Symposium review: One-carbon metabolism and methyl donor nutrition in the dairy cow*

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ABSTRACT

The present review focuses on methyl donor metabolism and nutrition in the periparturient and lactating dairy cow. Methyl donors are involved in one-carbon metabolism, which includes the folate and Met cycles. These cycles work in unison to support lipid, nucleotide, and protein synthesis, as well as methylation reactions and the maintenance of redox status. A key feature of one-carbon metabolism is the multi-step conversion of tetrahydrofolate to 5-methyltetrahydrofolate. Homocysteine and 5-methyltetrahydrofolate are utilized by vitamin B₁₂-dependent Met synthase to couple the folate and Met cycles and generate Met. Methionine may also be remethylated from choline-derived betaine under the action of betaine hydroxymethyltransferase. Regardless, Met is converted within the Met cycle to *S*-adenosylmethionine, which is universally utilized in methyl-group transfer reactions including the synthesis of phosphatidylcholine. Homocysteine may also enter the transsulfuration pathway to generate glutathione or taurine for scavenging of reactive oxygen metabolites. In the transition cow, a high demand exists for compounds with a labile methyl group. Limited methyl group supply may contribute to inadequate hepatic phosphatidylcholine synthesis and hepatic triglyceride export, systemic oxidative stress, and compromised milk production. To minimize the perils associated with methyl donor deficiency, the peripartum cow relies on *de novo* methylneogenesis from tetrahydrofolate. In addition, dietary supplementation of rumen-protected folic acid, vitamin B₁₂, Met, choline, and betaine are potential nutritional approaches to target one-carbon

pools and improve methyl donor balance in transition cows. Such strategies have merit considering research demonstrating their ability to improve milk production efficiency, milk protein synthesis, hepatic health, and immune response. This review aims to summarize the current understanding of folic acid, vitamin B₁₂, Met, choline, and betaine utilization in the dairy cow. Methyl donor co-supplementation, fatty acid feeding strategies that may optimize methyl donor supplementation efficacy, and potential epigenetic mechanisms are also considered.

Key words: dairy cow, liver, methyl donor

INTRODUCTION

One-carbon metabolism is a set of biochemical reactions that support nucleic acid synthesis and serve to activate and transfer methyl groups for methylation reactions. Folate and Met metabolism are features of one-carbon metabolism that drive the production of the universal methyl donor *S*-adenosylmethionine (**SAM**). Methyl group transfer supports the synthesis of the glycerophospholipid phosphatidylcholine (**PtdChol**), AA homeostasis, redox defense, and methylation of DNA and histone proteins. In humans, one-carbon metabolism has received attention in the study of alcoholic and nonalcoholic fatty liver disease, maternal nutrition and fetal programming, cancer, and fertility (Laanpere et al., 2010; Anderson et al., 2012; Corbin and Zeisel, 2012; Ducker and Rabinowitz, 2017). In the dairy cow, the focus on one-carbon metabolism within the context of animal health and lactation has also accelerated with the advent of methyl donor feeding technologies and breakthrough experimental approaches to study methyl donor metabolism including transcriptomics and lipidomics.

At the onset of lactation, a high demand for methylated compounds exists because their incorporation into milk (e.g., milk choline and protein) is high. However, the supply of preformed labile methyl groups, Met, cho-

Received July 23, 2019.

Accepted February 10, 2020.

*Presented as part of the Ruminant Nutrition: Dietary Methyl Donor Supplementation and Hepatic Health in Transition Dairy Cows Symposium at the ADSA Annual Meeting, Cincinnati, Ohio, June 2019.

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line, and betaine in diets is low in ruminants because of extensive rumen degradation (Xue and Snoswell, 1986a) and reduced DMI. To overcome the deficit in methyl groups, ruminant animals rely on methylneogenesis (i.e., *de novo* synthesis of methyl groups from the one-carbon pool). These processes depend on an adequate supply of B vitamins (e.g., folates, vitamins B₆, and B₁₂). In addition, ruminants have developed mechanisms to reduce the rate of physiologically nonessential methylations, mostly sarcosine synthesis (Xue and Snoswell, 1986a; Snoswell and Xue, 1987). The turnover rate of the choline moiety is also decreased (Dawson et al., 1981), whereas its recycling rate from phospholipids is increased (Dawson et al., 1981; Emmanuel and Kennelly, 1984). Dietary methyl donor supplementation in the form of rumen-protected nutrients has potential to improve methyl group “economy” and methylneogenesis in the early-lactation cow.

Methionine and choline represent types of methyl donors that have been studied extensively in the periparturient dairy cow. Methionine is an EAA and immediate precursor for SAM production. Choline can be oxidized to betaine, which can subsequently participate to regenerate Met. Rumen-protected Met (**RPM**) supplementation has been shown to improve milk protein synthesis, reduce oxidative stress, and enhance immune function (Osorio et al., 2014; Zhou et al., 2016a; Batistel et al., 2018). Rumen-protected choline (**RPC**) supplementation has been demonstrated to increase milk production and improve metabolic health (reviewed by Pinotti et al., 2002; Sales et al., 2010; Shahsavari et al., 2016). Dietary betaine supplementation has also been considered in early-lactation cows (Davidson et al., 2008); however, our understanding of hepatic betaine utilization within one-carbon metabolism is rather limited in comparison to choline or Met. Co-supplementation of Met, choline, betaine, or a combination of these has also received consideration and warrants further investigation (Zhou et al., 2016d; Zang et al., 2019).

The primary objective of this review is to define the role or lack thereof for folates, B vitamins, Met, choline, and betaine within the context of one-carbon metabolism, transsulfuration, and transmethylation-mediated PtdChol synthesis in transition dairy cattle. The secondary objective is to describe the effects of folic acid, vitamin B₁₂, Met, choline, and betaine supplementation on health and milk production during lactation. Methyl donor co-supplementation, fatty acid feeding strategies that may optimize methyl donor supplementation efficacy, and potential epigenetic mechanisms are also considered. Limitations in our understanding of methyl donor nutrition are clearly stated with the intent to provoke continued research focused on methyl donor nutrition and metabolism in the dairy cow.

ONE-CARBON METABOLISM AND TRANSULFURATION

Folate Cycle

Dietary folic acid is reduced by the NADPH-dependent enzyme dihydrofolate reductase to form dihydrofolate followed by tetrahydrofolate (**THF**; Figure 1). In turn, THF is transformed into 5,10-methylenetetrahydrofolate (**5,10-CH₂-THF**) by the vitamin B₆-dependent enzyme serine hydroxymethyltransferase. In the same reversible reaction, the NEAA Ser (derived in part from the glycolytic intermediate 3-phosphoglycerate) is converted to Gly. The flavoenzyme methylenetetrahydrofolate reductase catalyzes the irreversible conversion of 5,10-CH₂-THF to 5-methyltetrahydrofolate (**5-mTHF**). The reaction requires flavin adenine dinucleotide, which is formed from the precursor riboflavin. In an alternative pathway directed toward *de novo* purine synthesis, 5,10-CH₂-THF is transformed into 10-formyl-THF in a series of reactions controlled by methylenetetrahydrofolate dehydrogenases. The demethylation of 5-mTHF by the cytosolic enzyme Met synthase (**MTR**) completes the folate cycle. Methionine synthase requires the cofactor vitamin B₁₂ and MTR reductase to maintain an adequate supply of activated vitamin B₁₂ and MTR activity. The major outcome of the folate cycle is the remethylation of homocysteine (**HCY**) to form Met.

Methionine Cycle

The function of the Met cycle is to produce SAM to aid in methyl group transfer (Figure 1). Specifically, Met adenosyltransferase (**MAT**) transfers an adenosyl moiety of ATP to Met generating SAM and triphosphate, which is cleaved to create pyrophosphate and phosphate. Multiple MAT isoenzymes exist. The isoenzymes MATI and MATIII are encoded by the *MAT1A* gene, which is predominantly expressed in liver (Martinov et al., 2010). The *MAT2A* gene is expressed across various tissues including liver and encodes the MATII isoenzyme. Of major importance, SAM that is produced by MAT is the source of methyl groups for most biological methylations (Mudd et al., 1995). Different types of methyltransferases promote methyl group transfer and convert SAM to *S*-adenosylhomocysteine (**SAH**). For example, glycine *N*-methyltransferase utilizes SAM to methylate glycine forming SAH and sarcosine. The DNA methyltransferases utilize SAM to methylate the 5-position of cytosine residues in DNA. Phosphatidylethanolamine *N*-methyltransferase (**PEMT**) transfers a total of 3 methyl groups from 3 SAM molecules to phosphatidyl-

ethanolamine (**PtdEth**) to form 3 SAH and a single PtdChol. In nonruminant animals, PtdChol synthesis by PEMT is a major consumer of SAM (Brosnan and Brosnan, 2006). The biochemistry is likely similar in ruminants (Lobley et al., 1996). Regardless, the product of methyltransferases, SAH, is hydrolyzed to HCY and adenosine in a reversible reaction catalyzed by *S*-adenosylhomocysteine hydrolase. In turn, HCY may be used by MTR to reform Met and complete the Met cycle. Alternatively, the vitamin B₆-dependent enzyme betaine-homocysteine *S*-methyltransferase (**BHMT**) catalyzes the transfer of a methyl group from betaine to HCY to produce Met and dimethylglycine. This reac-

tion is important because choline can be transformed into betaine via the intermediate betaine aldehyde by choline dehydrogenase and betaine aldehyde dehydrogenase.

Transsulfuration Pathway

The transsulfuration pathway connects Met with glutathione and taurine synthesis and plays an important role in the maintenance of cellular redox homeostasis (Figure 1). The first step involves the condensation of HCY with Ser to produce cystathionine by cystathionine β -synthase. The second step is the hydrolysis of

Figure 1. One-carbon metabolism, the transsulfuration pathway, and phosphatidylcholine (PtdChol) synthesis. BAD = betaine aldehyde dehydrogenase; BHMT = betaine-homocysteine methyltransferase; CBS = cystathionine β -synthase; CCT = CTP:phosphocholine cytidyltransferase; CDH = choline dehydrogenase; CDO = cysteine dioxygenase; CDP = cytidine diphosphate; C γ L = cystathionine γ -lyase; CK = choline kinase; CPT = cholinephosphotransferase; CSD = cysteine sulfinic acid decarboxylase; DAG = diacylglycerol; DHF = dihydrofolate; DMG = dimethylglycine; ECT = CTP:phosphoethanolamine cytidyltransferase; EK = ethanolamine kinase; EPT = ethanolaminephosphotransferase; FAD = flavin adenine dinucleotide; GCS = γ -glutamylcysteine synthase; GPR = glutathione-disulfide reductase; GPX = glutathione peroxidase; GS = glutathione synthetase; GSH = reduced glutathione; GSSG = oxidized glutathione; HCY = homocysteine; HDH = hypotaurine dehydrogenase; MATI/III = methionine adenosyltransferase I/III (liver specific isoenzymes); 5-mTHF = 5-methyltetrahydrofolate; MTHFR = methylenetetrahydrofolate reductase; MTR = methionine synthase; PEMT = phosphatidylethanolamine *N*-methyltransferase (other types of methyltransferases may utilize SAM; only PEMT is shown for simplicity); PtdEth = phosphatidylethanolamine; SAH = *S*-adenosylhomocysteine; SAM = *S*-adenosylmethionine (3 SAM are required to convert PtdEth to PtdChol); SHMT = serine hydroxymethyltransferase; THF = tetrahydrofolate. Associated B vitamin cofactors are denoted in parentheses.

cystathionine to cysteine, ammonia, and α -ketobutyrate by cystathionine γ -lyase (i.e., γ -cystathionase). In turn, cysteine is utilized by γ -glutamylcysteine synthase to form γ -glutamylcysteine. Glutathione synthetase transforms γ -glutamylcysteine and glycine into glutathione. Reduced glutathione (**GSH**) plays a critical role in protecting macromolecules from reactive oxygen and nitrogen species. Cysteine may also be utilized to form cysteine sulfate by cysteine dioxygenase. In turn, cysteine sulfinic acid decarboxylase converts cysteine sulfate to hypotaurine, which is then converted to taurine by hypotaurine dehydrogenase. Taurine is a nonproteinogenic AA and antioxidant that protects cells from oxidant-induced injury by forming taurine chloramine. Interestingly, oxidative conditions appear to downregulate MTR activity and upregulate cystathionine β -synthase to shift HCY utilization toward the transsulfuration pathway (Chen et al., 1995; Taoka et al., 1998).

IMPORTANCE OF FOLIC ACID AND VITAMIN B₁₂ FOR ENDOGENOUS METHYL DONOR PRODUCTION

Methylnogenesis for Labile Methyl Group Supply in Ruminants

As stated previously, dietary sources of preformed labile methyl groups are Met, choline, and betaine. In dairy cow diets, Met is frequently reported as the most limiting AA (NRC, 2001). In liver, HCY can be remethylated using the methyl group provided by choline and betaine catabolism under the action of the BHMT as described previously. Because the rumen degradation of choline and betaine limits their intestinal absorption, this metabolic pathway is probably of limited importance in the transition dairy cow unsupplemented with RPC or betaine (Xue and Snoswell, 1986a,b; Preynat et al., 2010). Consequently, the cow has a high dependence on folate metabolism to support methylnogenesis during the peripartum period and early lactation. The one-carbon unit essential to methyl group formation is provided by catabolism of serine, glycine, histidine, and formate. In humans, the major source of one-carbon units is serine (Davis et al., 2004); however, the relative importance of the different sources of one-carbon units in ruminants is still unknown. The function of HCY remethylation is to ensure an adequate supply of SAM when intake of preformed labile methyl groups is low. Such action also reduces the need for Met by ensuring intracellular cycling of the homocysteinyl moiety. Nevertheless, even in the presence of methylnogenesis, there is a minimal absolute requirement for Met as the homocysteinyl moiety cannot be synthesized in cows or other mammals. Remethylation of the homocyste-

inyl moiety is controlled by SAM, which allosterically inhibits the activity of methylenetetrahydrofolate reductase (Jencks and Mathews, 1987). High supply in preformed labile methyl groups reduces cycling of the homocysteinyl moiety promoting catabolism of HCY through the transsulfuration pathway (Brosnan and Brosnan, 2006). In lactating ewes, more than half of the methyl groups required to maintain normal metabolic functions are obtained through remethylation and folate metabolism (Snoswell and Xue, 1987). Because production of 5-mTHF is irreversible, the sole reaction for regeneration of THF is through the vitamin B₁₂-dependent MTR. Consequently, an inadequate supply of vitamin B₁₂ traps folic acid under its methylated form leading to a secondary deficiency in folic acid in spite of an adequate folate supply (Bässler, 1997).

In an attempt to estimate the importance of de novo synthesis of methyl groups in the transition cow, 24 multiparous cows, from the last 6 blocks of a larger study described by Preynat et al. (2009b), were fed the same basal pre-calving and lactation diets supplemented with or without rumen-protected Met (**RPM**) from 3 wk before calving until 12 wk of lactation (Preynat et al., 2009a). The lactation diets were calculated to provide Met at 1.83 and 2.23% of MP for the diets not supplemented and supplemented with RPM, respectively (NRC, 2001). Within each group, cows received either no vitamin supplement or weekly i.m. injections of 160 mg of folic acid and 10 mg of vitamin B₁₂. At 12 wk of lactation, Met kinetics were measured by isotope dilution using continuous infusions of [¹³C]-NaHCO₃ and L-[1-¹³C, methyl-²H₃]-Met. Increasing dietary Met supply enhanced Met entry rate (i.e., the sum of Met absorbed, Met from body protein degradation, and Met from remethylation of HCY). Supplementary folic acid plus vitamin B₁₂ tended to increase Met entry rate by approximately 20% in cows fed the lower Met diets but had no further effect for cows fed RPM (Figure 2). Nevertheless, vitamin supplementation increased Met utilization for protein synthesis, independent of dietary Met supply. It is noteworthy that on the week of these measurements, supplementary B vitamins increased milk production from 34.7 to 38.9 $\pm$ 1.0 kg/d as well as milk lactose, protein, and TS yields, regardless of dietary Met supply (Preynat et al., 2009a). Trying to quantify the contribution of methylnogenesis to labile methyl group supply in lactating cows by following the fate of molecules marked with stable isotopes is challenging because the information is limited to exchange through plasma. Indeed, remethylation occurs within cells at a different rate than exchange with extracellular fluid (Lobley et al., 1996). Under these experimental conditions, calculated remethylation values represent only the flux of remethylated HCY released in the plas-

ma and cannot be used to infer the number of cycles performed by each homocysteiny moiety within cells. Even if the remethylation rate within tissues cannot be quantified, the combined supplementation of folic acid and vitamin B₁₂ increased plasma Met concentration of cows fed the diet with 1.83% of MP as Met to the level observed in cows fed the diet with 2.23% of MP as Met (~25 μ M), while decreasing plasma concentrations of HCY by 20% (Preynat et al., 2009a). In cows fed RPM, the vitamin supplement had no effect on plasma Met concentrations but decreased plasma HCY and cysteine concentrations by 29 and 15%, respectively, and lowered Met oxidation by 42% (Figure 2). As DMI did not differ among cows fed or not fed a supplement of RPM, these observations support an increase in remethylation of HCY and de novo synthesis of methyl groups when the dietary supply of folic acid and vitamin B₁₂ was increased.

Effects of Adequate Folic Acid and Vitamin B₁₂ Supply on Health and Milk Production

Girard and Matte (2005) observed that supplementary folic acid does not improve lactation performance in dairy cows when plasma concentrations of vitamin B₁₂ are under 200 pg/mL. When vitamin B₁₂ supply is sufficient to maintain plasma concentrations of vitamin B₁₂ over 200 pg/mL, due to ruminal synthesis or supplementation, the major effect of increasing folic acid supply is an augmentation of milk protein yield (Girard and Matte, 1998; Graulet et al., 2007; Preynat et al., 2009b). For instance, folic acid supplementation has been shown to increase milk casein yield and decrease milk NPN, suggesting that an adequate folate supply improves efficiency of nitrogen utilization for milk casein synthesis (Girard and Matte, 1998). This effect of folates on milk protein synthesis is supported by a

Figure 2. Effects of dietary supplements of rumen-protected Met and intramuscular injections of folic acid and vitamin B₁₂ given to dairy cows beginning at 3 wk before the expected calving date on (a) Met entry rate; SE $\pm$ 1.1, methionine $\times$ vitamin interaction, P = 0.09, (b) Met oxidation; SE $\pm$ 0.06, methionine $\times$ vitamin interaction, P = 0.06, (c) plasma Met concentration; SE $\pm$ 1.7, methionine $\times$ vitamin interaction, P = 0.10, and (d) plasma Cys concentration; SE $\pm$ 5.4, methionine $\times$ vitamin interaction, P = 0.02 measured at 12 wk of lactation. Met- = diet with 1.83% of MP as Met; Met+ = diet with 2.23% of MP as Met; V- = no folic acid and vitamin B₁₂ supplement; V+ = weekly intramuscular injections of 160 mg of folic acid and 10 mg of vitamin B₁₂.

comparative functional genomic study across species including cow, fur seal, and tammar wallaby (Menzies et al., 2009a). This study showed that the expression of folate receptor α , which mediates the entry of 5-mTHF into cells, exhibits the greatest positive change in mammary tissue of the 3 species with elevations in milk protein synthesis. Moreover, stimulation of milk protein synthesis in cow mammary explants increases expression of folate receptor α and *ALDH1L1*, which encodes for formyl-THF dehydrogenase, the enzyme involved in the acquisition of a one-carbon unit from formate (Menzies et al., 2009b). These findings emphasize that folate metabolism plays a critical role in supporting milk protein synthesis.

Moreover, in early lactation, a combined dietary supplementation with folic acid and vitamin B₁₂, when compared with a dietary supplement of folic acid given alone, increased plasma concentration of glucose by 10% and decreased by half the hepatic concentrations of total lipids and triglycerides (TG) at 4 wk of lactation in spite of similar DMI, milk production, and milk protein yield (Graulet et al., 2007). Co-supplementing folic acid and vitamin B₁₂ (weekly intramuscular injections of 160 mg of folic acid and 10 mg of vitamin B₁₂) tended to increase whole-body glucose rate of appearance by 160 mg/d and was associated with a similar increment in lactose yield (170 g/d; Preynat et al., 2009a). A supplement of folic acid and vitamin B₁₂ (weekly intramuscular injections of 320 mg of folic acid and 10 mg of vitamin B₁₂) given from 3 wk before calving until 8 wk of lactation increased milk production and milk protein yield, maintained BCS and BW, and decreased plasma concentrations of BHB and nonesterified FA even if DMI was unchanged (Ghaemi-alehashemi, 2013). Indeed, a similar treatment applied in commercial farms decreased the fat-to-protein ratio and maintained BCS and BW in early-lactation cows without an observed effect on milk yield (Duplessis et al., 2014). The decrease of the fat-to-protein ratio was due to a lower milk fat concentration and a greater milk protein concentration, suggesting a decreased release of preformed fatty acids from adipose tissue (Duplessis et al., 2014).

Altogether, increasing supply in folic acid and vitamin B₁₂ to meet dairy cow needs appears to promote milk protein synthesis and enhance the efficiency to produce milk in early lactation. However, these effects cannot be entirely attributable to de novo synthesis of methyl groups. Increasing Met supply, when folate and vitamin B₁₂ status was low, increased milk protein content but failed to increase milk protein yield (Girard et al., 2005; Preynat et al., 2009b). Consequently, an effect of an adequate supply of folic acid on nucleic acid synthesis cannot be ruled out. The role of vitamin B₁₂,

as coenzyme of the methylmalonyl-CoA mutase in the entry of propionate into the Krebs cycle, which increases energy supply, also cannot be ruled out. Moreover, an adequate folate supply is critical for coordination of the distribution of vitamin B₁₂ between cytosol (methionine synthase) and mitochondria (methylmalonyl-CoA mutase; Riedel et al., 1999). In dairy cows, increasing supply in both vitamins increased the affinity of methylmalonyl-CoA mutase for vitamin B₁₂ (Graulet et al., 2007) and *MUT* expression (Preynat et al., 2010; Duplessis et al., 2017). These observations confirmed that in dairy cows, as in humans (Selhub et al., 2007), an inadequate folate supply leads to a secondary deficiency in vitamin B₁₂, which provides an explanation for the enhanced efficiency of energy metabolism observed in early-lactating cows receiving the combined vitamin supplement.

Once ruminal synthesis of B vitamins had been demonstrated (Bechdel et al., 1928), there was a generalized loss of interest for studies focused on dietary B-vitamin supply and requirements for dairy cows. We emphasize that the apparent ruminal synthesis of folates and vitamin B₁₂ are extremely variable among studies, ranging from 0.5 to 3.3 mg/d for folates and 0.1 to 4.8 mg/d for vitamin B₁₂ (Girard and Duplessis, 2017). Knowledge of factors affecting their synthesis and supply is still limited, and given the variability reported, it is conceivable that situations occur when folate and vitamin B₁₂ supply are below demand. The interactions between folate and vitamin B₁₂ supplementation within the context of RPM, RPC, and betaine supplementation is warranted. Additionally, the ability of one-carbon donors serine, glycine, and formate to influence acquisition of one-carbon units through folate metabolism and methyl group transfer also deserves further investigation in the dairy cow. These studies are deemed necessary to optimize nutrient utilization, milk production efficiency, and health in early-lactation dairy cows.

INTEGRAL ROLE OF METHIONINE

Methionine is an essential sulfur-containing AA and methyl donor associated with various key physiological processes. During the transition from gestation to lactation, the requirement for Met increases in dairy cows due to inadequate DMI as well as the increased protein demand to support the exponential fetal growth of late gestation and milk protein synthesis at the onset of lactation. Due to extensive microbial degradation in the rumen, Met supplemented to dairy cows is either rumen protected or in the form of Met analogs for the subsequent conversion to Met in tissues. Although earlier studies with various Met supplementation dosage

(17 g, Overton et al., 1996; 10.5 g, Socha et al., 2005; 15.3 g, Preynat et al., 2009b) did not detect significant improvements of periparturient lactation performance after Met supplementation, recent results supplementing RPM at 0.07% intake (~9 g/d prepartum and ~11 g/d postpartum; Osorio et al., 2013), 0.08% intake (~14 g/d prepartum and ~16 g/d postpartum; Zhou et al., 2016d), and 0.09% intake (Batistel et al., 2017) during the transition period improved performance of transition cows by influencing the plasma AA profile, fine-tuning immunometabolism, and reducing oxidative stress (Osorio et al., 2014; Zhou et al., 2016a; Batistel et al., 2018). This section discusses the underlying metabolic alterations that likely lead to improved lactation performance observed during the transition period.

Methionine deficiency has often been reported in cows around parturition (Zhou et al., 2016b). Circulating Met concentrations decrease markedly around parturition and do not restore to prepartum levels until 28 d postpartum (Zhou et al., 2016b). Apart from the effect of Met deficiency on decreasing milk protein synthesis because of its status as a limiting AA, in typical dairy cattle diets, Met deficiency likely also compromises the circulating AA profile. This potentially results in limited utilization of other circulating AA, thus promoting protein degradation and decreasing the use of these AA for milk protein synthesis (Li et al., 2017). Therefore, the greater circulating levels of some EAA (i.e., Arg, Lys, Met, Thr, and Trp), NEAA (Ala, Asn, Asp, Glu, Gln, and Pro), and nonproteinogenic AA (Cit) observed in Met-fed cows could be a result of an improved AA profile in response to Met supplementation (Zhou et al., 2016c). Although increases in DMI are rarely observed in earlier studies of RPM (Zanton et al., 2014), increased DMI were observed in recent studies when RPM was supplemented both before and after calving (Osorio et al., 2013; Zhou et al., 2016d; Batistel et al., 2017). The increase in DMI likely contributed to the elevation in circulating AA, the higher proportion of Met in EAA and total AA, as well as the higher proportion of Lys in total AA, indicating that RPM supplementation during the transition period optimizes AA supply for the cow (Zhou et al., 2016c). Such profile changes likely account for the greater observed milk yield and milk protein yield (Zhou et al., 2016d; Batistel et al., 2017), since recent *in vitro* studies revealed that AA taste receptors (TAS1R1/TAS1R3) in mammary epithelial cells sense extracellular changes in Met and activate mTOR signaling in bovine mammary cells (Zhou et al., 2018a). Additionally, the increasing availability of other AA in circulation may also contribute to enhanced milk protein synthesis, as β -casein (*CSN2*) mRNA abundance was increased in response to increasing availability of other AA (e.g., Val; Dong et al., 2018a,b).

Methionine, Methyl Group Transfer, and Phosphatidylcholine Synthesis

As a nutritionally important methyl donor, Met supplementation during the transition period is expected to promote overall production and health of dairy cows by promoting synthesis of important methylated compounds. After rats receive a Met-rich diet, activities of MAT were increased in the liver (Matsumoto et al., 1984), suggesting a greater production of the global methyl donor SAM. Similarly, mRNA abundance of MAT in the ruminant liver was also greater in response to RPM supplementation during the periparturient period (Zhou et al., 2017a). Phosphatidylcholine is one of the most important and high-demand methylated compounds in dairy cows during the transition period. Apart from the well-characterized demand for PtdChol in milk, transition dairy cows also are expected to have high PtdChol requirements for very low density lipoprotein (VLDL) synthesis and subsequent secretion of TG within VLDL to prevent fatty liver (Pinotti et al., 2002). *In vivo* synthesis of PtdChol from Met can be achieved by sequentially transferring 3 methyl groups from SAM to PtdEth via the actions of PEMT (Zeisel, 1992). Increases in MAT1A and PEMT expression in primary liver cells (Zhou et al., 2018b) and in RPM-supplemented cows (Zhou et al., 2017a) support the idea that Met supplementation enhances PtdChol synthesis. Further investigation is required to determine the extent to which subsequent VLDL synthesis would benefit from such changes. However, it is noteworthy that liver function was improved without lowering TG level in response to Met supplementation (Zhou et al., 2017b).

Sulfur-Containing Compound Pool and Transsulfuration

Around parturition, the increased demand for energy leads to enhanced hepatic oxygen uptake for production of energy through oxidation, resulting in greatly augmented production of reactive oxygen metabolites (ROM; Reynolds et al., 2003). Although antioxidants in the liver are capable of neutralizing ROM, depletion of such antioxidants leads to substantial tissue damage and inflammation (Sordillo and Aitken, 2009). Taurine and GSH are sulfur-containing intracellular antioxidants with a marked ability to scavenge ROM and free radicals (Young and Woodside, 2001). As a sulfur-containing AA, Met is the principal precursor for GSH and taurine synthesis (Brosnan and Brosnan, 2006). Similar to plasma Met levels, hepatic GSH concentrations also decrease soon after parturition and do not return to prepartum levels until 21 d postpartum (Osorio et al.,

2014). Furthermore, RPM supplementation increases hepatic GSH levels, suggesting that Met supplementation contributes to GSH synthesis in cow liver during the transition period. Nonetheless, biomarker analysis from cows revealed a reduced oxidative stress status and inflammation in Met-supplemented cows (Zhou et al., 2016a), which at least in part contributes to the overall better liver function in these cows during the transition period (Zhou et al., 2017b). These data suggest that Met could improve cellular response to inflammatory challenge. Supplementing primary bovine hepatocytes with Met increased GSH and ameliorated the LPS-induced cytokine response in vitro (Zhang and White, 2017), supporting the role of Met to mitigate inflammatory challenges.

Circulating neutrophils contribute to the first line of defense against invading pathogens. Neutrophils execute their bactericidal function by producing oxidants, and lower levels of neutrophil antioxidants is associated with bactericidal dysfunction (Roos et al., 1979). Upon pathogen challenge, blood from RPM-supplemented cows had higher neutrophil and monocyte oxidative burst capacity, potentially because Met is a critical precursor for GSH and taurine synthesis (Zhou et al., 2018c). These data suggest that higher Met-mediated neutrophil antioxidant content might help mount effective protection (phagocytosis and oxidative burst) against invading pathogens in dairy cows (Kinnula et al., 2002).

INVOLVEMENT OF CHOLINE IN ONE-CARBON METABOLISM AND PHOSPHATIDYLCHOLINE SYNTHESIS

Supplementation of RPC to peripartum dairy cows has been of interest due to the consistent increase in yield of milk or FCM [Elek et al., 2008 (25 and 50 g/d choline); Zom et al., 2011 (14.4 g/d choline); Lima et al., 2012 (15 g/d choline peripartum or prepartum only); Zenobi et al., 2018c (17.3 g/d choline)]. A recent meta-analysis (Arshad et al., 2020) of RPC supplementation included 23 experiments (74 treatment means; 1,938 cows) with a mean supplementation of 12.7 ± 4.3 g/d (3.2 to 25.2 g/d) choline ion demonstrates a significant increase in pre- and postpartum DMI [0.28 and 0.47 kg/d weighted mean difference (WMD), respectively], increased ECM (1.61 kg/d WMD), and increased fat and protein yield (0.08 and 0.06 kg/d WMD, respectively). Incidence of retained placenta and mastitis, but not displaced abomasum, ketosis, or metritis, were also reduced across studies. Benefits of RPC supplementation do not appear to be dependent on prepartum dietary energy (Zenobi et al., 2018c) or BCS (Bollatti et al., 2019). Interestingly, benefits of

RPC supplementation on milk production may persist beyond the supplementation period, as demonstrated by tendencies for improved milk yield for 15 and 40 wk postpartum and improved milk components for 15 wk postpartum after supplementation during the 3 wk before and 3 wk after parturition (Zenobi et al., 2018c). Also of interest is that prepartum supplementation of RPC appears to have beneficial effects on offspring, with improved ADG from calving or weaning to 50 wk of age, as well as improved immune status and response to challenge (Zenobi et al., 2018a,b).

Although these production responses have been consistent and positive, the mechanism was not fully understood and recent efforts to elucidate the mechanism of action have provided valuable insight. Although choline can be taken up and utilized by many tissues within the body, metabolism primarily occurs within the liver (Pelech and Vance, 1984) which necessitates use of liver biopsies or hepatocyte cell culture models. A decrease in liver TG accumulation has been observed with peripartum RPC supplementation (14.4 to 15 g/d choline) in several (Cooke et al., 2007; Elek et al., 2008; Zom et al., 2011; Goselink et al., 2013) but not all (15 to 19 g/d choline; Piepenbrink and Overton, 2003; Zahra et al., 2006; Zhou et al., 2016d; Zenobi et al., 2018c) transition cow studies. The variable response of liver TG to RPC supplementation may be reflective of the variance of basal liver TG across cohorts of cows or relationship with postpartum DMI (Humer et al., 2019). A meta-analysis suggests that when increased milk or ECM production is not accompanied with an increase in DMI, improvements in parameters associated with metabolic health may not be observed (Humer et al., 2019). Attenuation of liver TG accumulation [Cooke et al., 2007 (15 g/d choline); Zenobi et al., 2018d (0, 6.5, 12.9, 19.4, and 25.8 g/d choline)] and reduction of TG after accumulation (Cooke et al., 2007), has also been observed in feed restricted nonlactating, pregnant cows supplemented with 15 g/d or linear increases from 6.5 to 25.8 g/d choline fed as RPC. Although the mechanism of RPC action to reduce liver TG was presumably through increased PtdChol synthesis and VLDL export, as is the case in nonruminants, differences in lipid density of VLDL particles have made VLDL quantification in ruminants much more challenging until recent employment of more advanced techniques. Markers of VLDL secretion, specifically *MTTP* and *APOB100* mRNA expression, were increased in transition cows supplemented with RPC that exhibited reduced liver TG accumulation (Goselink et al., 2013). Quantification of VLDL export from primary bovine hepatocytes by ELISA indicated an increase in VLDL export with increased choline supplementation (Chandler and White, 2017). In addition, the recent application of fast

protein liquid chromatography and MS has confirmed the ability of RPC supplementation to increase PtdChol (e.g., PtdChol 34:5 and 36:6) concentrations in TG-rich lipoproteins isolated from plasma (Myers et al., 2019b).

In addition to phosphorylation of dietary choline to PtdChol via the cytidine diphosphate (CDP)-choline pathway, choline synthesized endogenously by sequential methylation of PtdEth to PtdChol via PEMT, also occurs (Figure 1). Although both pathways are biochemically possible, the PEMT pathway is energetically expensive and relies heavily on SAM methyl-donation, which may also be limited outside of an experimental methyl-supplementation diet, possibly contributing to variation in dependence on the PEMT pathway by species and diet. In primary bovine hepatocytes, *PEMT* gene expression was decreased with both choline and Met supplementation, yet an increase in VLDL export with choline supplementation was observed across a range of Met concentrations (Chandler and White, 2017). Conversely, supplementation of Met increased *PEMT* gene expression immediately pre- and postpartum (Zhou et al., 2017a); however, PtdChol and VLDL were not quantified to determine if this increased hepatic lipid export. Although recent data provide support for choline supplementation to increase VLDL export, the role of PEMT-generated PtdChol to contribute to the VLDL pool remains unclear in ruminants.

The PEMT pathway is one of more than 100 known methyltransferases dependent on SAM (Mato et al., 1997). Choline can contribute to the methyl group pool through oxidization to betaine (Zeisel, 1990); betaine can then contribute methyl groups through the transmethylation pathways described above. Supplementation of choline in primary bovine hepatocytes upregulated expression of genes involved in transmethylation (*BHMT*, *MTR*, methylenetetrahydrofolate reductase), indicating that choline supplementation was likely contributing to Met remethylation (Chandler and White, 2017). Interestingly, this effect was observed across Met supplementation (no interaction), suggesting there is a biological priority for Met regeneration when methyl-donors are available. In vivo evidence indicates that supplementation with RPC is oxidized to betaine; however, this was not accompanied by an increase in *BHMT* or *MTR* expression (Zhou et al., 2017a). The authors suggest that the inability of choline supplementation to increase Met regeneration may have been due to insufficient HCY in vivo, which is the required sulfur donor in Met regeneration (Zhou et al., 2017a). Given that the in vivo cell culture media used in the primary bovine hepatocyte studies (Chandler and White, 2017) have sufficient homocysteinyl moiety, this difference may highlight the need for optimal precursor (sulfur

and methyl carbon) availability to maximize Met regeneration in vivo.

Beneficial production responses with RPC supplementation may also be associated with improvements in hepatic gluconeogenesis associated with decreased hepatic lipidosis (Drackley, 1999; Goselink et al., 2013); however, presence of sustained production responses without a decrease in liver TG (Zenobi et al., 2018c) suggests improvements in gluconeogenesis may not be dependent on decreased hepatic TG. Increased hepatic glycogen has been observed with RPC supplementation in vivo (Piepenbrink and Overton, 2003; Zenobi et al., 2018d) and in vitro (Chandler and White, 2019) and may reflect greater rates of gluconeogenesis (Piepenbrink and Overton, 2003; Chandler and White, 2019). Just as supply of glucose to the mammary gland via increased hepatic glucose production can support increased milk yield, increased mammary TG uptake may support improved milk or milk fat yield. The ability of choline within a phospholipid fraction was suggested to improve TG uptake by the mammary gland and increased milk fat, milk yield, or both in these early studies (Grummer et al., 1987; Sharma and Erdman, 1988; Erdman and Sharma, 1991). Improvements in lipidomics techniques will allow for further understanding of the role of choline in VLDL transport and mammary gland uptake.

POTENTIAL ROLE OF BETAINES

Betaine, also called tri-methylglycine, is an essential nutrient for humans and animals. The main functions of betaine are to act as an organic osmolyte and chemical chaperone to regulate cell volume and protect cells from stress but also serve as a source of methyl groups in one-carbon metabolism (Craig, 2004; Bertolo and McBreairey, 2013). In addition to the endogenous synthesis through choline oxidation (Craig, 2004; Day and Kempson, 2016), betaine can be provided in diets. Common feed ingredients that contain high concentrations of betaine include condensed sugar beet solubles, wheat, and wheat byproducts (Eklund et al., 2005).

Betaine in the Rumen

Rumen degradation of unprotected betaine is a modest concern. When infused into the rumen of a ram, betaine disappeared from the rumen 4 to 6 h after feeding and the estimated turnover rate was 45%/h with a half-life of 1.5 h (Mitchell et al., 1979). In contrast, 75 to 95% of betaine remained in rumen fluid after 4 to 6 h incubation in vitro (Mitchell et al., 1979; Löest et al., 2001; Nakai et al., 2013). Therefore, it is possible that a major proportion of rumen-unprotected betaine

escapes from microbial degradation and flows into the lower gut. Dietary betaine supplementation may have a positive effect on total-tract nutrient digestibility in ruminants. Wiedmeier et al. (1992) compared the nutrient value of sugar beet molasses and concentrated sugar beet byproduct, which contains more betaine, in beef cattle fed diets containing either forage or a mixture of grain and forage. Treatment had no effect on nutrient digestibility when the forage diet was fed, whereas cattle fed a grain-forage diet containing concentrated sugar beet byproduct had greater DM and NDF digestibility compared with those fed sugar beet molasses (Wiedmeier et al., 1992). Wang et al. (2010) examined the effect of feeding different levels of rumen unprotected betaine (0, 50, 100, and 150 g/d) on nutrient digestibility and reported that betaine supplementation increased the digestibility of DM, OM, CP, ether extract, NDF, and ADF, relative to an unsupplemented control.

Betaine As a Methyl Donor

Betaine is catalyzed by BHMT to donate one methyl group and form *N,N*-dimethylglycine, which then is demethylated by flavoprotein dimethylglycine dehydrogenase to form sarcosine. Sarcosine can be further demethylated to glycine by mitochondrial sarcosine dehydrogenase. The latter 2 demethylation reactions donate methyl groups to methylneogenesis (Ducker and Rabinowitz, 2017). In all, betaine has the potential to donate 3 methyl groups. Demethylation by BHMT is the only known pathway to dispose betaine in the mammalian body. Different from MTR which is ubiquitously expressed across tissues, BHMT is primarily expressed in liver, kidney, and pancreas in ruminants (Xue and Snoswell, 1985; Zhou et al., 2012; Ducker and Rabinowitz, 2017), suggesting a major functional role for betaine as a methyl donor in these tissues. Because BHMT is highly expressed in liver, betaine exerts critical metabolic functions that are related to hepatic health with implications for preventing or treating fatty liver disease. In nonruminants, the loss of BHMT is associated with accumulation of hepatic fat, partially due to reductions in hepatic concentration of PtdChol and VLDL secretion rate (Teng et al., 2011). Dietary supplementation of betaine increases the hepatic SAM:SAH ratio and alleviates the increases in liver TG and total fat content in rodents susceptible to fatty liver disease (Kharbanda et al., 2007; Kathirvel et al., 2010; Deminice et al., 2015). It is still unclear whether dietary betaine supplementation during the transition period has similar effects on alleviating hepatic fat accumulation in dairy cattle.

Effects of Betaine Supplementation on Milk Production in Cows

Due to limited research, the effective feeding level and form (rumen-protected vs. unprotected) of supplemental betaine to improve cow performance are not conclusive. Because betaine may positively influence nutrient digestibility and there may be a significant portion of supplemental betaine that escapes from rumen degradation, feeding rumen-unprotected betaine may improve performance of lactating or transition dairy cows. In a study conducted by Monteiro et al. (2017), feeding liquid supplement containing betaine (approximately ≥ 100 g/d) to cows from dry off to 8 wk of lactation increased milk yield and milk fat percentage, without affecting intake, compared with cows supplemented with a liquid supplement without betaine. In mid-lactation dairy cows, Wang et al. (2010) observed a positive, linear effect of supplemental rumen-unprotected betaine (0, 50, 100, 150 g/d) on milk yield. Peterson et al. (2012) reported that supplementation of rumen unprotected betaine at 100 g/d, but not 25 or 50 g/d, improved milk yield compared with unsupplemented mid-lactating cows. Similarly, Hall et al. (2016) reported that supplementation of unprotected betaine (114 mg/kg of BW, ~ 71 g/d) increased milk yield of mid-lactation cows, relative to those without supplementation under thermoneutrality. However, this positive effect on milk yield was lost when cows were exposed to extreme heat (Hall et al., 2016). In contrast, during summer, dietary supplementation of unprotected betaine at 15 g/d, but not 10 and 20 g/d, improved milk yield of mid-lactation dairy cows (Zhang et al., 2014). A similar improvement in milk yield by feeding rumen unprotected betaine (15 g/d) was also observed in heat-stressed lactating cows under a partial grazing system (Dunshea et al., 2019). Therefore, when betaine is supplemented in an unprotected form, a feeding rate equal to or over 75 g/d may be required to ensure a positive milk yield response of the transition or lactating dairy cow under non-heat-stressed condition. However, the effective supplemental rate to improve performance seems to be lower when cows exposed to environmental conditions that promote heat stress. The mechanism is unknown but deserves further investigation.

Few studies have evaluated the effects of rumen-protected betaine in dairy cattle. In early-lactation cows, supplementation of rumen-protected betaine (45 g/d) in cows fed a Met-deficient diet had no effect on performance (Davidson et al., 2008). Similarly, dietary supplementation of rumen-protected betaine (20 g/d) from 4 wk before expected calving to 6 wk postpartum had no effect on DMI, milk yield, or milk composition (Wang et al., 2019a). The ineffectiveness of rumen pro-

tected betaine on performance may be due to (1) lack of a positive effect on rumen fermentation; (2) over or underprotection that results in lower betaine release in the abomasum and lower gut; and (3) a low and inadequate supplementation rate. Further research needs to determine whether rumen-bypass betaine improves liver health and milk production in dairy cattle. Such information is critical to identify effective feeding levels of rumen-protected betaine.

CURRENT CONSIDERATIONS

Methyl Donor Requirements

Although methyl donor requirements are yet to be characterized for dairy cattle, lactating dairy cows are expected to experience negative methyl group balance. This is due to the high output of methylated compounds (e.g., PtdChol and choline) in the milk with extensive microbial degradation of dietary methyl donors in the rumen (Pinotti et al., 2002). The overall methyl group deficiency is unlikely to be methyl donor-specific, since the interrelated metabolism of nutritionally important methyl donors allows methyl group transfer among methyl donors (Figure 1). Although perturbations in one-carbon metabolism from deficiency in one methyl donor usually results in compensatory changes in the others (Niculescu and Zeisel, 2002), such compensatory syntheses are usually limited in vivo even with supplementation of an alternative methyl donor (Zhou et al., 2017a). To establish dietary methyl donor requirements for dairy cows, additional studies are needed to characterize limiting factors (e.g., substrate availability, enzyme activity) for compensatory synthesis of one methyl donor from another.

Methyl Donor Co-Supplementation

Dietary methyl donor co-supplementation may be a means to maximize liver lipid export and milk production in dairy cattle. Phosphatidylcholine synthesis is promoted by the CDP-choline and PEMT pathways and choline and Met are preferentially utilized by these pathways, respectively, thus co-supplementing choline and Met may augment hepatic PtdChol synthesis, VLDL secretion, and TG disposal. In agreement, a recent in vitro study with cow liver cells treated with Met or choline demonstrated that transmethylation and transsulfuration pathways are more responsive to Met supply, whereas the CDP-choline pathway is more responsive to choline supply (Zhou et al., 2018b). In transition dairy cows, the combined dietary inclusion of RPM (Met ~14 g/d prepartum and ~16 g/d postpartum) and RPC (12.4 g/d of metabolizable choline

chloride) did not provide additional gains in milk yield, ECM, or the efficiency to produce milk, relative to a diet supplemented only with RPM (Zhou et al., 2016d). However, in this particular study, dietary RPM, RPC, or combined RPM and RPC supplementation did not modify liver TG concentrations, relative to unsupplemented cows. Consistent production responses were observed when RPC was supplemented (17.3 g/d choline chloride) to Met-balanced peripartum diets (Zenobi et al., 2018a,c), and liver TG were decreased with increased RPC supplementation in a Met-balanced diet fed to feed-restricted dry cows (Zenobi et al., 2018d). In a recent study by Zang et al. (2019), transition Holstein dairy cows were fed diets containing Met in excess of predicted requirements and supplemented with a rumen-protected encapsulate containing Met (22 g/d), choline chloride (10 g/d), betaine (3 g/d), riboflavin (96 mg/d), and vitamin B₁₂ (1.4 mg/d). The outcome was an increase in serum Met concentrations, a decrease in the serum Met to Lys ratio, a tendency for lower plasma fatty acid concentrations, and a reduction in liver lipid content during the postpartum period. Because this study did not compare co-supplementation with Met, choline, betaine, riboflavin, or vitamin B₁₂ supplementation alone, it is challenging to determine whether the observed decrease in liver TG was due greater methyl donor concentration or the unique combination of methyl donors. Future studies will need to determine whether production responses observed with rumen-protected methyl donor co-supplementation are robust enough to justify the nutritional practice.

Fatty Acid Feeding to Optimize Methyl Donor Efficacy

In cows and humans, fatty liver disease develops with hepatic PUFA depletion (Douglas et al., 2007; Puri et al., 2007; Arendt et al., 2015). In transition cows, hepatic concentrations of arachidonic acid and docosahexaenoic acid are low, relative to saturated or shorter-chain PUFA (Douglas et al., 2007). Moreover, PUFA supply is limited in all cows because of rumen biohydrogenation. It is hypothesized that an insufficient supply of these types of PUFA may limit PtdChol synthesis. The mechanism may involve inadequate PEMT activation. In rodent hepatocytes, the PEMT pathway prefers PtdEth enriched in very-long chain n-3 fatty acids including arachidonic acid and DHA (DeLong et al., 1999). These findings are supported by observations that circulating PtdChol that contain DHA are positively associated with PEMT activity (da Costa et al., 2011). Saed Samii et al. (2017, 2018) demonstrated that hepatic concentrations of highly unsaturated PC are low in transition cows with high liver lipid content. The

CDP-choline pathway prefers diacylglycerol containing SFA and MUFA (e.g., palmitic acid and oleic acid, respectively; (DeLong et al., 1999), which are types of fatty acids mobilized from adipose tissue in transition cows experiencing increases in hepatic TG deposition (Contreras et al., 2010). A reduction in PUFA supply for PEMT activation potentially explains in part why some studies fail to observe a hepatic lipid-lower effect with methyl donor feeding in transition cows (Zenobi et al., 2018a). In lactating cows, Myers et al. (2019a) established that the abomasal infusion of an algal oil rich in DHA enhanced the incorporation of DHA and other PUFA in PtdChol, relative to cows infused with palmitic acid. Dietary co-supplementation of methyl donors and DHA in transition cows may be a means to maximize PEMT activity, PtdChol synthesis, and liver TG disposal. The influence of fatty acid nutrition and lipolysis on CDP-choline and PEMT pathway activation deserves further consideration in transition cows.

Epigenetics

Covalent modifications of DNA or histone proteins that modify gene expression are epigenetic mechanisms. This includes the addition of a methyl group or hydroxymethylation of carbon 5 of cytosine within DNA. Folic acid, vitamin B₁₂, Met, choline, and betaine are dietary components that may induce epigenetic modification. Our understanding of epigenetic regulation in dairy cattle is limited. Relationships between DNA methylation and milk production, and between DNA methylation and liver phenotypic variation have been recently revealed (Kiefer et al., 2016; Wang et al., 2019b). The current consensus is that diet has the potential to influence health and lactation in a manner dependent on DNA methylation (Singh et al., 2010; Feeney et al., 2014). Global hepatic DNA methylation on d 21 of lactation was lower in RPM-supplemented cows, relative to unsupplemented cows, albeit methylation within the nuclear receptor peroxisome proliferator-activated receptor- α was increased by RPM (Osorio et al., 2016). Supplemental Met feeding also decreased embryo DNA methylation in transition dairy cows (Acosta et al., 2016). The ability of dietary Met supplementation to divergently modify global and region-specific DNA methylation is unclear; however, Osorio et al. (2016) suggested that the regulation of DNA methyltransferase 1 is likely involved. In support, feeding RPM during late gestation has been shown to modify DNA methyltransferase expression in newborn calves (Jacometo et al., 2017). The effects of dietary methyl donor supplementation on hepatic DNA methylation within the context of fatty liver disease and the offspring epigenome should be evaluated. Potential

interactions between diet and other epigenetic modifiers including environment also deserve investigation when considering the ability of intrauterine heat stress to alter the methylation profile of liver and mammary DNA in neonatal calves (Skibieli et al., 2018).

CONCLUSIONS

Inadequate methyl donor supply may contribute to oxidative stress, inflammation, hepatic TG accumulation, and compromised milk production in the dairy cow transitioning from gestation to lactation. To address potential methyl donor deficiencies in the peripartum cow, supplementation with dietary folic acid, B vitamins, Met, choline, and betaine is a nutritional practice that deserves consideration; however, health and milk production outcomes most likely depend on the type and amount of methyl donor supplemented as well as dietary total Met, folate, and B vitamin status. Although there are commonalities within methyl donor pathways and function, there are also apparent biological priorities for each of the methyl donors. Future work needs to define individual methyl donor requirements and whether methyl donor co-supplementation benefits the cow. The development of fatty acid feeding regimens that work in unison with methyl donor supplementation strategies, and the effect of maternal methyl donor nutrition on the offspring epigenome and lifetime performance, also deserve attention.

ACKNOWLEDGMENTS

The authors have not stated any conflicts of interest.

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