Choline supply during negative nutrient balance alters hepatic cystathionine β -synthase, intermediates of the methionine cycle and transsulfuration pathway, and liver function in Holstein cows

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ABSTRACT

Although choline requirements for cows are unknown, enhanced postruminal supply may decrease liver triacylglycerol and increase flux through the Met cycle to improve immunometabolic status during a negative nutrient balance (NNB). Our objectives were to investigate the effects of postruminal choline supply during a feed restriction–induced NNB on (1) hepatic activity cystathionine β -synthase and transcription of enzymes in the transsulfuration pathway and Met cycle; (2) hepatic metabolites in the Met cycle and the transsulfuration pathway, bile acids, and energy metabolism; and (3) plasma biomarkers of liver function, inflammation, and oxidative stress. Ten primiparous rumen-cannulated Holstein cows (158 ± 24 d postpartum) were used in a replicated 5×5 Latin square design with 4-d treatment periods and 10 d of recovery (14 d/period). Treatments were unrestricted intake with abomasal infusion of water, restricted intake (R; 60% of net energy for lactation requirements) with abomasal infusion of water, or R plus abomasal infusion of 6.25, 12.5, or 25 g/d choline ion. Liver tissue was collected on d 5 after infusions ended, and blood was collected on d 1, 3, and 5. Statistical contrasts were A0 versus R0 (CONT1), R versus the average of choline doses (CONT2), and tests of linear and quadratic effects of choline dose. Activity of cystathionine β -synthase was lower with R (CONT1) and decreased linearly with choline. Hepatic glutathione was not different with R or choline, but taurine tended to be greater with choline (CONT2). Betaine and carnitine were greater with R (CONT1) and further increased with choline

(CONT2). Concentrations of NAD^+ were greater with choline (CONT2). Cholic and glycol-chenodeoxycholic acids were decreased by R and choline, while taurocholic and tauro-chenodeoxycholic acids were not altered. Plasma aspartate aminotransferase and bilirubin were greater with R (CONT1) but decreased with choline (CONT2). Paraoxonase was lower with R and increased with choline (CONT2). Data suggest that enhanced supply of choline during NNB decreases entry of homocysteine to the transsulfuration pathway, potentially favoring remethylation to Met by acquiring a methyl group from betaine. As such, Met may provide methyl groups for synthesis of carnitine. Along with production data indicating that 12.5 g/d choline ion improved milk yield and liver fatty acid metabolism during NNB, the changes in blood biomarkers also suggest a beneficial effect of choline supply on liver function and oxidative stress.

Key words: 1-carbon metabolism, lactation, methyl donors, oxidative stress

INTRODUCTION

During the periparturient period, dairy cows enter a period of negative nutrient balance (NNB) in which lipid mobilization increases, resulting in greater non-esterified fatty acid (NEFA) uptake by the liver and increased triacylglycerol (TAG) accumulation (Loor et al., 2013; Du et al., 2018). This period of NNB is also associated with increased disease incidence and sub-optimal production (Roche et al., 2013). While many studies have investigated the potential for choline to alleviate hepatic TAG accumulation in periparturient cows (Cooke et al., 2007; Zom et al., 2011; Elek et al., 2013), choline may also have immunomodulatory effects via Met (Vailati-Riboni et al., 2017) and may be beneficial in alleviating the inflammatory response that

Received January 30, 2019.

Accepted March 18, 2019.

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production of taurine and GSH via increased SAM. Increasing antioxidant production may alleviate oxidative stress and inflammation in the liver due to increased reactive oxygen metabolite (**ROM**) production (Osorio et al., 2014b; Zhou et al., 2016b; Batistel et al., 2018). Choline has been shown to alleviate oxidative stress in nonruminants (Sachan et al., 2005; Mehta et al., 2009; Wu et al., 2014). However, effects in dairy cattle are not entirely clear. One study with rumen-protected choline (**RPC**) reported no changes in markers of oxidative stress (Zhou et al., 2016a), whereas another reported enhanced antioxidant capacity (Sun et al., 2016).

The choline requirement for dairy cattle is unknown (NRC, 2001). Previous studies with RPC have suggested that the optimal amount needed for periparturient cows is 12.5 g/d (Zom et al., 2011; Zhou et al., 2016c). Recent work by de Veth et al. (2016) with lactating cows in positive nutrient balance reported that abomasal infusions of choline chloride for 5 d at 12.5 and 25 g/d increased the appearance of choline and its metabolites betaine and phosphocholine in the portal vein. In the context of physiologic adaptations during an NNB, a clear benefit would follow from determining not only optimal amounts of postruminally delivered choline, but also mechanisms in methyl donor

Figure 1. Methionine and choline metabolism. BADH = betaine aldehyde dehydrogenase; CBS = cystathionine β -synthase; CDO = cysteine dioxygenase; CEPT1 = choline/ethanolamine phosphotransferase 1; CHDH = choline dehydrogenase; CHK = choline kinase; CSAD = cysteine sulfinic acid decarboxylase; DMG = dimethylglycine; DMGDH = dimethylglycine dehydrogenase; GLC = glutamate-cysteine ligase catalytic subunit; GPX1 = glutathione peroxidase 1; GSH = reduced glutathione; GSR = glutathione reductase; GSS = glutathione synthase; GSSG = glutathione disulfide; Hcy = homocysteine; MAT = methionine adenosyltransferase; MTHFR = methylenetetrahydrofolate reductase; MTR = 5-methyltetrahydrofolate-homocysteine methyltransferase; PC = phosphatidylcholine; PCYT = phosphate cytidyltransferase; PE = phosphatidylethanolamine; PEMT = phosphatidylethanolamine methyltransferase; SAH = *S*-adenosylhomocysteine; SAHH = *S*-adenosylhomocysteine hydrolase; SAM = *S*-adenosylmethionine; SAR = sarcosine; SARDH = sarcosine dehydrogenase; THF = tetrahydrofolate; BHMT = betaine homocysteine methyltransferase; CDP = cytidine diphosphate.

metabolism responsive to choline that may improve liver function.

Our general hypothesis was that incremental post-ruminal supply of choline during a feed restriction-induced NNB would improve liver function and alleviate inflammation and oxidative stress. Our objectives were to investigate the effects of post-ruminal choline supply during a feed restriction-induced NNB on (1) hepatic activity of CBS and transcription of enzymes in the transsulfuration pathway and Met cycle; (2) hepatic concentration of metabolites in the Met cycle and transsulfuration pathway, bile acid synthesis, and energy metabolism; and (3) concentration of plasma biomarkers of liver function, inflammation, and oxidative stress.

MATERIALS AND METHODS

Experimental Design and Treatments

All procedures involving animals received approval from the Institutional Animal Care and Use Committee at the University of Illinois (protocol no. 16176). Ten primiparous rumen-cannulated Holstein cows (158 ± 24 postpartum) were used in a 5×5 replicated Latin square design. Periods were 14 d in length, with 4-d treatment periods (d 1–4) and 10 d of recovery (d 5–14). Treatment periods of 4 d were chosen based on preliminary observations that suggested concentrations of NEFA and BHB stabilized and began to return to normal after 4 d of feed restriction at 60% of NE_L requirements (unpublished data). The 10-d recovery periods were also chosen based on preliminary observations that suggested that milk yields and concentrations of NEFA and BHB had returned to baseline levels 10 d after feed restriction at 60% of NE_L requirements (unpublished data). Treatments in the present study were ad libitum intake with abomasal infusion of water (**A0**), restricted intake (**R**; 60% of net energy for lactation requirements) with abomasal infusion of water (**R0**) or **R** plus abomasal infusion of 6.25 (**R6.25**), 12.5 (**R12.5**), or 25 (**R25**) g/d choline ion. The diet was provided as TMR and formulated to meet NRC (2001) requirements (Table 1). Diets were fed once daily at 0800 h. The restricted intakes were calculated based on NRC equations to provide 60% of the NE_L at the start of each treatment period as described previously (Moyes et al., 2009). All cows returned to ad libitum intake during the 10-d recovery period.

The choline was provided as aqueous choline chloride (70%, Balchem Corp., New Hampton, NY). The doses of 6.25, 12.5, and 25 g/d represent the amount of choline ion that was supplied post-ruminally, and they were achieved by providing 12.0, 23.9, and 47.9 g/d of

choline chloride. Doses above and below the suggested requirement of 12.5 g/d of choline allowed for the determination of linear and quadratic responses of post-ruminal choline ion supplementation. Abomasal infusions were accomplished by passing an infusion line through the rumino-omasal orifice into the abomasum by way of the rumen cannula. An infusion line (Tygon tubing), fitted with a 60-mL plastic bottle with the bottom removed and with an approximately 8-cm rubber flange, was inserted into the abomasum. The tubing was in place for the duration of the study and was checked daily for proper placement. The infusion apparatus was connected to an external infusion line via a hole in the rumen cannula plug. The internal and external lines were connected by way of quick-disconnect fitting to allow disconnection for milking. The treatment solutions were provided by mixing the 70% choline chloride with deionized tap water and were infused continuously using rotary peristaltic pumps with their corresponding 1,200-mL bag (Sentinel Enteral Feeding Pump, Alcor Scientific Inc., Smithfield, RI). A daily volume of 3,600 mL/cow (three 1,200-mL bags per day) was infused during the 4-d treatment period. Infusions were initiated after cows returned from the 0400 h milking on d 1, and ran continuously until they were ended on the morning of d 5 at the 0400 h milking. Because the cows were milked 3 times a day (0400, 1200, and 1930 h), a new infusion bag was hooked up after each milking.

Table 1. Ingredient composition (% of DM) of the diet

Item	Content
Ingredient	
Alfalfa hay	9.26
Ajipro-L-G3 ¹	0.11
Canola meal	15.15
Corn gluten meal	4.56
Corn silage	41.33
Ground shelled corn	23.24
ProVAAL2 AADvantage ²	0.96
Smartamine M ³	0.07
Soy hulls	1.69
Vitamin-mineral mix ⁴	3.57
Zinpro Availa-Dairy ⁵	0.06
Chemical analysis	
CP	16.00
ADF	20.00
NDF	18.19
Ether extract	3.05
NE_L , Mcal/kg	1.71

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⁴Contained a minimum of 12.5% Ca, 10.4% Na, 2.2% Mg, 8.0% K, 0.1% S, 7.1% Se, 244.5 kIU of vitamin A/kg, 48.9 kIU of vitamin D₃/kg, and 0.922 kIU of vitamin E/kg.

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Blood Collection and Analyses

Blood was sampled from the coccygeal vein after the morning milking, approximately 3 h before feeding, on d 1, 3, and 5. Samples were collected into vacutainer tubes containing lithium heparin (BD Vacutainer, Becton, Dickinson and Co., Franklin Lakes, NJ) and placed on ice. Plasma was obtained by centrifugation at $2,000 \times g$ for 15 min at 4°C, and aliquots were stored at -80°C until further analysis. Glucose, albumin, total cholesterol, total bilirubin, creatinine, urea, aspartate aminotransferase (**AST**), γ -glutamyl transpeptidase (**GGT**), alkaline phosphatase, total plasma ROM, ferric reducing ability of plasma (**FRAP**), haptoglobin, ceruloplasmin, nitric oxide and nitric oxide metabolites, paraoxonase (**PON**) activity, vitamin A, vitamin E, and β -carotene were analyzed as described by Trevisi et al. (2013). Myeloperoxidase was measured as described by Bionaz et al. (2007).

Liver Biopsy and Tissue Analysis

Liver biopsies were conducted on the morning of d 5 when cows returned from the 0400 h milking (within 1 h of the infusions ending) using a similar technique described by Vailati Riboni et al. (2015). An 18-gauge by 10.2-cm bone marrow probe (Monoject-8881-247087; Medtronic, Minneapolis, MN) was used to obtain approximately 2 g (wet weight) of liver. Samples were snap frozen in liquid nitrogen and subsequently stored at -80°C.

Approximately 150 mg of frozen tissue was extracted for metabolomics analysis using the 2-step protocol described by Wu et al. (2008). Targeted metabolomics (LC/MS/MS) was performed to quantify metabolites related to 1-carbon metabolism, the transsulfuration pathway, the tricarboxylic acid (**TCA**) cycle, and bile acid synthesis. Samples were analyzed with the 5500 QTRAP LC/MS/MS system (Sciex, Framingham, MA) in the Metabolomics Laboratory of the Roy J. Carver Biotechnology Center, University of Illinois at Urbana-Champaign. Details of the system and analysis are provided in Supplemental File and Table S1 (<https://doi.org/10.3168/jds.2019-16406>). Total RNA was extracted from liver tissue and quantitative PCR performed as described previously (Osorio et al., 2014a). Quantitative PCR performance and primer information are included in Supplemental Tables S2 and S3 (<https://doi.org/10.3168/jds.2019-16406>). Activity of CBS was measured as described by Zhou et al. (2017). Total and oxidized GSH in liver tissue were measured using a commercial kit (cat. no. NWH-GSH01; Northwest Life Science Specialties LLC, Vancouver, WA). Reduced

GSH was calculated as reduced GSH = total GSH - oxidized GSH.

Statistical Analyses

Data were analyzed as a replicated 5×5 Latin square using the MIXED procedure of SAS 9.4 (SAS Institute Inc., Cary, NC). The model tested the random effects of square and cow within square and the fixed effects of period and treatment. Covariance structures compared were compound symmetry, autoregressive order 1, autoregressive heterogeneous order 1, unstructured, and Toeplitz. The compound symmetry structure was used based on the corrected Akaike information criterion. The Kenward-Roger degrees of freedom approximation was used to determine the denominator degrees of freedom for tests of fixed effects. The CONTRAST statement of SAS was used to perform orthogonal contrasts: CONT1 = A0 compared with R0; CONT2 = R0 compared with the average of the 3 choline treatments; and the linear and quadratic effects of R0, R6.25, R12.5, and R25. Contrasts were adjusted using the IML procedure to reflect uneven spacing of treatments.

The mRNA abundance data were $\log_2$ -scale transformed to fit normal distribution of residuals. Tables and graphs contain the $\log_2$ back-transformed means that resulted from the statistical analysis. Least squares means and standard errors were determined using the LSMEANS statement of SAS.

RESULTS

Met and Choline Metabolism Pathway

The hepatic mRNA abundance for genes in the Met and choline metabolism pathways are presented in Table 2. Abundance of choline dehydrogenase (*CHDH*) was lower with R ($P = 0.07$; CONT1) and choline supply ($P = 0.08$; CONT2). The abundance of dimethylglycine dehydrogenase (*DMGDH*) was greater with choline supply ($P = 0.02$; CONT2). Sarcosine dehydrogenase was lower with R ($P < 0.001$; CONT1). Abundance of betaine aldehyde dehydrogenase did not differ with R or choline supply ($P > 0.10$).

Transsulfuration Pathway

The activity of CBS and the mRNA abundance results for genes in the transsulfuration pathway are reported in Table 2. Activity of CBS was lower with R ($P = 0.07$; CONT1) and decreased linearly with increasing choline dose ($P = 0.03$). However, abundance of *CBS* was not different with R or choline supply ($P > 0.10$).

The abundance of cysteine dioxygenase 1 (*CDO1*) and cysteine sulfinic acid decarboxylase (*CSAD*) was reduced with R ($P = 0.02$ and $P < 0.001$, respectively; CONT1). A tendency for a quadratic effect was also observed for *CDO1* ($P = 0.07$), with R12.5 having the greatest abundance. Choline supply reduced the mRNA abundance of glutamate-cysteine ligase (*GCL*) catalytic subunit (*GCLC*) compared with R0 ($P = 0.07$; CONT2). A quadratic effect was also observed for *GCLC* ($P = 0.04$); the abundance decreased from R0 to R6.25, but then increased from R12.5 to R25. Restriction (CONT1) reduced the abundance of glutathione synthase (*GSS*; $P < 0.001$) and glutathione reductase (*GSR*; $P < 0.001$). Additionally, a quadratic tendency existed, with the abundance of *GSS* decreasing from R0 through R12.5 and then increasing with R25 ($P = 0.10$). Last, glutathione peroxidase 1 (*GPX1*) mRNA abundance was lower with choline supply compared with R0 ($P = 0.01$; CONT2), and a quadratic effect ($P = 0.06$) of choline dose was observed.

Liver Metabolites

Methionine and Choline Metabolism. The effects of R and choline on hepatic metabolites involved

in Met and choline metabolism are presented in Table 3. Liver betaine was greater with R ($P = 0.02$; CONT1) and increased further with choline supply ($P < 0.001$; CONT2). This increase was linear across choline dose ($P < 0.001$). Choline concentrations in liver tissue increased linearly across choline dose ($P = 0.001$). The concentration of Gly was greater with R ($P = 0.04$; CONT1). Liver acetylcholine/butyrobetaine, which could not be detected separately, increased linearly across choline dose ($P = 0.03$).

Transsulfuration Pathways. The concentrations of metabolites in the transsulfuration pathway are presented in Table 3. No differences were detected in the concentrations of total GSH or reduced GSH with R or choline ($P > 0.10$). Restriction (CONT1) reduced the concentrations of cystathionine ($P = 0.001$), hypotaurine ($P < 0.001$), and Ser ($P = 0.001$). Concentrations of Ser also tended to decrease with choline compared with R0 ($P = 0.10$; CONT2). Last, taurine concentrations in the liver tended to increase with choline ($P = 0.09$; CONT2).

Energy Metabolism. The results for metabolites involved in energy metabolism and the TCA cycle are presented in Table 3. Of the 23 metabolites measured in these pathways, only 7 were detected. The concen-

Table 2. Least squares means and pooled SEM for hepatic activity of cystathionine β -synthase (CBS) and mRNA abundance of genes in the methionine cycle and transsulfuration pathways in Holstein cows fed 5 different treatments¹ over a period of 4 d

Item ²	Treatment						P-value ³			
							Contrast		Treatment	
	A0	R0	R6.25	R12.5	R25	SEM	1	2	L	Q
Enzyme activity, nmol of product·h ⁻¹ ·mg of protein ⁻¹										
CBS	36.99	29.44	29.81	26.29	22.15	3.07	0.07	0.29	0.03	0.70
Met and choline metabolism, log ₂ back transformed										
<i>BADH</i>	1.17	1.17	1.20	1.19	1.17	0.06	0.99	0.69	0.80	0.47
<i>CHDH</i>	1.37	1.06	0.87	0.86	0.91	0.17	0.07	0.08	0.39	0.14
<i>DMGDH</i>	0.89	1.00	1.18	1.13	1.10	0.10	0.13	0.02	0.42	0.06
<i>SARDH</i>	1.29	0.87	0.91	0.95	0.92	0.05	<0.001	0.12	0.29	0.17
Transsulfuration pathway, log ₂ back transformed										
<i>CBS</i>	1.07	1.05	1.06	1.07	1.05	0.06	0.75	0.82	0.95	0.67
<i>CDO1</i>	1.14	0.94	0.98	1.04	0.89	0.09	0.02	0.72	0.36	0.07
<i>CSAD</i>	1.37	0.70	0.69	0.73	0.70	0.08	<0.001	0.89	0.90	0.81
<i>GCLC</i>	1.25	1.05	0.85	0.86	0.96	0.09	0.11	0.07	0.70	0.04
<i>GPX1</i>	0.97	0.98	0.89	0.91	0.91	0.04	0.78	0.01	0.13	0.06
<i>GSR</i>	1.47	1.07	1.00	1.00	0.98	0.06	<0.001	0.24	0.29	0.61
<i>GSS</i>	1.36	0.95	0.91	0.89	0.93	0.05	<0.001	0.21	0.73	0.10

¹A0 = ad libitum intake, abomasal infusion of water; R0 = restricted intake, abomasal infusion of water; R6.25 = restricted intake, abomasal infusion of 6.25 g/d of choline; R12.5 = restricted intake, abomasal infusion of 12.5 g/d of choline; R25 = restricted intake, abomasal infusion of 25 g/d of choline. Choline was provided via 70% aqueous solution of choline chloride (Balchem Corporation, New Hampton, NY) mixed with water.

²*BADH* = betaine aldehyde dehydrogenase; *CBS* = cystathionine β -synthase; *CHDH* = choline dehydrogenase; *CDO1* = cysteine dioxygenase; *CSAD* = cysteine sulfinic acid decarboxylase; *DMGDH* = dimethylglycine dehydrogenase; *GCLC* = glutamate-cysteine ligase, catalytic subunit; *GPX1* = glutathione peroxidase 1; *GSR* = glutathione reductase; *GSS* = glutathione synthase; *SARDH* = sarcosine dehydrogenase.

³Contrasts: 1 = A0 compared with R0; 2 = R0 compared with the average of the 3 treatments (6.25, 12.5, and 25 g/d of choline). Linear (L) and quadratic (Q) effects of treatments R0, R6.25, R12.5, and R25.

Table 3. Least squares means and associated SEM for liver concentrations of glutathione and of metabolites (measured via liquid chromatography-tandem MS, LC-MS/MS) in the transmethylation, transsulfuration and bile acid synthesis pathways, and the tricarboxylic acid (TCA) cycle in Holstein cows in 5 treatments¹ applied for 4 d

Item ^{2,3}	Treatment						P-value ⁴			
							Contrast		Treatment	
	A0	R0	R6.25	R12.5	R25	SEM	1	2	L	Q
Glutathione, $\mu\text{mol/g}$ of protein										
Total glutathione	55.02	54.08	47.42	51.14	49.32	3.56	0.84	0.21	0.50	0.51
Reduced glutathione	54.40	53.36	46.80	50.44	48.63	3.52	0.82	0.21	0.50	0.51
Met and choline metabolism, ng/mg of total protein										
Adenosine	6,168.28	6,152.49	6,717.69	6,313.50	6,199.07	719.31	0.99	0.74	0.89	0.69
Acetylcholine/butyrobetaine	13.68	17.53	19.36	18.81	24.58	2.50	0.12	0.16	0.03	0.57
Betaine	371.67	2,552.78	5,349.58	6,263.75	10,893.00	682.89	0.02	<0.001	<0.001	0.91
Choline	1,297.40	1,453.73	1,459.47	1,520.73	2,347.57	182.64	0.54	0.12	0.001	0.09
Glycine	11,971	14,047	13,532	13,796	14,768	841	0.04	0.98	0.31	0.34
5-Methyltetrahydrofolic acid	2.50	2.20	2.12	2.08	2.01	0.74	0.74	0.95	0.72	0.82
N,N-dimethylglycine	23.39	22.63	24.20	22.69	23.56	1.00	0.47	0.28	0.64	0.80
Transsulfuration pathway, ng/mg of total protein										
Cystathionine	498.88	294.85	243.56	291.88	307.26	61.84	0.001	0.64	0.43	0.40
Cysteinylsulfenic acid	881.49	718.12	578.34	670.00	654.00	102.88	0.15	0.34	0.82	0.51
Hypotaurine	680.94	387.02	338.32	392.98	407.33	62.68	<0.001	0.89	0.56	0.68
Serine	1,983.80	1,428.96	985.98	1,353.80	1,340.84	146.64	0.001	0.10	0.71	0.14
Taurine	11,603.8	13,976.6	16,368.2	16,857.8	17,194.2	2,094.1	0.17	0.09	0.15	0.37
Energy/TCA, ng/mg of total protein										
cAMP	121.65	107.69	103.35	112.91	111.62	17.79	0.57	0.89	0.93	0.94
Carnitine	105.45	176.94	187.61	165.82	224.83	21.48	0.01	0.39	0.04	0.15
FAD	2,241.09	2,092.59	2,464.29	2,384.74	2,447.49	214.92	0.58	0.10	0.26	0.33
Fumarate	807.12	939.99	712.79	690.88	946.48	102.41	0.31	0.13	0.64	0.01
Glutamate	42,453	38,858	40,334	39,248	40,270	1,719	0.13	0.55	0.66	0.92
NAD ⁺	33,358.0	24,986.2	28,041.4	29,546.1	25,686.2	2904.2	0.001	0.10	0.91	0.02
NADH	4,914.79	2,443.64	3,512.46	4,056.10	2,824.29	657.30	0.004	0.11	0.77	0.03
NAD ⁺ :NADH	10.46	12.32	11.68	8.83	12.23	1.89	0.35	0.38	0.88	0.08
Bile acid synthesis, ng/mg of total protein										
Cholic acid	651.48	1,792.26	758.58	445.86	956.97	738.70	0.10	0.07	0.37	0.05
Glycocholic acid	26,817	17,667	22,337	17,941	21,741	2,093	0.004	0.21	0.34	0.98
Glyco-chenodeoxycholic acid	988.20	654.46	575.17	366.08	494.27	66.63	0.001	0.02	0.05	0.03
Lithocholic acid	8,855.2	6,760.8	11,378.9	10,437.6	9,397.2	1,947.0	0.29	0.04	0.37	0.07
Taurocholic acid	2,462.50	2,370.37	3,139.91	2,385.03	3,202.36	461.49	0.89	0.30	0.32	0.86
Tauro-chenodeoxycholic acid	178.69	219.38	252.76	161.36	242.16	46.97	0.45	0.92	0.94	0.31

¹A0 = ad libitum intake, abomasal infusion of water; R0 = restricted intake, abomasal infusion of water; R6.25 = restricted intake, abomasal infusion of 6.25 g/d of choline; R12.5 = restricted intake, abomasal infusion of 12.5 g/d of choline; R25 = restricted intake, abomasal infusion of 25 g/d of choline. Choline was provided via 70% aqueous solution of choline chloride (Balchem Corporation, New Hampton, NY) mixed with water.

²Metabolites not detected by LC-MS/MS: betaine aldehyde, folic acid, tetrahydrofolic acid, N-methylglycine, cysteine, γ -glutamylcysteine, vitamin B₆, vitamin B₁₂, acetyl-CoA, α -ketoglutaric acid, AMP, ATP, citrate/isocitrate, glutamine, guanosine 5'-triphosphate, malic acid, NAD⁺, NADH, nicotinic acid, pyruvate, succinyl-CoA, tri-methyl-lysine, uridine diphosphate, and deoxycholic/chenodeoxycholic acids.

³cAMP = cyclic adenosine monophosphate; FAD = flavin adenine dinucleotide.

⁴Contrasts: 1 = A0 compared with R0; 2 = R0 compared with the average of the 3 treatments (6.25, 12.5, and 25 g/d of choline). Linear (L) and quadratic (Q) effects of treatments R0, R6.25, R12.5, and R25.

tration of carnitine was greater with R ($P = 0.01$; CONT1) and increased linearly with choline ($P = 0.04$); however, R6.25 had the lowest concentration. Choline supply tended to increase the concentration of flavin adenine dinucleotide (FAD; $P = 0.10$; CONT2) relative to R0. A quadratic effect ($P = 0.01$) was observed for hepatic fumarate concentration, with concentration decreasing from R0 through R12.5, but then increasing with R25. Restriction (CONT1) led to lower concentrations of NAD^+ ($P = 0.001$; CONT1) and NADH ($P = 0.004$). Choline supply also tended to increase the concentration of NAD^+ relative to R0 ($P = 0.10$; CONT2). However, a quadratic increase occurred in both NAD^+ ($P = 0.02$) and NADH ($P = 0.03$) across choline dose, with R12.5 having the greatest concentration. A quadratic effect was also observed for the ratio of NAD^+ to NADH ($P = 0.08$), with R12.5 having the lowest ratio.

Bile Acid Synthesis. The data for concentrations of hepatic metabolites in bile acid synthesis are presented in Table 3. Restriction (CONT1) led to lower concentrations of glycocholic acid ($P = 0.004$) and glyco-chenodeoxycholic acid ($P = 0.001$). Glyco-chenodeoxycholic acid decreased with choline supply ($P < 0.001$; CONT2) and the decrease was quadratic ($P = 0.03$), with R12.5 having the lowest concentration. Cholic acid tended to be greater with R ($P = 0.10$; CONT1) but tended to be lower with choline compared with R0 ($P = 0.07$; CONT2). The decrease in cholic acid with choline was quadratic ($P = 0.05$), with the R12.5 having the least hepatic cholic acid. Last, lithocholic acid increased with choline relative to R0 ($P = 0.04$; CONT2). A quadratic effect was also observed for lithocholic acid ($P = 0.07$), with concentrations increasing from R0 to R6.25, but then decreasing with R12.5 and R25.

Plasma Biomarkers

Energy Metabolism. Plasma biomarker results related to energy metabolism are presented in Table 4. Concentrations of creatinine in plasma were greater with R ($P = 0.07$; CONT1), while plasma glucose was lower with R ($P < 0.001$; CONT1) and was further reduced with choline supply ($P < 0.001$; CONT2). A treatment $\times$ day interaction was also observed (Supplemental Figure S1; <https://doi.org/10.3168/jds.2019-16406>) for plasma glucose ($P = 0.002$) and urea ($P = 0.01$).

Liver Function. Results for the plasma biomarkers related to liver function are presented in Table 4. Alkaline phosphatase in plasma was lower with R ($P = 0.05$; CONT1) and increased linearly with increasing choline ($P = 0.02$), except for R12.5, which had the

lowest plasma alkaline phosphatase. The level of AST was greater with R ($P = 0.01$; CONT1) but decreased with choline relative to R0 ($P = 0.001$; CONT2). The decrease in AST with choline was quadratic ($P = 0.001$), with R12.5 being associated with the lowest level. Concentrations of cholesterol tended to be greater in plasma with R ($P = 0.09$; CONT1), whereas γ -glutamyl transferase in plasma was greater with R ($P = 0.01$; CONT1). Plasma PON was lower with R ($P = 0.02$; CONT1) but increased with choline supply compared with R0 ($P = 0.04$; CONT2). Total bilirubin in plasma was greater with R ($P < 0.001$; CONT1), while choline decreased total bilirubin compared with R0 ($P = 0.01$; CONT2). However, the concentration of total bilirubin changed quadratically in response to choline supply ($P = 0.06$), with a decrease from R0 to R6.25 and then increases with R12.5 and R25. Treatment $\times$ day interactions (Supplemental Figure S2; <https://doi.org/10.3168/jds.2019-16406>) were observed for plasma alkaline phosphatase ($P < 0.001$), AST ($P = 0.003$), GGT ($P = 0.02$), and total bilirubin ($P = 0.06$).

Inflammation and Oxidative Stress. Results for plasma biomarkers of inflammation and oxidative stress are reported in Table 4. No differences ($P > 0.10$) were observed for the markers of inflammation: albumin, ceruloplasmin, haptoglobin, and myeloperoxidase. The biomarker of antioxidant capacity ferric reducing ability was lower with R ($P = 0.01$; CONT1). Plasma nitrogen oxide was lower with R ($P = 0.04$; CONT1). The concentration of nitrite (NO_2^-) decreased linearly with choline ($P = 0.06$). In contrast, nitrate (NO_3^-) concentration was greater with R ($P = 0.06$; CONT1) but tended to decrease with choline ($P = 0.06$; CONT2). The decrease in nitrate with choline was quadratic across choline dose ($P = 0.04$); concentrations decreased from R0 to R6.25, but then increased with R12.5 and R25. Plasma α -tocopherol decreased linearly with choline ($P = 0.08$), while β -carotene decreased quadratically ($P = 0.08$). However, R12.5 led to the greatest concentrations of both. Additionally, plasma concentrations of retinol decreased with R ($P = 0.03$; CONT1). A treatment $\times$ day interaction (Supplemental Figure S3; <https://doi.org/10.3168/jds.2019-16406>) was observed for retinol ($P = 0.03$), nitrogen oxide ($P = 0.08$), and FRAP ($P = 0.06$).

DISCUSSION

Markers of Energy Metabolism

Data on BW, energy balance, milk yield, and plasma concentrations of NEFA, BHB, and glucose clearly confirmed that our feed restriction model successfully

induced an NNB (Coleman et al., 2019). Carnitine is essential for the transport of long-chain fatty acyl-CoA into the mitochondria via carnitine palmitoyltransferase-1 (**CPT1**; Rebouche and Seim, 1998). As such, the observed increase in carnitine with R may have helped support increased fatty acid oxidation due to the increase in NEFA mobilization. The change in carnitine with choline (especially R12.5) fits our hypothesis that the decrease in liver TAG content and the upregulation of *CPT1A* with choline were associated with increased fatty acid oxidation (Coleman et al., 2019). Although the major source of carnitine within the liver is via uptake as a result of transport from skeletal muscle (Bremer, 1983), hepatic carnitine could also be synthesized endogenously from butyrobetaine via γ -butyrobetaine hydroxylase or from metabolism of the amino acids Met and Lys; Met serves as a methyl donor to synthesize trimethyllysine using SAM (Carter et al., 1995). Greater remethylation of homocysteine

(discussed below) may have supported synthesis of carnitine, resulting in the linear increase detected here. Although we did not measure enzymes involved in the synthesis of carnitine, the fact that trimethyllysine could not be detected in the present study may suggest a fast metabolism and partly support our hypothesis. In addition, while we could not detect butyrobetaine separately from acetylcholine, the linear increase in their concentration with increasing doses of choline may also have contributed to an increase in carnitine synthesis.

Nicotinamide adenine dinucleotide is a redox carrier that is involved in many oxido-reductive pathways such as amino acid catabolism, β -oxidation, the TCA cycle, electron transport chain, and the urea cycle (Strömmand et al., 2019). The decreases in NAD^+ and NADH that we observed with R are likely due to the reduction in energy status with NNB. Although the ratio of NAD^+/NADH was not altered, the greater NAD^+ and NADH

Table 4. Least squares means and associated pooled SEM for plasma concentrations of biomarkers of energy metabolism, liver function, inflammation, and oxidative stress in Holstein cows fed 5 different treatments¹ over a period of 4 d

Item ²	Treatment					SEM	Contrast ³			
	A0	R0	R6.25	R12.5	R25		1	2	L	Q
Energy metabolism										
Creatinine, $\mu\text{mol/L}$	87.64	89.17	90.83	89.36	89.57	1.42	0.07	0.26	0.86	0.39
Glucose, ⁴ mmol/L	4.71	4.43	4.39	4.40	4.44	0.06	<0.001	0.67	0.80	0.85
Urea, ⁴ mmol/L	4.64	4.95	4.90	4.85	4.72	0.18	0.12	0.43	0.23	0.95
Liver function										
Alkaline phosphatase, ⁴ U/L	76.07	69.06	69.77	68.99	77.40	4.07	0.05	0.29	0.02	0.20
AST, ⁴ U/L	108.32	118.96	114.02	98.79	108.42	9.47	0.01	0.001	0.01	0.001
Cholesterol, mmol/L	5.74	6.61	5.87	6.20	5.96	0.32	0.09	0.64	0.84	0.71
GGT, ⁴ U/L	42.42	47.82	47.36	47.73	47.01	8.32	0.01	0.24	0.60	0.15
Paraoxonase, U/mL	102.56	96.69	100.46	100.49	100.21	5.77	0.02	0.04	0.39	0.11
Total bilirubin, ⁵ $\mu\text{mol/L}$	2.44	3.09	2.75	2.91	2.92	0.14	<0.001	0.01	0.46	0.06
Inflammation										
Albumin, g/L	36.45	36.31	36.63	36.40	36.44	0.46	0.69	0.51	0.92	0.64
Ceruloplasmin, $\mu\text{mol/L}$	3.43	3.39	3.37	3.40	3.39	0.14	0.73	0.99	0.91	0.99
Haptoglobin, g/L	0.36	0.43	0.33	0.39	0.31	0.08	0.38	0.22	0.25	0.86
Myeloperoxidase, U/L	458.07	459.30	450.33	451.30	437.17	17.73	0.95	0.43	0.28	0.95
Oxidative stress										
α -Tocopherol, $\mu\text{g/mL}$	5.77	6.23	5.85	6.33	5.58	0.47	0.13	0.21	0.08	0.35
β -Carotene, $\text{mg}/100 \text{ mL}$	0.36	0.37	0.36	0.41	0.35	0.04	0.61	0.99	0.46	0.08
FRAP, ⁵ $\mu\text{mol/L}$	135.83	126.41	124.37	124.97	123.90	3.64	0.01	0.46	0.51	0.80
NO_2^- , $\mu\text{mol/L}$	4.30	4.47	4.43	4.26	4.11	0.17	0.41	0.24	0.06	0.98
NO_3^- , $\mu\text{mol/L}$	14.47	15.03	14.39	14.55	14.78	0.29	0.06	0.06	0.74	0.04
NOx , ⁵ $\mu\text{mol/L}$	18.58	19.28	19.15	18.65	18.89	0.31	0.04	0.17	0.19	0.22
Retinol, ⁴ $\mu\text{g}/100 \text{ mL}$	38.55	35.28	37.78	35.26	38.31	2.10	0.03	0.13	0.11	0.69
ROMt, $\text{mg of H}_2\text{O}_2/100 \text{ mL}$	15.84	15.78	15.41	15.85	15.49	0.58	0.92	0.72	0.80	0.93

¹A0 = ad libitum intake, abomasal infusion of water; R0 = restricted intake, abomasal infusion of water; R6.25 = restricted intake, abomasal infusion of 6.25 g/d of choline; R12.5 = restricted intake, abomasal infusion of 12.5 g/d of choline; R25 = restricted intake, abomasal infusion of 25 g/d of choline. Choline was provided via 70% aqueous solution of choline chloride (Balchem Corporation, New Hampton, NY) mixed with water.

²AST = aspartate aminotransferase; GGT = γ -glutamyl transpeptidase; FRAP = ferric reducing ability of plasma; NO_2^- = nitrite; NO_3^- = nitrate; NOx = nitric oxide metabolites; ROMt = reactive oxygen species, total.

³Contrasts: 1 = A0 compared with R0; 2 = R0 compared with the average of the 3 treatments (6.25, 12.5, and 25 g/d of choline). Linear (L) and quadratic (Q) effects of treatments R0, R6.25, R12.5, and R25.

⁴Treatment $\times$ day, $P \leq 0.05$.

⁵Treatment $\times$ day, $P \leq 0.10$.

with choline supply could also indicate an increase in energy production, for example, as a result of greater β -oxidation (Rogers et al., 2014). The fact that R12.5 had the greatest concentration of NADH further supports the hypothesis that those cows experienced the greatest transport of fatty acids into the mitochondria for oxidation.

Met and Choline Metabolism

The linear increase in hepatic choline with increasing dose indicates that our abomasal infusions successfully delivered choline postruminally. During its metabolism to betaine, choline is first converted to betaine aldehyde via *CHDH*. Subsequently, betaine aldehyde is converted to betaine via *BADH* (Slow and Garrow, 2006). Although mRNA abundance of *CHDH* was lower with choline compared with R0 and *BADH* did not differ with infusion, the increase in hepatic betaine concentration with choline indicated that oxidation of choline to betaine was increased. This increase was likely used to support the remethylation to Met, followed by production of SAM to maintain synthesis of carnitine during an NNB. The changes in *CHDH* abundance in the present study contrast with previous work on periparturient cows in which long-term (−28 through 30 d around calving) RPC supplementation led to greater hepatic *BADH* and *CHDH* expression (Zhou et al., 2017). The abundance of these genes was also increased in vitro when isolated bovine hepatocytes were supplied with choline (Zhou et al., 2018). Thus, we speculate that the increased supply of choline during a feed restriction-induced NNB had an inhibitory effect on *CHDH* abundance. Additionally, betaine aldehyde has been observed to be a competitive inhibitor of *CHDH* activity (Haubrich and Gerber, 1981; Perrino and Pierce, 2000). While betaine aldehyde was not detected in the present study, suggesting rapid metabolism, changes in *CHDH* may have been mediated by increased betaine aldehyde synthesis with increased choline supply.

Transsulfuration Pathway

Cystathionine β -synthase converts homocysteine to cystathionine using Ser. We have previously reported that homocysteine production may be enhanced by choline supply, and that Met production via MTR and BHMT was enhanced with R and increasing choline supply (Coleman et al., 2019). Therefore, the decrease in CBS activity with R and choline supply corresponds with changes in MTR and BHMT activity; more homocysteine was being used to synthesize Met rather than cystathionine for the transsulfuration

pathways during R and with choline supply during a feed restriction-induced NNB. This interpretation is also supported by the decrease in hepatic cystathionine concentration with R compared with A0. This result suggests a decreased flux in the transsulfuration pathway with R and choline, underscoring the importance of maintaining Met production when Met intake (or its supply from microbial protein) is limited during a feed restriction-induced NNB.

The regulation of expression and activity of CBS occurs at multiple levels via hormones and growth factors (Zhu et al., 2018), but of interest here is the regulation by SAM, which can allosterically activate CBS (Banerjee et al., 2003). *S*-Adenosylmethionine is synthesized from Met via methionine adenosyltransferase (Corrales et al., 2002). Thus, we hypothesized that enhanced Met production with increased choline supply would enhance SAM production, leading to greater CBS activity. However, the decrease in CBS activity indicates that SAM was not being used to activate CBS. Instead, the increase in carnitine concentration in the present study suggests that SAM was being used as a methyl donor in the synthesis of carnitine to support fatty acid oxidation (Grum et al., 1996).

The next step in the transsulfuration pathway is the synthesis of cysteine from cystathionine, which can then be used to produce taurine and GSH. Both taurine and GSH are potent antioxidants that help alleviate ROM damage in the liver (Zhou et al., 2016b). Glutathione is synthesized via 2 enzymes, *GCL* and *GSS* (Lushchak, 2012). Glutamate cysteine ligase converts the cysteine to γ -glutamylcysteine and is the rate-limiting step of GSH synthesis (Chen et al., 2013). This enzyme is a heterodimer made up of a catalytic subunit (*GCLC*) and a modifier subunit, with *GCLC* controlling the catalytic activity of the enzyme (Chen et al., 2013). In the second step of GSH synthesis, γ -glutamylcysteine is used to synthesize reduced GSH (Chen et al., 2013). The reduced GSH that is produced can then be oxidized via the enzyme *GSR*, and the oxidized GSH can be reduced via *GPX1* (Lushchak, 2012). The lack of difference in GSH concentration in liver tissue along with lower mRNA abundance of *GCLC* and *GPX1* with choline suggests that GSH metabolism was not altered significantly by choline supply. Additionally, these results suggest that production of GSH may not be highly regulated at the transcriptional level.

Taurine is also synthesized in the transsulfuration pathway. Cysteinesulfinic acid is synthesized from cysteine by *CDO1* and can then be used by *CSAD* to produce taurine (Park et al., 2017). While *CDO1* and *CSAD* were decreased with R, the decrease in hypotaurine concentration with R suggests rapid conversion

to taurine. Despite the lack of change in mRNA abundance of *CDO1* and *CSAD* with choline supply, the greater hepatic taurine concentration suggests increased synthesis with choline supply. In rat liver, changes in CDO activity with variation in sulfur amino acid intake have occurred at the protein rather than mRNA level (Bella et al., 1999a, b). This may partly explain why the difference in taurine concentrations were not related to mRNA expression of *CDO1* and *CSAD*. A change in efficiency of the reaction due to differences in the supply of trace minerals also cannot be discounted, especially taking into account that some (Fe, Zn) can enhance catalytic activity (at least in the mouse; McCoy et al., 2006). The fact that concentrations of taurocholic acid and tauro-chenodeoxycholic acid were not increased by the greater taurine production (discussed below) suggest that bile acid metabolism might also have contributed to the accumulation of taurine with choline supply.

Together, the changes in GSH and taurine concentrations indicate that when cows were supplemented with choline, the transsulfuration pathway was altered toward production of taurine, rather than GSH. However, it is not clear why cows were synthesizing taurine rather than GSH. A possibility exists that increased taurine was a “tissue-protective” mechanism induced in response to oxidative stress or immune status (Schuller-Levis and Park, 2004). In addition, CDO is known to be regulated by cysteine concentration such that high concentrations of cysteine increase CDO activity (Yin et al., 2016). As CBS activity was decreased with choline and R, less cysteine would have been available. Because low cysteine would be expected to reduce taurine synthesis, the change in CBS suggests that something besides cysteine was mediating the production of taurine rather than GSH.

Glutamate cysteine ligase is also regulated by feedback inhibition of GSH (Chen et al., 2013). Although GSH concentrations were not different in the present study, it is possible that they initially increased under low cysteine concentrations, resulting in feedback inhibition of GCL, leading to cysteine being converted to cysteinesulfinic acid, rather than γ -glutamylcysteine. Finally, the changes in synthesis of taurine and GSH could have been related to bile acid metabolism. In mice, when bile acid recycling was disrupted, changes in hepatic *CDO*, *CSAD*, *GCL*, and metabolites indicated that GSH production was decreased, whereas taurine increased (Wang et al., 2018). Although bile acid recycling itself was not measured in the present study, a possibility exists that this process was inhibited by choline during NNB, which resulted in enhanced taurine rather than GSH synthesis. Additionally, secretion of bile is partly stimulated by cholecystokinin, a hormone

that is released in response to macronutrient digestion, particularly fat (Dockray, 2012). Thus, it is also possible that DMI restriction per se reduced the amount of bile secreted and subsequently recycled. As such, it could have contributed to enhanced taurine synthesis. The increase in plasma bilirubin with R and the modest mitigating effect of choline supply suggest that the capacity of the liver to excrete bilirubin into bile was not diminished.

Bile Acid Synthesis Metabolites

The primary bile acids formed in liver are cholic acid and chenodeoxycholic acid, and they are subsequently conjugated with Gly or taurine for secretion into bile (Chiang, 2013). Ruminants secrete more taurine-conjugated bile acids than Gly-conjugated bile acids (Moore and Christie, 1984). Conjugation of bile acids is a 2-step process, catalyzed by cholesteryl-CoA synthetase and cholesteryl-CoA-amino acid *N*-acyltransferase (Vessey, 1978). While chenodeoxycholic acid could not be detected in the present study, the tendency for decreased cholic acid concentration suggests that bile acid synthesis was decreased with choline supply. Additionally, the decrease in glyco-chenodeoxycholic acid with choline suggests that conjugation of chenodeoxycholic acid with Gly was also decreased. Surprisingly, the conjugation with Gly also decreased with R because hepatic Gly was increased with R and it was not being used in the synthesis of GSH either. If Gly was not being used for these 2 functions, it may have been directed toward its other functions, such as creatine, purine, and heme synthesis (Wang et al., 2013). Despite the greater hepatic concentration of taurine with choline, the lack of change in concentrations of taurocholic acid and tauro-chenodeoxycholic acid indicated that the increased taurine was being used for other processes, such as its antioxidant functions neutralizing hypochlorous acid and enhancing antioxidant enzymes (Marcinkiewicz and Kontny, 2014). It is also worth noting that in guinea pig, rat, and human liver, Gly and taurine were observed to be mutually inhibitory of conjugation reactions (Vessey, 1978). Thus, elevated taurine concentrations may have been exerting an inhibitory effect on conjugation reactions, which may have contributed to the decrease in glyco-chenodeoxycholic acid with increased choline supply.

Biomarkers of Liver Function

Paraoxonase, a marker of liver function, is synthesized and released from the liver into the bloodstream, where it associates with high-density lipoproteins to protect them from oxidation (Ferré et al., 2002). The

greater plasma PON concentration with choline supply contrasts with previous work in which RPC was fed during the periparturient period (Zhou et al., 2016a). Additionally, the enzyme AST is primarily found in the liver and is used clinically as an indicator of liver function (Huang et al., 2006). Bilirubin is produced during heme degradation, and increases in circulating bilirubin during the periparturient period reflects impaired clearance by the liver (Assenat et al., 2004). Although plasma AST and bilirubin decreased with choline supply relative to R0, Zahra et al. (2006) observed no difference in plasma AST with RPC supplementation, while Zhou et al. (2016a) found no differences in AST or bilirubin with RPC supplementation. Overall, changes in the plasma concentrations of AST, PON, and bilirubin suggest that liver function was improved with postruminal choline supply during a feed restriction-induced NNB. These improvements in liver function may have been enhanced by the potential increase in fatty acid oxidation described earlier. This is because TAG accumulation is associated with reduced liver function, and increasing fatty acid oxidation would have limited the deposition of TAG.

Biomarkers of Inflammation and Oxidative Stress

We found few differences in the plasma concentrations of biomarkers of inflammation and oxidative stress with our feed restriction-induced NNB, which is contrary to work with periparturient cows (Bertoni et al., 2008). Increases in FRAP and retinol are positively associated with antioxidant activity (Benzie and Strain, 1996; Trevisi et al., 2011), whereas nitric oxide increases are associated with greater inflammation and oxidative stress (Trevisi et al., 2013). Therefore, the changes in FRAP, retinol, and nitric oxide indicate that our feed restriction model did induce some oxidative stress. The lack of overall choline effects on these biomarkers is consistent with the lack of changes in ROM, FRAP, haptoglobin, ceruloplasmin, and albumin previously observed by Zhou et al. (2016a) when 12.5 g/d of choline was supplemented during the periparturient period. Both α -tocopherol and β -carotene can serve as antioxidants (Sordillo and Aitken, 2009). Therefore, the increase in the concentration of both α -tocopherol and β -carotene with R12.5 could indicate greater antioxidant capacity in those cows.

Overall, the lack of changes in the plasma concentrations of biomarkers associated with inflammation and oxidative stress may be attributable to several aspects of our experimental design. One aspect is that first lactation heifers were used in our study. It is well documented that cows in their first lactation are less susceptible to disease incidence than multiparous cows

(Ingvarsen, 2006), suggesting that heifers may inherently have limited the inflammatory response. Another aspect is the length of our NNB. During the 3 wk of the pre- and postpartum period (i.e., the periparturient period), cows experience immense physiological and immunological changes (Trevisi and Minuti, 2018). Therefore, our 4-d feed restriction-induced NNB may not have been long enough to elicit major changes in inflammation and oxidative stress. Additionally, the cows used in the present study were in late lactation and therefore not experiencing the physical stress of calving and the physiological and immunological changes associated with it. This circumstance may also explain the lack of changes in biomarkers of inflammation and oxidative stress.

CONCLUSIONS

Postruminal choline supply during a feed restriction-induced NNB decreased activity of CBS, while increasing the production of betaine to maintain Met production. As such, Met may provide methyl groups for carnitine synthesis to support fatty acid oxidation. Choline supply altered flux of the transsulfuration pathway toward production of taurine, rather than GSH. Although choline was not associated with improvements in inflammatory status, it could play a role in reducing oxidative stress and improving certain aspects of liver function. Together, these data underscore the importance of regenerating Met during an NNB, especially when choline supply increases. In such circumstances, exogenous choline supply could have a positive effect on liver function.

ACKNOWLEDGMENTS

The authors acknowledge Ajinomoto Co. Inc. (Tokyo, Japan) for financial support of this research. Abdulrahman S. M. Alharthi is the recipient of a PhD fellowship from King Saud University (Rihadh, Saudi Arabia).

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