PERSPECTIVE: How to address the root cause of milk fat depression in dairy cattle

Timothy J. Hackmann* and Payam Vahmani
Department of Animal Science, University of California, Davis, CA 95168

Milk fat depression is a costly and complex nutritional disorder of dairy cattle. It occurs when feeding cattle diets high in fermentable carbohydrates or unsaturated fat, and yield of milk fat can drop by 50% (Davis and Brown, 1970; Bauman and Griinari, 2003). It has been studied for over 175 yr (Boussingault, 1845), yet there is still no way to directly address the root cause—microbes that form antilipogenic fatty acids in the rumen (Bauman and Griinari, 2003; Dewanckele et al., 2020). In this Perspective article, we propose that enzyme inhibitors could directly target enzymes in these microbes and be effective feed additives for alleviating this disorder.

Approximately 2 centuries of research have painted a detailed picture of how milk fat depression develops (Figure 1A). Early experiments showed the disorder can be triggered by an abrupt shift from forage to grain (i.e., decrease in forage to concentrate ratio; Davis and Brown, 1970; Bauman and Griinari, 2003). Similar experiments identified other risk factors in the diet (Davis and Brown, 1970; Bauman and Griinari, 2003). In later experiments, researchers illuminated events in the rumen and mammary gland behind the disorder. After following many false leads, researchers turned their attention to unique fatty acids formed by rumen microbes (Bauman and Griinari, 2003). These would prove responsible for the disorder, and they remain key to solving it today.

Rumen microbes form a number of unique fatty acids through a process known as biohydrogenation (Figure 1B). In this process, microbes take dietary UFA and isomerize cis (c) to trans (t) double bonds (Dewanckele et al., 2020). They then reduce these double bonds, forming SFA (Dewanckele et al., 2020). One pathway of biohydrogenation proceeds under normal conditions of the rumen, and it has no negative effect on milk fat synthesis by the mammary gland (Bauman and Griinari, 2003; Dewanckele et al., 2020). However, a second, alternate pathway can be triggered by risk factors mentioned and forms distinct fatty acid intermediates containing a t10 double bond. These and other intermediates can depress milk fat synthesis, resulting in classic milk fat depression (Bauman and Griinari, 2003; Dewanckele et al., 2020). The first intermediate in the pathway (t10,c12-18:2) has received the most study, though others contribute to the disorder (Dewanckele et al., 2020). Collectively, t10,c12-18:2 and other intermediates that depress milk fat synthesis are called antilipogenic fatty acids.

Though the onset of milk fat depression has been studied in detail, there are currently few ways to alleviate or prevent this disorder. The most obvious solution is to limit risk factors (see Figure 1A). Given the nature of the risk factors, availability of feed ingredients, and feed cost, this solution is not always practical. For example, low fiber is a risk factor (Davis and Brown, 1970; Bauman and Griinari, 2003), but nutritionists keep fiber low to maximize energy intake. Even diets that appear relatively safe (28% NDF, 18% starch, 3% fat) can still trigger milk fat depression (Stoffel et al., 2015). Milk fat depression is thus inherently difficult to avoid, and many dairy herds still show evidence of this disorder (McCarthy et al., 2018).

Rumen buffers are widely used and practical feed additives for alleviating milk fat depression, but even these have shortcomings. Sodium bicarbonate, for example, is fed to over 50% of dairy herds in California (Silva del Rio et al., 2010). Despite their ubiquity, buffers do not completely alleviate milk fat depression (Davis et al., 1964; Kalscheur et al., 1997). In one trial, milk fat from cows fed buffers was still depressed by 11% compared to normal values (Davis et al., 1964). A meta-analysis shows buffers increase milk fat yield, but further increases appear possible; yield does not plateau as inclusion of buffer (dietary cation anion difference) increases (Iwaniuk and Erdman, 2015). Other trials show that buffers reduce t10 fatty acids in digesta, but concentrations still remain elevated above normal (Piperova et al., 2002; Jenkins et al., 2014). Together, these studies suggest that buffers can partly, but not fully, alleviate milk fat depression.
Figure 1. Milk fat depression is a disorder caused by antilipogenic fatty acids formed by rumen microbes, and we propose enzyme inhibitors could alleviate it. (A) Overview of the onset of milk fat depression, showing where enzyme inhibitors would be useful. CHO = fermentable carbohydrates; unsat = unsaturated. (B) Pathways of biohydrogenation for linoleic acid (c9,c12-18:2), showing how an enzyme of the alternate pathway (linoleic acid C9 isomerase) leads to production of an antilipogenic fatty acid (t10,c12-18:2). (C) Structure of one potential inhibitor (polyethylene glycol 400; PEG) and its target enzyme (linoleic acid C9 isomerase). The structure is PDB 2B9W and drawn with PyMOL version 2.5.5 (Schrödinger, LLC; New York, NY).
Other feed additives for milk fat depression exist, but they also have limits. HMTBα, an analog of the AA methionine, alleviates milk fat depression but only partly (Baldin et al., 2018). Plant secondary metabolites have also been explored, but they were unable to lower t10 fatty acids in rumen digesta (Lourenço et al., 2008).

We propose enzyme inhibitors can target the root cause of the disorder and thus serve as effective feed additives (Figure 1A). The inhibitors would act directly against microbial enzymes forming antilipogenic fatty acids, slowing or stopping their production. Enzyme inhibitors have been developed to help solve other difficult problems in animal agriculture. 3-Nitrooxypropionol, for example, is an inhibitor developed to reduce methane production in the gut. It targets the enzyme catalyzing the last step of methane formation, doing so by entering the active site and modifying (oxidizing) it (Duin et al., 2016). By irreversibly inhibiting the enzyme, it reduces methane in vivo by 30% (Duin et al., 2016). We envision similar use of inhibitors for alleviating milk fat depression.

To develop effective inhibitors, we should look for compounds that enter the active site of enzymes producing antilipogenic fatty acids. Fortunately, one compound appears to have already been found. Polyethylene glycol, a long molecule resembling a fatty acid, has been found to enter the active site of the enzyme producing t10,c12-18:2 (Figure 1C). Further, this compound stops the usual substrate (linoleic acid) from entering the active site. As such, this compound should inhibit enzyme catalysis and slow production of t10,c12-18:2. The structural biologists who made this discovery did not pursue it further; it was contrary to their original goal of determining the structure of the normal enzyme-substrate complex (Liavonchanka et al., 2006). Thus, the opportunity is open to test this and other potential inhibitors in the context of milk fat depression.

Our proposal to develop enzyme inhibitors will involve overcoming some challenges. First, we need to isolate more bacteria from the rumen that produce antilipogenic fatty acids. These bacteria would be natural targets for screening inhibitors in vitro, but none had been available in culture collections. We have recently isolated a strain, Cutibacterium acnes AP1, that produces t10,c12-18:2 and would be useful for experiments. Second, we need to identify important antilipogenic fatty acids with more certainty. The evidence for r10,c12-18:2 acting as an antilipogenic fatty acid is strong, but it is more equivocal for others (Dewanckele et al., 2020). Third, inhibitors must be selective and not disrupt the normal pathway of biohydrogenation. The normal pathway does not cause milk fat depression, and it is essential to remove UFA that are toxic to rumen microbes (Dewanckele et al., 2020). Fourth, in vitro screening of many compounds is a logical start, but efficacy will have to be proven with extensive in vivo trials.

New feed additives for alleviating milk fat depression, such as enzyme inhibitors, hold great significance to dairy farmers. Over 50% of farms feed buffers, despite their limits. Thus, more targeted and effective feed additives should be adopted widely. It remains to be seen just how targeted and cost-effective enzyme inhibitors will be. The possibility exists that inhibitors will not fully alleviate milk fat depression, but another merit, such as high specificity, will make them useful. New additives would be useful not just with current rations; they would enable nutritionists to push new boundaries in feeding fermentable carbohydrates and unsaturated fat. Feeding more unsaturated fat is more important than ever, given public concerns over feeding palmitic acid, a saturated fat supplement widely fed to dairy cows (Harvatine, 2021). Though milk fat depression has been studied for 175 years, some of the most exciting advances may be yet to come.

ACKNOWLEDGMENTS

This work was supported by the USDA National Institute of Food and Agriculture under project CA-D-ASC-2756-RR. The authors acknowledge that T. Hackmann is a section editor for the Journal of Dairy Science. The authors have not stated any conflicts of interest.

REFERENCES


Boussingault, J. B. 1845. Rural Economy, In Its Relations with Chemistry, Physics, and Meteorology; Or, an Application of the Principles of Chemistry and Physiology to the Details of Practical Farming. H. Bailliere, London, UK.


Lourenço, M., P. W. Cardozo, S. Calsamiglia, and V. Fievé. 2008. Effects of saponins, quercetin, eugenol, and cinnamic acid on fatty acid biohydrogenation of forage polyunsaturated fatty acids in du-