

Article

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

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Abstract

Postpartum dairy cows fed more rapidly fermentable starch sources have depressed dry matter intake (DMI), compounding the risk of negative energy balance and hepatic lipid accumulation. Rumen-protected choline (RPC) is supplemented to periparturient dairy cows to facilitate hepatic lipid export. Our objective was to evaluate the interaction of dietary starch fermentability (DF) and RPC supplementation on postpartum DMI and performance. Prepartum supplementation of a low dose of RPC (no RPC [C−] vs. RPC [C+; 30 g/d]) began 21 d before the expected calving date for Holstein cows with at least one parity. Postpartum, cows were assigned to 1 of 4 postpartum treatments for 21 d with a 2 × 2 factorial arrangement of starch fermentability rate (low [dry-rolled corn; LFERM] vs. high [dry-rolled wheat; HFERM]) and RPC (C− vs. C+). Prepartum, C+ decreased DMI by 2.3 kg compared with C−, but there was no evidence of treatment effect on DMI postpartum. Time and DF interacted on milk yield, with HFERM increasing milk yield after d 3 compared with LFERM. Compared with LFERM, HFERM decreased milk fat content but not fat yield. For blood metabolites, C+ decreased plasma beta-hydroxybutyrate by 0.3 mmol/L and tended to increase glucose concentration compared to C−. In conclusion, supplementation with RPC at a low rate of inclusion reduced DMI in prepartum cows and decreased postpartum plasma BHB concentrations. Further work is required to elucidate potential mechanisms of action for RPC-mediated reductions in DMI.

Keywords: transition period; feeding behavior; diet fermentability

1. Introduction

Increasing the dietary starch fermentability (DF) rate in postpartum dairy cow diets depresses DMI and milk energy output in early-lactation cows [1–3]. This depression in intake may exacerbate negative energy balance (NEB) naturally experienced after calving, further challenging postpartum metabolic adaptation. The reduction in DMI for these rapidly fermentable starch sources 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 [4]. Supporting this concept, postpartum DMI is negatively associated with hepatic acetyl-CoA concentration [5], and increases in hepatic acetyl-CoA and plasma free fatty acid (FFA) concentration after feeding are negatively related to short-term dry matter intake (DMI) in both postpartum and late-lactation cows [6].

Excessive lipid mobilization during the transition period contributes to hepatic lipid accumulation and impaired liver function [7,8]. Rapid influx of FFA to the liver can exceed



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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 [8,9]. Strategies that enhance hepatic lipid export and reduce intracellular triglyceride accumulation may therefore help sustain intake and improve metabolic efficiency in postpartum cows fed highly fermentable starch sources.

Supplementation with rumen-protected choline (RPC) has been shown to mitigate these challenges by supporting hepatic lipid metabolism [10]. 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 [11]. Phosphatidylcholine supply supports the export of hepatic triacylglycerol (TAG) in VLDL, reducing the risk of hepatic lipidosis and improving liver function in transition cows [10,12,13]. Meta-analyses further support these benefits, reporting increased postpartum DMI and milk yield in cows supplemented with RPC during the transition period [14,15].

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 dietary starch source. Despite being intricately linked, the combined effects of DF and RPC supplementation on postpartum feed intake and performance have not been previously evaluated. Therefore, the objective of this study was to evaluate the 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.

2. Materials and Methods

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

2.1. Animals and Experimental Design—Prepartum

Holstein cows with at least 1 parity ($n = 65$; 2.1 ± 1.13 parities) were enrolled on a rolling basis and blocked by expected calving date (ECD), body condition score (BCS; ≤ 1 -unit using a 5-point scale; [16]), and previous lactation 305 d mature equivalent milk yield (≤ 5000 kg). While the overall sample size was determined primarily for the postpartum portion of the study, 50 cows ($n = 25$ /treatment) is sufficient to observe a 2.1 kg/d difference in prepartum DMI ([17,18]; $\alpha = 0.05$; $\beta = 0.80$; $\sigma = 2.39$). Cows were enrolled 21 days before ECD and randomly assigned to treatment utilizing a random number generator (Microsoft Excel, Microsoft Corporation, Redmond, WA, USA). The treatments included two factors: (1) RPC supplementation (Control [C−; 30 g/d soy hulls] vs. RPC [C+; 30 g/d ReaShure supplying 8.8 g choline chloride or 6.6 g choline ion [14]; Balchem Corporation]) fed throughout the study period, 21 d prepartum to 21 d postpartum, and (2) postpartum dietary starch fermentability initiated on the day of calving (low [supplied via dry-rolled corn; LFERM] vs. high [supplied via dry-rolled wheat; HFERM]). The prepartum study design was a randomized complete block design with only RPC supplementation as a treatment factor. Prepartum treatments were mixed into a total mixed ration (TMR). The diet was formulated to meet the dietary requirements of a 726 kg cow (1.43 Mcal net energy of lactation/kg dry matter) and to contain 13.0% crude protein (CP), 46.7% amylase-treated neutral detergent fiber organic matter (aNDFom), and 12.4% starch. The diet was

formulated to supply 30 g of metabolizable methionine per day (2.38% of metabolizable protein), which met or exceeded estimated requirements. Prepartum dietary and chemical compositions are described in Table 1. Diets were reformulated after week 12 of the study to maintain a consistent nutrient composition across treatments following a change in corn silage source.

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

Item	Treatment ^{1,2}			
	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 ³	20.52	26.20	20.52	26.20
RPC	-	-	0.22	0.20
Soyhulls	0.22	0.20	-	-
Nutrient, % of DM				
DM	55.48	57.45	55.53	57.58
CP	13.83	13.72	14.35	14.25
aNDFom ⁴	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− = 30 g/d of soy hulls; C+ = 30 g/d of RPC (ReaShure, Balchem Corp., Montvale, NJ, USA); ² Diets were reformulated after wk 12 to maintain similar nutrient composition across treatments following a change in corn silage source; ³ Common grain mix (average % of DM): 7.92% soybean meal, 7.11% dry-ground corn, 7.42% SoyChlor (Landus Cooperative, Jefferson, IA, USA), 0.12% NutriTek (Diamond V, Cedar Rapids, IA, USA), 0.17% salt, 0.13% NiaShure (ReaShure, Balchem Corp., Montvale, NJ, USA), 0.04% Zinpro 4 Plex C (Zinpro, Eden Prairie, MN, USA), 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, USA), 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; ⁴ aNDFom = amylase NDF OM; EE = ether extract.

Animals were housed in a bedded pack barn and individual feed intake was recorded using a Roughage Intake Control (RIC) computerized feeding system (Hokofarm Group, Emmeloord, The Netherlands) equipped with radio frequency identification collars linked to Apollo Farm Management software (Hokofarm Group, Emmeloord, The Netherlands). Collars enabled individual, access-controlled entry to the RIC bunk 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. The number of visits to each bunk and the 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. Technicians were not blinded to treatment assignments at any point during the study. Bunk stocking density was maintained at 1 to 2 cows/bunk throughout the duration of the study to minimize behavioral effects on feeding behavior variables, as pen stocking density changed throughout the study according to animal availability.

2.2. Animals and Experimental Design—Postpartum

The postpartum dataset included 55 cows across 14 blocks after cows were excluded for calving difficulties ($n = 1$) or other postpartum health events ($n = 9$; Supplemental Table S1). According to a power analysis, 13 cows per treatment is sufficient to detect a 3.9 kg difference in postpartum DMI ([3]; $\alpha = 0.10$; $\beta = 0.80$; $\sigma = 4.0$). After calving, cows continued the same RPC treatment as prepartum (C− vs. C+) with the addition of the secondary factor of starch fermentability as described in the previous section (LFERM vs. HFERM). As such, the postpartum treatment structure constituted a randomized complete block design with a 2×2 factorial arrangement. Treatment combinations were LFERM C−, LFERM C+, HFERM C−, and HFERM C+. The postpartum diets were formulated for a 628 kg cow producing 34 kg milk per day (1.61 Mcal net energy of lactation/kg DM), with 17.8% CP, 29.4% aNDFom, and 24.9% starch. The diet was formulated to supply 59 g of metabolizable methionine per day (2.37% of metabolizable protein), which met estimated requirements. Diet fermentability rate was modified by partially replacing dry-rolled corn in LFERM with dry-rolled wheat in HFERM diets to increase starch fermentability. According to Herrera-Saldana et al. [19], this substitution was estimated to increase the 7 h *in vitro* starch digestibility (IVSD7) rate from 44.6% (LFERM) to 60.1% (HFERM). This was also similar to that achieved in Alborno et al. [3] which was effective in depressing DMI in postpartum cows; however, actual differences in our diets were only about 5% IVSD7 (Table 2). Manipulation of dietary RPC inclusion rates was conducted by replacing soyhulls in C− with RPC in C+. A basal diet with common feed ingredients for all treatments was mixed daily in a Forage Express Feed Mixer (414-41B; Roto-Mix, Dodge City, KS, USA), 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 and chemical compositions are described in Table 2. 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 the DM content of wet ingredients accordingly. Diets were reformulated after week 12 of the study 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 as described in Table 2. Forty-five prepartum and 48 postpartum cows were enrolled after the reformulation.

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 [20]. 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. Postpartum health evaluations were conducted daily by evaluating rectal temperature, urine ketones, previous 24 h feed disappearance, and general appearance. Animals showing moderate or greater ketones on two consecutive urine samples (Aspen Veterinary Resources, Loveland, CO, USA) 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; Portachek, Carlsbad, CA, USA). Cows meeting these criteria received 300 mL propylene glycol or keto-gel once daily for 3 to 4 d. If blood BHB concentration exceeded 3.0 mmol/L, treatment was supplemented with 250 mL intravenous dextrose in accordance with DTRC protocol. The number of postpartum cows treated for ketosis included LFERM C− ($n = 7$), LFERM C+ ($n = 4$), HFERM C− ($n = 2$), and HFERM C+ ($n = 2$).

Table 2. Ingredients and 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 diet DM.

Item	Treatment ^{1,2}							
	LFERM C−		LFERM C+		HFERM C−		HFERM C+	
	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 ³	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 ⁴	-	-	0.14	0.13	-	-	0.13	0.13
Soyhulls	0.15	0.14	-	-	0.14	0.14	-	-
Nutrient, % of DM								
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 ⁵	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+ = low fermentability, RPC; HFERM C− = high fermentability, no RPC; HFERM C+ = high fermentability, RPC; ² Diets were reformulated after wk 12 to maintain similar nutrient composition across treatments following a change in corn silage source; ³ 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, USA), 0.41% blood meal, 0.06% Smartamine M (Adisseo Inc., Antony, France), 1.12% micromineral and vitamin premix (MKC Cooperative, Moundridge, KS, USA), 0.99% sodium bicarbonate, 0.68% calcium carbonate; ⁴ ReaShure (Balchem Corp., Montvale, NJ, USA); ⁵ aNDFom = amylase NDF OM; EE = ether extract.

2.3. 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 [21], except meal-based behaviors were determined using a 12 min intermeal interval as the minimum time between feed bin visits to qualify as a separate meal [20]. Prepartum variables of interest included DMI, number of meals, meal size, meal length, total eating time, eating rate, largest meal, and meal interval. 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, USA) macro according to [20]. Postpartum meal-based feeding behaviors were determined using a 12 min threshold as the minimum time between feeding activity for separation of individual meals, and all meals < 0.2 kg were excluded [20].

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 h to determine DM%. Samples were ground through a 1 mm screen using a Thomas Wiley Mill (Thomas Scientific, Swedesboro, NJ, USA) and were analyzed for chemical composition at a commercial laboratory (Dairyland Laboratories, Arcadia, WI, USA). The CP content was determined using the combustion method according to the

AOAC Official Method 990.03 [22]. According to the AOAC Official Method 2002.04 [23], 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 [24]. Ether extract (EE) was determined according to the AOAC Official Method 920.39 [25] 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 the 30th Annual MW AOACI Meeting and Exposition [26], with the modification that glucose analysis was performed using a YSI 2700 Select Biochemistry Analyzer (YSI, Yellow Springs, OH, USA). The procedure of Richards et al. [27] 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, USA). Milk samples were obtained weekly at 6 consecutive milkings using in-line milk sampling equipment. Samples were analyzed at AgSource Laboratories (Menomonie, WI, USA) 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 was calculated according to [28] using the formula: Milk net energy (Mcal/kg) = $(9.29 \times \text{kg Fat/kg Milk}) + (5.85 \times \text{kg True Protein/kg Milk}) + (3.95 \times \text{kg Lactose/kg Milk})$. Energy-corrected milk (ECM) and fat-corrected milk (FCM) were calculated according to the Dairy Herd Improvement Glossary [29] using the formulas: ECM = $(0.327 \times \text{milk lbs.}) + (12.95 \times \text{fat lbs.}) + (7.65 \times \text{protein lbs.})$; FCM = $(0.432 \times \text{milk lbs.}) + (16.216 \times \text{fat lbs.})$. Somatic cell score (SCS) was calculated using the following formula: $\log_2 (\text{SCC}/100,000) + 3$.

Blood samples (30 mL) were collected via coccygeal venipuncture on d 3, 7, 14, and 21 (± 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, USA). The tube was maintained at room temperature to allow for clot formation for 30 min prior to centrifugation for 15 min at 20 °C and $2500 \times g$ (Fisherbrand accuSpin Max; Thermo Fisher Scientific, Waltham, MA, USA). Two additional tubes containing potassium-EDTA or sodium fluoride/potassium oxalate were used for plasma harvest (BD Vacutainer; Franklin Lakes, NJ, USA). Immediately following collection, tubes for plasma separation were placed on ice before centrifugation at 4 °C and $2000 \times g$ (Fisherbrand accuSpin Max; Thermo Fisher Scientific, Waltham, MA, USA). Serum and plasma were subsequently harvested and frozen for future analysis. The intra-assay and inter-assay CV were, respectively, FFA: 4.91% and 10.49%; insulin: 4.33% and 4.71%; glucose: 1.35% and 5.72%; BUN: 2.30% and 4.58%; and BHB: 0.92% and 4.43%.

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 [16] and scores were averaged.

2.4. Statistical Analyses

Data were analyzed using the MIXED procedure of SAS (version 9.4; SAS Institute, Cary, NC, USA). Prepartum data included $n = 32$ cows for C− and $n = 33$ cows for C+, and outcomes were analyzed relative to the actual calving date. To ensure a complete, balanced prepartum dataset across cows, including cows that calved earlier than ECD, prepartum data were analyzed and reported for −14 to −1 d relative to calving. Pre-supplementation baseline data was not included in the statistical model. Postpartum data included $n = 14$ cows for all treatments except HFERM C+ ($n = 13$). The data were analyzed as a split-plot design, with cow as the whole plot and days in milk (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 $\times$ 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 was evaluated via visual inspection of studentized residual panels, histograms, and QQ plots, and data transformations were applied if necessary. Specifically, the following transformations were applied prior to analysis: (i) natural log transformation: MUN, saturated fatty acid (SFA) yield, BHB, NEFA, insulin; (ii) square-root transformation: meal size, meal length, largest meal, protein yield, SCC, SCS, poly-unsaturated fatty acid (PUFA) yield, short-chain fatty acid (SCFA) yield, medium-chain fatty acid (MCFA) yield, trans-fatty acid (TFA) yield, C14:0 yield, C16:0 yield, C18:0 yield, C18:1 yield, de novo fatty acid (FA) yield, mixed FA yield; (iii) squared transformation (χ^2): lactose yield, lactose percent, BCS prepartum; (iv) inverse-square transformation ($1/\chi^2$): protein percent; and (v) reciprocal transformation ($1/\chi$): meal interval. Due to displaced abomasum events, two cows on LFERM C− were removed from the 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. Due to unanticipated differences in dietary nutrient composition after the corn silage source year change, models for DMI and production variables were tested with a fixed effect of corn silage source. There was no evidence of statistical significance for corn silage source year and the factor was subsequently removed from all models. 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 $0.05 < p \leq 0.10$.

3. Results

3.1. 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 3), 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 ($p < 0.001$; Supplemental Figure S4). 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 3) and largest meal (3.8 kg [3.42, 4.19] vs. 4.3 kg [3.91, 4.74]; $p = 0.02$; Table 3) 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 the number of meals, meal interval, meal length, or total eating time ($p > 0.23$).

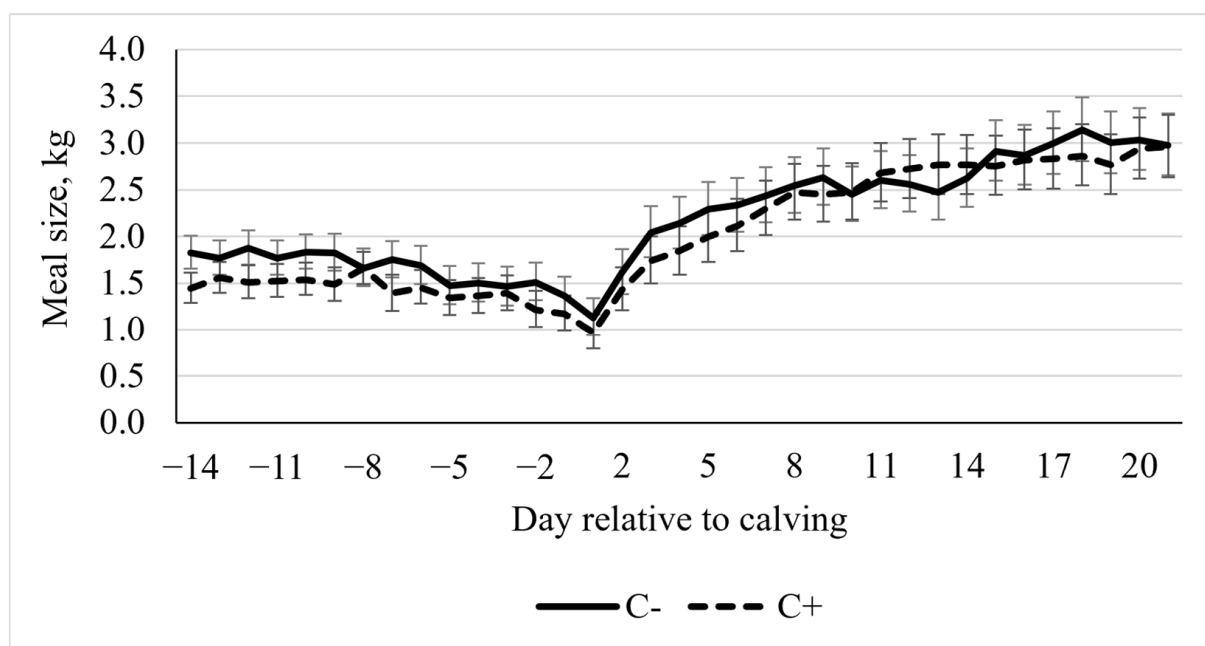


Figure 1. Interaction of rumen-protected choline (RPC) and time on meal size of multiparous Holstein cows fed a control diet (C−) or diet containing RPC (C+) during the last 3 weeks prepartum and 3 weeks postpartum. Time and RPC tended to interact on meal size prepartum ($p = 0.08$) and the interaction was significant postpartum ($p = 0.04$). The data are presented as LSM with 95% CI.

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

Item	Treatment ¹		Trt	<i>p</i> -Value	
	C− ²	C+ ³		Time	Trt × 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− = 30 g/d of soy hulls;

³ C+ = 30 g/d of RPC (ReaShure, Balchem Corp., Montvale, NJ, USA).

3.2. Postpartum

3.2.1. Feeding Behavior

From calving date through 21 DIM, DMI increased with time ($p < 0.001$; Table 4) with no evidence of treatment effect ($p > 0.19$). The number of meals decreased over time

($p < 0.001$; Table 4), 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 4; Figure 1), with C+ decreasing meal size through d 10 and after d 15 compared with C−. The HFERM diets tended to increase the 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 4). There was no evidence of treatment effect on the number of meals, meal length, total eating time, and meal interval ($p \geq 0.28$).

3.2.2. Milk Production

Milk yield and components results are listed in Table 5. Milk fat 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 2), 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 3), but there was no difference across wk for LFERM. Time and DF interacted for milk true protein content ($p = 0.04$; Figure 3), 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]; $p = 0.06$).

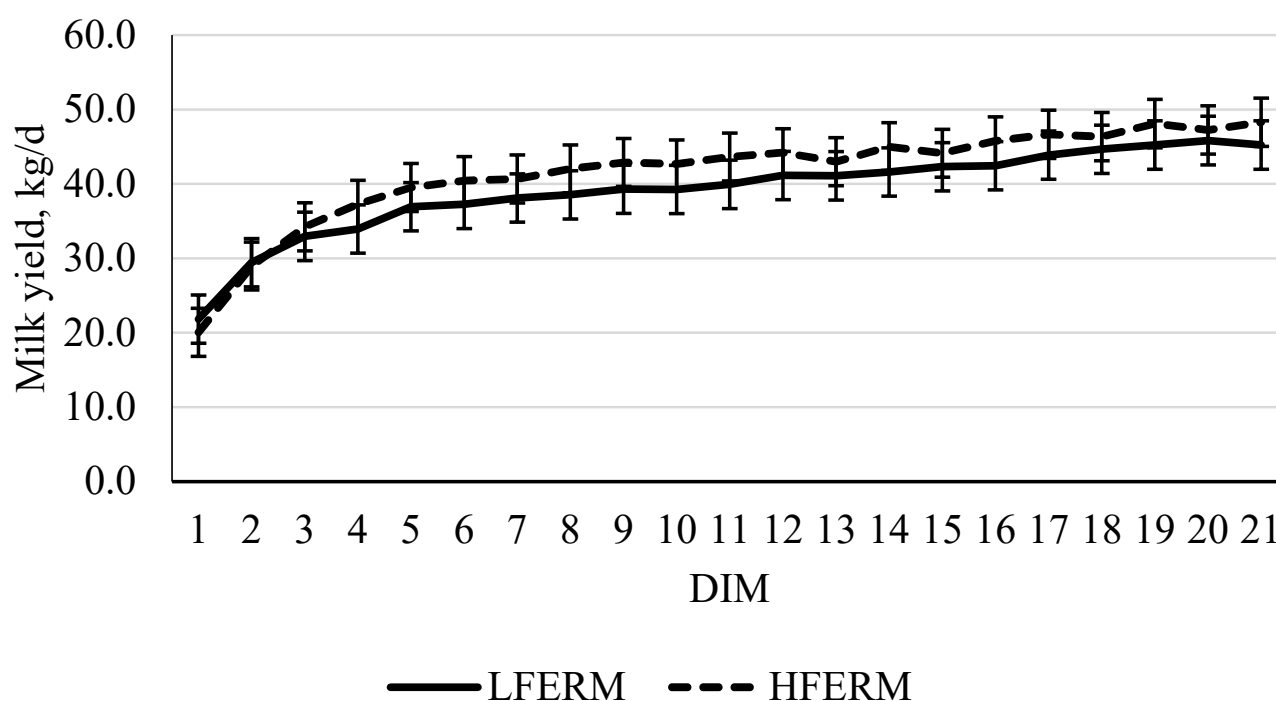


Figure 2. Interaction of diet fermentability and days in milk (DIM) on milk yield of multiparous Holstein cows 3 weeks postpartum fed a low (LFERM; dry-rolled corn) or high (HFERM; dry-rolled wheat) fermentable diet. An interaction between diet fermentability and time was detected ($p = 0.02$) with HFERM producing more milk after d 3. The data are presented as LSM with 95% CI.

Diet fermentability, RPC, and time interacted on SCS ($p = 0.04$; Table 5; Supplemental Figure S1) 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 5). 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 5). Regardless of treatment, MUN decreased

with time ($p < 0.01$). The HFERM diet decreased milk net energy compared to LFERM (0.78 Mcal/kg [0.74, 0.82] vs. 0.83 Mcal/kg [0.79, 0.86]; $p = 0.02$; Table 5). Milk net energy decreased with time ($p < 0.01$). There was an overall time effect on ECM and FCM, which increased from wk 1 to wk 2 ($p < 0.01$) 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 ($p > 0.35$; Table 4).

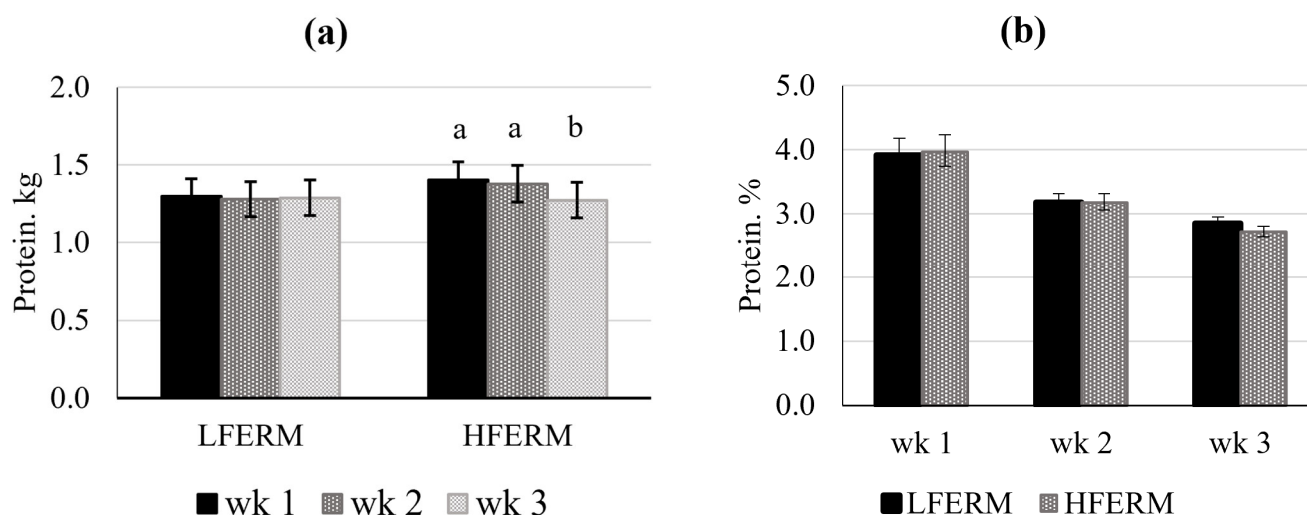


Figure 3. Interaction of diet fermentability and time on milk true protein yield (a) and percent (b) of multiparous Holstein cows 3 weeks postpartum fed a low (LFERM; dry-rolled corn) or high (HFERM; dry-rolled wheat) fermentable diet. (a) An interaction between diet fermentability and time was detected ($p = 0.04$). Within HFERM, milk true protein yield decreased from wk 1 to 3 ($p = 0.01$), but for LFERM, there was no evidence of difference in protein yield across weeks ($p > 0.05$). Within a treatment, means with differing superscripts differ ($p < 0.05$). The data are presented as LSM with 95% CI. (b) An interaction between diet fermentability and time was detected ($p = 0.05$). Milk true protein percentage decreased more rapidly over time for HFERM than for LFERM. The data are presented as LSM with 95% CI.

Table 4. 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}				<i>p</i> -Value ³						
	LFERM C−	LFERM C+	HFERM C−	HFERM C+	Ferm	Chol	Time	F × C	F × T	C × T	F × C × T
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	0.80	<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, h	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
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+ = low fermentability, RPC; HFERM C− = high fermentability, no RPC; HFERM C+ = high fermentability, RPC; ² Treatment means presented as least square means with 95% confidence intervals; ³ F × C: Fermentability × choline; F × T: Fermentability × time; C × T: Choline × time; F × C × T: fermentability × choline × time; ⁴ Feed efficiency = ECM/DMI.

Table 5. 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).

Item	Treatment ^{1,2}				<i>p</i> -Value ⁴						
	LFERM C−	LFERM C+	HFERM C−	HFERM C+	Ferm	Chol	Time	F × C	F × T	C × T	F × C × T
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

Table 5. Cont.

Item	Treatment ^{1,2}				<i>p</i> -Value ⁴						
	LFERM C−	LFERM C+	HFERM C−	HFERM C+	Ferm	Chol	Time	F × C	F × T	C × T	F × C × T
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	0.50	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	0.50	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

¹ LFERM C− = low fermentability, no RPC; LFERM C+ = low fermentability, RPC; HFERM C− = high 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 F × C: Fermentability × choline; F × T: Fermentability × time; C × T: Choline × time; F × C × T: fermentability × choline × time.

3.2.3. Milk Fatty Acids

Milk fatty acid (FA) concentrations are reported in Table 6. The HFERM diets decreased the milk 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 ($p > 0.05$).

Table 6. Milk fatty acid (FA) 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).

Item ³	Treatment ^{1,2}				<i>p</i> -Value ⁴		
	LFERM C−	LFERM C+	HFERM C−	HFERM C+	Ferm	Chol	Wk
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
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
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
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
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
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
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
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
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
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
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
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
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
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
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

¹ LFERM C− = low fermentability, no RPC; LFERM C+ = low fermentability, RPC; HFERM C− = high 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; ⁴ Mixed FA: Fermentability × choline × time interaction, $p = 0.03$.

Diet fermentability, RPC, and time interacted to affect mixed FA content ($p = 0.03$; Supplemental Figure S2), where LFERM C+ increased mixed FA in 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 7), 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 ($p = 0.08$).

Table 7. 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).

Item ³	Treatment ^{1,2}				p-Value ⁴		
	LFERM C−	LFERM C+	HFERM C−	HFERM C+	Ferm	Chol	Wk
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
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
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
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
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
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
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
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
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
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
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
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

Table 7. Cont.

Item ³	Treatment ^{1,2}				p-Value ⁴		
	LFERM C–	LFERM C+	HFERM C–	HFERM C+	Ferm	Chol	Wk
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
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
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

¹ LFERM C– = low fermentability, no RPC; LFERM C+ = low fermentability, RPC; HFERM C– = high fermentability, no RPC; HFERM C+ = high fermentability, RPC; ² Treatment means presented as least square means with 95% confidence intervals; ³ 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; ⁴ MCFA: Fermentability × choline interaction, $p = 0.08$; C14:0: Fermentability × choline interaction, $p = 0.05$; De novo FA: Fermentability × choline interaction, $p = 0.08$; Mixed FA: Fermentability × choline interaction, $p = 0.07$; Mixed FA: Fermentability × choline × time interaction, $p = 0.09$.

Individual milk FA content for C14 to C18 chain lengths is presented in Table 6 and yields are presented in Table 7. 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 in wk 1 compared to HFERM C+ ($p = 0.08$) but did not differ from the other 2 treatments. Pairwise differences were not detected in 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]; $p = 0.10$). The 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]; $p = 0.01$). The 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.

3.2.4. Blood Metabolites

Results for blood metabolite concentrations are presented in Table 8. 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 ($p < 0.01$; Supplemental Figure S3), 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 over 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 ($p > 0.44$).

Table 8. 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}				<i>p</i> -Value						
	LFERM C−	LFERM C+	HFERM C−	HFERM C+	Ferm	Chol	Time	F × C ³	F × T ³	C × T ³	F × C × T ³
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, μ g/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+ = low fermentability, RPC; HFERM C− = high fermentability, no RPC; HFERM C+ = high fermentability, RPC; ² Treatment means presented as least square means with 95% confidence intervals; ³ F × C: Fermentability × choline; F × T: Fermentability × time; C × T: Choline × time; F × C × T: fermentability × choline × time.

3.2.5. Body Weight and Body Condition

Body weight (BW), BW change, and body condition score (BCS) changes are reported in Tables 9 and 10 for the pre- and postpartum periods, respectively. 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.

Table 9. 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−) diet or a diet containing rumen-protected choline (RPC; C+).

Item	Treatment ^{1,2}		<i>p</i> -Value
	C−	C+	
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− = 30 g/d of soy hulls; C+ = 30 g/d of RPC (ReaShure, Balchem Corp., Montvale, NJ, USA); 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 10. 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).

Item	Treatment ^{1,2}				<i>p</i> -Value		
	LFERM C−	LFERM C+	HFERM C−	HFERM C+	Ferm	Chol	F × C
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+ = low fermentability, RPC; HFERM C− = high 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; ⁵ change from calving to 21 DIM.

4. Discussion

4.1. Prepartum

In the present study, prepartum DMI decreased progressively as calving date approached (Supplemental Figure S4), consistent with previous observations in transition cows [30–32]. However, C+ consumed less DMI compared with C− (14.8 vs. 17.1 kg/d) throughout the prepartum period. This is in stark contrast with the larger body of transition cow RPC supplementation research. In a recent meta-analysis, RPC linearly increased prepartum DMI across 19 experiments, with 12.9 g/d of choline ion increasing prepartum DMI 0.2 kg/d [14]. It is unclear why our experiment elicited such contrasting results. Interestingly, since the meta-analysis was published, a Wisconsin experiment [17] and

another study in our lab [33] fed RPC prepartum and also reduced DMI by about 2 kg/d. A major difference among these 3 studies, in which RPC dramatically depressed prepartum DMI, is that the RPC was fed as part of a TMR rather than using the historical experimental approach of top-dressing. Whether the feeding strategy is causative of the prepartum hypophagia is unclear, so further work is necessary to determine whether this pattern persists in other laboratories. If the pattern persists, evaluation of palatability differences, satiety signaling, or the pattern of RPC metabolism may be warranted. Another consideration may be whether the excess estimated methionine supply in the diet may have interacted with choline supplementation in some way to influence dry matter intake. Greater methionine content increases DMI prepartum, but it does not interact with choline [14], so the excess supply of methionine in the present study is not likely a factor for our prepartum feeding behavior results.

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 [1,4], so the reduction in 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 [34,35]. 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. This raises the possibility that lower intake prepartum may reflect improved feed efficiency. While feed efficiency was not calculated in this study, the observation that cows consumed less yet performed similarly postpartum suggests that such an effect may be economically and physiologically beneficial. The decline in feed intake as parturition approaches, mentioned previously, is accompanied by shifts in feeding behaviors, including the number of meals, meal length, meal size, and total eating time [17,36]; 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.

4.2. Postpartum

Postpartum DMI increased with DIM, as expected during early lactation, but was not affected by DF, RPC supplementation, or their interaction. While a recent study also failed to observe differences in DMI in postpartum cows fed ground corn or crushed wheat [37], a different study reduced DMI up to 3 kg/d through the substitution of dry ground corn with high moisture corn [2]. The starch fermentability difference in our study was only ~5% compared with the targeted 15% established by Alborno et al. [2]. In general, more fermentable starch sources reduce DMI to a greater extent in cows fed high-starch (>27%) vs. low-starch diets (<22%) [1,2]. The ability of the fermentability differences in our study to influence DMI may have been hindered by relatively lower starch concentrations of 23–25%. Feeding a higher-starch diet in our study likely would have improved our ability to observe treatment differences.

The lower starch digestibility rate than anticipated in our study may have been a byproduct of the 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 [19]. Smaller particle sizes enhance microbial attachment and enzymatic access to starch granules, thereby increasing the rate and extent of ruminal starch degradation [38]. Future work evaluating the effect of fermentability differences between diets may benefit from using grain sources with similar particle size or reducing particle size to the maximum extent possible. Ideally, processing grain through ensiling as a high-moisture product can reduce protein matrices surrounding starch and maximize the utility of comparing starch fermentability without confounding factors of differing grain types. Alternatively, selecting forage sources that inherently lack starch [2] enables greater manipulation of diet fermentability through grain treatments alone.

Additionally, IVSD7 in the present study shifted after wk 12, reducing the intended fermentability contrast across periods. This factor was controlled in our model as a block was included as a random effect to capture variability associated with the diet reformulation. A fixed effect of diet change was added to the models to determine the effect of diet change, but was later removed due to a lack of significance, indicating that the corn silage source change was not a relevant factor in the interpretation of these results, despite the shift in starch content. The lack of significance of corn silage change in the statistical models supports that these data can be interpreted with minimal concern as to the effects of corn silage changes in this study. Taking these factors into consideration, starch concentrations may have been too low and there may have been insufficient differences in fermentation rates and corresponding portal-drained propionate influx between diets to produce differences in HQT-mediated hypophagic response [34].

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 [12,39,40]. 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 [14]. 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 [15]. 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 represents 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. The low dose of RPC in the present study was inadvertent due to a ration formulation error; nonetheless, it provides valuable data as field nutritionists consider the value of lower feeding rates of RPC in an effort to reduce costs while maintaining benefits. Doses this low have been evaluated in only a few studies [14]. Nonetheless, this lower choline ion dose in combination with the lesser divergence in starch fermentability achieved between DF treatments, as discussed earlier, 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, and future studies should ensure adequate starch content and divergence in starch fermentability rates to achieve adequate capability to truly test the ability of RPC to mitigate hypophagia from highly fermentable diets.

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 lower for HFERM than LFERM in a corresponding manner

to the expected daily DMI, consistent with Oba and Allen [1], because greater starch fermentability increases the rate of propionate production and can suppress intake via hepatic oxidation feedback. The moderate starch inclusion rate (25%) in the present study may have been insufficient to elicit a HOT-mediated reduction in meal size [34], especially considering that more fermentable postpartum diets have a greater hypophagic response at greater dietary starch concentrations [3]. Furthermore, the largest meal size was greater in HFERM compared to LFERM, although average meal size did not differ. This is the first report of the largest meal size for postpartum cows in the literature. Lactating dairy cows consume almost twice as much feed in the first 2 h after feeding as at any other time throughout the day [21], with 20 to 35% of total daily feed intake occurring in the first hour [41]. This likely means that the largest meal occurs immediately after feeding and could serve as a predictive variable for estimating daily DMI. Liang et al. [42] used accumulated DMI over the first 2 h after feeding to estimate daily DMI with modest success, so more research is needed in this area to leverage the ability of post-feeding DMI and meal size to develop our understanding of DMI predictions and feeding behavior in dairy cows.

Feeding RPC is a key tool on dairy farms to reduce ketone body formation through a reduction in 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 [43,44]. 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 [14]. 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 [45,46]. 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; [13,17,47]). 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, while having relatively average DMI compared with similar studies [14]. While there were instances of elevated ketones in the present study that required treatment, care was taken to ensure that treatment was administered after blood samples were obtained to avoid confounding blood metabolite results. Overall, the reduction in BHB observed in the present study at 6.4 g/d of choline ion supports that even minimal supplementation may benefit postpartum dairy cow metabolic health.

Beyond effects on ketone body formation, RPC tended to increase plasma glucose concentration postpartum. A recent meta-analysis showed that increasing choline ion supplementation linearly increased postpartum plasma glucose concentrations [14]. At the hepatic level, RPC supplementation upregulates hepatic genes related to glucose export (GLUT2) and gluconeogenesis during early lactation, which could potentially contribute to greater circulating glucose [48]; however, the mechanisms of the greater circulating glucose content in our study are unclear without more robust measurements. The observed tendency for greater glucose concentration observed in our study supports that even low-dose RPC may increase circulating glucose concentrations during early lactation in very high-producing cows.

Despite differences in plasma glucose and BHB concentrations and their connection to energy balance, 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 the greater MUN observed for HFERM. The altered BUN and MUN may reflect the 0.5% greater CP content of the HFERM diet due to the greater CP content of the wheat than corn, and the

more rapid ruminal degradability of wheat protein compared with corn [19]. This increased degradability would provide more rapidly available ruminal ammonia to support microbial growth or could 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 increased ruminal 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. Another study using wheat to increase diet fermentability in postpartum cows failed to observe milk yield responses [37]. In further contrast, postpartum cows fed more rapidly fermentable high-moisture corn had decreased milk yield compared with those fed dry-ground corn [2], likely due to reduced DMI. Feeding more fermentable starch sources in peak lactation has had similarly variable results on milk yield; wheat increased milk yield 1.7 kg/d compared to cows fed corn [49], while high-moisture corn did not affect milk yield compared with dry-ground corn [50]. Given the variation in previously reported milk yield responses of postpartum cows fed more fermentable diets, it is unclear why greater milk yield was observed in the present study.

Our study failed to increase ECM despite broader evidence that RPC typically increases ECM [14]. The meta-analysis by Arshad et al. [14] evaluated the interaction of RPC and dietary methionine concentrations. At higher levels of dietary methionine as a percentage of metabolizable protein, responses in production to supplemental choline appear blunted as dietary choline inclusion increases. Our diets were formulated to meet the estimated needs of the cows in this study, but are on the higher end of metabolizable methionine concentration calculated by Arshad et al. [14] in their meta-analysis. We may have observed greater production responses to choline supplementation (and greater quantities of choline) had the metabolizable methionine concentration in our diets been lower.

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 and magnitude of change were observed in early postpartum cows fed wheat vs. corn [49], and in cows fed steam-flaked corn compared to cracked corn [51]. In the present study, the increase in milk yield without a concomitant increase in fat yield 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 [3].

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 [52,53]. In classical milk fat depression, the proportion of de novo FA synthesized in the mammary gland declines, whereas preformed FA from plasma uptake typically increases [52]. 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. In terms of milk FA yield, HFERM tended to decrease preformed FA yield, but there was no evidence of difference for de novo FA, mixed FA, or overall fat yield. 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, neither dietary fat supply nor greater adipose mobilization are likely explanatory factors for the milk fat responses observed herein. 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.

One thing to consider is that treatment of hyperketonemia in this study may have influenced the observed results. All treatments were administered immediately after the collection of blood samples, when possible, to avoid confounding effects on metabolic results. While metabolic changes are most dramatically observed within hours of treatment [54], alteration of blood metabolites can persist for up to a week depending on treatment strategy [55]. The primary factor influencing ketosis incidence in the present study was diet fermentability (LFERM = 11, HFERM = 4), although there was no significant effect of diet fermentability on plasma BHB, FFA, or glucose concentrations. Additionally, ketosis treatment can increase milk production for an extended period after treatment [56,57], although this has not been observed in all instances [54,57]. Given the ability of ketosis treatment protocols to influence metabolic and production responses, consideration should be given to this factor when interpreting these results.

5. Conclusions

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 very 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, even at low doses, supports metabolic adaptation without altering total feed intake in high-producing cows. 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.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/dairy7040053/s1>, Figure S1: Interaction of rumen-protected choline (RPC), diet fermentability, and time on somatic cell score (SCS) of multiparous Holstein cows 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); Figure S2: Interaction of rumen-protected choline (RPC), diet fermentability, and time on mixed FA percent of multiparous Holstein cows 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); Figure S3: Interaction of diet fermentability and time on blood urea nitrogen (BUN) concentration of multiparous Holstein cows 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); Figure S4: Prepartum DMI of multiparous Holstein cows fed a control diet (C−) or diet containing RPC (C+) during the last 3 weeks prepartum; Table S1: Summary of multiparous Holstein cow exclusions from the postpartum dataset by treatment, including reason and timing of removal (DIM).

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Abbreviations

The following abbreviations are used in this manuscript:

AHF	Acid hydrolysis fat
aNDFom	Amylase neutral detergent fiber organic matter
BCS	Body condition score
BHB	β -hydroxybutyrate
BUN	Blood urea nitrogen
BW	Body weight
C–	No choline treatment
C+	Choline treatment
CP	Crude protein
DIM	Days in milk
DF	Diet fermentability
DM	Dry matter
DMI	Dry matter intake
DTRC	Dairy Teaching and Research Center
ECD	Expected calving date
ECM	Energy-corrected milk
EE	Ether extract
FCM	Fat-corrected milk
FA	Fatty acid
FFA	Free fatty acid
HFERM	High-fermentability treatment
HOT	Hepatic oxidation theory
IVSD7	7-h in vitro starch digestibility
LFERM	Low-fermentability treatment
MCFA	Medium-chain fatty acid
MUFA	Monounsaturated fatty acid
NE	Net energy
NEB	Negative energy balance
PUFA	Polyunsaturated fatty acid
RIC	Roughage intake control
RPC	Rumen-protected choline
SCC	Somatic cell count
SCFA	Short-chain fatty acid
SFA	Saturated fatty acid

SCS	Somatic cell score
TAG	Triacylglycerol
TFA	Trans fatty acid
TMR	Total mixed ration
UFA	Unsaturated fatty acid
VLDL	Very low-density lipoprotein

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