FATTY ACID NUTRITION AND MILK FAT DEPRESSION

K.J. Harvatine, Ph.D.
Department of Animal Science
The Pennsylvania State University

INTRODUCTION

Milk fat concentration is variable and very responsive to many factors including genetics, season of the year, and physiological state, but is especially responsive to diet. Synthesis of milk fat is an energy demanding process, but also represents a significant portion of the economic and nutritional value of dairy products. First described over one and a half centuries ago, diet-induced milk fat depression (**MFD**) is characterized by a decrease in milk fat yield of up to 50% with no change in milk yield or yield of other milk components. MFD is classically observed in ruminants fed highly fermentable diets or diets high in plant oils. Varying levels of MFD are commonly experienced today in both intensively and extensively managed dairy herds, and this represents a level of milk fat production below the genetic potential of the cow. MFD is also a useful variable for evaluating herd management; in many cases onset of diet-induced MFD is an indication of modified ruminal fermentation and in more pronounced cases this can be associated with ruminal acidosis and reduced efficiency. Therefore, maintaining optimal milk fat synthesis has value beyond the milk fat sold. Although we know extensively the cause of MFD we continue to experience MFD because of the high-energy requirements of cows and the desire to maintain optimal milk production. Numerous dietary factors commonly interact to cause MFD making prediction difficult. Recently we have investigated the time course of induction and recovery of MFD that provides insight into identifying causative factors and setting expectations for correction of MFD.

Historical Theories of Milk Fat Depression

The investigation of diet-induced MFD has a rich history that has included many theories to explain reduced milk fat synthesis. Most of these theories postulated that limitations in substrate supply for milk fat synthesis caused MFD, generally based on changes in absorbed metabolites as a consequence of alterations in ruminal fermentation. For example, the alterations in the ruminal environment typically include decreased pH and decreased acetate to propionate molar ratio (Bauman and Griinari, 2001). This formed the basis for one of the most widely known substrate supply limitation theories that proposed that acetate supply was limiting milk fat synthesis. However, the reduced ratio of acetate to propionate with highly fermentable diets is predominantly due to increased ruminal production of propionate (Bauman and Griinari, 2001, 2003), and ruminal infusion of acetate to cows that during MFD has only a marginal impact on milk fat yield (Davis and Brown, 1970). Overall, several decades of research has tested numerous theories based on substrate limitations and found little to no evidence in their support (extensively reviewed by Bauman and Griinari, 2003 , Shingfield and Griinari, 2007, Bauman et al., 2011).

Davis and Brown (1970) recognized that *trans*-C18:1 fatty acids (**FA**) were increased in milk fat of cows with low-milk fat syndrome. They suggested that these *trans*-FA originated from incomplete ruminal biohydrogenation of unsaturated FA and might contribute to the development of MFD. Subsequent studies have demonstrated a clear relationship between *trans*-FA and MFD (see reviews by Bauman and Griinari, 2003, Shingfield and Griinari, 2007, Bauman et al., 2011). Investigations over the past dozen years have clearly established that diet-induced MFD is associated with rumen production of unique FA from ruminal metabolism of dietary polyunsaturated fatty acids (**PUFA**). Referred to as the "biohydrogenation theory," the basis for diet-induced MFD relates to an inhibition of mammary lipid synthesis by specific FA that are intermediates in the biohydrogenation of dietary PUFA, and these are only produced under certain conditions of altered ruminal fermentation (Figure 1, Bauman and Griinari, 2003). *Trans*-10, *cis*-12 conjugated linoleic acid (CLA) was the first of these to be recognized and it has been extensively investigated at the whole animal and molecular level (reviewed in Bauman et al., 2011).

Ruminal Biohydrogenation

Ruminant diets are low in total fat, although forages, oilseeds, fat supplements, and some byproducts can result in a significant intake of PUFA. Dietary FA are metabolized in the rumen resulting in a large difference between the dietary FA pattern and the profile of FA absorbed from the small intestine. Most FA in the diet are esterified and these are hydrolyzed in the rumen and the resulting unsaturated FA are isomerized (double bond position changed) and biohydrogenated (double bond removed; Figure 1). The extent of biohydrogenation and the intermediates formed are determined by the properties of the fat source, retention time in the rumen, and characteristics of the microbial population (Allen, 2000, Palmquist et al., 2005). Dietary factors that modify ruminal fermentation (ex. high starch, high oil, rumensin) also modify ruminal FA metabolism through associative effects that presumably result in a microbial population that utilizes the alternative pathway of PUFA biohydrogenation.

Ruminal biohydrogenation may be simply described as a function of the available FA pool size, ruminal retention time, and bacterial biohydrogenation capacity (Harvatine and Bauman, 2007). Microbial biohydrogenation is a multi-step process for which the kinetics are not well documented. Harvatine and Allen (2006b) used the pool and flux method (Firkins et al., 1998) to observe *in vivo* ruminal FA kinetics of a cottonseed-based diet that included a fat supplement. Dietary FA had a slow ruminal passage rate (6.4 to 7.4%/h) indicating a long average rumen retention time. In contrast, the fractional biohydrogenation rate of linoleic acid was high (14.6 to 16.7%/h). Interestingly, the biohydrogenation of *trans* C18:1 FA was also very high (33.4 to 48.4%/h), although a decrease in the biohydrogenation rate of *trans*-C18:1 FA was associated with an increased duodenal flow of biohydrogenation intermediates and diet-induced MFD. *In vivo* ruminal FA kinetics clearly demonstrates that ruminal FA metabolism is responsive to associative dietary factors and that the long retention time

provides ample time for metabolism of fat sources that are not rapidly available in the rumen.

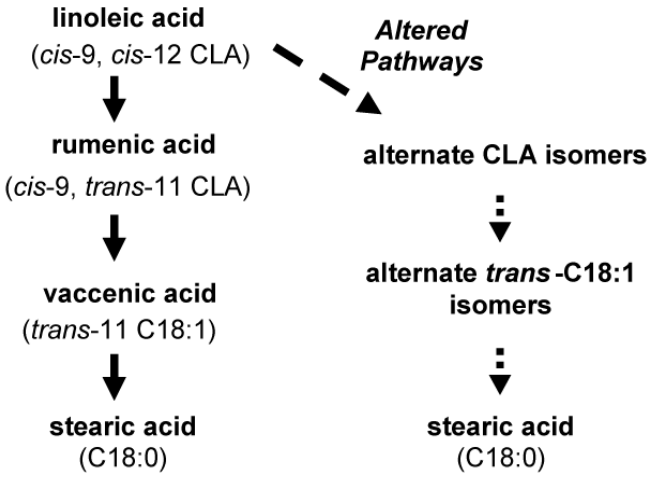

Figure 1. Biohydrogenation pathways during normal and altered ruminal fermentation. Adapted from Griinari and Bauman (1999)

DIETARY RISK FACTORS FOR MILK FAT DEPRESSION

Prediction of the occurrence of MFD is complex because it is not directly caused by a single dietary factor, rather is the result of numerous factors that reduce the rate of biohydrogenation and shift biohydrogenation to the alternate pathway. It is preferable to think of dietary "risk factors" that move a diet along a continuum from low to high risk. Below is a summary of major risk factors. This is not a complete list, but highlights the most important issues.

1. Diet Fermentability

The microbial population is driven by the substrate available and by the rumen environment and is directly dependent on the concentration of starch and NDF and the rates and extent of ruminal digestion. Maximizing fermentablity is important for energy intake, but care should be given to minimizing sub-acute ruminal acidosis. Milk fat depression more commonly occurs with corn silage compared to haylage based rations and with more rapidly digested starch sources such as high moisture corn compared to dry ground corn. Providing multiple sources of starch and fiber with overlapping rates of digestion is the safest approach. Additionally, sugar substituted for dietary starch reduces risk without loss of digestibility (Mullins and Bradford, 2010).

Low milk fat is commonly associated with sub-clinical and clinical ruminal acidosis, but MFD is frequently observed without a reduction in rumen pH (Harvatine and Allen, 2006a). Rumen pH is dependent on the VFA profile, rate of production, and rate of absorption, buffer secretion, and presence of dietary buffers and varies by

approximately 1 to 1.2 pH units over the day (Allen, 1997). It appears that the microbial shift causing MFD occurs before changes in a rumen pH are apparent, but may be related to more subtle changes such as the timing of low pH.

2. Diet Polyunsaturated Fatty Acids

Unsaturated fatty acids have a dual impact on ruminal biohydrogetion in that they modify the microbial population and increase the amount of substrate that must be biohydrogenated. It is important to know the total amount of unsaturated fat and also the source since this dictates the fatty acid profile and rate of ruminal availability. Fish oil has the greatest impact, but is not commonly found in diets in the USA. Cotton, soy, corn and many other plant oils are high in linoleic acid and incorporation of these grains, oils, and their byproducts increases the risk of MFD. The concept of Rumen Unsaturated Fatty Acid Load (RUFAL, Jenkins, 2011) is a simple and insight calculation that is complemented by consideration of the fat source. There are significant differences in the rate of ruminal availability, for instance cottonseed and whole roasted soybeans are expected to have a much slower release of fatty acids in rumen than distillers grains, ground sources, or oil supplements.

Fat is commonly supplemented to increase diet energy density and many protected fat supplements are available. Supplements that are high in saturated fat (palmitic and stearic) do not increase the risk of MFD, however calcium salts of fatty acids are available in the rumen and can reduce milk fat (Lundy et al., 2004, Harvatine and Allen, 2006b). The calcium salt slows the release of unsaturated fat in the rumen and does reduce the impact of these oils compared to free oil, but does not provide a high level of rumen inertness. The impact of calcium salts depends on the profile of the fat supplement and interaction with other factors. For instance, we have observed in two experiments that calcium salts of palm FA reduced milk fat in high producing cows, but not in low producing cows presumably because of differences in intake, passage rate, and rumen environment (Harvatine and Allen, 2006a, Rico and Harvatine, 2011).

3. Rumen Modifiers

Many supplements have a large impact on the rumen microbial population. Monensin is the most common rumen modifier associated with MFD (Jenkins, 2011). However, it is only a risk factor and can be safely used in many diets. Other rumen modifiers may reduce risk, although their effectiveness generally has not been specifically tested. We have ongoing work demonstrating that HMTBa (Alimet, Novus International) reduces the risk of milk fat depression in high risk situations, although the exact mechanism is not yet clear. Additionally, direct fed microbials have been shown to stabilize rumen biohydrogenation during a high diet fermentability challenge (Longuski et al., 2009), although a clear role for these supplements in preventing milk fat depression has not been well investigated.

4. Feeding Strategies

Slug feeding grains is commonly associated with sub-clinical rumen acidosis and MFD. Many assume that TMR feeding eliminates this issue since every bite has the same nutrient composition. However, the rate of intake of fermentable organic matter is very variable over the day due to sorting and variable rates of intake. Generally, cows sort for more fermentable feed particle early in the day, but also consume feed at approximately a three times higher rate after delivery of fresh feed. We recently compared feeding cows 1x/d or in four equal meals very six hours (Rottman et al., 2011, Rottman et al., 2014). The frequent feeding treatment decreased the concentration of alternate biohydrogenation FA and increased milk fat yield and concentration. This experimental treatment highlights the potential to increase milk fat through management of feed delivery.

HOW TO PREDICT THE OCCURANCE OF MILK FAT DEPRESSION

The complexity of predicting dietary fermentability and associative effects makes prediction of MFD difficult. It is arguably impossible to balance a diet that maximizes milk yield and energy intake without incorporation of numerous risk factors. Ruminant nutrition is best practiced as a continuous experiment that monitors cow response to diet modification (Allen, 2011). It is important to monitor nutrient concentrations and model predicted benchmarks that are applicable to your region and logical based on previous experience with similar diets. However, even with the best feed analysis, software, and experience the interaction of diet ingredients and effectiveness of the diet is best determined by the cow and observed by titration and observation.

Diet fermentability is much more extensively handled by feed analysis and software prediction than dietary fat. Dietary FA have typically been consolidated in ration balancing and simply reported as total ether extract or fat concentration. More recently the FA profile of feedstuffs has been included in feed libraries and a more detailed approach of FA nutrition has been taken (Moate et al., 2004). Effectively utilizing this information in diet formulation represents a challenge because of rumen alterations of dietary FA and the fact that individual FA isomers differ in their biological effect. Thus, based on the current understanding of bioactive FA, effective models must predict ruminal outflow of individual FA, including specific *trans*-FA isomers. Secondly, the metabolism of FA by rumen bacteria is extremely dynamic and difficult to integrate into prediction algorithms. Ruminal FA models must account for dietary associative effects that modify the predominant pathways and rates of ruminal biohydrogenation thereby altering the pattern of FA outflow. This may require a mechanistic rather than empirical approach to adequately model. Book values are expected to accurately represent the FA profile of forages and grains and testing of individual lots should not be required for most feedstuffs. However, more variability exists in byproducts, which may require frequent testing of FA concentration depending on the byproduct and source. An understanding and quantification of all factors that induce altered ruminal fermentation is not currently available and development of prediction equation that consider dietary risk factors will require further experimentation and more advanced modeling.

THE TIME COURSE OF INDUCTION AND RECOVERY

Dietary factors that cause low milk fat have almost exclusively been studied through induction of MFD. This is useful because it tells us what dietary factors cause MFD, but it does not directly tell how to recover or accelerate recovery once you have MFD. We recently conducted a high-resolution time course experiment to characterize the timing of induction and recovery of diet induced MFD (Rico and Harvatine, 2013). We induced milk fat depression by feeding a low fiber and high soybean oil diet and then recovered by feeding a higher fiber and low oil diet. We took milk samples every other day to observe milk fat change over time. Milk fat yield decreased progressively when the low fiber and high oil diet was fed and was significantly decreased after 7 days (Figure 2). When switched to the recovery diet, milk fat yield progressively increased and was not different from control until day 11. A key insight from the experiment is the expected lag between making diet adjustments and recovery of milk fat synthesis. Addition of a risk factor may cause milk fat depression in 7 to 10 days and elimination of a risk factor is expected to take 10 to 14 days to observe a benefit. Knowing the time course is very important to identify what may have caused milk fat depression and knowing how long to wait to determine if a diet correction has been effective in improving milk fat.

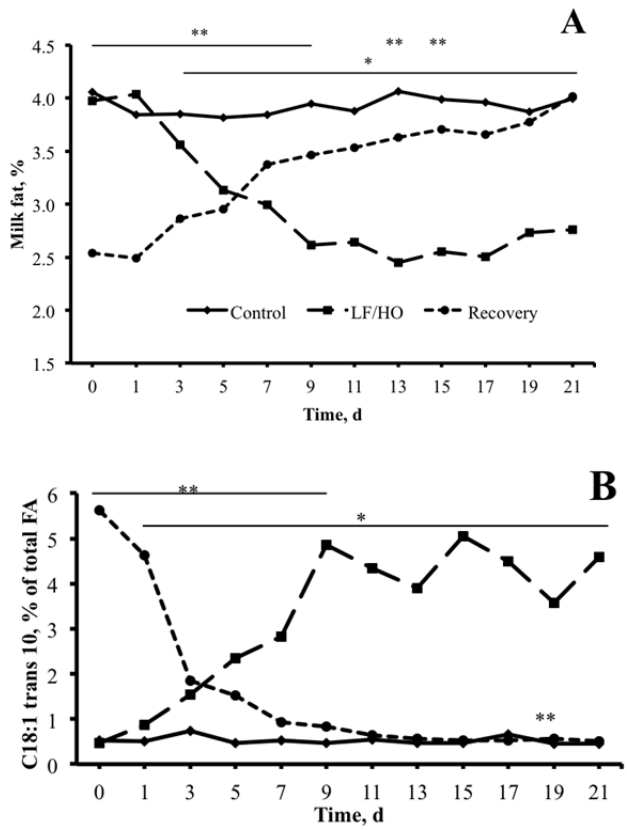

Figure 2. Temporal changes during induction of and recovery from milk fat depression. Panel A. Milk fat percent and Panel B. Milk fat concentration of the bioactive *trans*-10 C18:1 fatty acid.

RAPIDLY RECOVERING MILK FAT

When milk fat moves below the herds goal the logical approach is to systemically remove risk factors. The challenge is which risk factors to remove without loss of milk or energy intake. A multistep approach may be best. First, determine the diet polyunsaturated fat level and availability. In the short term, minimizing PUFA intake is the best first step and is expected to have little effect on milk yield. Secondly, determine if diet fermentability is higher than optimal. In some cases reducing fermentability may reduce sub-clinical acidosis and improve rumen function without loss of milk. If diet fermentability appears within safe limits a reduction may result in lost milk yield so monitor production closely after a diet modification. Lastly, determine if a rumen modifier can be added to stabilize fermentation. For example, if a direct fed microbial is not being used it may be a good opportunity to try a supplement in the herd. It is important to have reasonable expectations on the time-course of recovery. Dietary changes are expected to result in observable improvements in 10 to 14 d, but complete recovery will require nearly 3 weeks and maybe longer with more modest dietary changes.

OTHER IMPORTANT REGULATORS OF MILK FAT YIELD

Seasonal Variation in Milk Fat

Most dairymen and nutritionists recognize a seasonal change in milk fat that is commonly attributed to changes in forage sources, weather, or herd days in milk. A very repeatable seasonal pattern is observed in milk fat and protein concentration at the milk market level. Milk fat and protein concentration peak around December and January and reach a nadir around July and August. This highly repeatable pattern appears to be independent of year-to-year differences in forage quality and weather. A similar pattern is observed in milk marketing orders in different regions. This seasonal variation should be incorporated into the expected milk fat concentration when setting production goals and troubleshooting milk fat production.

Circadian Patterns

Circadian rhythms are changes that occur over the day and repeat every day. Dairymen commonly recognized that morning and evening milking differ in milk yield and composition. Gilbert et al. (1972) reported 0.65 kg higher milk yield at the morning milking, but 0.32 and 0.09 percentage unit higher milk fat and protein, respectively, at the evening milking in cows milked at 12 h intervals. More recently Quist et al. (2008) conducted a large survey of the milking-to-milking variation in milk yield and composition on 16 dairy farms. Milk yield and milk fat concentration showed a clear repeated daily pattern over the 5 days sampled in herds that milked 2 and 3 x/d. Surprisingly milk yield was highest and milk fat lowest in the AM milking of herds milked 2 x/d, but milk yield and milk fat concentration was lowest at the AM milking and highest at the night milking of herds milked 3 x/d. The difference in these rhythms may be due to differences in the length of time represented by each milking interval. However, their

data demonstrated a rhythm of milk and milk fat. We have recently observed milk yield and milk composition at each milking while milking every 6 h and feeding cows 1 x/d at 0800 h or in 4 equal feedings every 6 h. We observed an effect of time of day on milk and milk fat yield and milk fat and protein concentration in cows milked every 6 h. This high resolution and well-controlled experiment demonstrates the circadian pattern of milk synthesis and the interaction of the timing of nutrient intake in high producing dairy cows (Mean MY = 47.7 kg/d). This variation is commonly observed with AM/PM DHIA testing and on large herds shipping multiple tankers per day. We continue to explore nutritional opportunities based on these rhythms.

CONCLUSIONS

Milk fat depression results from an interaction between ruminal fermentation processes and mammary tissue metabolism. Investigation of milk fat synthesis over the past 100 years has resulted in numerous theories based on observational differences in dietary associations, alterations in ruminal fermentation, and adaptations in animal metabolism. To date, the biohydrogenation theory is the only proposed mechanism that has provided causative evidence and withstood rigorous examination. The mechanism by which biohydrogenation intermediates reduce milk fat synthesis has and will continue to provide insight into the regulation of milk fat synthesis. Milk fat depression continues to be a real-world condition that reduces the efficiency and productivity of dairy cows, but understanding its fundamental basis will allow for effective management and intervention strategies. Management of the risk factors associated with MFD is required to reach both milk and milk fat yield goals. The time course of induction and recovery can be utilized to both identify contributing factors and set expectations for recovery. Lastly, the seasonal and circadian pattern of milk fat synthesis explains variation observed between summer and winter and between milkings and should be considered in monitoring and setting production goal.

ACKNOWLEDGEMENTS

This work is partially supported by the Agriculture and Food Research Initiative Competitive Grant no. 2010-65206-20723 from the USDA National Institute of Food and Agriculture [PI Harvatine], USDA Special Grant 2009-34281-20116 [PI Harvatine], Novus International, ELANCO Animal Health, Berg&Schmidt Company, and Penn State University.

LITERATURE CITED

Allen, M. S. 1997. Relationship between fermentation acid production in the rumen and the requirement for physically effective fiber. J. Dairy Sci. 80:1447-1462.

Allen, M. S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. J. Dairy Sci. 83:1598-1624.

Allen, M. S. 2011. Mind over models. Pages 29-44 in Proceeding of the 20th Annual Tri-State Dairy Nutrition Conference. Fort Wayne, IN.

Bauman, D. E. and J. M. Griinari. 2001. Regulation and nutritional manipulation of milk fat: Low-fat milk syndrome. Livest. Prod. Sci. 70:15-29.
Bauman, D. E. and J. M. Griinari. 2003. Nutritional regulation of milk fat synthesis. Annu. Rev. Nutr. 23:203-227.
Bauman, D. E., K. J. Harvatine, and A. L. Lock. 2011. Nutrigenomics, rumen-derived bioactive fatty acids, and the regulation of milk fat synthesis. Annu. Rev. Nutr. 31:299-319.
Davis, C. L. and R. E. Brown. 1970. Low-fat milk syndrome. Pages 545-565 in Physiology of digestion and metabolism in the ruminant. A. T. Phillipson, ed. Oriel Press, Newcastle upon Tyne, UK.
Firkins, J. L., M. S. Allen, B. S. Oldick, and N. R. St-Pierre. 1998. Modeling ruminal digestibility of carbohydrates and microbial protein flow to the duodenum. J. Dairy Sci. 81:3350-3369.
Gilbert, G. R., G. L. Hargrove, and M. Kroger. 1972. Diurnal variation in milk yield, fat yield, milk fat percentage, and milk protein percentage of holstein-friesian cows. J. Dairy Sci. 56:409-410.
Harvatine, K. J. and M. S. Allen. 2006a. Effects of fatty acid supplements on milk yield and energy balance of lactating dairy cows. J. Dairy Sci. 89:1081-1091.
Harvatine, K. J. and M. S. Allen. 2006b. Fat supplements affect fractional rates of ruminal fatty acid biohydrogenation and passage in dairy cows. J. Nutr. 136:677-685.
Harvatine, K. J. and D. E. Bauman. 2007. Recent advances in milk fat depression:1 time course of milk fat depression and 2. Adipose tissue lipogenesis duiring milk fat depression. Pages 135-142 in Proceedings cornell nutrition conference for feed manufacturers. Syracuse, NY.
Jenkins, T. C. 2011. Managing the rumen environment to control milk fat depression. Pages 31-37 in Proc. Penn State Dairy Nutrition Workshop, Grantville, PA.
Longuski, R. A., Y. Ying, and M. S. Allen. 2009. Yeast culture supplementation prevented milk fat depression by a short-term dietary challenge with fermentable starch. J. Dairy Sci. 92:160-167.
Lundy, F. P., E. Block, W. C. Bridges, Jr., J. A. Bertrand, and T. C. Jenkins. 2004. Ruminal biohydrogenation in holstein cows fed soybean fatty acids as amides or calcium salts. J. Dairy Sci. 87:1038-1046.
Moate, P. J., W. Chalupa, T. C. Jenkins, and R. C. Boston. 2004. A model to describe ruminal metabolism and intestinal absorption of long chain fatty acids. Animal Feed Science and Technology 112:79-105.
Mullins, C. R. and B. J. Bradford. 2010. Effects of a molasses-coated cottonseed product on diet digestibility, performance, and milk fatty acid profile of lactating dairy cattle. J. Dairy Sci. 93:3128-3135.
Palmquist, D. L., A. L. Lock, K. J. Shingfield, and D. E. Bauman. 2005. Biosynthesis of conjugated linoleic acid in ruminants and humans. Adv. Food Nutr. Res. 50:179-217.
Quist, M. A., S. J. LeBlanc, K. J. Hand, D. Lazenby, F. Miglior, and D. F. Kelton. 2008. Milking-to-milking variability for milk yield, fat and protein percentage, and somatic cell count. J. Dairy Sci. 91:3412-3423.

Rico, D. E. and K. J. Harvatine. 2011. Effect of a high palmitic acid fat supplement on ruminal fermentation and milk production in high- and low-producing dairy cows. J. Dairy Sci. 94:1333.
Rico, D. E. and K. J. Harvatine. 2013. Induction of and recovery from milk fat depression occurs progressively in dairy cows switched between diets that differ in fiber and oil concentration. J. Dairy Sci. 96:6621-6630.
Rottman, L. W., Y. Ying, and K. J. Harvatine. 2011. Effect of timing of feed intake on circadian pattern of milk synthesis. J. Dairy Sci. 94:830.
Rottman, L. W., Y. Ying, K. Zhou, P. A. Bartell, and K. J. Harvatine. 2014. The daily rhythm of milk synthesis is dependent on the timing of feed intake in dairy cows. Physiological reports 2
Shingfield, K. J. and J. M. Griinari. 2007. Role of biohydrogenation intermediates in milk fat depression. Eur. J. Lipid Sci. Technol. 109:799-816.