Allen, 2020). As reductions in DMI occur due to hepatic and hormonal signaling, there are significant implications for animal health and metabolic disease prevalence during the transition period.

Consequences of Declining DMI on Metabolic Disorders

Declining DMI during the transition period exacerbates NEB, leading to excessive lipid mobilization and elevated FFA concentrations, which increases risks of metabolic disorders. The blood metabolites most commonly measured to assess NEB and related disorders include FFA and BHB (Duffield et al., 2009). Early detection of metabolic diseases has gained interest due to their substantial impact on animal health and herd profitability. Metabolic diseases influenced by nutritional management include ketosis, metritis, displaced abomasum, hypocalcemia, retained placenta, and lameness (Oetzel, 2014). Changes in DMI and feeding behaviors leading up to calving have been linked to increased risk of metabolic disorders and disease incidence (Pérez-Báez et al., 2019a; b). For every 1 kg decrease in DMI during the week before calving, cows are 3 times more likely to subsequently be diagnosed with metritis postpartum (Huzzey et al., 2007). Additionally, cows that later developed metritis had exhibited significant reductions in feeding time and DMI beginning 2 weeks before clinical diagnosis and continued to consume less during the 3-week postpartum period (Huzzey et al., 2007). Similarly, cows that developed subclinical ketosis (SCK) following calving had reduced feed intake, fewer visits and time spent at the feed bunk, and less competitive behavior during the transition period compared to healthy cows (Goldhawk et al., 2009). These changes in feeding behavior impacted metabolic health, as each 1 kg drop in intake or 10-minute reduction in feeding time significantly increased the risk of SCK by 2.2 and 1.9 times respectively, underscoring the importance of maintaining feed intake and feed bunk access before parturition (Goldhawk et al., 2009). These findings illustrate the potential for feeding behavior monitoring as a tool for early detection, while also emphasizing that maintaining DMI is critical to prevent metabolic diseases. They next sections explore

nutritional and management tools that target maintaining DMI and metabolic challenges occurring in the transition period.

Dietary and Management Strategies to Optimize Feed Intake

Controlled Energy Intake Through Fiber and Forage

Industry-standard dry cow nutritional management strategies consist of a dry period divided into far-off and close-up phases, each with distinct dietary energy targets aimed at supporting changes in metabolic demands. In early lactation, dietary energy intake rarely meets the combined demands for milk synthesis and maintenance, resulting in NEB. To adapt, cows undergo a coordinated metabolic shift characterized by insulin resistance in muscle and adipose tissue, mobilization of FFA, and increased hepatic reliance on gluconeogenic precursors to meet the demands for milk synthesis (Bell, 1995). Consequently, elevated lipid mobilization and ketone production increases the susceptibility to metabolic disorders like ketosis, hepatic lipidosis, and displaced abomasum (Drackley, 1999). Since cows experience a natural decline in DMI around calving, it was once considered logical to increase dietary energy density prepartum to compensate for the anticipated intake depression (Grummer, 1995). However, subsequent research has shown that excessive prepartum energy intake, even at similar DMI levels, leads to excessive body condition gain and predisposes cows to greater lipid mobilization and metabolic dysfunction after calving (Janovick and Drackley, 2010; Mann et al., 2015; Richards et al., 2020). These insights highlight the importance of prepartum dietary energy supply on feeding behavior and metabolic health, prompting strategies to control prepartum energy intake and align DMI with energy requirements while minimizing postpartum disorders.

Controlled-energy diets are formulated to meet but not exceed prepartum energy requirements through inclusion of less energy-dense forages such as straw or grass hay (Janovick et al., 2011). This fiber dilution approach enables ad libitum DMI while controlling the amount of energy intake, ultimately minimizing the risk of excessive body condition gain, lipid mobilization, and metabolic disorders during the transition period. Adequate effective fiber supports rumen health by stimulating salivation and chewing activity, maintaining rumen fill, and balancing fermentation dynamics. The concept of physically effective neutral detergent fiber (peNDF), a measure that accounts for particle size and chemical fiber content, provides a framework to evaluate dietary fiber adequacy and impact of fiber particle size on prepartum feeding behavior and DMI (Mertens, 1997; Zebeli et al., 2012).

These concepts have been evaluated in controlled trials and large-scale analyses, which consistently show that controlling prepartum dietary energy supply improves postpartum outcomes. Feeding a reduced-energy diet (1.5 Mcal NEL/kg DM) during the far-off period and a moderate-energy diet (1.69 Mcal NEL/kg DM) during the last 28 d leading up to parturition resulted in 2.9 to 4.3 kg/d lower prepartum DMI compared to cows fed a high-energy diet (1.75 Mcal NEL/kg DM) for 28 d or no dry period, with less of a decline in intake near calving (Rastani et al., 2005). Similarly, cows fed a controlled-energy diet (100% of NEL requirements) during the entire dry period had lower DMI compared to intermediate (125% of NEL requirements) or high (150% of NEL requirements) energy diets, yet experienced a less pronounced decline in intake near calving (Mann et al., 2015). A similar pattern was observed in cows fed a high-straw diet (32% of DM) during the entire dry period where DMI remained more constant up until calving (Janovick and Drackley, 2010). Along with changes in DMI, overfeeding energy in the dry period increased postpartum FFA and BHB concentrations,

suggesting a more severe NEB postpartum and greater risk of metabolic disorders (Mann et al., 2015; Richards et al., 2020). A recent analysis across 72 commercial herds confirmed that feeding a controlled-energy diet during the far off period is associated with lower metabolic biomarkers and improved reproductive outcomes (Kerwin et al., 2023). Cunha et al. (2025) extended these findings by evaluating dietary energy manipulation prior to the dry period and its implications on the subsequent dry period and lactation. Cows fed a low-energy diet (1.50 Mcal NEL/kg DM) during late lactation had higher DMI during the last 10 d prepartum and 21 d postpartum and increased daily rumination time compared to cows fed a high-energy diet (1.74 Mcal NEL/kg DM; Cunha et al., 2025). This work suggests that dietary energy management strategies implemented even before the dry period may influence cow performance across the transition period.

The role of forage particle size is also a critical aspect when feeding a controlled-energy diet. Excessively long forage particles can limit intake by increasing rumen fill and encouraging sorting behavior, whereas shorter particles can improve uniform intake and stabilize rumen pH (Miller-Cushon and DeVries, 2017; Coon et al., 2018). Feeding shorter wheat straw particles in prepartum diets increased prepartum DMI (15.0 vs. 15.6 kg/d), reduced sorting against long particles, and supported a more stable reticulorumen pH postpartum (Havekes et al., 2020). Reducing wheat straw particle length (2.54 vs. 5.08 cm) decreased sorting against long particles and increased sorting in favor of the medium and short particles, maintained more stable rumination, and increased milk yield in early lactation (Coon et al., 2018). While these behavioral changes can support intake and rumen stability, excessive sorting against long fiber particles and overconsumption of rapidly fermentable carbohydrates can predispose cows to subacute ruminal acidosis (SARA), which negatively impacts DMI, fiber digestion, and milk

composition (Plaizier et al., 2008). The consequences of sorting and SARA have been reviewed extensively (Plaizier et al., 2008; Miller-Cushon and DeVries, 2017).

In addition to diet dilution with fiber and forage, restricting total energy intake by limiting feed availability has also been investigated. Dann et al. (2006) found that cows fed diets meeting 80% or 100% of NEL requirements during the far off dry period had lower postpartum FFA (0.627, 0.787, and 0.793 mmol/L) and BHB (6.61, 8.13, and 9.05 mg/dL) concentrations, as well as increased DMI (2.5, 2.46, and 2.16 % of BW) compared with cows overfed at 150% of NEL requirements. Similarly, restricting energy intake to 80% of NEL requirements during the prepartum period improved postpartum DMI (22.6 vs. 20.5 kg/d), reduced postpartum hepatic TAG accumulation, and improved metabolic function compared to cows allowed ad libitum intake at 160% of NEL requirements (Douglas et al., 2006). However, feed restriction should be balanced with recognition that stressors during the close-up period, such as overcrowding, can limit feed intake and exacerbate postpartum metabolic risk (Dann et al., 2006). Overall, controlled-energy diets achieved through inclusion of low-energy forages represent a practical and effective prepartum feeding strategy to stabilize DMI, reduce lipid mobilization, and minimize metabolic disruptions during the transition period.

Diet Fermentability

Fermentability of the diet plays a central role in optimizing DMI, rumen function, and energy supply during the transition period. Diet fermentability is influenced by the type and amount of grain included within the diet, while starch digestibility is controlled by the starch type, particle size, and processing method (NRC, 2001). As a result, variation in starch fermentability directly modulates rumen dynamics, satiety signals, and DMI (Bradford and

Allen, 2007). Ruminal starch fermentability varies between starch sources, with high-moisture corn (HMC), wheat, and barley fermenting more rapidly compared to dry-ground corn (DGC) and dry-rolled corn (Knowlton et al., 1998; Oba and Allen, 2003c; Doepel et al., 2009). These feedstuffs differ in starch fermentability, with more rapidly fermentable sources like HMC producing greater ruminal propionate compared to DGC (Bradford and Allen, 2007). According to the hepatic oxidation theory, this increased propionate supply stimulates hepatic oxidation of fuels, which generates satiety signals via the vagus nerve and reduces feed intake (Allen et al., 2009).

In prepartum cows, moderate grain inclusion has gained attention for its potential role in increasing insulin concentration and influencing lipolysis. Grain provides readily fermentable carbohydrates that serve as energy precursors for rumen microorganisms (Doepel et al., 2009). Feeding additional grain prior to calving has been proposed as a strategy to improve energy status and reduce lipid-related metabolic disorders through greater ruminal propionate production and enhanced insulin secretion, which together can reduce adipose tissue lipolysis and support energy balance (Grummer, 2008). However, while moderate grain inclusion can improve diet digestibility and energy supply, excessive starch fermentability may reduce rumen fill and depress DMI due to rapid fermentation and reduced rumen distension (Allen et al., 2009). Reported effects of prepartum grain inclusion on DMI, milk yield, and hepatic lipid accumulation remain inconsistent across studies (Grummer, 2008).

In postpartum cows, rapidly fermentable starches can depress feed intake, likely due to increased supply of propionate to the liver and oxidation of acetyl-CoA sending satiety signals to the brain (Allen et al., 2009). Depressed DMI in early lactation exacerbates NEB and compromises peak milk yield. Albornoz and Allen (2018) demonstrated that feeding HMC as a

rapidly fermentable starch source compared to DGC in early postpartum diets depressed DMI and milk yield, with the greatest reductions in DMI occurring when combined with a higher starch concentration. These carryover effects diminished over time, but highlight the risk of excessive starch fermentability in the immediate postpartum period. Similarly, feeding HMC during the early postpartum period decreased DMI and NEL compared with DGC when included in a high starch diet (3.9 kg/d and 3.2 Mcal/d) than in a low starch diet (0.9 kg/d and 0.6 Mcal/d; Albornoz et al., 2019). More rapidly fermentable feed sources also reduced DMI in mid-lactation cows with HMC depressing DMI by as much as 8% without reducing digestible DMI (Bradford and Allen, 2007).

Beyond effects on intake, variation in starch fermentability has important metabolic consequences. Increasing dietary starch elevates ruminal propionate production. This is problematic during the transition period as inadequate propionate prepartum may limit energy supply, whereas excessive propionate postpartum enhances hepatic acetyl-CoA oxidation and satiety signaling, suppressing DMI (Allen et al. 2005). Since propionate is the major gluconeogenic precursor in ruminants, reduced DMI compromises glucose supply to support lactation, exacerbates NEB, and increases lipid mobilization. Excessive FFA influx to the liver, combined with greater hepatic acetyl-CoA availability from ruminal propionate, increases the risk of hepatic triglyceride accumulation when VLDL export is insufficient.

Accordingly, nutritional strategies that maintain rumen fermentability and enhance hepatic lipid export are of particular interest. Rumen protected choline (RPC) plays a central role in lipid metabolism and has been extensively reviewed (Zenobi et al., 2018). As a precursor for phosphatidylcholine, choline is required for VLDL assembly and subsequent hepatic TAG export (Overton and Waldron, 2004). Inadequate choline supply reduces phosphatidylcholine synthesis,

impairs hepatic TAG export as VLDL, and leads to excessive hepatic lipid accumulation (Yao and Vance, 1988). By facilitating VLDL export, RPC reduces the hepatic accumulation of acetyl-CoA generated from FFA oxidation and propionate metabolism. By limiting the buildup of hepatic acetyl-CoA, RPC may reduce satiety signaling derived from hepatic fuel oxidation and mitigate the hypophagic effects of highly fermentable diets. Indeed, supplementation of RPC during the transition period has been associated with improved liver function, enhanced feed intake, and metabolic adaptation (Zenobi et al., 2018b; Arshad et al., 2020a). Thus, RPC has the potential to complement starch fermentability strategies, particularly in situations where greater ruminal propionate production would otherwise intensify hepatic oxidation and suppress DMI. Collectively, this underscores the importance of balancing starch fermentability with adequate forage NDF to maintain rumen fill and stabilize DMI, thereby supporting optimal metabolic health during early lactation (Allen et al., 2009).

Calcium Status and Rumen Motility

While diet fermentability modulates propionate supply and energy balance, calcium status represents another critical determinant of DMI and metabolic resilience during the transition period. As calving approaches, demand for calcium (Ca) increases to support colostrum production and the onset of lactation. However, when blood Ca concentrations are deficient, smooth muscle function is impaired which reduces ruminal contractions and passage of digesta, the primary driver of depressed DMI observed in cows with hypocalcemia (Martinez et al., 2014; Goff et al., 2020). Reduced digesta flow limits nutrient absorption and prolongs energy deficits, which creates a challenge during the transition period as energy demands for lactation increase. Likewise, subclinical hypocalcemia compromises rumen motility, leading to depressed

DMI and impaired metabolic recovery (Martinez et al., 2012; Rodríguez et al., 2017; Seely et al., 2021). Feeding a negative dietary cation-anion difference (DCAD) diet during the prepartum period is a well-adopted strategy to induce metabolic acidosis, which stimulates Ca mobilization to meet lactation demands and reduce the incidence of hypocalcemia postpartum (Glosson et al., 2020; Zhang et al., 2022). Several meta-analyses and controlled trials confirm that negative DCAD diets improve Ca homeostasis and increase postpartum DMI by 1-2 kg/d (Charbonneau et al., 2006; Santos et al., 2019). Therefore, strategic manipulation of DCAD during the prepartum period enhances Ca availability, supports rumen motility, and ultimately improves DMI and transition cow success.

Conclusion

Nutritional management strategies such as controlling dietary energy intake through fiber and forage management, moderating starch fermentability, and optimizing calcium status each contribute to stabilizing DMI and supporting metabolic resilience during the transition period. Despite extensive research, gaps remain in connecting these nutritional approaches to underlying hepatic mechanisms that regulate satiety signaling. Highly fermentable diets depress feed intake through rapid propionate absorption and acetyl-CoA oxidation, while excessive lipid mobilization further exacerbates hepatic fuel overload. At the same time, RPC has been shown to facilitate hepatic lipid export and increase DMI, yet its interaction with diet fermentability remains unclear. Therefore, the potential for RPC to mitigate hypophagia in cows fed highly fermentable diets warrants further evaluation, and will be explored in detail in the following chapter.

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Chapter 2 - Effect of Dietary Choline and Diet Fermentability on Performance and Feeding Behavior of Postpartum Dairy Cows

Abstract

Depressed intake and hepatic lipid accumulation are major contributors to metabolic stress in transition dairy cows. Highly fermentable diets have also been shown to depress dry matter intake (DMI), compounding the risk of metabolic stress. Choline is commonly supplemented to periparturient dairy cows to facilitate hepatic lipid metabolism and support postpartum health and performance. Our objective was to evaluate effects of diet fermentability (DF) and rumen-protected choline (RPC) on postpartum DMI and performance. Multiparous Holstein cows (n = 65) were enrolled on a rolling basis according to body condition score, expected calving date (ECD), and 305-d mature equivalent milk yield, and were randomly assigned to 1 of 4 postpartum treatments in a randomized complete block design with a 2x2 factorial arrangement of starch fermentability rate (low [dry-rolled corn; LFERM] vs. high [wheat; HFERM]) and RPC (no RPC [C-] vs. RPC [C+; 30 g/d]). Prepartum RPC supplementation began 21 d before ECD in a total mixed ration for C+ cows and continued postpartum, whereas C- cows received no RPC. Cows were fed with a Roughage Intake Control (RIC) computerized feeding system during the prepartum period (Hokofarm Group, Emmeloord, The Netherlands). Postpartum cows (n = 55) were fed for 21 d postpartum in a tie-stall with a computerized feeding behavior system. Feeding behaviors, DMI, and milk yield were collected daily, and milk components were collected 2 d/wk. Data were analyzed using linear mixed models for the fixed effects of treatment, time, and their interactions using PROC MIXED in SAS (v 9.4). Means are presented with 95% confidence interval. Prepartum, C+ decreased DMI

compared with C- (14.8 kg [13.8, 15.9] vs. 17.1 kg [16.0, 18.2]). Postpartum, there was no evidence of treatment effect on DMI. Time and RPC interacted for meal size with C- increasing meal size more rapidly than C+ through d 10 and after d 15. Time and DF interacted to influence milk yield with HFERM increasing milk yield after d 3 compared with LFERM. Compared with LFERM, HFERM decreased milk fat content (4.4 % [4.0, 4.8] vs 4.8 % [4.4, 5.2]) but not fat yield. Time and DF interacted for protein content and yield; HFERM decreased protein yield from wk 1 to 3 (1.5 kg [1.3, 1.5] vs. 1.3 kg [1.2, 1.4]) but there was no difference across wk for LFERM. Compared to LFERM, HFERM decreased milk fatty acid concentrations and preformed fatty acids. There was no evidence of treatment difference on ECM and FCM. For blood metabolites, C+ decreased plasma BHB (1.0 mmol/L [0.9, 1.2] vs. 1.3 mmol/L [1.1, 1.5]) and tended to increase glucose concentration (49.0 mg/dL [46.4, 51.5] vs. 46.0 mg/dL [43.5, 48.5]) compared to C-. Time and DF interacted on BUN with HFERM increasing BUN concentration (13.5 mg/dL [12.4, 14.5] vs. 12.6 mg/dL [11.5, 13.6]). In conclusion, supplementation of RPC, even at a low rate of inclusion, decreased plasma BHB concentrations suggesting improved metabolic status postpartum. Prepartum DMI was reduced in cows supplemented with RPC, which requires further work to elucidate potential mechanisms of action showing similar results in TMR-fed cows. Diet fermentability and RPC supplementation did not interact to affect feeding behavior, which may be reflective of relatively lesser differences in starch digestibility between treatments than anticipated. Formulated starch digestibility was 60.1% for HFERM and 44.6% for LFERM, whereas actual analyzed values were 53.8% and 46.7%, respectively, suggesting that modest differences in starch fermentability may have limited treatment responses.

Introduction

Increasing the fermentability of postpartum diets depresses dry matter intake (DMI) and milk energy output in early-lactation cows (Oba and Allen, 2003a; Albornoz and Allen, 2018; Albornoz et al., 2019). This depression in intake may exacerbate negative energy balance (NEB) naturally experienced after calving, further challenging metabolic adaptation. This is likely connected to the hepatic oxidation theory (HOT), where rapid production and absorption of propionate, the primary gluconeogenic precursor in ruminants, increases hepatic fuel oxidation and acetyl-CoA oxidation, generating satiety signals and hypophagia (Allen et al., 2009). Supporting this concept, postpartum DMI is negatively associated with hepatic acetyl-CoA concentration (Stocks and Allen, 2012), and increases in hepatic acetyl-CoA and plasma free fatty acid (FFA) concentration after feeding are negatively related to short-term DMI in both postpartum and late-lactation cows (Piantoni et al., 2015).

Excessive lipid mobilization during the transition period contributes to hepatic lipid accumulation and impaired liver function (Drackley, 1999; Ospina et al., 2010). Rapid influx of FFA to the liver can exceed its oxidative capacity, leading to incomplete oxidation of fatty acids and elevated hepatic acetyl-CoA concentration. The alternative fate of acetyl-CoA is for ketone body production, which increases the risk for ketosis and related metabolic disorders (Goff and Horst, 1997; Ospina et al., 2010). Strategies that enhance hepatic lipid export and reduce intracellular triglyceride accumulation and ketone body production may therefore help sustain intake and improve metabolic efficiency in cows fed highly fermentable diets.

Supplementation with rumen-protected choline (RPC) has been shown to mitigate these challenges by supporting hepatic lipid metabolism (Zenobi et al., 2018b). Mechanistically, choline acts as a methyl donor and a precursor for phosphatidylcholine synthesis, which is

required for the assembly and secretion of very low-density lipoprotein (VLDL) particles (Overton and Waldron, 2004). Phosphatidylcholine supply supports methylation reactions and the export of hepatic triacylglycerol (TAG) as VLDL, reducing the risk of hepatic lipidosis and improving liver function in transition cows (Piepenbrink and Overton, 2003; Zenobi et al., 2018b; Arshad et al., 2023). Meta-analyses further support these benefits, reporting increased postpartum DMI and milk yield in cows supplemented with RPC during the transition period (Arshad et al., 2020; Hosseini Ghaffari et al., 2025).

Nutritional strategies that limit hepatic lipid accumulation may therefore decrease acetyl-CoA availability for oxidation during postprandial influx of propionate and alleviate hypophagic responses. By supporting hepatic lipid export, supplementation of RPC may mitigate DMI depression and performance losses in postpartum dairy cows fed a highly fermentable diet. Despite being intricately linked, the combined effects of diet fermentability (DF) and RPC supplementation on postpartum feed intake and performance have not been previously evaluated. Therefore, the objective of this study was to evaluate interactive effects of DF and RPC supplementation on postpartum dairy cow DMI, metabolism, and milk production. We hypothesized that supplementation of RPC would attenuate postpartum DMI depression resulting from highly fermentable diets.

Materials and Methods

Animals and Experimental Design

This study was conducted from January 11, 2024 to September 8, 2024, at the Kansas State University Dairy Cattle Research and Teaching Center (DTRC; Manhattan, KS). All experimental procedures were approved by the Kansas State University Institutional Animal

Care and Use Committee. Multiparous Holstein cows (n = 65) were enrolled on a rolling basis and blocked by expected calving date (ECD), body condition score (BCS; ≤ 1-unit using a 5-point scale; Wildman et al., 1982), and previous lactation 305-d mature equivalent milk yield (≤ 5,000 kg).

Prepartum

Cows were enrolled 21 d before ECD and fed a prepartum diet with RPC (30 g/d; C+) or without (C-) RPC supplementation. Treatments were mixed into a total mixed ration (TMR). The diet was formulated to meet dietary requirements of a 726 kg cow (1.43 Mcal NEL/kg DM) and to contain 13.0% CP, 46.7% aNDFom, and 12.4% starch. The RPC replaced soyhulls in C+ diets compared with C-. Actual prepartum dietary ingredients are described further in Table 1 and chemical compositions are described in Table 2.

Animals were housed in a bedded pack barn and fed individually using a Roughage Intake Control (RIC) computerized feeding system (Hokofarm Group, Emmeloord, the Netherlands) equipped with RFID collars linked to Apollo Farm Management software (AFM; Hokofarm Group, Emmeloord, the Netherlands). Collars enabled RIC bunk access according to the assigned treatment. As-fed feed intake was quantified daily from changes in starting and ending bunk weights recorded during individual visits of each cow to the RIC bunk to the nearest 0.1 kg as-fed. Number of visits to each bunk and time of individual visits were also quantified. Orts were removed daily at 1200 h, and fresh feed was offered at 1300 h and 2000 h to permit ad libitum feed intake. Bunk stocking density was maintained throughout the duration of the study between 1 and 2 cows/bunk to minimize behavioral effects on feeding behavior variables as pen stocking density changed throughout the study according to animal availability.

Postpartum

After calving, cows were randomly assigned to 1 of 4 postpartum treatments in a randomized complete block design with a 2 x 2 factorial arrangement, including starch fermentability (low [supplied via dry-rolled corn; LFERM] vs. high [supplied via dry-rolled wheat; HFERM]) and RPC (0 g/d [C-] vs. 30 g/d [C+]; ReaShure, Balchem Corp.). The postpartum dataset included 55 cows across 14 blocks after cows were excluded for calving difficulties (n = 1), or other health events (n = 9). The postpartum diets were formulated for a 628 kg cow producing 34 kg milk per day (1.61 Mcal NEL/kg DM), with 17.8% CP, 29.4% aNDFom, and 24.9% starch. The RPC replaced soyhulls in C+ diets compared with C-. Diet fermentability was modified by partially replacing dry-rolled corn with dry-rolled wheat in HFERM diets to increase starch fermentability. This substitution was estimated to increase 7-h *in vitro* starch digestibility (IVSD7) rate from 44.6% (LFERM) to 60.1% (HFERM), similar to that achieved in Albornoz et al. (2019) which was effective in depressing DMI in postpartum cows. A basal diet with common feed ingredients to all treatments was mixed daily in a Forage Express Feed Mixer (414-41B; Roto-Mix, Dodge City, KS), and treatment grain mixes (approximately 15% of total DM) were top-dressed and mixed into the basal diet by hand for each cow. Actual postpartum dietary ingredients are described further in Table 3 and chemical compositions are described in Table 4. Dry matter content of the wet corn gluten feed was determined once weekly, and that of corn silage was determined 3 times weekly. Diets were adjusted for DM content of wet ingredients accordingly. Diets were reformulated in wk 13 to maintain a consistent nutrient composition across treatments following a change in corn silage source.

Although the formulation aimed to standardize nutrient supply, decreases in crude protein and IVSD7 were observed after the reformulation due to natural variation in the new silage.

Postpartum cows were housed in a tie-stall barn and individually fed their assigned treatment diet ad libitum for 21 d. Each stall was equipped with an individual feed bin suspended from load cells capable of electronic recording of feed weights and feeding behavior as described in Mullins et al. (2012). Stalls were also equipped with individual water cups with meters. Daily orts were weighed and removed at 1000 h, and cows were fed twice daily at 1100 h and 1900 h for 110% of expected intake. Cows were milked three times daily at 0600 h, 1100 h, and 1900 h in a milking parlor. Cows were monitored daily for common postpartum metabolic disorders and infectious diseases according to DTRC protocols. Animals showing moderate or greater ketones on two consecutive urine samples (Aspen Veterinary Resources, Loveland, CO) collected 24 h apart were further evaluated with a blood β-hydroxybutyrate (BHB) test to confirm subclinical ketosis (BHB > 1.2 mmol/L; BHBCheck Plus; Portacheck, Carlsbad, CA). Cows meeting these criteria received 300 mL propylene glycol or keto-gel once daily for up to 3-4 d. If blood BHB concentration exceeded 3.0 mmol/L, treatment was supplemented with 250 mL intravenous dextrose in accordance with DTRC protocol.

Data Collection and Analyses

Daily DMI was quantified by multiplying the total feed disappearance by the calculated diet DM. Prepartum feeding behaviors were quantified as defined by Brown et al. (2022), although meal-based behaviors were determined using a 12-minute intermeal interval as the minimum time between feed bin visits to qualify as a separate meal (Mullins et al., 2012). Prepartum variables of interest included DMI, number of meals, meal size, meal length, total

eating time, eating rate, largest meal, and meal interval, and postpartum meal variables included DMI, number of meals, meal size, meal length, total eating time, largest meal, and meal interval. Postpartum raw feeding behavior data were exported and processed using a custom Microsoft Excel (Microsoft Corp, Redmond, WI) macro according to Mullins et al. (2012). Postpartum meal-based feeding behaviors were determined using a 12-minute threshold as the minimum time between feeding activity for separation of individual meals, and all meals <0.2 kg were excluded (Mullins et al., 2012).

Individual feed ingredient samples were collected once weekly throughout the experiment and frozen for subsequent analysis. Feed ingredient samples were dried in a 55°C forced air oven for 48 hours to determine DM%. Samples were ground through a 1-mm screen using a Thomas Wiley Mill (Thomas Scientific, Swedesboro, NJ) and were analyzed for chemical composition at a commercial laboratory (Dairyland Laboratories, Arcadia, WI). Crude protein (CP) content was determined using the combustion method according to the AOAC Official Method 990.03 (AOAC International, 2012). According to the AOAC Official Method 2002.04 (AOAC International, 2005), amylase-treated neutral detergent fiber (aNDF) was quantified with the modification of a sea sand filter aid and Whatman GF/C filter paper for residue collection. Values were expressed on an ash-corrected basis (aNDFom) by correcting for residual ash after fiber analysis. Ash content was measured according to the AOAC Official Method 942.05 (AOAC International, 1996). Ether extract (EE) was determined according to the AOAC Official Method 920.39 (AOAC International, 2016) using an automated Soxtec 2047 machine (Foss Analytics, Hilleroed, Denmark) and diethyl ether. Crude fat was quantified by acid hydrolysis fat (AHF) analysis using the SoxCap 2047 in combination with Soxtec extraction systems (Foss Analytics, Hilleroed, Denmark) as described in Foss Analytical AB Soxtec System

Application Note AN3902 (Foss Analytics, 2006). Dietary starch content was determined according to Starch Analysis in Animal Feed: Method workshop from 30th Annual MW AOACI Meeting and Exposition (AOAC International, 2015), with the modification of glucose analysis being completed on YSI 2700 Select Biochemistry Analyzer (YSI, Yellow Springs, OH). The procedure of Richards, et al. (1995) was utilized to determine IVSD7; modifications included inoculum made from a composite of strained ruminal fluid and strained fluid from blended ruminal solids, combined with an equivalent volume of reduced media.

Milk yield was recorded at each milking daily using the SmartDairy in-parlor monitoring system (BouMatic, Madison, WI). Milk samples were obtained weekly at 6 consecutive milkings using in-line milk sampling equipment. Samples were analyzed at AgSource Laboratories (Menomonie, WI) using a CombiFoss 7 analyzer that integrates the MilkoScan 7 RM and Fossomatic 7 modules (Foss Analytics, Hilleroed, Denmark). The MilkoScan 7 RM was used to determine milk fat, true protein, lactose, non-fat solids, milk urea nitrogen (MUN) and milk fatty acids. The Fossomatic 7 was used to determine somatic cell count (SCC). Milk net energy (NE) was calculated according to NASEM (2021). Energy correct milk (ECM) and fat corrected milk (FCM) were calculated according to the DHI Glossary (Dairy Records Management Systems, 2014). Somatic cell score (SCS) was calculated using the following formula: $\log_2$ (SCC/100,000) + 3.

Blood samples (30 mL) were collected via coccygeal venipuncture on d 3, 7, 14, and 21 ($\pm$ 1 d) postpartum before feeding (approximately 1030 h). Samples were obtained using 20-gauge needles into 10 ml evacuated tubes. A red-top tube without anticoagulants was used for serum collection (BD Vacutainer; Franklin Lakes, NJ). The tube was maintained at room temperature to allow for clot formation for 30 minutes prior to centrifugation for 15 minutes at

20°C and 2,500 x g (Fisherbrand accuSpin Max; Thermo Fisher Scientific, Waltham, MA). Two additional tubes containing potassium-EDTA or sodium fluoride/potassium oxalate were used for plasma harvest (BD Vacutainer; Franklin Lakes, NJ). Immediately following collection, tubes for plasma separation were placed on ice before centrifugation at 4°C and 2,000 x g (Fisherbrand accuSpin Max; Thermo Fisher Scientific, Waltham, MA). Serum and plasma were subsequently harvested and frozen for future analysis. Serum FFA concentration was determined using a 96-well plate colorimetric assay with commercial reagent kits (Catachem C514-0A; Catachem Inc., Oxford, CT) and absorbance was measured by spectrophotometry (BioTek PowerWave HT; Agilent, Santa Clara, CA) according to Martin et al. (2021) and Pralle et al. (2021). Insulin concentration was measured using a commercial ELISA assay (Mercodia, Uppsala, Sweden). A ChemWell-T autoanalyzer (Awareness Technology Inc., Palm City, FL) and Catachem reagent kits (Catachem Inc., Oxford, CT) were utilized to measure plasma glucose (Catachem C124-06; Pralle et al., 2021), blood urea nitrogen (BUN) (Catachem C264-03; Caputo Oliveira et al., 2020), and BHB (Catachem V444-0B; Martin et al., 2021 and Pralle et al., 2021).

Body weight (BW) was recorded at enrollment (-21 d relative to ECD), at calving, and on d 21 postpartum using a walkover scale. At the same timepoints, BCS was determined by 3 trained individuals using a 5-point scale (Wildman et al., 1982) and scores were averaged.

Statistical Analyses

Data were analyzed using the MIXED procedure of SAS (version 9.4; SAS Institute, Cary, NC). Data were analyzed as a split-plot design, with cow as the whole plot and DIM as the subplot. The model included the fixed effects of DF, RPC, time, and their interactions, and the random effects of block and cow nested within block x treatment. Denominator degrees of

freedom were approximated using the Kenward-Rogers method, and pairwise least-squares means were adjusted for multiple comparisons using the Tukey-Kramer procedure. Normality of residuals were evaluated via visual inspection of studentized residual panels, histograms, and QQ plots, and data transformations were applied if necessary. Due to health issues, two cows on LFERM C- were removed from study on d 14 and d 17, and one cow on HFERM C+ was removed on d 19. Data for these cows were included up until the day of removal. Results are reported as least-squares means with 95% confidence intervals. Treatment effects and interactions were declared significant at $P \leq 0.05$ and tendencies at $0.5 < P \leq 0.10$.

Results

Prepartum

Supplementation of C+ decreased DMI compared to C- (14.8 kg/d [13.8, 15.9] vs. 17.1 kg/d [16.0, 18.2]; $P < 0.01$; Table 5), while there was no treatment by time interaction observed ($P = 0.22$). Dry matter intake decreased with time, particularly within 14 days of the calving date ($P < 0.001$; data not shown). A tendency existed for an interaction between time and RPC for meal size ($P = 0.08$; Figure 1), with C+ decreasing meal size more rapidly over time compared to C-. Similarly, C+ decreased eating rate (0.09 kg/min [0.087, 0.099] vs. 0.10 kg/min [0.094, 0.108]; $P = 0.02$; Table 5) and largest meal (3.8 kg [3.42, 4.19]) vs. 4.3 kg [3.91, 4.74]; $P = 0.02$; Table 5) compared to C-. Concomitant with daily DMI, meal length, total eating time, largest daily meal, and eating rate decreased over time ($P \leq 0.05$). There was no evidence of treatment effects on number of meals, meal interval, meal length, or total eating time ($P > 0.23$).

Postpartum

From calving date through 21 DIM, DMI increased with time ($P < 0.001$; Table 6) with no evidence of treatment effect ($P > 0.19$). Number of meals decreased over time ($P < 0.001$; Table 6), whereas meal size, meal length, total eating time, largest meal, and meal interval increased with time ($P < 0.001$). Time and RPC interacted for meal size ($P = 0.04$; Table 6; Figure 2), with C+ decreasing meal size through d 10 and after d 15 compared with C-. The HFERM diets tended to increase largest meal size compared to LFERM (6.8 kg [6.2, 7.5] vs. 6.2 kg [5.6, 6.8]; $P = 0.07$; Table 6). There was no evidence of treatment effect on number of meals, meal length, total eating time, and meal interval ($P \geq 0.28$).

Milk yield and components results are listed in Table 7. Milk yield, milk fat content and yield, milk protein content and yield, and lactose yield increased with time ($P \leq 0.04$). Time and DF interacted to influence milk yield ($P = 0.02$; Figure 3), with HFERM increasing milk yield after d 3 compared with LFERM. Cows fed HFERM diets had decreased milk fat content (4.4 % [4.0, 4.8] vs 4.8 % [4.4, 5.2]; $P < 0.01$) compared to LFERM diets, but there was no treatment effect on milk fat yield ($P = 0.16$). Time and DF interacted to impact milk true protein yield, where HFERM decreased protein yield from wk 1 to 3 (1.5 kg [1.3, 1.5] vs. 1.3 kg [1.2, 1.4]; $P = 0.05$; Figure 4) compared to LFERM, but there was no difference across wk for LFERM. Time and DF interacted for milk true protein content ($P = 0.04$; Figure 5), where HFERM decreased milk protein content more rapidly from wk 1 to 3 than LFERM. There was no observed treatment effect on lactose percent ($P > 0.18$), although HFERM tended to increase lactose yield compared to LFERM (1.9 kg/d [1.8, 2.1] vs. 1.8 kg/d [1.7, 2.0]; $P = 0.06$).

Diet fermentability, RPC, and time interacted on SCS ($P = 0.04$; Table 7; Figure 6) whereby HFERM C+ and LFERM C- decreased from wk 1 and 2, and SCS for the other 2

treatments continued declining after wk 2. Compared to LFERM, HFERM increased MUN (11.6 mg/dL [10.8, 12.5] vs. 10.5 mg/dL [9.7, 11.2]; $P < 0.01$; Table 7). Additionally, C+ increased MUN concentration compared to C- (11.4 mg/dL [10.6, 12.2] vs. 10.6 mg/dL [9.9, 11.4]; $P = 0.04$; Table 7). Regardless of treatment, MUN decreased with time ($P < 0.01$). The HFERM diet decreased milk NE compared to LFERM (0.78 Mcal/kg [0.74, 0.82] vs. 0.83 Mcal/kg [0.79, 0.86]; $P = 0.02$; Table 7). Milk NE decreased with time ($P < 0.01$). There was an overall time effect on ECM and FCM which increased from wk 1 to wk 2 ($P < 0.01$; data not shown) and plateaued after wk 2. There was no evidence of treatment difference on ECM or FCM ($P = 0.20$). There was no evidence of treatment effect on feed efficiency ($P > 0.35$).

Milk fatty acid (FA) concentrations are reported in Table 8. The HFERM diets decreased the content of monounsaturated fatty acid (MUFA), saturated fatty acids (SFA), short chain fatty acid (SCFA), medium chain fatty acid (MCFA), long chain fatty acid (LCFA), and preformed FA compared with LFERM ($P < 0.05$). Similarly, HFERM tended to decrease unsaturated fatty acid (UFA; $P = 0.07$) and mixed FA ($P = 0.06$) concentration compared with LFERM. Diet fermentability and RPC tended to interact for de novo FA content ($P = 0.10$); pairwise comparisons among individual treatments were not significant ($P > 0.10$). Diet fermentability, RPC, and time interacted to affect mixed FA content ($P = 0.03$; Figure 7), where LFERM C+ increased mixed FA on wk 1 compared to HFERM C+ ($P = 0.05$) with no difference across other treatments or wks. The proportions of MUFA, UFA, and preformed FA all changed with time ($P < 0.01$) with a significant reduction in concentration from wk 2 to wk 3 ($P < 0.01$), whereas polyunsaturated fatty acid (PUFA), SCFA, and de novo FA composition decreased from wk 1 and wk 2 ($P < 0.001$) and stabilized thereafter. There was a time effect for SFA and mixed FA content ($P < 0.001$) whereby their content progressively declined with time ($P < 0.05$). The

percentage of MCFA and LCFA in the milk fat tended to decrease from wk 1 to wk 2 ($P < 0.10$), and further declined from wk 2 to wk 3 ($P < 0.01$). Although trans fatty acid (TFA) content differed over time ($P < 0.01$), no pairwise differences were detected between weeks.

For milk FA yields (Table 9), diet fermentability and RPC tended to interact for de novo FA and mixed FA yields ($P < 0.08$), although pairwise comparisons among individual treatments were not significant ($P > 0.10$). The HFERM diets tended to decrease preformed FA yield ($P = 0.09$) compared to LFERM. Diet fermentability, RPC, and time tended to interact for mixed FA yield ($P = 0.09$); pairwise comparisons among individual treatments were not significant ($P > 0.10$). There was no evidence of diet fermentability or RPC treatment effect ($P > 0.10$) on MUFA, PUFA, SFA, UFA, SCFA, MCFA, LCFA, or TFA yields. There was a time effect ($P < 0.001$) on MUFA, SFA, total UFA, MCFA, and LCFA, with overall yields of these FA categories increasing from wk 1 to wk 2 ($P < 0.001$) and stabilized thereafter. There was a time effect on SCFA yield ($P < 0.001$) with a tendency for yields to increase from wk 1 to wk 2 ($P = 0.10$), and from wk 2 to wk 3 ($P = 0.08$).

Individual milk FA content for C14 to C18 chain lengths are presented in Table 8 and yields are presented in Table 9. Diet fermentability, RPC, and time tended to interact for milk C14:0 content ($P = 0.10$), where LFERM C+ tended to have greater milk C14:0 content on wk 1 compared to HFERM C+ ($P = 0.08$) but did not differ from the other 2 treatments. Pairwise differences were not detected on wk 2 or wk 3. Time affected milk C16:0 content ($P = 0.06$) whereby C16:0 decreased from wk 2 to wk 3 ($P = 0.05$). Compared to LFERM, HFERM decreased milk C16:0 content ($P = 0.02$). Supplementation of C+ tended to increase milk C16:0 content compared to C- (1.01 % [0.89, 1.14] vs. 0.93 % [0.81, 1.05]; $P = 0.10$). Percentage of milk C18:0 content differed by wk ($P < 0.01$), where milk C18:0 content decreased from wk 2 to

wk 3 ($P = 0.01$). The HFERM diets decreased milk C18:0 content compared to LFERM (0.65 % [0.59, 0.71] vs. 0.72 % [0.66, 0.78]; $P = 0.01$). Concentration of milk C18:1 content decreased with time ($P < 0.01$). The HFERM diets tended to decrease C18:1 compared to LFERM (1.28 % [1.15, 1.42] vs. 1.40 % [1.26, 1.53]; $P = 0.07$). For individual milk FA yields, diet fermentability and RPC interacted for milk C14:0 yield ($P = 0.05$), although pairwise comparisons among individual treatments were not significant ($P > 0.10$). From wk 1 to wk 2, yields of C14:0, C16:0, C18:0, and C18:1 increased ($P < 0.01$) and stabilized thereafter.

Results for blood metabolite concentrations are presented in Table 10. Supplementation of C+ decreased plasma BHB concentration compared to C- (1.02 mmol/L [0.87, 1.19] vs. 1.31 mmol/L [1.12, 1.52]; $P = 0.02$). There was no evidence of difference in BHB concentration across time ($P = 0.14$) or between DF treatments ($P = 0.16$). Time and DF interacted to impact BUN ($P < 0.01$; Figure 8) where HFERM increased BUN compared to LFERM on d 7 (15.01 mg/dL [13.76, 16.26] vs. 12.57 mg/dL [11.33, 13.80]; $P = 0.01$) but not on any other day. Supplementation of C+ tended to increase plasma glucose concentration compared to C- (49.0 mg/dL [46.4, 51.5] vs. 46.0 [43.5, 48.5]; $P = 0.07$). Concentration of blood metabolites including BUN, glucose, FFA, and insulin changed with time ($P < 0.01$). Concentration of BUN changed with time ($P < 0.01$), increasing from d 3 to 7, decreasing from d 7 to 14, and increasing from d 14 to 21. Concentrations of glucose and insulin changed with time ($P < 0.01$), decreasing from d 3 to 7 and increasing after d 7. Concentration of FFA changed with time ($P < 0.01$), increasing from d 3 to 7 and decreasing after d 7. There was no evidence of treatment differences in plasma insulin or serum FFA concentrations ($P > 0.44$).

Body weight (BW), BW change, and body condition score (BCS) changes are reported in Table 11 for the prepartum period and in Table 12 for the postpartum period. There was no

evidence of difference between treatments for prepartum BW at enrollment ($P = 0.40$) or for BW change ($P = 0.50$), and BCS change ($P = 0.89$) during the prepartum period. Furthermore, there was no evidence of treatment effects on postpartum BW at study completion ($P = 0.16$) or for BW change ($P = 0.37$) and BCS change ($P = 0.14$) during the postpartum period.

Discussion

Prepartum

In the present study, prepartum DMI decreased progressively as calving date approached, consistent with previous observations in transition cows (Hayirli et al., 2003; Grummer et al., 2004; Santos et al., 2024). Cows fed C+ consumed less DMI compared to C- (14.8 vs. 17.1 kg/d) throughout the prepartum period, which aligns with Holdorf et al. (2023). Prepartum DMI responses to RPC supplementation have been inconsistent across studies. Most reports show no effect of RPC on prepartum DMI (Zenobi et al., 2018a; Bollatti et al., 2020a; Hosseini Ghaffari et al., 2025), whereas a meta-analysis indicated a modest increase (Arshad et al., 2020). In those studies, RPC was typically top-dressed rather than incorporated into the TMR (Zenobi et al., 2018a; Bollatti et al., 2020a; Arshad et al., 2023). In contrast, both the present study and that of Holdorf et al. (2023) mixed RPC directly into the TMR. It is unclear if the inclusion of RPC directly into a TMR may be a contributing factor to the depression in overall DMI, but further investigation may be required if other studies support this result.

The reduction in DMI among cows receiving RPC was accompanied by smaller meal size, slower eating rate, and reduced largest meal, while there was no evidence of difference in the number and duration of meals. This pattern suggests that the decrease in intake was not driven by fewer or shorter feeding events, but by a reduction in the quantity of feed consumed

per meal. Total DMI is a function of meal size and intermeal interval (Oba and Allen, 2003a; Allen et al., 2009), so the reduction of DMI within each meal suggests that the mechanism of DMI reduction is occurring within a meal. The smaller meal size and lesser eating rate in C+ cows, despite no change in meal duration, may reflect differences in RPC palatability when mixed in a TMR, or could suggest that there are other mechanisms relaying satiety signals within a meal for cows offered RPC (Allen, 2000; Allen and Bradford, 2012). Although the exact mechanism remains unclear, this is an area that merits further investigation.

Interestingly, despite reduced DMI in the prepartum period, cows receiving RPC maintained similar performance postpartum. Although this pattern initially suggested the possibility of improved feed efficiency, calculated feed efficiency indicated no evidence of treatment differences. The decline in feed intake as parturition approaches mentioned previously is accompanied by shifts in feeding behaviors, including number of meals, meal length, meal size, and total eating time (Huzzey et al., 2005; Holdorf et al., 2023); however, limited research exists that quantifies detailed feeding behavior metrics in prepartum dairy cows. Given the relationship we have observed between meal size, eating rate, and overall DMI in prepartum dairy cows, further research into this area is warranted.

Postpartum

Postpartum DMI increased with DIM, as expected during early lactation, but was not affected by DF, RPC supplementation, or their interaction. From the perspective of diet fermentability, more rapidly fermentable starch sources typically depress DMI postpartum, likely through more rapid ruminal starch degradation and propionate production (Oba and Allen, 2003a; Albornoz and Allen, 2018). Feeding more rapidly fermentable high-moisture corn vs.

dry-ground corn to postpartum cows decreased DMI 2.4 kg/d, which corresponded with a 15% difference in 7-h *in vitro* starch digestibility between diets (Albornoz and Allen, 2018). The absence of DMI reduction in HFERM for the present study compared with previous experiments may be partially explained by the lesser divergence in 7-hr starch fermentability than anticipated (~5% vs. targeted 15%), which likely limited the postprandial propionate production and hepatic oxidation. In alignment with our data, DMI was not different between grazing cows in early lactation fed ground corn or crushed wheat, despite a 7% difference in 7-h *in vitro* starch digestibility between grains (Albornoz et al., 2024). The lesser starch digestibility rate than anticipated in our study may have been a byproduct of coarser particle size of the dry-rolled wheat used within the present study compared with the 1-mm ground wheat used in the *in vitro* assay that informed diet formulation (Herrera-Saldana et al., 1990). Smaller particle sizes enhance microbial attachment and enzymatic access to starch granules, hereby increasing the rate and extent of ruminal starch degradation (Gallo et al., 2018). Taking these factors into consideration, there may have been insufficient differences in fermentation rates and corresponding portal-drained propionate influx between diets to produce differences in HOT-mediated hypophagic response (Allen and Bradford, 2012).

The absence of RPC effect on postpartum DMI aligns with previous studies supplementing RPC during the transition period, which typically report no differences in postpartum feed intake (Piepenbrink and Overton, 2003; Zenobi et al., 2018a; Bollatti et al., 2020a). A meta-analysis of RPC supplementation during the transition period found a linear increase in postpartum DMI with increasing choline ion dose, with an increase of 0.5 kg/d in postpartum DMI when 12.9 g/d of choline ion is supplemented (Arshad et al., 2020). In a more recent meta-analysis, a nonlinear increase in overall DMI across the transition period was

observed, with a maximal response of 0.48 kg/d greater DMI at doses of 13 to 14 g/d of choline chloride (Hosseini Ghaffari et al., 2025). In the present study, the diets were formulated to supply about 30 g/d of rumen-protected product (6.4 g/d of choline ion), which is one of the lowest supplemental doses of choline ion reported in the literature and is below the dose range predicted to elicit the maximal DMI response. This lower choline ion dose, in combination with the lesser divergence in starch fermentability achieved between DF treatments, may have limited the overall metabolic response needed to increase feed intake as hypothesized. Consequently, these factors together likely contributed to the absence of a detectable interaction effect of RPC and DF on postpartum DMI.

Feeding behavior variables provide additional insight into the regulation of total DMI postpartum. An interaction between RPC and time was detected for meal size, where C+ had smaller meals through d 10 and after d 15 compared with C-, despite no evidence of differences between treatment main effects for daily DMI and eating time. To our knowledge, feeding behavior responses to RPC supplementation have not been evaluated previously in postpartum cows beyond measurement of total DMI. We hypothesized average meal size would be lesser for HFERM than LFERM in a corresponding manner to expected daily DMI, consistent with Oba and Allen (2003d), because greater starch fermentability increases the rate of propionate production and can suppress intake via hepatic oxidation feedback. The moderate starch inclusion in HFERM was likely insufficient to elicit a HOT-mediated reduction in meal size (Allen and Bradford, 2012). Furthermore, largest meal size was greater in HFERM compared to LFERM, although average meal size did not differ. This is the first report of largest meal size for postpartum cows in the literature. While Brown et al. (2022) reported largest meal in mid-

lactation Holstein cows, its biological relevance for the transition period remains unclear given the absence of overall difference in daily DMI.

Feeding RPC is a key tool on dairy farms to reduce ketone body formation through reduction of hepatic lipid accumulation. In our study, the average BHB concentration for C+ was below the subclinical ketosis threshold of 1.2 mmol/L, whereas C- cows exceeded this value, suggesting meaningful reduction in ketosis risk (Duffield et al., 2009; McArt et al., 2012). In a recent meta-analysis, feeding 8.1 g/d of dietary choline ion (range of 5.6-25.2 g/d) to postpartum dairy cows resulted in the lowest concentration of circulating BHB (Arshad et al., 2020). Few studies have evaluated choline ion doses less than 8.5 g/d. In those studies, choline ion was supplemented between 6.0 and 8.4 g/d, but BHB was not reported (Hartwell et al., 2000; Xu et al., 2006). Interestingly, mean BHB concentrations in the present study (1.0-1.3 mmol/L) were greater than those typically reported in other RPC studies (0.5-0.9 mmol/L; Bollatti et al., 2020b; Arshad et al., 2023; Holdorf et al., 2023). The difference cannot be fully explained by diet composition as diets across these studies and the present study were similar. The greater BHB concentrations observed in our study may instead reflect the fact that our cows produced more milk, fat, and protein than the cows in almost all of the other studies that have evaluated RPC supplementation (Arshad et al., 2020). Overall, the reduction in BHB observed in the present study at 6.4 g/d of choline ion supports that even minimal supplementation can enhance hepatic lipid metabolism.

Beyond effects on ketone body formation, RPC tended to increase plasma glucose concentration postpartum, suggesting improved hepatic glucose output or reduced peripheral glucose utilization. Although other studies have reported no difference in plasma glucose concentrations across varying RPC doses (Piepenbrink and Overton, 2003; Bollatti et al., 2020b),

a recent meta-analysis showed that increasing choline ion supplementation linearly increased postpartum plasma glucose concentrations (Arshad et al., 2020). Mechanistically, RPC supplementation has been shown to upregulate hepatic genes related to glucose export (GLUT2) and gluconeogenesis while reducing pyruvate carboxylase expression during early lactation, suggesting improved hepatic energy status and reduced need of pyruvate carboxylase for gluconeogenic activity (Goselink et al., 2013). The observed tendency for greater glucose concentration observed in our study supports that even low-dose RPC may enhance hepatic glucose metabolism during early lactation.

Despite differences in plasma glucose and BHB concentrations, there was no evidence of treatment effects on plasma FFA, BW change, or BCS change, indicating similar adipose mobilization across treatments. Furthermore, plasma BUN was greater for HFERM compared with LFERM, consistent with greater MUN observed for HFERM. This may reflect the 0.5% greater CP content of the HFERM diet and the more rapid ruminal degradability of wheat starch and protein compared with corn (Herrera-Saldana et al., 1990). This increased degradability would provide more rapidly available ruminal ammonia to support microbial growth or to be absorbed and converted to urea in the liver, resulting in elevated plasma BUN concentration. Collectively, these responses indicate that the more fermentable diet altered ruminal nitrogen dynamics and may have improved the synchrony between ruminal energy and nitrogen availability.

Consistent with these differences in nitrogen metabolism, milk production and milk components in the present study were primarily affected by DF. In particular, HFERM progressively increased milk yield more with time than LFERM through the first 3 wk of lactation. This is a similar response as that observed in post-transition early-lactation cows fed

wheat grain, which increased milk yield 1.5-1.7 kg/d compared to cows fed barley or corn (Mosavi et al., 2012). However, our data contrasts with Albornoz and Allen (2018), where postpartum cows housed in confinement systems and fed high moisture corn had decreased milk yield compared with those fed dry-ground corn. Cows in that study had depressed DMI, which was likely a primary causative agent of the reduced milk yield. In post-transition early-lactation cows, milk yield did not differ between cows fed high moisture corn or dry-ground corn, although high moisture corn slightly reduced DMI at a higher starch concentration, consistent with greater ruminal starch fermentability and hepatic oxidation-mediated satiety (Oba and Allen, 2003a). As mentioned previously, the 7-hr digestibility rates of the experimental diets in our study (~5%) was less divergent than that reported by Albornoz and Allen (15%; 2018), which may be why we observed different results. The moderate starch concentrations and difference in fermentability rates in the present study likely improved ruminal starch utilization without triggering hepatic satiety responses when propionate accumulation is excessive (Oba and Allen, 2003b), resulting in greater milk yield without intake depression.

The corresponding reduction in milk fat concentration and fatty acid concentration in HFERM cows reflects a dilution effect from increased milk yield. A similar pattern was observed in early postpartum cows fed steam-flaked corn compared to cracked corn, where milk yield increased by 2.3 kg/d and milk fat concentration decreased, resulting in unchanged fat yield (Dann et al., 1999). In contrast, more rapidly fermentable high-moisture corn compared to dry-ground corn did not alter milk fat concentration, but rather decreased overall milk yield and corresponding fat yield (Oba and Allen, 2003d). Furthermore, the increase in milk yield without an increase in fat yield in the present study reduced milk energy output per kg of milk for HFERM compared to LFERM (0.78 vs. 0.83 Mcal/kg). Similar reductions in milk energy

concentration have been reported when dietary starch fermentability increases, without classical milk fat depression (Albornoz et al., 2019).

Considering these changes in milk fat content, we considered whether the HFERM diet resulted in milk fat depression. High dietary starch concentrations and rapidly fermentable diets can depress milk fat and alter milk fatty acid profile due to shifts in ruminal fermentation and biohydrogenation (Bauman and Griinari, 2003; Liu et al., 2023). In classical milk fat depression, the proportion of de novo FA synthesized in the mammary gland declines, whereas preformed FA from plasma uptake typically increase (Bauman and Griinari, 2003). Interestingly, HFERM decreased the proportion of milk preformed FA in the present study without an effect on de novo FA content, opposite to this typical pattern. Lactose yield tended to be greater for HFERM than LFERM, suggesting that increased starch fermentability may have redirected energy toward lactose synthesis and milk volume. With similar DMI and dietary EE content, and no evidence of treatment differences in plasma FFA, dietary supply of fat nor greater adipose mobilization are likely explanatory factors to explain the milk fat responses observed herein. Although fatty acid yields were not statistically evaluated, these patterns suggest that differences in milk fat composition likely reflect dilution from increased milk yield rather than altered lipid synthesis, and highlight lactose synthesis as an area for further investigation.

Conclusion

Supplementing RPC reduced DMI prepartum, which requires further investigation to elucidate potential mechanisms underlying similar responses in TMR-fed cows. Contrary to our hypothesis, postpartum diet fermentability and RPC supplementation did not interact to affect DMI or feeding behavior, which may reflect relatively lesser differences in starch digestibility

rates and reduced rates of RPC supplementation compared with formulated rates. Supplementation of RPC at only 30 g/d decreased postpartum plasma BHB concentration below subclinical ketosis thresholds, demonstrating the utility of even low doses of choline to support metabolic adaptation in high-producing dairy cows. In addition, feeding a more rapidly fermentable diet using wheat as a substitute for corn increased milk yield, but failed to alter fat yield. Overall, these findings suggest that moderate increases in starch fermentability rates using wheat can enhance milk yield without compromising intake, and that RPC supports metabolic adaptation without altering total feed intake. Future studies should evaluate greater contrasts in starch fermentability and increased choline supply to determine potential interactive effects on feed intake, feeding behavior, and performance.

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Tables

Table 1. Ingredients of prepartum experimental diets for multiparous Holstein cows fed a control (C-) or diet containing rumen-protected choline (C+; RPC), expressed as % of diet DM

Item	Treatment[1]			
	C-		C+	
	WK 1-12	WK 13-34	WK 1-12	WK 13-34
Ingredient, % of DM				
Corn silage	17.64	13.66	17.64	13.66
Wet corn gluten feed	15.51	13.84	15.51	13.84
Grass hay	46.11	46.10	46.11	46.10
Grain mix				
Common grain mix ingredients[2]	20.52	26.20	20.52	26.20
RPC	-	-	0.22	0.20
Soyhulls	0.22	0.20	-	-

[1] C− = 0 g/d of RPC; C+ = 30 g/d of RPC (ReaShure, Balchem Corp., Montvale, NJ)
[2] Common grain mix (average % of DM): 7.92% soybean meal, 7.11% dry-ground corn, 7.42% SoyChlor (Landus Cooperative, Jefferson, IA), 0.12% NutriTek (Diamond V, Cedar Rapids, IA), 0.17% salt, 0.13% NiaShure (ReaShure, Balchem Corp., Montvale, NJ), 0.04% Zinpro 4 Plex C (Zinpro, Eden Prairie, Minnesota), 0.02% Zinpro Avail Zn 120 (zinc amino acid complex; Zinpro), 0.07% magnesium oxide, 0.02% selenium, 0.01% Rumensin (Elanco Animal Health, Greenfield, IN), 0.01% Biotin, 0.28% Vitamin E 20,000 IU/lb, 0.03% Vitamin A 30,000 IU/gram, 0.01% Vitamin 30,000 IU/gram

Table 2. Nutrient composition of prepartum experimental diets for multiparous Holstein cows fed a control (C-) or diet containing rumen-protected choline (C+; RPC), expressed as % of diet DM

	Treatment[1]			
	C-		C+	
Item	WK 1-12	WK 13-34	WK 1-12	WK 13-34
Nutrient, % of DM[2]				
DM	55.48	57.45	55.53	57.58
CP	13.83	13.72	14.35	14.25
aNDFom	43.96	42.89	43.69	42.91
Forage NDF	36.92	35.47	36.92	35.47
Starch	10.63	12.89	10.43	12.24
EE	3.19	3.04	3.26	3.16

[1] C− = 0 g/d of RPC; C+ = 30 g/d of RPC (ReaShure, Balchem Corp., Montvale, NJ)
[2] aNDFom = amylase NDF OM; EE = ether extract

Table 3. Ingredients of postpartum experimental diets fed to multiparous Holstein cows, formulated with low (LFERM; dry-rolled corn) or high (HFERM; dry-rolled wheat) starch fermentability, with (C+) or without (C-) rumen-protected choline (RPC), expressed as % of diet DM

	Treatment[1]							
	LFERM C-		LFERM C+		HFERM C-		HFERM C+	
Item	WK 1-12	WK 13-34	WK 1-12	WK 13-34	WK 1-12	WK 13-34	WK 1-12	WK 13-34
Ingredient, % of DM								
Corn silage	25.32	22.52	25.24	22.41	25.41	22.54	25.27	22.42
Alfalfa hay	19.65	20.25	19.52	20.15	19.66	20.26	19.55	20.16
Grass hay	7.29	7.19	7.27	7.15	7.32	7.19	7.28	7.15
Wet corn gluten feed	3.45	3.51	3.44	3.50	3.46	3.52	3.44	3.50
Cottonseed	3.95	3.88	3.94	3.86	3.96	3.88	3.94	3.86
Base mix[2]	24.92	27.63	24.85	27.50	25.02	27.65	24.88	27.51
Top dress								
Dry-rolled corn	15.28	14.88	15.62	15.30	3.90	3.84	4.02	3.96
Dry-rolled wheat	-	-	-	-	11.13	10.97	11.49	11.31
RPC[3]	-	-	0.14	0.13	-	-	0.13	0.13
Soyhulls	0.15	0.14	-	-	0.14	0.14	-	-

[1] LFERM C- = low fermentability, no RPC; LFERM C+ = high fermentability, RPC; HFERM C- = low fermentability, no RPC; HFERM C+ = high fermentability, RPC

[2] Base mix (average % of DM): 9.53% dry-ground corn, 9.45% Soy Plus, 3.38% soybean meal, 0.62% Megalac (Arm and Hammer Nutrition, Princeton, NJ), 0.41% blood meal, 0.06% Smartamine (Adisseo Inc., Antony, France), 1.12% micromineral and vitamin premix (MKC Cooperative, Moundridge, KS), 0.99% sodium bicarbonate, 0.68% calcium carbonate

[3] ReaShure (Balchem Corp., Montvale, NJ)

Table 4. Nutrient composition of postpartum experimental diets fed to multiparous Holstein cows, formulated with low (LFERM; dry-rolled corn) or high (HFERM; dry-rolled wheat) starch fermentability, with (C+) or without (C-) rumen-protected choline (RPC), expressed as % of DM

	Treatment[1]							
	LFERM C-		LFERM C+		HFERM C-		HFERM C+	
Item	WK 1-12	WK 13-34	WK 1-12	WK 13-34	WK 1-12	WK 13-34	WK 1-12	WK 13-34
Nutrient, % of DM[2]								
DM	60.47	64.95	60.80	65.49	60.10	64.83	60.66	65.42
CP	17.18	16.65	17.11	16.57	17.71	17.14	17.64	17.09
aNDFom	27.88	27.59	27.67	27.04	28.34	27.66	27.99	27.41
Forage NDF	21.86	20.46	21.77	20.36	21.92	20.48	21.80	20.37
Starch	25.05	24.96	23.83	25.42	23.42	24.96	23.33	24.79
IVSD7	50.18	43.68	48.21	43.94	56.43	50.94	55.21	51.44
EE	4.72	4.65	4.72	4.65	4.52	4.37	4.53	4.46

[1] LFERM C- = low fermentability, no RPC; LFERM C+ = high fermentability, RPC; HFERM C- = low fermentability, no RPC; HFERM C+ = high fermentability, RPC
[2] aNDFom = amylase NDF OM; IVSD7 = in-vitro starch digestibility 7 hr; EE = ether extract

Table 5. Dry matter intake and feeding behavior of multiparous Holstein cows during the last 3 weeks prepartum fed a control (C-) or diet containing rumen-protected choline (C+; RPC)

	Treatment[1]		*P*-value		
Item	C−[2]	C+[3]	Trt	Time	Trt x Time
DMI, kg/d	17.1 [16.0, 18.2]	14.8 [13.8, 15.9]	<0.01	<0.001	0.22
Meals, n/d	10.5 [9.97, 11.04]	10.5 [9.92, 10.98]	0.86	0.51	0.35
Meal interval, min	117.0 [110.6, 122.8]	118.0 [111.8, 123.9]	0.77	0.42	0.95
Meal length, min	16.7 [14.56, 18.95]	15.5 [13.51, 17.68]	0.23	<0.001	0.27
Meal size, kg	1.7 [1.51, 1.82]	1.4 [1.29, 1.57]	0.01	<0.001	0.08
Eating rate, kg/min	0.1 [0.094, 0.108]	0.09 [0.087, 0.099]	0.02	0.05	0.59
Total eating time, min	172.0 [154.3, 189.5]	162.0 [144.7, 179]	0.23	<0.001	0.58
Largest meal, kg	4.3 [3.91, 4.74]	3.8 [3.42, 4.19]	0.02	<0.001	0.48

[1] Treatment means presented as least square means with 95% confidence intervals
[2] C− = 0 g/d of RPC (ReaShure, Balchem Corp., Montvale, NJ)
[3] C+ = 30 g/d of RPC (ReaShure, Balchem Corp., Montvale, NJ)

Table 6. Dry matter intake and feeding behavior of multiparous Holstein cows during the first 3 weeks postpartum fed a low (LFERM; dry-rolled corn) or high (HFERM; dry-rolled wheat) fermentable diet, with (C+) or without (C-) rumen-protected choline (RPC)

Item	Treatment[1, 2]				*P*-value						
	LFERM C-	LFERM C+	HFERM C-	HFERM C+	Ferm	Chol	DIM	FxC	FxD	CxD	FxCxD
DMI, kg/d	19.1 [17.5, 20.6]	18.9 [17.3, 20.5]	20.4 [18.8, 22.0]	19.1 [17.4, 20.7]	0.28	0.29	<0.001	0.40	0.30	0.19	0.87
Meals, n/d	13.9 [12.9, 14.8]	14.3 [13.3, 15.2]	13.9 [12.9, 14.8]	13.9 [12.9, 14.9]	0.73	0.61	<0.001	0.71	0.95	0.92	0.92
Meal size, kg	2.4 [2.1, 2.7]	2.3 [2.0, 2.6]	2.6 [2.3, 2.9]	2.4 [2.1, 2.7]	0.17	0.35	<0.001	0.69	0.41	0.04	0.68
Meal length, min	17.6 [15.8, 19.5]	16.6 [14.8, 18.4]	16.9 [15.1, 18.7]	17.1 [15.2, 19.0]	0.89	0.63	<0.001	0.50	0.28	0.80	0.44
Total eating time, min/day	237.0 [218.8, 255.2]	231.0 [212.8, 249.2]	230.1 [211.9, 248.2]	231.9 [213.1, 250.8]	0.72	0.8	<0.001	0.64	0.55	0.96	0.38
Largest meal, kg	6.0 [5.3, 6.8]	6.4 [5.6, 7.1]	7.1 [6.3, 8.0]	6.5 [5.8, 7.4]	0.07	0.76	<0.001	0.18	0.83	0.16	0.89
Meal interval, hr	1.4 [1.3, 1.5]	1.4 [1.3, 1.5]	1.5 [1.4, 1.6]	1.4 [1.3, 1.5]	0.67	0.61	<0.001	0.92	0.92	0.98	0.95

[1] LFERM C- = low fermentability, no RPC; LFERM C+ = high fermentability, RPC; HFERM C- = low fermentability, no RPC; HFERM C+ = high fermentability, RPC

[2] Treatment means presented as least square means with 95% confidence intervals

Table 7. Milk yield and components of multiparous Holstein cows during the first 3 weeks postpartum fed a low (LFERM; dry -rolled corn) or high (HFERM; dry-rolled wheat) fermentable diet, with (C+) or without (C-) rumen-protected choline (RPC)

	Treatment[1, 2]				*P*-value						
Item	LFERM C-	LFERM C+	HFERM C-	HFERM C+	Ferm	Chol	Wk	FxC	FxW	CxW	FxCxW
Milk, kg/d	39.3 [36.0, 42.5]	38.9 [35.6, 42.2]	42.9 [39.7, 46.2]	40.1 [36.7, 43.4]	0.04	0.15	<0.001	0.26	0.02	0.61	0.44
Fat, %	4.8 [4.3, 5.2]	4.9 [4.4, 5.3]	4.3 [3.9, 4.7]	4.4 [4.0, 4.9]	0.01	0.50	<0.001	0.89	0.86	0.78	0.85
Fat, kg	1.9 [1.6, 2.1]	1.9 [1.7, 2.1]	1.8 [1.6, 2.0]	1.7 [1.5, 2.0]	0.16	0.61	<0.001	0.42	0.77	0.41	0.71
True protein, %	3.2 [3.1, 3.4]	3.2 [3.1, 3.4]	3.2 [3.1, 3.4]	3.1 [3.0, 3.3]	0.36	0.48	<0.001	0.29	0.04	0.5	0.97
True protein, kg	1.3 [1.2, 1.4]	1.3 [1.2, 1.4]	1.4 [1.3, 1.5]	1.3 [1.2, 1.4]	0.17	0.09	0.04	0.27	0.05	0.33	0.81
Lactose, %	4.6 [4.5, 4.7]	4.6 [4.5, 4.7]	4.6 [4.5, 4.7]	4.6 [4.5, 4.7]	0.97	0.79	0.58	0.5	0.71	0.18	0.67
Lactose, kg	1.8 [1.7, 2.0]	1.8 [1.7, 2.0]	2.0 [1.9, 2.2]	1.9 [1.7, 2.0]	0.06	0.11	<0.001	0.11	0.14	0.35	0.22
SCS[3]	1.9 [1.3, 2.5]	2.0 [1.5, 2.7]	2.2 [1.7, 2.9]	2.5 [1.8, 3.2]	0.20	0.52	<0.001	0.98	0.80	0.93	0.04
MUN, mg/dL	10.0 [9.2, 10.9]	10.9 [10.0, 11.8]	11.3 [10.4, 12.2]	11.9 [11.0, 13.0]	<0.01	0.04	<0.001	0.76	0.21	0.31	0.92
Milk NE, Mcal/kg	0.82 [0.78, 0.87]	0.82 [0.78, 0.87]	0.78 [0.74, 0.83]	0.79 [0.74, 0.83]	0.02	0.69	<0.001	0.94	0.98	0.56	0.91
ECM, kg/d	46.9 [42.7, 51.2]	46.8 [42.5, 51.0]	48.5 [44.2, 52.7]	45.5 [41.2, 49.8]	0.92	0.20	<0.001	0.26	0.35	0.23	0.73
FCM, kg/d	47.1 [42.7, 51.6]	47.1 [42.7, 51.5]	48.0 [43.5, 52.4]	45.4 [40.9, 49.9]	0.73	0.31	<0.001	0.30	0.54	0.33	0.64
Feed efficiency[4]	2.5 [2.3, 2.6]	2.5 [2.4, 2.7]	2.4 [2.3, 3.6]	2.4 [2.3, 3.6]	0.35	0.75	<0.001	0.97	0.96	0.73	0.70

[1] LFERM C- = low fermentability, no RPC; LFERM C+ = high fermentability, RPC; HFERM C- = low fermentability, no RPC; HFERM C+ = high fermentability, RPC

[2] Treatment means presented as least square means with 95% confidence intervals

[3] SCS = $\log_2$ (SCC/100,000) + 3

[4] Feed efficiency = ECM/DMI

Table 8. Milk fatty acid concentrations of multiparous Holstein cows during the first 3 weeks postpartum fed a low (LFERM; dry-rolled corn) or high (HFERM; dry-rolled wheat) fermentable diet, with (C+) or without (C-) rumen-protected choline (RPC)

	Treatment[1, 2]				*P*-value						
Item[3]	LFERM C-	LFERM C+	HFERM C-	HFERM C+	Ferm	Chol	Wk	FxC	FxW	CxW	FxCxW
MUFA, %	1.62 [1.4, 1.8]	1.55 [1.4, 1.7]	1.38 [1.2, 1.6]	1.49 [1.3, 1.7]	0.04	0.74	<0.001	0.24	0.54	0.85	0.74
PUFA, %	0.12 [0.11, 0.14]	0.12 [0.10, 0.13]	0.11 [0.09, 0.13]	0.12 [0.10, 0.14]	0.33	0.44	<0.001	0.13	0.82	0.59	0.76
SFA, %	2.73 [2.5, 3.0]	2.86 [2.6, 3.1]	2.53 [2.3, 2.8]	2.55 [2.3, 2.8]	0.01	0.39	<0.001	0.56	0.24	0.95	0.53
Total UFA, %	1.69 [1.5, 1.9]	1.65 [1.5, 1.8]	1.47 [1.3, 1.6]	1.63 [1.5, 1.8]	0.07	0.38	<0.001	0.14	0.75	0.68	0.63
SCFA, %	0.32 [0.29, 0.35]	0.34 [0.31, 0.37]	0.31 [0.28, 0.34]	0.30 [0.27, 0.33]	0.05	0.65	<0.001	0.18	0.45	0.66	0.22
MCFA, %	1.50 [1.3, 1.7]	1.67 [1.5, 1.9]	1.43 [1.2, 1.6]	1.45 [1.3, 1.6]	0.02	0.11	<0.001	0.21	0.69	0.98	0.62
LCFA, %	2.13 [1.9, 2.3]	2.08 [1.9, 2.3]	1.86 [1.7, 2.1]	1.98 [1.8, 2.2]	0.02	0.63	<0.001	0.29	0.36	0.84	0.86
TFA, %	0.04 [0.03, 0.05]	0.04 [0.03, 0.05]	0.04 [0.03, 0.05]	0.04 [0.03, 0.05]	0.96	0.77	0.01	0.59	0.28	0.74	0.48
C14:0, %	0.28 [0.24, 0.32]	0.32 [0.27, 0.36]	0.27 [0.23, 0.32]	0.27 [0.22, 0.31]	0.06	0.28	0.08	0.12	0.73	0.82	0.10
C16:0, %	0.98 [0.84, 1.11]	1.09 [0.95, 1.22]	0.89 [0.75, 1.03]	0.94 [0.80, 1.08]	0.02	0.10	0.06	0.52	0.73	0.52	0.65
C18:0, %	0.71 [0.64, 0.78]	0.72 [0.65, 0.79]	0.63 [0.56, 0.70]	0.67 [0.59, 0.74]	0.01	0.43	<0.001	0.63	0.39	0.98	0.91
C18:1, %	1.43 [1.28, 1.59]	1.36 [1.21, 1.52]	1.23 [1.08, 1.39]	1.34 [1.18, 1.50]	0.07	0.77	<0.001	0.16	0.41	0.87	0.45
De novo FA, %	0.69 [0.61, 0.78]	0.76 [0.67, 0.84]	0.70 [0.61, 0.78]	0.68 [0.59, 0.76]	0.16	0.40	<0.001	0.10	0.92	0.54	0.14
Mixed FA, %	1.22 [1.07, 1.37]	1.36 [1.22, 1.51]	1.21 [1.06, 1.35]	1.20 [1.05, 1.35]	0.06	0.16	<0.001	0.12	0.61	0.77	0.03
Preformed FA, %	2.61 [2.32, 2.90]	2.51 [2.23, 2.80]	2.18 [1.89, 2.46]	2.40 [2.10, 2.70]	0.02	0.59	<0.01	0.17	0.55	0.89	0.57

[1] LFERM C- = low fermentability, no RPC; LFERM C+ = high fermentability, RPC; HFERM C- = low fermentability, no RPC; HFERM C+ = high fermentability, RPC

[2] Treatment means presented as least square means with 95% confidence intervals

[3] % of total milk; SCFA = short-chain fatty acids; MCFA = medium-chain fatty acids; LCFA = long-chain fatty acids; TFA = trans fatty acids. De novo fatty acids chain lengths ≤ C14; mixed fatty acids chain lengths = C16; preformed fatty acids chain lengths ≥ C18

Table 9. Milk fatty acid yields of multiparous Holstein cows during the first 3 weeks postpartum fed a low (LFERM; dry-rolled corn) or high (HFERM; dry-rolled wheat) fermentable diet, with (C+) or without (C-) rumen-protected choline (RPC)

	Treatment[1, 2]				*P*-value						
Item	LFERM C-	LFERM C+	HFERM C-	HFERM C+	Ferm	Chol	Wk	FxC	FxW	CxW	FxCxW
MUFA, kg	0.63 [0.54, 0.72]	0.60 [0.51, 0.69]	0.58 [0.49, 0.67]	0.58 [0.49, 0.67]	0.16	0.59	<0.001	0.59	0.57	0.74	0.68
PUFA, kg	0.05 [0.04, 0.06]	0.04 [0.04, 0.05]	0.05 [0.04, 0.05]	0.05 [0.04, 0.06]	0.84	0.71	0.62	0.30	0.92	0.57	0.87
SFA, kg	1.03 [0.90, 1.18]	1.06 [0.93, 1.22]	1.03 [0.89, 1.17]	0.98 [0.86, 1.13]	0.28	0.88	<0.001	0.35	0.60	0.74	0.78
Total UFA, kg	0.66 [0.58, 0.73]	0.64 [0.57, 0.71]	0.61 [0.54, 0.68]	0.64 [0.56, 0.71]	0.30	0.90	<0.001	0.36	0.89	0.44	0.57
SCFA, kg	0.12 [0.11, 0.14]	0.13 [0.11, 0.15]	0.13 [0.11, 0.15]	0.12 [0.10, 0.13]	0.71	0.57	<0.001	0.11	0.86	0.47	0.56
MCFA, kg	0.57 [0.48, 0.67]	0.63 [0.53, 0.73]	0.60 [0.51, 0.70]	0.56 [0.47, 0.66]	0.44	0.65	<0.001	0.08	0.85	0.75	0.80
LCFA, kg	0.83 [0.73, 0.94]	0.81 [0.70, 0.91]	0.79 [0.68, 0.89]	0.78 [0.67, 0.88]	0.21	0.56	<0.001	0.77	0.50	0.75	0.79
TFA, kg	0.02 [0.01, 0.02]	0.02 [0.01, 0.02]	0.02 [0.01, 0.02]	0.02 [0.01, 0.02]	0.36	0.79	0.13	0.91	0.57	0.82	0.42
C14:0, kg	0.11 [0.09, 0.13]	0.12 [0.10, 0.14]	0.11 [0.09, 0.13]	0.10 [0.08, 0.12]	0.52	0.80	<0.001	0.05	0.94	0.60	0.18
C16:0, kg	0.37 [0.31, 0.43]	0.41 [0.35, 0.48]	0.37 [0.31, 0.43]	0.36 [0.30, 0.43]	0.20	0.29	<0.001	0.22	0.95	0.44	0.81
C18:0, kg	0.27 [0.24, 0.31]	0.27 [0.24, 0.31]	0.26 [0.23, 0.30]	0.26 [0.22, 0.29]	0.16	0.91	<0.001	0.82	0.76	0.81	0.90
C18:1, kg	0.54 [0.47, 0.62]	0.52 [0.44, 0.59]	0.51 [0.44, 0.59]	0.51 [0.44, 0.59]	0.38	0.57	0.01	0.45	0.49	0.88	0.48
De novo FA, kg	0.27 [0.22, 0.31]	0.29 [0.24, 0.33]	0.29 [0.25, 0.34]	0.26 [0.22, 0.31]	0.93	0.83	0.04	0.08	0.94	0.23	0.41
Mixed FA, kg	0.46 [0.40, 0.53]	0.51 [0.45, 0.57]	0.50 [0.43, 0.56]	0.47 [0.40, 0.53]	0.83	0.73	0.41	0.07	0.97	0.46	0.09
Preformed FA, kg	1.02 [0.88, 1.16]	0.98 [0.84, 1.12]	0.92 [0.78, 1.06]	0.94 [0.80, 1.09]	0.09	0.82	<0.001	0.47	0.53	0.88	0.51

[1] LFERM C- = low fermentability, no RPC; LFERM C+ = high fermentability, RPC; HFERM C- = low fermentability, no RPC; HFERM C+ = high fermentability, RPC

[2] Treatment means presented as least square means with 95% confidence intervals

[3] % of total milk; SCFA = short-chain fatty acids; MCFA = medium-chain fatty acids; LCFA = long-chain fatty acids; TFA = trans fatty acids. De novo fatty acids chain lengths ≤ C14; mixed fatty acids chain lengths = C16; preformed fatty acids chain lengths ≥ C18

Table 10. Concentrations of blood β-Hydroxybutyrate (BHB), blood urea nitrogen (BUN), glucose, free fatty acid (FFA), and insulin of multiparous Holstein cows during the first 3 weeks postpartum fed a low (LFERM; dry-rolled corn) or high (HFERM; dry-rolled wheat) fermentable diet, with (C+) or without (C-) rumen-protected choline (RPC)

Item	Treatment[1, 2]				*P*-value						
	LFERM C-	LFERM C+	HFERM C-	HFERM C+	Ferm	Chol	DIM	FxC	FxD	CxD	FxCxD
BHB, mmol/L	1.44 [1.16, 1.79]	1.08 [0.87, 1.34]	1.19 [0.96, 1.47]	0.96 [0.77, 1.20]	0.16	0.02	0.14	0.73	0.75	0.23	0.23
BUN, mg/dL	12.44 [11.25, 13.62]	12.69 [11.52, 13.86]	13.30 [12.13, 14.47]	13.64 [12.44, 14.84]	0.05	0.51	0.01	0.92	<0.01	0.16	0.91
FFA, mmol/L	0.30 [0.25, 0.36]	0.32 [0.27, 0.39]	0.29 [0.24, 0.36]	0.30 [0.24, 0.36]	0.54	0.64	<0.001	0.72	0.61	0.58	0.76
Glucose, mg/dL	43.76 [40.39, 47.12]	48.79 [45.57, 52.11]	48.22 [44.89, 51.55]	49.17 [45.71, 52.62]	0.14	0.07	<0.001	0.21	0.27	0.45	0.57
Insulin, µg/L	0.09 [0.07, 0.11]	0.09 [0.07, 0.11]	0.08 [0.06, 0.10]	0.08 [0.07, 0.11]	0.48	0.78	<0.001	0.52	0.44	0.48	0.51

[1] LFERM C- = low fermentability, no RPC; LFERM C+ = high fermentability, RPC; HFERM C- = low fermentability, no RPC; HFERM C+ = high fermentability, RPC

[2] Treatment means presented as least square means with 95% confidence intervals

Table 11. Body weight (BW) at enrollment 21 d prior to expected calving date and change in prepartum BW and body condition score (BCS) of multiparous Holstein cows fed a control (C-) or diet containing rumen-protected choline (C+; RPC)

	Treatment[1,2]		*P*-value
Item	C-	C+	Chol
BW, kg[3]	752.8 [728.1, 777.5]	738.0 [713.6, 762.3]	0.40
BW change, kg[4]	-29.3 [-42.1, -16.5]	-35.3 [-47.7, -22.9]	0.50
BCS change, units[5]	-0.05 [-0.11, 0]	-0.06 [-0.12, -0.01]	0.89

[1] C− = 0 g/d of RPC; C+ = 30 g/d of RPC (ReaShure, Balchem Corp., Montvale, NJ)
[2] Treatment means presented as least square means with 95% confidence intervals
[3] BW at -21 d relative to expected calving date
[4] change from -21 d relative to expected calving date to calving
[5] change from -21 d relative to expected calving date to calving

Table 12. Body weight (BW) at unenrollment 21 d postpartum and change in postpartum BW and body condition score (BCS) of multiparous Holstein cows fed a low (LFERM; dry-rolled corn) or high (HFERM; dry-rolled wheat) fermentable diet, with (C+) or without (C-) rumen-protected choline (RPC)

	Treatment[1,2]				*P*-value		
Item	LFERM C-	LFERM C+	HFERM C-	HFERM C+	Ferm	Chol	FxC
BW, kg[3]	608.6 [574.4, 642.8]	602.0 [570.3, 633.7]	628.9 [597.2, 660.6]	588.6 [555.7, 621.5]	0.83	0.16	0.31
BW change, kg[4]	-92.2 [-108.4, -76.1]	-99.5 [-114.4, -84.5]	-94.9 [-109.9, -80.0]	-101.5 [-117.1, -86.0]	0.76	0.37	0.97
BCS change, units[5]	-0.35 [-0.44, -0.25]	-0.34 [-0.43, -0.25]	-0.40 [-0.48, -0.31]	-0.42 [-0.51, -0.33]	0.14	0.88	0.74

[1] LFERM C- = low fermentability, no RPC; LFERM C+ = high fermentability, RPC; HFERM C- = low fermentability, no RPC; HFERM C+ = high fermentability, RPC
[2] Treatment means presented as least square means with 95% confidence intervals
[3] BW at 21 DIM
[4] change from calving to 21 DIM
[3] change from calving to 21 DIM

Figures

Figure 1. Interaction of rumen-protected choline (RPC) and time on meal size of multiparous Holstein cows fed a control diet (C-) or diet containing RPC (C+) during the last 3 weeks prepartum.

A tendency for an interaction between RPC and time was detected ($P = 0.08$). Data are presented as LSM with 95% CI.

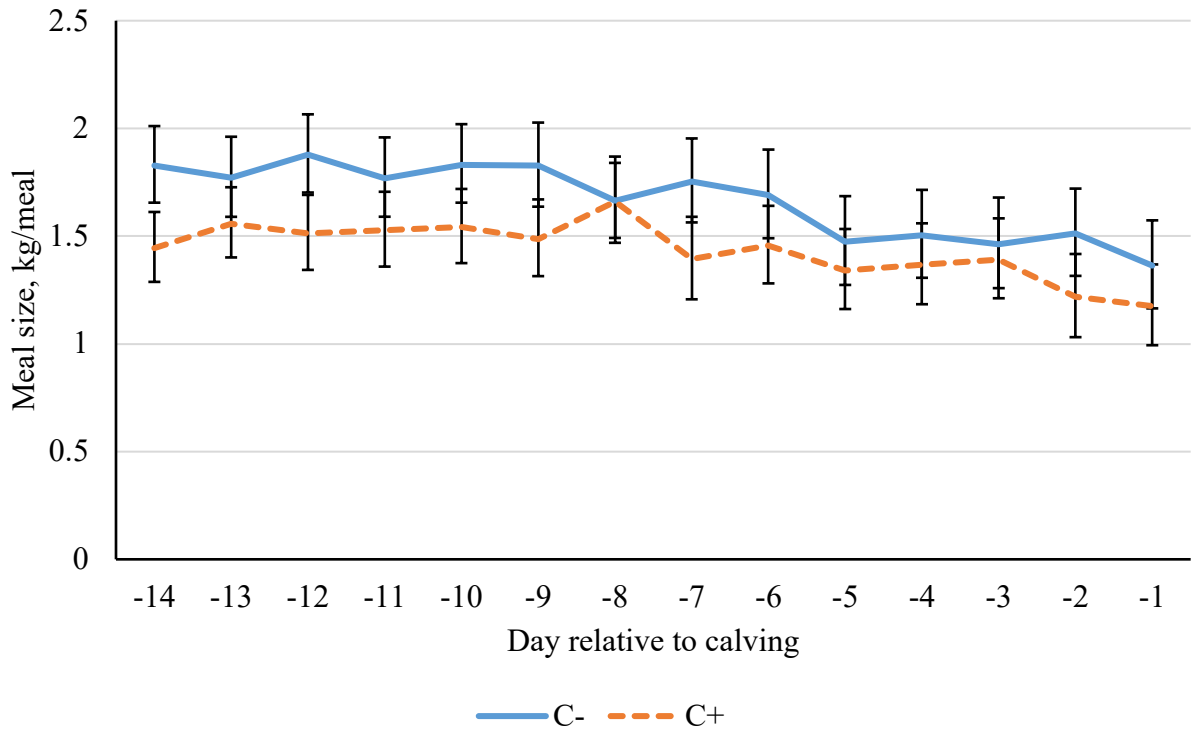

Figure 2. Interaction of rumen-protected choline (RPC) and time on meal size of multiparous Holstein cows during the first 3 weeks postpartum fed a low (LFERM; dry-rolled corn) or high (HFERM; dry-rolled wheat) fermentable diet, with (C+) or without (C-) rumen- protected choline (RPC).

An interaction between RPC and time was detected ($P = 0.04$). Data are presented as LSM with 95% CI. Pairwise means did not separate.

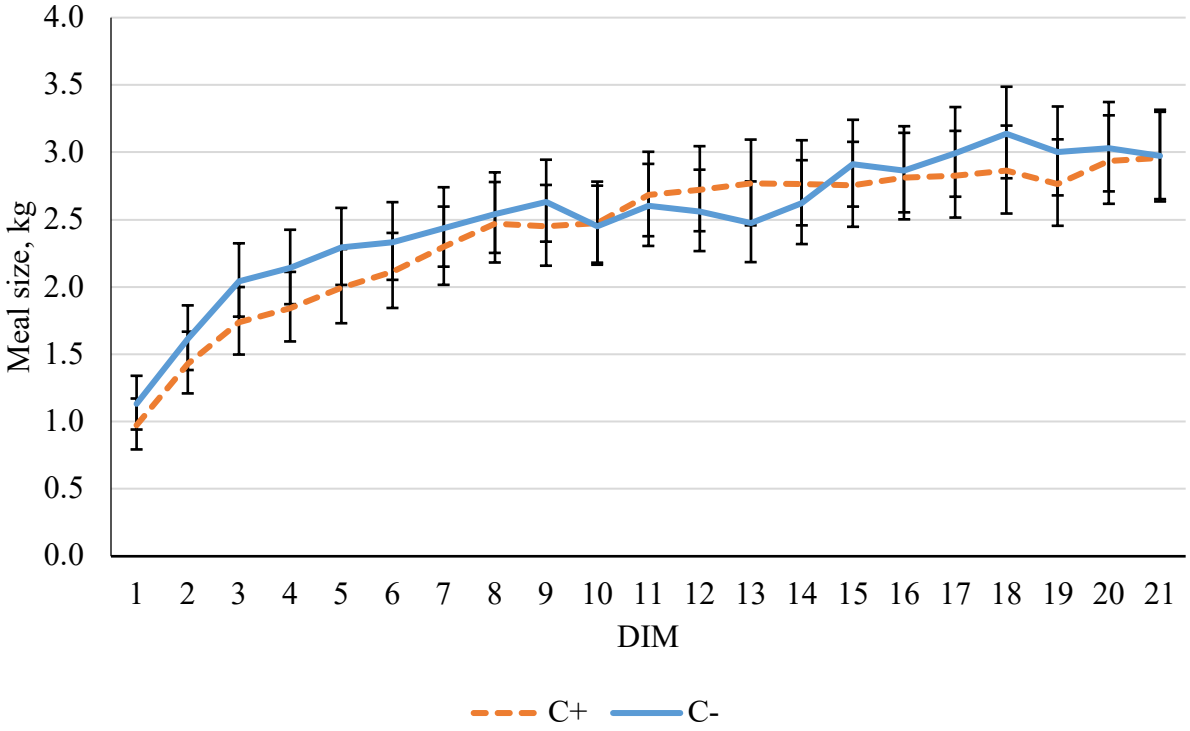

Figure 3. Interaction of diet fermentability and time on milk yield of multiparous Holstein cows during the first 3 weeks postpartum fed a low (LFERM; dry-rolled corn) or high (HFERM; dry-rolled wheat) fermentable diet, with (C+) or without (C-) rumen-protected choline (RPC).

An interaction between diet fermentability and time was detected ($P = 0.02$). Data are presented as LSM with 95% CI. Pairwise means did not separate.

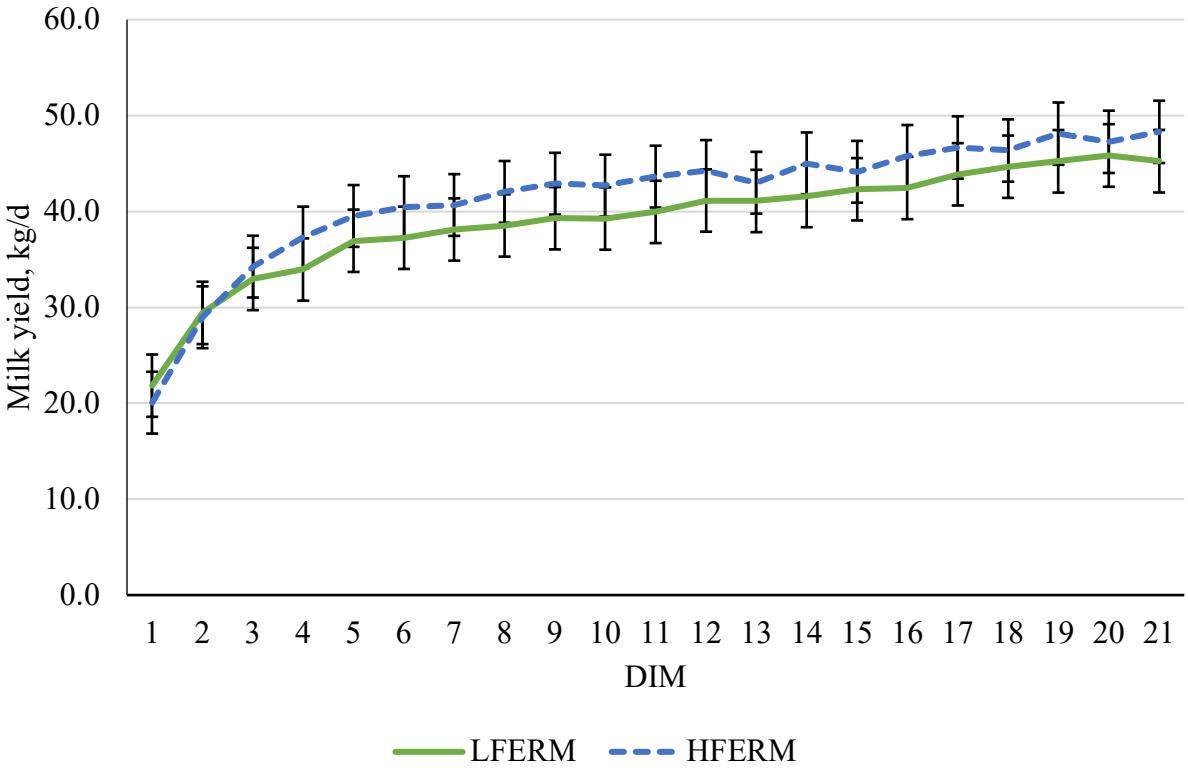

Figure 4. Interaction of diet fermentability and time on milk true protein yield of multiparous Holstein cows during the first 3 weeks postpartum fed a low (LFERM; dry-rolled corn) or high (HFERM; dry-rolled wheat) fermentable diet, with (C+) or without (C-) rumen-protected choline (RPC).

An interaction between diet fermentability and time was detected ($P = 0.04$). Within HFERM, milk true protein yield decreased from wk 1 to 3 ($P = 0.01$) and tended to decrease from wk 2 to 3 ($P = 0.08$); no differences between diets were detected within any week. Data are presented as LSM with 95% CI.

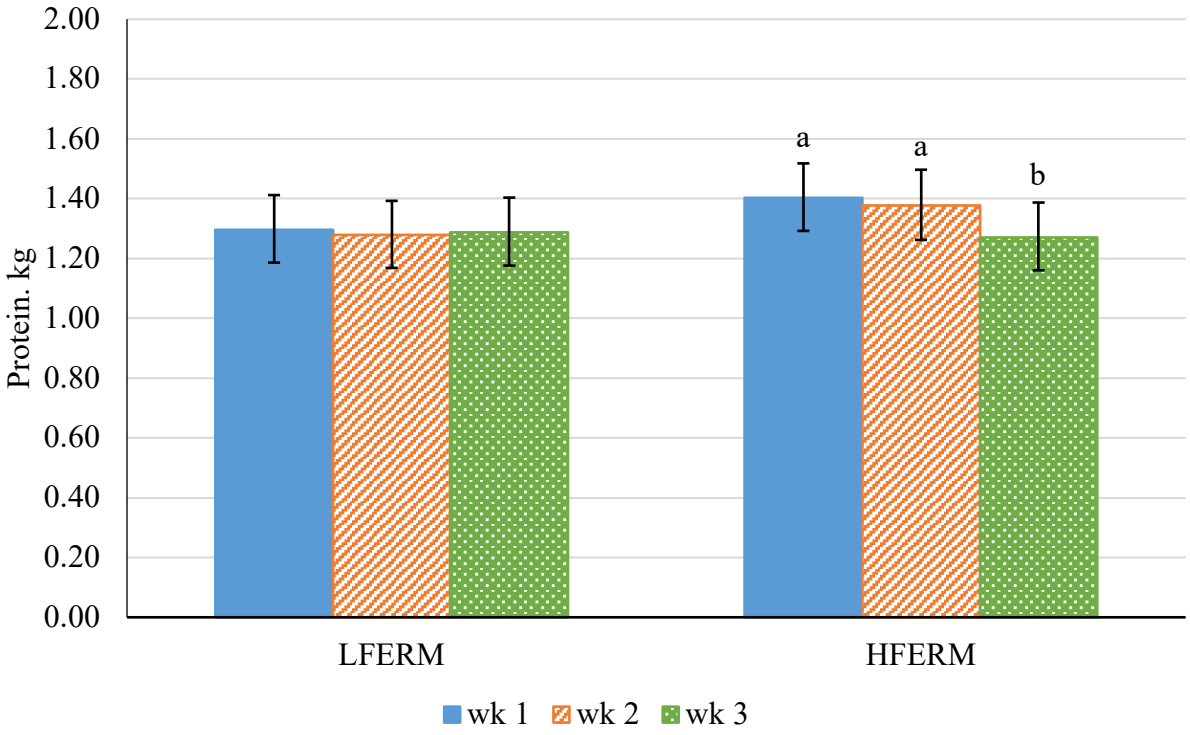

Figure 5. Interaction of diet fermentability and time on milk true protein percent of multiparous Holstein cows during the first 3 weeks postpartum fed a low (LFERM; dry-rolled corn) or high (HFERM; dry-rolled wheat) fermentable diet, with (C+) or without (C-) rumen-protected choline (RPC).

An interaction between diet fermentability and time was detected ($P = 0.05$). Milk true protein percentage decreased over time ($P < 0.001$); however, no differences between diets were detected within week. Data are presented as LSM with 95% CI.

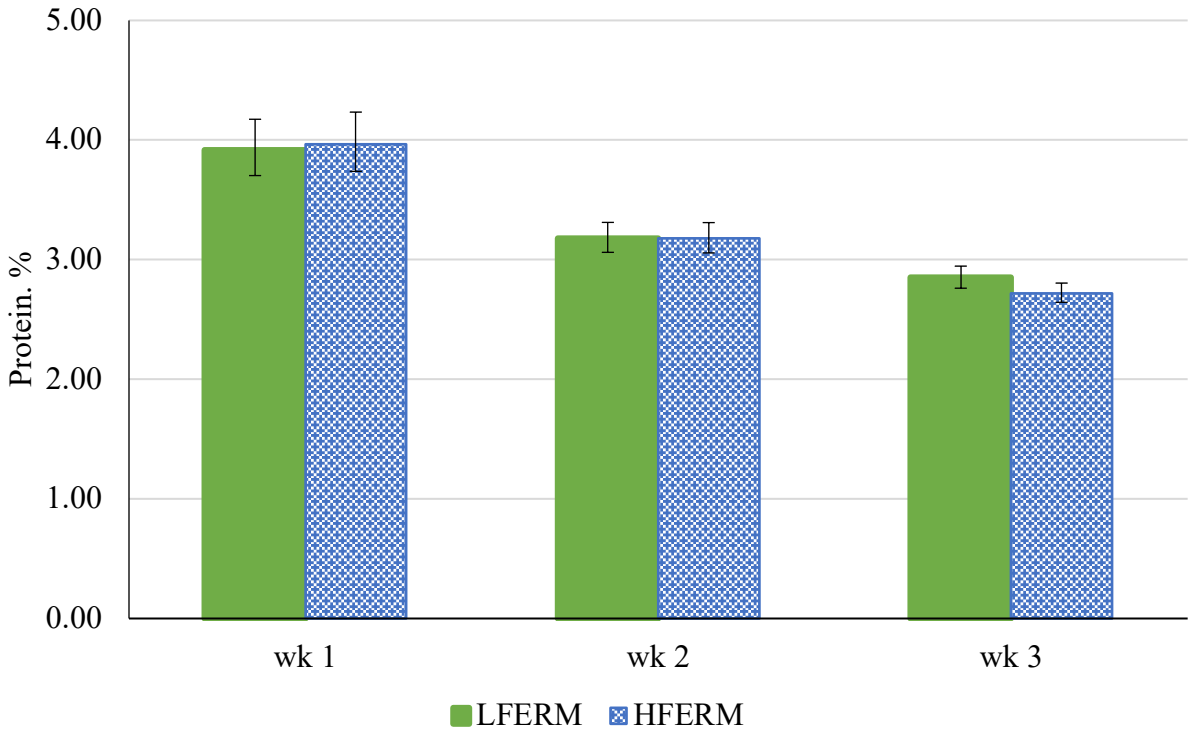

Figure 6. Interaction of rumen-protected choline (RPC), diet fermentability, and time on somatic cell score (SCS) of multiparous Holstein cows during the first 3 weeks postpartum fed a low (LFERM; dry-rolled corn) or high (HFERM; dry-rolled wheat) fermentable diet, with (C+) or without (C-) rumen-protected choline (RPC).

An interaction between diet fermentability, RPC, and time was detected ($P = 0.04$). Data are presented as LSM with 95% CI. Pairwise means did not separate.

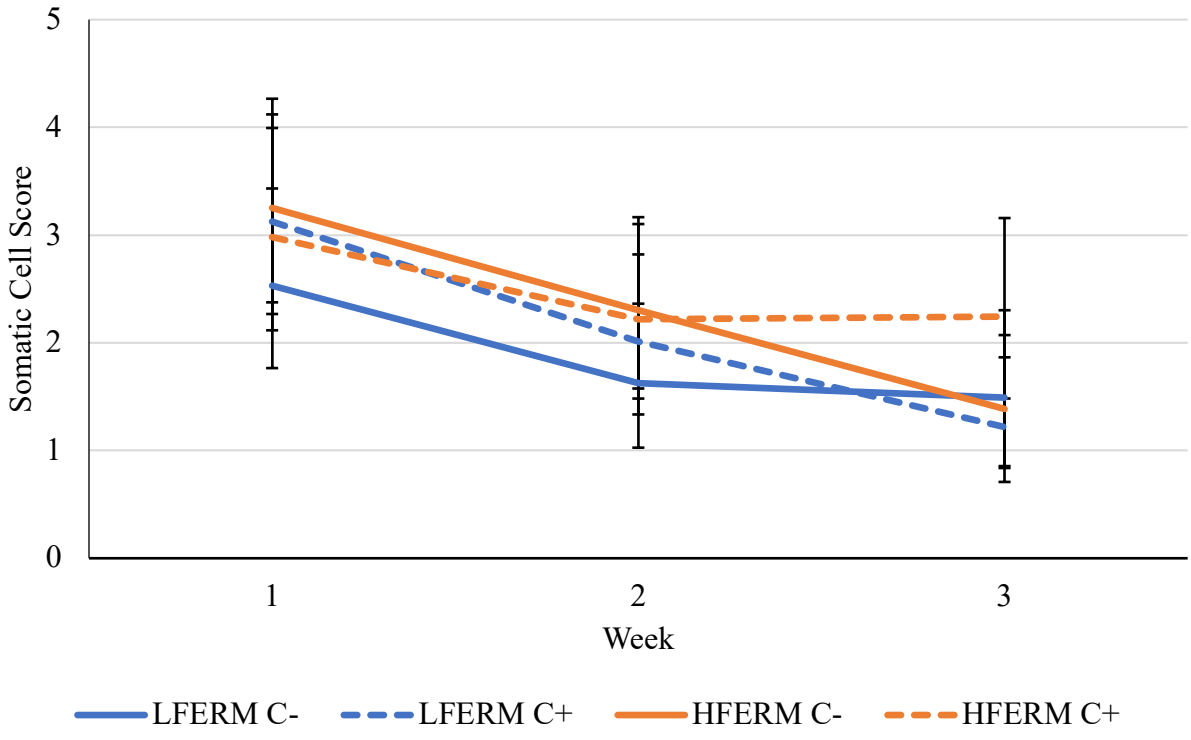

Figure 7. Interaction of rumen-protected choline (RPC), diet fermentability, and time on mixed FA percent of multiparous Holstein cows during the first 3 weeks postpartum fed a low (LFERM; dry-rolled corn) or high (HFERM; dry-rolled wheat) fermentable diet, with (C+) or without (C-) rumen-protected choline (RPC).

An interaction between diet fermentability, RPC, and time was detected ($P = 0.03$). An adjusted difference was detected between LFERM C+ and HFERM C+ at wk 1 ($P < 0.05$); no other pairwise differences within week were significant. Data are presented as LSM with 95% CI.

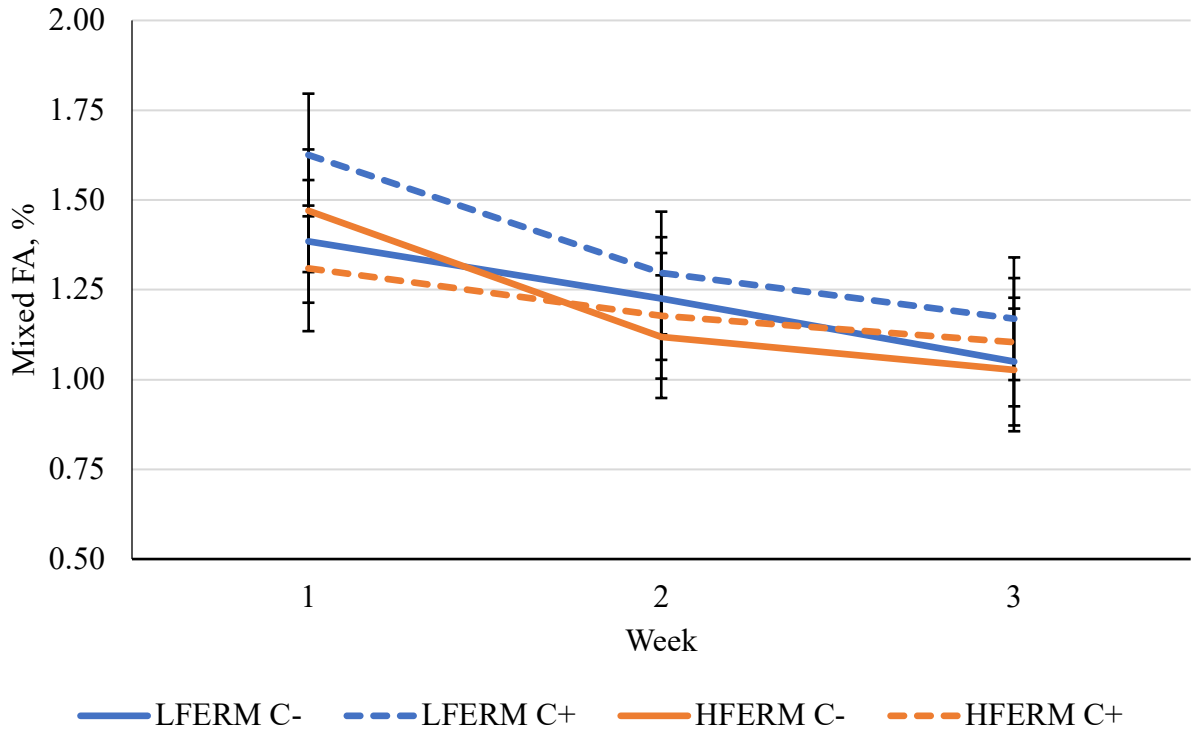

Figure 8. Interaction of diet fermentability and time on blood urea nitrogen (BUN) concentration of multiparous Holstein cows during the first 3 weeks postpartum fed a low (LFERM; dry-rolled corn) or high (HFERM; dry-rolled wheat) fermentable diet, with (C+) or without (C-) rumen-protected choline (RPC).

Data are presented as LSM with 95% CI. An interaction between diet fermentability and time was detected ($P < 0.01$). An adjusted difference was detected between LFERM and HFERM at d 7 ($P = 0.01$); no other pairwise differences within day were significant.