

Fatty Acid Biology and Nutrition to Optimize Health and Production

Joseph W. McFadden and J. E. Rico¹
Cornell University

Introduction

The onset of the periparturient period is characterized by a series of coordinated metabolic adaptations to support fetal and neonatal development in dairy cattle (see review by (McFadden and Rico, 2019)). These processes are controlled by endocrine signals including placental lactogen, somatotropin, and insulin. The consequence of their action is the fine-tuned control of metabolism to spare key nutrients such as glucose, fatty acids, and amino acids for growth of the fetus and milk production. The demand for glucose by the mammary gland for the synthesis of milk lactose, the osmotic regulator of milk volume, is supported by (i) increased hepatic gluconeogenesis, ketogenesis, and glycogen breakdown, (ii) increased blood flow to the mammary gland, (iii) decreased skeletal muscle protein synthesis and adipose tissue lipogenesis, (iv) elevated adipose tissue lipolysis and circulating fatty acid supply, and (v) increased utilization of fatty acids and amino acids for oxidative metabolism. These changes in nutrient metabolism are due in part to reductions in pancreatic insulin secretion and the effectiveness of insulin. Specifically, lactation is supported by a decrease in insulin sensitivity (i.e., enhanced insulin concentration to achieve half-maximal response) and responsiveness (i.e., a decrease in maximal response at a specific insulin concentration; Debras et al., 1989; Vernon et al., 1990; Baumgard et al., 2017). Early lactation is also characterized by the uncoupling of the somatotrophic axis and low circulating concentrations of insulin-like growth factor-I, an insulin-sensitizer. Although the mechanisms of maternal insulin resistance are not completely defined, this review discusses the potential interplay of fatty acids and the implications nutrient partitioning may have for milk production and health.

Defining the role of fatty acids within the context of metabolism, nutrient partitioning and lactation has scientific merit for several reasons. Fatty acids derived from the diet or adipose tissue lipolysis constitute an important energy source for the dairy cow, especially during early lactation when the cow produces milk equivalent to 50 to 70% of total daily requirements for energy. Mitochondrial or peroxisomal fatty acid oxidation produce reducing equivalents (i.e., FADH₂ and NADH) and acetyl-CoA, which may be used to support electron transport and adenosine triphosphate synthesis. Acetyl-CoA may also be utilized to support the production of ketones, which when used as a fuel source spares glucose. Fatty acids from circulation or those synthesized *de novo* are also utilized to generate major constituents of milk including triacylglycerols, glycerophospholipids, and sphingolipids (e.g., ~30% of milk solids in a modern Holstein cow; Jensen, 2002). Fatty acids may also directly influence nutrient utilization in the mammary gland (Cant et al., 1993). Increased fatty acid availability holds potential to

¹ Contact at: Department of Animal Sciences, 149 Morrison Hall, Ithaca, NY 14853; Tel: (607) 255-5497; E-mail: jwm43@cornell.edu.

preserve acetate, a building block for mammary *de novo* fatty acid synthesis, for oxidation in extra-mammary and -hepatic tissues (Cant et al., 1993). One concern for the transition dairy cow is that exacerbated body fat mobilization with inadequate hepatic fatty acid oxidation and triacylglycerol secretion are predisposing factors for fatty liver disease, ketosis, and other metabolic maladies that may compromise milk production, fertility, and the cow's productive lifespan. This review focuses on the role of fatty acids as agonists and antagonists of insulin-stimulated glucose utilization and lipogenesis. The effects of nutrient restriction, and saturated and unsaturated fatty acid feeding are highlighted. To provide a contemporary perspective, the emerging role of the sphingolipid ceramide is considered within the framework of somatotropin action, nutrient partitioning, lactation, and health. Lastly, the potential interplay of somatotropin, adiponectin and fibroblast growth factor-21 (**FGF21**) are also discussed.

Fatty Acids as Modulators of Insulin Sensitivity

Fatty acids modulate insulin signaling and sensitivity in non-ruminants. In pregnant women for example, the increase in plasma fatty acid concentrations during late pregnancy is considered a potential cause of insulin resistance (Sivan and Boden, 2003). The increase in lipolysis is due in part to the actions of placental lactogen and growth hormone. Women with gestational diabetes experience defective insulin secretion and fatty acids play a key role in mediating skeletal muscle insulin resistance. The ability of specific fatty acids to modulate insulin-stimulated glucose utilization in the cow was characterized at the start of the 21st century. At the University of Wisconsin, Pires et al. (2007) demonstrated that the abomasal infusion of nicotinic acid, a potent suppressor of lipolysis, was able to lower circulating fatty acids and insulin following an intravenous glucose challenge. The investigators also confirmed that nicotinic acid administration increased glucose clearance rate following the administration of an intravenous glucose bolus. In non-pregnant and non-lactating Holstein cows fasted to trigger hepatic lipidosis, elevations in circulating fatty acids occurred with a decrease in insulin-stimulated glucose disposal (Oikawa and Oetzel, 2006). In late-gestation dairy cows, feed deprivation increased circulating fatty acids and reduced glucose clearance rate following a glucose challenge, relative to ad libitum fed cows (Schoenberg et al., 2012). It has also been established that overconditioning during gestation increases postpartum circulating fatty acids from lipolysis, which occurs concomitantly with reductions in insulin sensitivity (Rico et al., 2015, 2017b; Davis et al., 2019). Indeed, cows prone to high weight loss during the periparturient period develop adipose tissue insulin resistance (Zachut et al., 2013). Collectively, these studies support the conclusion that adipose-derived fatty acids reduce insulin-mediated glucose utilization in dairy cows.

The current scientific consensus is that the directional change in insulin sensitivity is specific for individual fatty acids in dairy cows. Saturated fatty acids deserve consideration because of their established ability to antagonize insulin action in non-ruminants (Kennedy et al., 2008). In transition dairy cows, the plasma fatty acid pool contains a greater proportion of saturated palmitic acid (C16:0), whereas the proportion of unsaturated linoleic acid (C18:2) and arachidonic acid (C20:4) are lower

(Douglas et al., 2007). Contreras et al. (2010) also demonstrated that palmitic acid is elevated within circulating non-esterified fatty acid and phospholipid fractions during the immediate postpartum. We can postulate that palmitic acid from adipose tissue lipolysis may be responsible for reductions in insulin-stimulated glucose disposal in the aforementioned feed-restricted cows. In support, the intravenous infusion of tallow was compared to linseed or fish oil infusion in non-pregnant and non-lactating Holstein cows (Mashek et al., 2005). The result was a respective increase in circulating concentrations of palmitic acid, linolenic acid (C18:3), and docosahexaenoic acid (**DHA**; C22:6 n-3). In addition, tallow infusion results in greater plasma glucose concentrations and total fatty acid concentrations, relative to fish oil or linseed oil infusion, respectively. Cows infused tallow also had higher circulating glycerol and insulin concentrations, relative to cows infused linseed or fish oil. The authors postulated that the intravenous saturated fat infusion may have increased insulin secretion and decreased insulin-stimulated glucose uptake and suppression of adipose tissue lipolysis. In support, Pires et al. (2008) concluded that the abomasal infusion of tallow impaired the ability of insulin to stimulate glucose uptake and prevent lipolysis in cows, relative to cows abomasally infused linolenic acid-rich linseed oil. These studies also suggested that unsaturated fatty acids may improve insulin action in cows. Such a hypothesis was considered by Gingras et al. (2007). The investigators demonstrated that the continuous abomasal infusion of long-chain omega-3 polyunsaturated fatty acids (**PUFA**) from fish oil increased insulin-stimulated whole-body disposal of amino acids and glucose, activated muscle insulin signaling intermediates protein kinase B and mammalian target of rapamycin, and increased muscle glucose transporter-4 protein abundance in growing steers. More recently, Laguna et al. (2019) abomasally infused oleic acid to postpartum cows and subcutaneous adipose tissue explants were collected, which were challenged with isoproterenol or insulin. The result was that oleic acid was able to enhance insulin sensitivity and reduce lipolysis. Because adipose tissue is enriched in palmitic acid and oleic acid, and both of these fatty acids contribute to the rise in circulating total fatty acids during the peripartum and feed restriction, the interactions between palmitic acid and oleic acid within the context of insulin sensitivity deserves further consideration. Regardless, transition dairy cows do experience decreases in the proportion of long- and very-long chain PUFA including arachidonic acid and DHA in plasma and tissues during the peripartum (Douglas et al., 2007). We postulate that periparturient increases in the ratio of available saturated fatty acids to unsaturated fatty acids may reduce insulin action, promote lipolysis and body weight loss, and increase a cow's risk for developing a metabolic disease; however, such outcomes would also enhance nutrient partitioning towards the mammary gland to support milk production.

Dietary Fatty Acid Feeding and Nutrient Partitioning

Supplemental dietary fat is a common dietary strategy to support the energy needs of high producing dairy cows. Given its high energy density per unit of weight, fat supplementation increases energy supply, and may modulate dry matter intake, and yields of milk and milk solids (i.e., fat and protein). The comprehensive reviews by Palmquist and Jenkins (1980), Grummer (1991), and Coppock and Wilks (1991), and a meta-analysis by Rabiee et al. (2012), summarize a large body of work and are

recommended for further reading. Although supplemental fat is typically included in lactating cow rations to increase energy density and support milk production, there is growing interest to utilize individual fatty acid feeding for non-caloric purposes. This is because not all fatty acids appear to induce the same biological response. In the meta-analysis using 68 comparisons described by Rabiee et al. (2012), responses to supplemental fat were highly heterogeneous with variation attributed to the type of dietary fat supplement (i.e., animal rendered fats, highly unsaturated oilseeds and calcium soaps of fatty acids, and highly saturated prilled fats). Relevant to this paper, we have come to understand that the degree of fatty acid saturation (e.g., saturated stearic vs. monounsaturated oleic, vs. polyunsaturated linoleic) influences milk production response (Relling and Reynolds, 2007), nutrient metabolism and body composition (Pires and Grummer, 2008; Zachut et al., 2010; de Souza et al., 2018), and immune system and cow health (Lessard et al., 2004; Contreras et al., 2012; Mavangira and Sordillo, 2018).

Although it is clear that fatty acid digestibility and absorption vary depending of fatty acid chain length and degree of saturation (Glasser et al., 2008; Boerman et al., 2015), less is known about the direct effects of specific fatty acids on energy metabolism and how specific fatty acids are preferentially partitioned across tissues throughout lactation. Such information may explain the underlying variation in production responses to fatty acid feeding. This question was recently considered in lactating dairy cows by de Souza et al. (2018), who fed fat supplements varying in the proportion of palmitic, stearic (C18:0), and oleic (C18:1 *cis*-9) acids, as follows: 1) PA (~80 C16:0), 2) PA+SA (40% C16:0 and 40% C18:0), and 3) PA+OA (46% C16:0 and 34% C18:1 *cis*-9). Although milk production was comparable across the fatty acid treatments, milk fat yield was highest in cows fed PA, as might be expected. However, body condition score and body weight change were positive and highest in cows fed PA+OA, relative to PA or PA+SA treatment. Further, the PA+OA diet resulted in body weight change 50% above that of PA+SA. These results indicate a preferential partitioning of palmitic acid towards milk energy output, while oleic acid feeding seemed to favor energy partitioning towards body weight storage. In support, palmitic acid feeding has been shown to increase energy partitioning towards milk without a change in energy partitioned towards body reserves in multiparous cows, relative to a no-added fat control (de Souza and Lock, 2018). In addition, palmitic acid feeding accelerated body weight and body condition loss in fresh cows when compared to cows fed a no-added fat control diet (de Souza et al., 2019). While the reasons for these unique responses need to be elucidated, the mechanisms may involve suppressed and enhanced insulin sensitivity with palmitic acid and oleic acid feeding, respectively. In support, we have reported that palmitic acid feeding results in increased milk energy output and plasma fatty acids, and reduced estimated insulin sensitivity and glucose-stimulated fatty acid disappearance, the latter being an indicator of increased lipolytic activity in adipose tissue (Mathews et al., 2016). As described above, the abomasal infusion of oleic acid to postpartum cows was shown to enhance adipose tissue insulin sensitivity and reduce lipolysis (Laguna et al., 2019).

Although the dairy cow's diet is rich in PUFA, mostly in the form of omega-6 linoleic (C18:2 *cis*-9, *cis*-12) and omega-3 linolenic (C18:3 *cis*-9, *cis*-12, *cis*-15) acids,

their ruminal biohydrogenation is so extensive (70 to 95% and 85 to 100% for linoleic and linolenic acids, respectively) that the duodenal fatty acid outflow is mostly saturated stearic acid (Lock et al., 2006). The consequence is that dairy cows typically have limited amounts of essential n-6 and n-3 PUFA available for absorption. Over the past few decades, attention has centered on n-3 PUFA supplementation (i.e., linolenic acid and DHA), because of their ability to modulate prostaglandin synthesis (e.g., prostaglandin F₂ α ; Staples et al., 1998; Mattos et al., 2004) to improve fertility and embryo survival (Santos et al., 2008; Sinedino et al., 2017), and modulate immunity (Lessard et al., 2004). The ability of PUFA to modulate nutrient partitioning during lactation is less clear. Omega-3 fatty acid feeding has been shown to improve insulin sensitivity in rodents (Andersen et al., 2008; Capel et al., 2015); however, a meta-analysis of randomized controlled trials was unable to demonstrate this relationship in humans (Akinkuolie et al., 2011). Cartiff et al. (2013) was able to show an improvement in insulin sensitivity in growing steers fed calcium salts of n-3 fatty acids. Feeding extruded flaxseed enriched in omega-3 linolenic acid to transition dairy cows reduced circulating palmitic acid concentrations, improved postpartum energy balance, and increased early lactation body weight (Zachut et al., 2010). Decreasing the ratio of n-6 to n-3 fatty acids in the diet by feeding calcium salts of fish oil enhanced milk yield and 3.5% FCM in Holstein cows (Greco et al., 2015). Approximately 28% could not be accounted for by increases in caloric intake. The authors explain that nutrient partitioning towards the mammary gland could have favored lactation. Future studies comparing saturated and omega-3 fatty acid feeding on nutrient partitioning, health, and milk production are needed.

The Role of the Sphingolipid Ceramide

The synthesis and accumulation of ceramide is a dominant feature that defines the mechanisms of insulin resistance in rodent models of type 2 diabetes mellitus and non-alcoholic fatty liver disease caused by surplus saturated fatty acids (Summers, 2006; Pagadala et al., 2012). Specifically, ceramide is a bioactive sphingolipid and mediator of insulin resistance (Summers, 2006). The modes by which ceramide inhibits insulin-stimulated glucose appears to involve the activation of protein kinase C- ζ , phosphatase and tensin homolog, and protein phosphatase 2A (Hajduch et al., 2008; Blouin et al., 2010; Chavez and Summers, 2012). Moreover, ceramide-dependent caveolin-enriched microdomain-recruitment of protein kinase C- ζ inhibits protein kinase B activation and glucose transporter translocation to the plasma membrane (Powell et al., 2003). The emerging role of ceramide in the dairy cow experiencing insulin antagonism was recently reviewed by McFadden and Rico (2019). **Table 1** summarizes the current body of work. Several studies have defined ceramide status in the transition dairy cow (Rico et al., 2015; Rico et al., 2017b; Davis et al., 2019). We demonstrated that ceramide accumulates in circulation, and liver and skeletal muscle tissue during the transition from gestation to lactation, regardless of prepartum adiposity status. However, overconditioned prepartum cows experienced greater postpartum body weight loss, hepatic lipid deposition, and circulating fatty acid and ceramide concentrations, relative to cows with moderate body condition. We also demonstrated that plasma and skeletal muscle ceramides were negatively associated with glucose clearance rate and insulin-

stimulated reductions in glucose following an intravenous insulin challenge after calving, respectively. Moreover, plasma glycosylated ceramide concentrations were inversely related to glucose-stimulated reduction in total fatty acids following an intravenous glucose challenge. These studies suggest a role for ceramide in the transition cow.

Our data suggested that fatty acids from adipose tissue lipolysis are utilized to stimulate de novo ceramide synthesis in bovine liver and skeletal muscle. We explored this possibility in Holstein cows following a feed deprivation protocol (Davis et al., 2017a). The result was an increase in circulating fatty acids, liver lipid accrual, impaired glucose disposal following an intravenous insulin challenge, and the accumulation of ceramide in serum and liver. In a subsequent study, Holstein cows were intravenously infused with a soybean oil emulsion (Rico et al., 2018a). The infusion of triacylglycerol increased circulating fatty acids, hepatic triacylglycerol accumulation, and plasma and liver ceramide concentrations. We observed marked increases in hepatic dihydro-ceramide concentrations and ceramide synthase 2 mRNA expression, which suggest that de novo ceramide synthesis was upregulated by hyperlipidemia induction. We postulated that saturated palmitate would activate de novo ceramide synthesis because the pathway is controlled by serine palmitoyltransferase. Indeed, culturing bovine primary neonatal hepatocytes with sodium palmitate increased intracellular ceramide concentrations, which was prevented by co-treating hepatocytes with a serine palmitoyltransferase inhibitor called myriocin (McFadden et al., 2018). More recently, we confirmed that intravenous myriocin administration inhibits ceramide accumulation in feed-restricted adult ewes (unpublished). Collectively, these data confirm in ruminants that hepatic de novo ceramide synthesis is activated during negative energy balance and fatty acids from lipolysis are involved.

Fatty acid feeding regimens that modulate ceramide supply have potential to influence insulin sensitivity and nutrient partitioning. Initial research focused on the effects of palmitic acid feeding on ceramide status in cows. Rico et al. (2016) demonstrated that feeding mid-lactation Holstein cows palm fat enriched in palmitic acid increased circulating fatty acids, ceramide concentrations, and milk yield and milk production efficiency, relative to cows fed a diet without supplemental palm fat. In the same experiment, cows fed palm fat also experienced reductions in glucose-stimulated fatty acid disappearance following an intravenous glucose-challenge. These data suggest that palmitic acid feeding may decrease the ability of insulin to suppress adipose tissue lipolysis via ceramide-dependent mechanisms. In support, Rico et al. (2018b) demonstrated that ceramide inhibited insulin-stimulated 2-deoxyglucose uptake in bovine primary differentiated adipocytes. In contrast, treating bovine adipocyte cultures with myriocin effectively lowered intracellular ceramide concentrations, and enhanced insulin signaling (i.e., protein kinase B activation) and insulin-stimulated 2-deoxyglucose uptake (Rico et al., 2018b). These findings demonstrated that ceramide causes insulin resistance in bovine adipocytes. The ability of palmitic acid to increase circulating ceramide concentrations was also observed in early lactation cows that experienced heightened body weight loss during the fresh period (Davis et al., 2017b; de Souza et al., 2019). Subsequent studies were performed to evaluate the effects of other fatty acids on plasma ceramide concentrations in lactating cows and lambs. In

brief, abomasal palmitic acid infusion elevates circulating ceramides in cows, relative to stearic acid or medium-chain fatty acids (i.e., C8:0 and C10:0) in the form of triacylglycerol (Rico et al., 2017a). When compared to a palmitic acid treatment, only the abomasal infusion of behenic acid (C22:0) has been shown to increase ceramides (Myers et al., 2019). Feeding a fish oil enriched in palmitoleic acid (C16:1), an insulin-sensitizing fatty acid, was shown to increase circulating eicosapentaenoic acid and DHA, and reduce serum ceramide concentrations in lambs, relative to a no-added fat control (Duckett et al., 2019). Lastly, the abomasal infusion of a fish oil highly-enriched in DHA was able to lower circulating ceramides in cows, relative to cows infused with palmitic acid or behenic acid (Myers et al., 2019). Across these studies, we have consistently demonstrated that circulating ceramides are associated with enhanced milk production and the efficiency to produce milk (McFadden and Rico, 2019). In ruminants, our studies suggest that saturated fatty acids (i.e., palmitic and behenic acids) increase ceramide synthesis, whereas PUFA (i.e., palmitoleic acid or DHA) decrease ceramide synthesis. A major implication is that saturated fat feeding has potential to exacerbate insulin resistance and accelerate body weight loss during the fresh period via the actions of ceramide, which may be prevented by increasing bioavailable unsaturated fatty acids and inhibiting ceramide synthesis. Additionally, saturated fat feeding may be a means to maintain or restore insulin antagonism as a means to spare glucose and fatty acids for milk production later in lactation.

Endocrine Control of Ceramide Synthesis in Cows

McFadden and Rico (2019) describes the potential interplay between somatotropin action, ceramide synthesis, and insulin resistance. In brief, uncoupling of the somatotrophic axis inhibits skeletal muscle glucose uptake by downregulating insulin signaling. Moreover, recombinant bovine somatotropin has the ability to increase adipose tissue lipolysis in dairy cows experiencing negative energy balance. Therefore, we posed the possibility that the ability of somatotropin to increase milk production during a catabolic state may involve the synthesis of ceramide from adipose-derived fatty acids, which would in turn downregulate skeletal muscle insulin signaling to spare glucose for milk production. In support, the McFadden lab confirmed that cows administered recombinant bovine somatotropin experience marked increases in circulating ceramides with the concomitant inhibition of insulin-stimulated glucose disposal (unpublished; in review).

Fibroblast growth factor-21 and adiponectin are endocrine signals involved in fatty acid metabolism (Steinberg and Kemp, 2007; Potthoff et al., 2009). The ability of FGF21 to improve glucose homeostasis during obesity involves the stimulation of adiponectin secretion (Holland et al., 2013). In diet-induced obese mice, the insulin-sensitizing effects of FGF21 is due in part to the ability of adiponectin to prevent ceramide accrual (Holland et al., 2011; Holland et al., 2013). This was not observed in ad libitum fed non-pregnant, non-lactating Holstein cows intravenously infused a soybean oil emulsion (Caixeta et al., 2017; Krumm et al., 2017; Rico et al., 2018a). In the postpartum cow experiencing negative energy balance, circulating FGF21 levels are elevated but adiponectin concentrations are low (Schoenberg et al., 2011; Giesy et al.,

2012). We hypothesize that suppressed adiponectin release from adipose tissue during early lactation may contribute to activation of ceramide synthesis from saturated fatty acids. If observed, these findings would suggest that FGF21 does not modulate ceramide supply or increase insulin sensitivity in postpartum cows because of unresponsive adiponectin secretion and enhanced ceramide production.

Conclusions

Our current understanding of lipid biology points to fatty acids, not merely as energy dense sources to sustain milk production, but as bioactive nutrients that influence metabolism and health. The current thought is that saturated fatty acid reduce insulin sensitivity and shift energy partitioning away from body fat reserves and towards milk production. Increasing unsaturated fatty acid availability is likely to increase insulin-stimulated glucose utilization and nutrient utilization for body weight gain. What is unclear is how changes in insulin action and nutrient partitioning influence liver health, inflammation and immune response. We could hypothesize that decreasing insulin sensitivity during early lactation has potential to accelerate body weight loss and increase a cow's risk for developing fatty liver, ketosis or associated disorder. Such challenges may reduce fertility success and productive lifespan. We can also argue that increasing insulin sensitivity during the immediate postpartum is likely to reduce body fat mobilization and inflammation to optimize health. Beyond peak milk production, reducing insulin sensitivity in the cow may enhance nutrient partitioning towards the mammary gland to boost milk production. Because energy balance is restored by this stage of lactation, any potential detriments on health are unlikely to be observed. Future research is likely to answer these questions and delineate the role of individual fatty acids.

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Table 1. Studies of ceramide biology in ruminants

Experiment	Approach	Main outcomes
Rico et al. (2015)	Lean vs. overweight cows during the transition period	Plasma ceramides increased during the transition period, more so for overweight cows. Ceramides were positively related to circulating fatty acids and estimated insulin resistance.
Mathews et al. (2016); Rico et al. (2016)	Palmitic acid feeding at 4% dry matter to mid-lactation cows, relative to no-added fat	Palmitic acid feeding increased milk yield, and plasma fatty acids and ceramide while reducing glucose-stimulated fatty acid disappearance. Milk yields were positively related to plasma ceramides.
Rico et al. (2017b)	Lean vs. overweight cows during the transition period	Postpartum ceramide status was elevated, more so for overweight cows. Muscle ceramides accumulate postpartum. Ceramides were associated with direct measures of impaired insulin sensitivity.
Rico et al. (2017a)	Abomasal infusions of medium-chain triglycerides (8:0+10:0), palmitic acid, or stearic acid to mid-lactation cows	Plasma ceramides were highest in cows infused palmitic acid, relative to other fatty acids tested. Milk yields were positively related to plasma ceramides.
Davis et al. (2017b); de Souza et al. (2019)	Palmitic acid feeding at 1.5% dry matter to early lactation cows, relative to no-added fat	Palmitic acid feeding increased plasma ceramides during early lactation with accelerated body weight and body condition loss. Milk yields were positively related to plasma ceramides.
Davis et al. (2017a)	Ad libitum fed vs. feed-restricted non-pregnant, non-lactating cows	Feed-restriction increased serum ceramides and impaired insulin-stimulated glucose disposal.
Phipps et al. (2017)	Characterization of ceramides in bovine lipoprotein fractions	Ceramides are concentrated in bovine low-density lipoproteins.
Rico et al. (2018a)	Intravenous triacylglycerol or saline infusion to non-pregnant, non-lactating cows	Plasma and hepatic ceramide concentrations increased with circulating fatty acids and hepatic triacylglycerol deposition. Hepatic ceramide synthase 2 mRNA expression increased with triacylglycerol infusion.
McFadden et al. (2018)	Bovine primary neonatal hepatocytes treated with bovine serum albumin (vehicle), palmitic acid, or palmitic acid plus serine palmitoyltransferase inhibitor (myriocin)	Palmitic acid increased hepatocyte de novo ceramide synthesis by activating serine palmitoyltransferase.
Rico et al. (2018b)	Bovine primary differentiated adipocytes treated with cell-permeable ceramide or serine palmitoyltransferase inhibitor (myriocin)	Ceramide inactivates insulin signaling and insulin-stimulated 2-deoxyglucose uptake in bovine adipocytes.
Davis et al. (2019)	Lean vs. overweight cows during the transition period	Circulating ceramides within low-density lipoproteins accumulate postpartum.
Duckett et al. (2019)	Lambs fed no-added fat (control), flaxseed oil, or fish oil enriched in palmitoleic acid	Palmitoleic acid reduced serum very-long chain ceramides, relative to control or flaxseed oil.
Myers et al. (2019)	Abomasal infusion of palmitic acid, behenic acid, or fish oil enriched in DHA	Plasma very-long chain ceramides were elevated, relative to palmitic acid or DHA. DHA lowered muscle ceramides (unpublished).
Unpublished	Subcutaneous injection of recombinant bovine somatotropin to mid-lactation cows	Recombinant bovine somatotropin increased milk yield, reduced insulin-stimulated glucose disposal, and enhanced plasma ceramide concentrations.

Unpublished

Intravenous infusion of serine palmitoyltransferase inhibitor (myriocin) in feed-restricted ewes.

Adipose tissue lipolysis enhances de novo ceramide synthesis.

SESSION NOTES