THE SYNERGY OF CHOLINE AND OMEGA-3 FATTY ACIDS FOR OPTIMIZED TRANSITION COW NUTRITION: A HYPOTHESIS

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INTRODUCTION

The development of postpartum fatty liver disease (**FLD**; i.e., hepatic steatosis or lipidosis) in dairy cattle continues to represent a nutrition and management challenge for the cow and producer. Of major concern, fatty liver is associated with decreased health status, fertility, and lactation performance (Wensing et al., 1997; Bobe et al., 2004). Hepatic lipid accrual, especially in the form of triacylglycerol (**TAG**), may limit gluconeogenesis and contribute to inflammation (Rukkwamsuk et al., 1999; Sordillo et al., 2009). Moreover, cows with fatty liver are at risk for acquiring other metabolic disorders including ketosis and displaced abomasum. For instance, fatty liver cows are predisposed to a heightened severity of infectious diseases including mastitis and metritis (Hill et al., 1985; Bobe et al., 2004). These outcomes may explain why cows with elevated hepatic lipid deposition are prone to infertility and compromised milk production later in lactation (Rajala-Schultz et al., 1999; Jorritsma et al., 2000). Herein, the current understanding of FLD mechanisms in dairy cows with a focus on very low density lipoprotein (**VLDL**) secretion is reviewed. Evidence is also provided to support the hypothesis that rumen-protected choline and docosahexaenoic acid (**DHA**) co-supplementation may represent a novel nutritional approach to minimize hepatic lipid accretion during the periparturient period.

MECHANISMS OF FATTY LIVER DISEASE

The advancement of negative energy balance during early lactation promotes adipose tissue lipolysis to enhance circulating concentrations of nonesterified fatty acids (**FA**). Dyslipidemia in turn elevates hepatic FA uptake where they are converted to fatty acyl-CoA by acyl-CoA synthetase. In the cow, the biochemical mechanisms of hepatic steatosis likely involve inadequate mitochondrial β-oxidation of fatty acyl-CoA, enhanced TAG esterification, and limited secretion of TAG within VLDL. First, the capacity to completely oxidize palmitoyl-CoA to CO_2 is not augmented during the transition from gestation to lactation (Litherland et al., 2011; McCarthy et al., 2015). In contrast, incomplete oxidation to acid-soluble products such as tricarboxylic acid cycle intermediates or ketones is maximum during peak lipolytic response (Dann et al., 2006; Litherland et al., 2011). In support, plasma lipidomic observations have also revealed marked elevations in circulating fatty acylcarnitines during the transition period (Rico et al., 2017b). As a consequence of increased hepatic FA uptake and inadequate oxidation, fatty acyl-CoA are partitioned towards TAG esterification which is mediated by various acyltransferases including glycerol-3-phosphate acyltransferase. In contrast to non-ruminant experimental models of non-alcoholic FLD in humans (Ferré and Foufelle,

2010), hepatic de novo lipogenesis of FA does not appear to contribute to TAG deposition in dairy cows with FLD (Pullen et al., 1990). Therefore, it is not surprising that observed postpartum elevations in liver palmitic (16:0) and oleic (*cis*-9 18:1) acids in dairy cows with FLD mimic observed elevations in plasma FA and the adipose tissue FA profile during the immediate postpartum period (Rukkwamsuk et al., 2000). The potential implications of these FA on VLDL synthesis and secretion are described below.

Although hepatic TAG esterification is upregulated during the transition period, concomitant elevations in VLDL-TAG secretion could limit the progression of FLD in periparturient dairy cows. In humans with simple steatosis and not the advanced inflammatory steatohepatitis form of non-alcoholic FLD, the secretion of TAG-containing VLDL increases linearly with TAG accrual (Donnelly et al., 2005; Choi and Ginsberg, 2011). However, the dairy cow has a reduced capacity to export TAG within VLDL from liver, relative to non-ruminants (Pullen et al., 1990). Although not a measure of VLDL secretion, our untargeted lipidomic approaches have confirmed dramatic reductions in circulating plasma TAG (e.g., TAG 60:1, 62:0, and 56:1) and TAG-rich lipoproteins in dairy cows transitioning from gestation to lactation (Davis et al., 2018; Saed Samii et al., 2018a). Assuming VLDL secretion is limited in postpartum dairy cows that develop FLD, relative to prepartum cows or clinically-healthy postpartum cows, then one possible explanation for limited VLDL export may be limited hepatic apolipoprotein (**Apo**) B_{100} concentrations. Apolipoprotein B_{100} is required for VLDL assembly and secretion (Sundaram and Yao, 2010); however, the mRNA expression of hepatic Apo B_{100} and circulating Apo B_{100} concentrations decrease as parturition approaches (Bernabucci et al., 2004; Bernabucci et al., 2009). Another possibility and focus of this review is a limited supply of hepatic phosphatidylcholine (**PC**), as proposed by Van den Top and coworkers (1995). Glycerophospholipids and some sphingolipids (i.e., choline-containing sphingomyelin) form a monolayer on the lipoprotein surface surrounding the TAG-rich hydrophobic core, and PC is the most abundant glycerophospholipid component of lipoprotein surface monolayers. Considerable evidence in biomedical non-ruminant experimental models demonstrate that reduced levels of hepatic PC impair the secretion of VLDL from liver (Yao and Vance, 1988; Fast and Vance, 1995). To the best of our knowledge, no additional evidence exists for any other glycerophospholipid or sphingolipid requirement for VLDL secretion including phosphatidylethanolamine (**PE**) or sphingomyelin, respectively.

PHOSPHATIDYLCHOLINE METABOLISM

Glycerophospholipid metabolism is complex. Two major types of glycerophospholipids include PC and PE. Of interest, the synthesis of PC involves the cytidine diphosphate (**CDP**)-choline pathway (i.e., Kennedy pathway) and the phosphatidylethanolamine *N*-methyltransferase (**PEMT**) pathway (Figure 1). Whereas the CDP-choline pathway utilizes choline as the key precursor, the PEMT pathway relies on the transmethylation cycle and the prerequisite methyl donor *S*-adenosylmethionine to methylate PE. It is generally recognized that PEMT is a liver-specific enzyme (Vance and Ridgway, 1988). Although choline may serve as a methyl donor for PE methylation, L-

methionine and betaine are other examples. Importantly, the CDP-choline and PEMT pathways appear to work in unison to synthesize PC in liver (Sundler and Akesson, 1975).

Phosphatidylcholine and choline-containing sphingomyelin are the predominant phospholipids in human VLDL with far less PE (Wiesner et al., 2009; Dashti et al., 2011). Indeed, PC comprises ~70% (mol %) of total phospholipids of rodent plasma VLDL (Agren et al., 2005). Earlier work by Fast and Vance (1995) demonstrated that the ratio of total PC to PE of nascent particles resembles hepatic organelle membrane phospholipid composition. Less clear is the specific types of PC (and maybe PE) that are preferentially required for VLDL assembly and secretion. Although attention has focused on the importance of choline within PC, we must be cognizant of the complete structure and potential functional importance of the two fatty acyl chains. Indeed, VLDL may contain hundreds of different types of PC, and early evidence suggests that PC 34:2 (number of carbons:number of double bonds; e.g., 16:0/18:2), 34:1 (e.g., 16:0/18:1), and 36:4 (e.g., 16:0/20:4 or 18:2/18:2) are prevalent on the surface of non-ruminant VLDL (Wiesner et al., 2009). The type of PC produced is likely governed by the preferentially utilization of specific fatty acids within the CDP-choline or PEMT pathways. For example, PC produced by the PEMT pathway are enriched in long-chain polyunsaturated FA (**PUFA**) including omega-6 arachidonic acid (20:4) and omega-3 DHA (DeLong et al., 1999; Pynn et al., 2011). Indeed, supplementing isolated rat hepatocytes with ethanolamine activates PEMT to promote the inclusion of DHA within PC (Samborski et al., 1993), and the quantitation of DHA-containing PC is utilized as surrogate measure for PEMT activity (da Costa et al., 2011). In contrast, PC synthesized by the CDP-choline pathway are enriched in saturated (e.g., 16:0 and 18:0) and unsaturated fatty acids with one or two double bonds including oleic and linoleic acids, respectively (DeLong et al., 1999; Pynn et al., 2011). It is also generally understood that saturated fatty acids are preferentially found at the *sn*-1 position of PC, whereas unsaturated fatty acids typically reside at the *sn*-2 position (MacDonald and Sprecher, 1991).

The synthesis and degradation of PC is further complicated by the involvement of other intermediary complex lipids including lysophosphatidylcholine (**LPC**), diacylglycerol, and ceramide. Liver LPC acyltransferase reacylates LPC to form PC, whereas PC is metabolized by the enzyme phospholipase A_2 releasing the fatty acid at the *sn*-2 position to form LPC. In circulation, LPC is a highly-abundant lyso-phospholipid, and LPC composition in plasma is a mixture of different species such as 16:0 (40%), 18:2 (20%), 18:1/18:0 (10–15%) and 20:4 (10%; Ojala et al., 2007). Diacylglycerol is utilized by choline phosphotransferase within the CDP-choline pathway, and sphingomyelin synthase transfers phosphocholine from PC to ceramide to form sphingomyelin. Although not the focus of this review, LPC, diacylglycerol, and ceramide are signaling lipids with diverse functions in inflammation, immunity, and insulin-mediated glucose utilization (Gräler and Goetzl, 2002; Summers, 2006; Erion and Shulman, 2010). Although the role of ceramide in the dairy cow has been extensively evaluated (Rico et al., 2016; Rico et al., 2017a; Rico et al., 2018b), the actions of LPC and diacylglycerol are less clear.

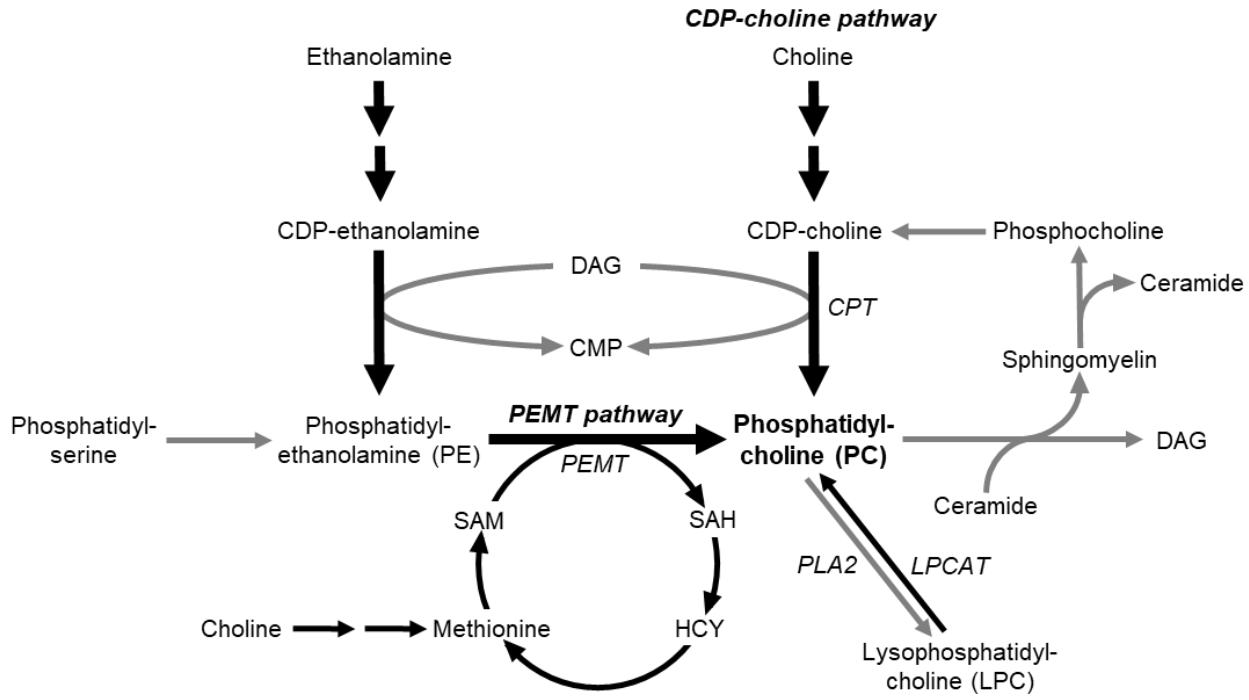

Figure 1. The complex synthesis and degradation of phosphatidylcholine. CDP, cytidine diphosphate; CMP, cytidine monophosphate; CPT, cholinephosphotransferase; DAG, diacylglycerol; HCY, homocysteine; LPCAT, LPC acyltransferase; PEMT, phosphatidylethanolamine *N*-methyltransferase; PLA2, phospholipase A_2; SAH, *S*-adenosylhomocysteine; SAM, *S*-adenosylmethionine.

Phosphatidylcholine Biomarkers for Fatty Liver Disease in Dairy Cow

Early evaluation of the bovine plasma lipidome has identified candidate PC biomarkers for FLD in postpartum dairy cows (Saed Samii et al., 2017; Saed Samii et al., 2018a,b). Notably, we have identified multiple plasma PC that decrease during the transition from gestation to lactation, which are associated with the development of postpartum FLD (Figure 2; Bobe et al., 2004). These include PC 36:6, 32:3, 34:4, 34:6, and 37:6. Similar reductions in these PC species were observed in liver biopsied from postpartum cows, relative to prepartum (e.g., PC 36:6, Saed Samii et al., 2018a,b). Our lab has utilized these lipidomic features as initial targets for the development of nutritional therapies aimed at enhancing VLDL secretion in dairy cows. For instance, PC 36:6 may contain DHA; therefore, we consider the possibility that DHA supplementation may increase hepatic PC 36:6 synthesis and TAG export. These findings are supported by observed increases in palmitic acid concentrations within the cellular membrane phospholipid layer of hepatocytes with parallel decreases in eicosapentaenoic acid (**EPA**; 20:5) and DHA during the periparturient period in dairy cows (Douglas et al., 2007).

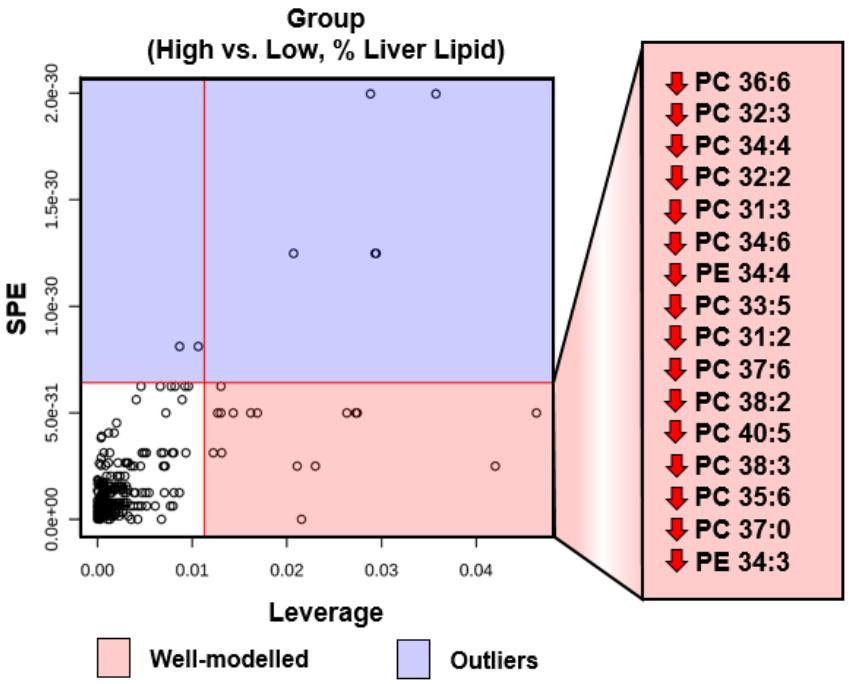

Figure 2. Suppressed plasma phosphatidylcholine (PC) levels are associated with fatty liver disease in periparturient Holstein dairy cows. Leverage/squared prediction error (SPE) plot of 301 complex lipids and their relationship with hepatic lipid accumulation. Normalized data represent plasma samples collected from periparturient Holstein dairy cows categorized into low (n = 7) or high (n = 7) mean (d 5 and 14 postpartum) liver lipid content (5 ± 1 vs. 12 ± 2 % of wet weight, respectively). Metabolites in lower right quadrant have high loadings and follow the expression pattern of the submodel (i.e., data demonstrate that out of 301 metabolites, the suppression of specific PC levels are most associated with fatty liver disease). Data were obtained using quadrupole time-of-flight mass spectrometry. PE = phosphatidylethanoline.

DIETARY CHOLINE AND OMEGA-3 FATTY ACID SUPPLEMENTATION

Choline Supplementation

Dietary choline supplementation has been considered a potential nutritional therapy to mitigate FLD in dairy cows and humans (Cooke et al., 2007; Guerrerio et al., 2012; Zenobi et al., 2018). For example, the supplementation of feed-restricted nonlactating multiparous Holstein cows during late gestation with rumen-protected choline ions (0 to 25.8 g/d) lowered liver TAG deposition in a dose dependent manner (Zenobi et al., 2018). To build on their findings, we applied fast protein liquid chromatography to demonstrate that this dietary approach increased circulating total TAG concentrations in TAG-rich and low density lipoproteins (**LDL**), and increased circulating total phospholipid levels in LDL (Table 1). The mechanisms of these outcomes may involve the ability of choline to stimulate hepatic PC synthesis, and thus provide key

glycerophospholipids for VLDL assembly. Recent findings obtained using bovine neonatal hepatocyte cultures suggests that choline chloride supplementation increases PC synthesis and VLDL secretion by predominantly activating the CDP-choline pathway (Chandler and White, 2017). Although choline may be transformed into betaine to support the transmethylation of PE, Chandler and White (2017) observed lower hepatocyte PEMT mRNA expression with choline chloride supplementation. Indeed, PC generated by the CDP-choline pathway was preferentially detected in VLDL fractions obtained from rodent hepatocyte cultures supplemented with radiolabeled choline (Vance and Vance, 1986). It is intriguing to postulate whether hepatocyte PEMT pathway activation can be enhanced during choline chloride supplementation while in the presence of preferential PE substrate containing very long chain PUFA such as DHA (DeLong et al., 1999; Pynn et al., 2011).

Table 1. Effect of increasing intake of ruminally protected choline ions on circulating concentrations of triacylglycerol (TAG) in plasma lipoprotein fractions collected using fast protein liquid chromatography.[1]

	TAG-rich lipoprotein fraction						**Low density lipoprotein fraction**					
	Intake of RPC, g/d				*P* =		Intake of RPC, g/d				*P* =	
Component	0	12.9	25.8	SEM	Linear	Quadratic	0	12.9	25.8	SEM	Linear	Quadratic
TAG, mg/dL	0.97	2.03	1.77	0.22	0.01	0.01	0.74	0.96	0.92	0.08	0.02	0.04
Cholesterol, mg/dL	0.65	1.04	1.11	0.13	0.01	0.21	34.0	38.5	41.2	3.10	0.09	0.80
Phospholipid, mg/dL	1.53	1.76	1.66	0.28	0.26	0.09	15.9	19.4	21.6	1.38	0.01	0.69

[1]Samples derived from lab of Dr. Charles Staples at the University of Florida following their evaluation of feeding increasing amounts of ruminally protected choline (RPC; ReaShure; Balchem Corporation) on hepatic health and lactation performance in nonlactating, pregnant Holstein dairy cows in negative energy balance (Zenobi et al., 2018). Lipoprotein fractions collected as described by Phipps et al. (2017).

Omega-3 PUFA Supplementation

Omega-3 long-chain PUFA include α-linolenic acid (**ALA**; 18:3), DHA, docosapentaenoic acid (**DPA**; 22:5), and EPA. Beneficial effects promoted by omega-3 PUFA are routinely observed in humans and various experimental models (Riediger et al., 2009; Kalupahana et al., 2011). These include reductions in circulating TAG in patients with hypertriglyceridemia (Mori et al., 2000; Schwellenbach et al., 2006), lower plasma levels of pro-inflammatory cytokines such as tumor necrosis factor-α and interleukin-1β (Endres et al., 1989; Caughey et al., 1996), and the prevention of atherosclerosis and hypertension (Hino et al., 2004; Erkkilä et al., 2006). Although uncertain in humans (Poudyal et al., 2011), the dietary intake of fish oil rich in omega-3 PUFA has an insulin-sensitizing effect in adipose and liver tissues (González-Périz et al., 2009). In rats fed a 12% canola oil-based diet, improvements in estimated insulin sensitivity were observed with the supplementation of EPA and DHA, relative to ALA supplementation (Andersen et al., 2008). Improvements in insulin action with EPA and DHA supplementation may be due in part to reductions in lipogenesis and heightened FA oxidation (Kalupahana et al., 2011). Moreover, omega-3 EPA and DHA serve as

substrate for the synthesis of resolvins and protectins which may enhance insulin signaling by alleviating chronic inflammation (Serhan and Petasis, 2011). Collectively, these benefits associated with dietary DHA and EPA consumption have stimulated interest in promoting omega-3 PUFA intake for the prevention of metabolic diseases (Kinsella et al., 1990; Mostad et al., 2006).

Omega-3 FA supplementation is also being studied as means to alleviate hepatic steatosis in humans. Although not completely defined, the mechanisms of action appear to be multifaceted. Omega-3 PUFA are potent peroxisome proliferator-activated receptor-α activators which in turn stimulates FA oxidation (Pawar and Jump, 2003). Additionally, omega-3 PUFA inhibit lipogenesis by downregulating sterol regulatory element-binding protein (Xu et al., 1999). Improvements in hepatic oxidative balance and inflammatory status are also observed with EPA or DHA supplementation (Li et al., 2005; Ishii et al., 2009). As described earlier, activation of the hepatic PEMT pathway for PC production preferentially utilizes DHA (Samborski et al., 1993; DeLong et al., 1999; Watkins et al., 2003). Moreover, reduced PEMT activity confers susceptibility to FLD in humans (Song et al., 2005). Therefore, DHA supplementation may be a means to selectively activate PEMT to promote PC synthesis and thus support the secretion of TAG within VLDL. Such an approach may benefit humans (and potentially cows) experiencing reductions in total plasma and liver DHA/EPA levels during inflammatory FLD (Puri et al., 2009; Arendt et al., 2015). In support, randomized controlled trials have shown reductions in liver fat and circulating liver enzymes (i.e., alanine or aspartate aminotransferase) in children and adults with advanced non-alcoholic FLD (Nobili et al., 2013; He et al., 2016; Nogueira et al., 2016). Unfortunately, we cannot yet discern whether these beneficial outcomes are due to enhanced hepatic TAG secretion or because of other aforementioned beneficial effects associated with omega-3 FA consumption.

Omega-3 Fatty Acid Supplementation in Dairy Cows

In dairy cows, the effects of dietary supplementation of omega-3 FA including DHA on fertility and lactation performance has been extensively studied. Regarding reproduction, pregnancy losses are lower in cows fed rolled flaxseed which is rich in ALA, relative to cows fed rolled sunflower seed (Ambrose et al., 2006). Moreover, feeding dairy cows EPA and DHA may inhibit uterine prostaglandin $F_{2\alpha}$ synthesis, delay regression of the corpus luteum, and promote fertility by enhancing embryo survival (Burke et al., 1997; Staples et al., 1998). With regard to lactation, heavy emphasis has focused on the ability of omega-3 FA supplementation to enrich their concentrations in milk fat (Kitessa et al., 2004). Such a response is a perceived benefit for the production of functional foods that benefit human health (Lock and Bauman, 2004). Indeed, the consumption of omega-3 FA in milk may represent a preventive approach for the prevention of type 2 diabetes and coronary heart disease (Thorsdottir et al., 2004). Other research has focused on the ability of unprotected DHA supplementation to modulate the ruminal microflora and milk fat synthesis (Petit et al., 2002; Shingfield et al., 2003; Maia et al., 2007). Our understanding of the effects of DHA supplementation on metabolic health in cows is also developing. For instance, omega-3 FA may enhance insulin sensitivity in cows (Gingras et al., 2007), and elevate circulating insulin and insulin-like growth factor-I during early

lactation (Heravi Moussavi et al., 2007). Although the effects of omega-3 FA on TAG secretion in cows is uncertain, a recent evaluation of dietary rumen-protected DHA demonstrated accumulation of DHA in plasma phospholipids in dairy cows (Stamey et al., 2012).

Effects of Abomasal Infusion of Various Phosphatidylcholine Precursors on Lactation Performance, Metabolic Status, and the Hepatic Phosphatidylcholine Lipidome

An interest of our research group is to discover novel dietary approaches that maximize hepatic PC synthesis, TAG secretion, and health in dairy cows. In a recent proof-of-principle study performed at the Cornell Dairy Research Center, five multiparous late lactation Holstein dairy cows were enrolled in a 5 × 5 Latin Square design experiment. Cows were continuously abomasally infused for 6 d with emulsion preps containing palmitic acid (PA; 98% 16:0; BergaFat F-100 HP; Berg + Schmidt GmbH & Co.), PA + choline (50 g of choline ion delivered as choline chloride), PA + L-serine (170 g; 1X estimated duodenal flow), behenic acid (BA; 92% 22:0; Berg + Schmidt), or omega-3 FA (44% DHA, 0.7% DPA; algae-sourced life'sDHA; DSM Nutritional Products, Inc.). Although each cow was infused 301 g of total FA each day (12.54 g/h), infusions were balanced for the amount of palmitic acid and glycerol within the omega-3 oil (40 and 19 g, respectively). Moreover, each emulsion prep contained whey protein, polysorbate 80, ethoxyquin, and water. To justify our treatments (Figure 1), palmitic acid is a potent inducer of ceramide synthesis (Rico et al., 2016), detected at the *sn*-1 position of PC (MacDonald and Sprecher, 1991), and preferentially utilized by the CDP-choline pathway (Vance and Vance, 1986). Choline is the principal substrate for the CDP-choline pathway, but also believed to serve as a methyl donor for PEMT activation in a limited capacity. We also considered the role of choline in sphingomyelin synthesis and degradation. Although a non-essential amino acid, serine may be used for PC and ceramide synthesis (Rico et al., 2015a). Behenic acid is a very-long chain saturated FA chosen because of its preferential utilization for ceramide synthesis (Rico et al., 2015a; Rico et al., 2018a); however, the potential low digestibility of very-long chain FA is a concern. To the best of our knowledge our work represents the first intensive evaluation of behenic acid in dairy cows. Lastly, our omega-3 FA treatment rich in DHA and DPA was selected because of their potential to improve health and PC synthesis via the PEMT pathway (DeLong et al., 1999; Pynn et al., 2011; Siriwardhana et al., 2012). We are cognizant that our study evaluated clinically healthy late lactation cows in positive energy balance, and the observed effects may or may not be observed in early lactation cows at risk for FLD. Of relevance to our investigation, circulating PC concentrations increase, and plasma ceramide concentrations and nutrient partitioning decrease as lactation advances (Artegoitia et al., 2014; Rico et al., 2016).

Omega-3 infusion lowered dry matter intake, relative to PA treatment ($P < 0.01$). Milk yields recorded for cows abomasally infused with PA, PA co-infused with serine, or BA were comparable (Table 2). These data suggest that BA may also increase circulating very-long chain ceramides to support nutrient partitioning (Rico et al., 2015b; Rico et al., 2017a). In contrast to our observations in PA cows, omega-3 FA infusion significantly decreased ($P = 0.03$) and PA co-infused with choline tended to lower milk yield ($P = 0.08$).

Observed reductions in dry matter intake with DHA treatment were likely the cause considering feed efficiency was not modified. This may be due to the ability of omega-3 FA to promote satiety (Parra et al., 2008). Additionally, a working theory is that both DHA and choline lower ceramide levels and improve insulin sensitivity to reduce glucose partitioning to the mammary gland. Docosahexaenoic acid supplementation does improve bovine insulin sensitivity in vitro (Gingras et al., 2007), and a tendency for lower milk yield with high choline intake in mid-lactation cows has been observed (Sharma and Erdman, 1988). Such a response may explain the observed tendency for reductions in milk yield during late lactation but we argue that this effect would be highly dependent on stage of lactation. During early lactation, improvements in insulin sensitivity hold the potential to reduce lipolysis and minimize hepatic FA uptake. Indeed, lower levels of circulating FA have been observed with choline supplementation during the peripartum (Pinotti et al., 2003). Therefore, clear evidence exists for the improvement in hepatic health and milk production during early lactation choline supplementation (Pinotti et al., 2003; Zahra et al., 2006).

Table 2. Effect of abomasal infusion of palmitic acid (PA; 16:0), PA and choline chloride (PA+C), PA and L-serine (PA+S), behenic acid (BA; 22:0), or a mixed omega-3 oil containing docosahexaenoic and docosapentaenoic acids (DHA/DPA) on dry matter intake and milk production in late lactation Holstein dairy cows.[1]

	Treatment						
	PA	PA+C	PA+S	BA	DHA/ DPA	SEM	*P-value*
Dry matter intake, kg/d	24.4	23.7	22.8	22.9	21.3*	1.61	0.06
Milk yield, kg/d	29.2	26.2†	28.2	28.5	25.6*	1.72	0.09
Milk components, kg/d							
Fat	1.34	1.33	1.18	1.23	0.92	0.15	0.28
Protein	0.97	0.82	0.94	0.96	0.93	0.06	0.66
Lactose	1.32	1.25	1.30	1.31	1.20	0.08	0.44
Milk composition, %							
Fat	4.57	4.32	4.31	4.22*	3.62*	0.14	0.04
Protein	3.43	3.29	3.41	3.43	3.65	0.13	0.77
Lactose	4.49	4.64†	4.55	4.54	4.69*	0.06	0.09
Feed efficiency[2]	1.17	1.10	1.24	1.22	1.25	0.13	0.72

[1]In a 5 × 5 Latin Square design experiment, late lactation Holstein dairy cows were continuously abomasally infused for 6 d with emulsion preps containing PA (98% 16:0; BergaFat F-100 HP; Berg + Schmidt GmbH & Co.), BA (92% 22:0; Berg + Schmidt), PA + Choline (50 g of choline ion delivered as choline chloride), PA + Serine (170 g; 1X estimated duodenal flow), or omega-3 FA (44% DHA, 0.7% DPA; life'sDHA; DSM Nutritional Products, Inc.). Although each cow was infused 301 g of total FA each day, infusions were balanced for the amount of 16:0 and glycerol within the omega-3 oil (40 and 19 g, respectively). Data reflect LS Means ± SE for d 5 and 6 of infusion. Relative to PA: *, $P < 0.05$. †, $P < 0.10$.
[2]Feed efficiency = kg of milk/kg of dry matter intake.

Although milk fat yield was maintained with BA infusion, relative to PA cows, BA significantly lowered milk fat content ($P = 0.04$). Relative to palmitic acid, our data suggest that behenic acid digestibility and/or incorporation of behenic acid into milk fat TAG is lower. However, omega-3 FA infusion exhibited a far greater suppression in milk fat content and a numerical decrease in milk fat yield. These findings are especially intriguing

because we utilized an abomasal delivery approach; therefore, we consider the effects of omega-3 FA on biohydrogenation were negligible. The potent ability of omega-3 FA to suppress lipogenesis may be the cause (Xu et al., 1999). It is important to mention that DHA dietary supplementation at a much lower level (29 g/d) did not modify milk yield or milk fat composition in mid-lactation Holstein cows (Stamey et al., 2012).

The described treatments did not modify circulating glucose or total FA concentrations; however, circulating TAG and cholesterol concentrations were greatest with PA treatment, whereas the levels of these metabolites were lowest with omega-3 FA infusion (unpublished). Such outcomes were expected based on the previously described benefits linked with DHA consumption in non-ruminants. We also biochemically mapped the bovine hepatic glycerophospholipidome. Profound changes across hundreds of lipids were observed with treatment (Figure 3). For instance, DHA was markedly enriched in hepatic PC and LPC (e.g., biomarker PC 36:6). Elevations in DHA-containing PC may be indicative of enhanced PEMT activity (da Costa et al., 2011). In support, PEMT activation prefers PUFA-containing PE (DeLong et al., 1999). Indeed, DHA/DPA infusion robustly increased over 100 PC containing more than 3 double bonds, relative to PA cows. In contrast, PA infusion significantly increased a similar number of PC containing saturated FA or FA containing 3 or less double bonds. These PC may be preferentially synthesized by the CDP-choline pathway (Vance and Vance, 1986). We also observed modest increases in hepatic PC with choline chloride or L-serine infusion, relative to PA. Although some exceptions were observed, omega-3 infusion enhanced the incorporation of PUFA (>3 double bonds) into PC, whereas saturated FA feeding, choline, and serine elevated hepatic PC containing saturated FA, or FA with 1 to 3 double bonds.

CONCLUSION

Current evidence supports the ability of dietary rumen-protected choline supplementation to enhance TAG disposal in postpartum dairy cows at risk for metabolic disease. Our data suggest that the ability of choline to activate the CDP-choline pathway to promote PC synthesis may be the cause. However, potential exists to refine nutritional practices as a means to optimize liver PC production and VLDL secretion for enhanced liver health during the peripartum. Although research should continue to focus on whether methyl donor supplementation (e.g., choline and L-methionine) provides added benefit, dairy science advancements should determine which dietary FA inhibit or promote hepatic PC synthesis and TAG export. Moreover, the identification of methyl donors and fatty acids that work in unison to upregulate both the CDP-choline and PEMT pathways to maximize PC production and VLDL secretion for the prevention of fatty liver disease would represent a paradigm shift in transition cow nutrition.

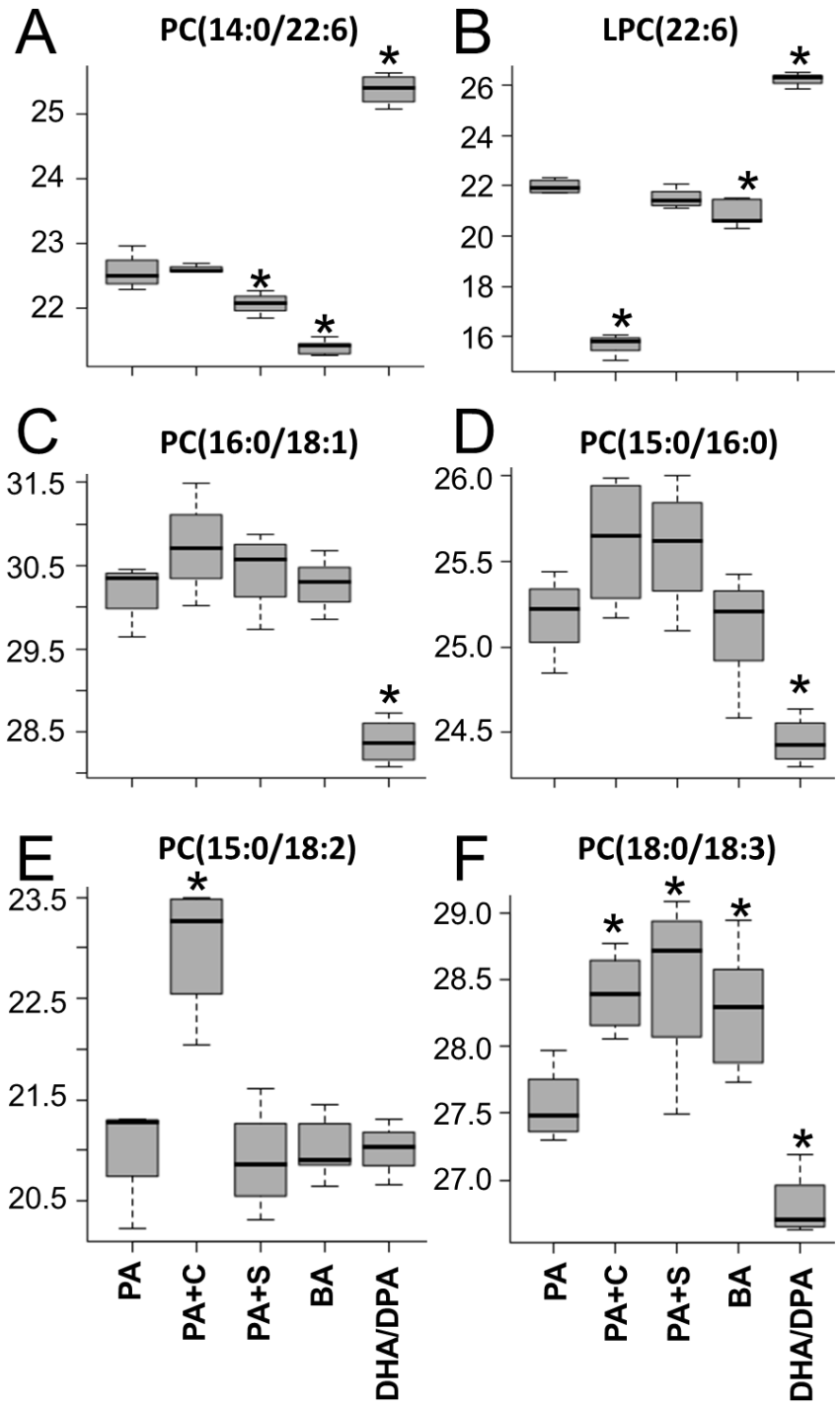

Figure 3. Effect of abomasal infusion of palmitic acid (PA; 16:0), PA and choline chloride (PA+C), PA and L-serine (PA+S), behenic acid (BA; 22:0), or a mixed omega-3 oil containing docosahexaenoic and docosapentaenoic acids (DHA/DPA) on select hepatic phosphatidylcholine (PC) and lysophosphatidylcholine (LPC) levels in late lactation Holstein dairy cows. Generalized log-transformed intensities (Y-axis) derived from time-of-flight mass spectrometry. Samples reflect liver biopsied at the end of d 6 of each infusion.

ACKNOWLEDGEMENTS

Abomasal infusion trial results were generated by a collaborative effort with Dr. Eduardo Rico, William Myers, Amanda Davis, and Ananda Fontoura within the McFadden lab. We thank Dr. Carmen Moraru within the Department of Food Science at Cornell University for assisting with emulsion preparation and the Cornell University Dairy Research Center personnel for cow care. Appreciation is also extended toward Dr. Charles Staples within the Department of Animal Sciences at the University of Florida for providing plasma samples for lipoprotein isolation work. Results were generated with support derived by the Northeast Agribusiness and Feed Alliance, the Agriculture and Food Research Initiative Competitive Grant 2016-67015-24582 from the USDA National Institute of Food and Agriculture, Cornell University Center for Advanced Technology, Vetagro, and Balchem Corporation. We thank Berg + Schmidt GmbH & Co. KG for providing the saturated palmitic and behenic acid supplements and DSM Nutritional Products, Inc. for supplying the polyunsaturated omega-3 FA oil.

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