

UPDATE ON MILK FAT: IDENTIFICATION OF RUMEN BIOHYDROGENATION INTERMEDIATES THAT INHIBIT SYNTHESIS

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INTRODUCTION

Fat is the most variable component in milk and is markedly affected by diet. Nutrition affects both the quantity and composition of milk fat and a striking example is milk fat depression (MFD). Diet-induced MFD represents a challenging problem that involves the interrelationship between bacterial fermentation processes in the rumen and the synthesis of milk fat by the mammary glands. The cause of MFD has perplexed scientists for over a century and various theories have been advanced and subsequently found inadequate (Bauman and Griinari, 2003). Of special importance in elucidating the basis for MFD was the observation by Davis and Brown (1970) that increases in the milk fat content of *trans* 18:1 fatty acids (TFA) coincided with MFD. As analytical techniques improved it became apparent that MFD was correlated with changes in the pattern of TFA and conjugated linoleic acid (CLA) isomers in milk fat.

The biohydrogenation theory hypothesized that under certain dietary conditions the pathways of rumen biohydrogenation are altered to produce unique fatty acid intermediates which are potent inhibitors of milk fat synthesis (Bauman and Griinari, 2001). TFA and CLA originate as intermediates in rumen biohydrogenation and the change in their pattern represents a clear indication that rumen fermentation is altered during MFD. Therefore, the induction of MFD requires both an altered rumen environment and the presence of unsaturated fat in the rumen. Subsequent work by other groups verified early results and support for the biohydrogenation theory has grown.

Our knowledge of the relationship between nutrition and milk fat is advancing rapidly and we have provided updates at previous Conferences (e.g. Perfield and Bauman, 2005). However, our understanding is incomplete and MFD remains a challenge for dairy producers and their advisors. Current research efforts can be divided into three distinct, but interrelated areas: 1) to identify fatty acids arising from rumen biohydrogenation that inhibit milk fat synthesis, 2) to establish cellular mechanisms by which these biohydrogenation intermediates inhibit mammary synthesis of milk fat, and 3) to apply this knowledge in troubleshooting on-farm problems associated with milk fat production. Updates on the first two of these are the subject of this paper and the paper that follows by Harvatine and Bauman (2006). The application for troubleshooting on commercial dairies was recently reviewed by Overton et al. (2006) and we will expand on this further at this conference by looking at the affect of specific feed components and their interaction on rumen lipid metabolism and milk fat content (Lock et al., 2006).

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BIOHYDROGENATION INTERMEDIATES AND MFD

The analytical advances that have enabled us to identify dietary affects on the fatty acid composition of milk fat have also revealed that the pathways of rumen biohydrogenation are considerably more complex than first thought. TFA and CLA isomers are generally not present in dietary ingredients fed to dairy cows, so their presence in milk fat is a consequence of their formation as intermediates in rumen biohydrogenation. Dietary situations causing MFD alter the pathways of rumen biohydrogenation resulting in changes in the TFA and CLA isomer profile in milk fat, and these changes are often correlated with MFD (for example see Loores et al., 2005). However, correlation does not demonstrate causality and once we identify fatty acids that vary in relation to milk fat it is important to examine their biological activity. Our ability to directly test effects on milk fat synthesis is limited by the availability of pure fatty acids, and often we have had to chemically synthesize candidate fatty acids to test them in dairy cows. Despite these obstacles, we have been able to examine several novel biohydrogenation intermediates.

CLA Isomers

Rumen production of CLA isomers and their concentration in milk fat are markedly effected by diet (Bauman and Lock, 2006). A list of these and their concentration range in milk fat are presented in Table 1. The first CLA isomer shown to effect milk fat synthesis was *trans*-10, *cis*-12 CLA (Baumgard et al., 2000). Effects are specific for milk fat and subsequent studies demonstrated a curvilinear relationship between increasing *trans*-10, *cis*-12 CLA dose and the reduction in milk fat yield (deVeth et al., 2004). Furthermore, *trans*-10, *cis*-12 CLA is a potent inhibitor of milk fat synthesis with as little as 2.0 g/d being sufficient to cause a 20% reduction in milk fat production. However, the observed reduction in milk fat in studies of diet-induced MFD is greater than can be accounted for by the increase in milk fat content of *trans*-10, *cis*-12 CLA when compared with dose-response studies using the pure CLA isomer (Bauman and Griinari, 2003; Peterson et al., 2003). Thus, there must be additional biohydrogenation intermediates that regulate milk fat synthesis.

The CLA isomers that have been examined for affects on milk fat synthesis are also listed in Table 1. These have typically been examined at concentrations similar to those found effective for *trans*-10, *cis*-12 CLA. All of the newly tested CLA isomers were taken up by the mammary gland and incorporated into milk fat, but only *trans*-9, *cis*-11 CLA (Perfield et al., 2005) and *cis*-10, *trans*-12 CLA (Sæbø et al., 2005) caused a reduction in milk fat synthesis. The predominant CLA isomer in milk fat is *cis*-9, *trans*-11 CLA (trivial name = rumenic acid) and it is the isomer that has anti-cancer properties. It's interesting that rumenic acid has no affect on milk fat synthesis, but *trans*-9, *cis*-11 CLA is a potent inhibitor (Perfield et al., 2005). While the double bonds are in the same position in both of these isomers (carbons 9 and 11), their orientation has been reversed (*trans/cis* vs. *cis/trans*). This emphasizes the critical importance of bond position and orientation in determining the biological activity of a fatty acid. Other CLA isomers that have been examined and found to have no effect on milk fat yield are

trans-8, *cis*-10 CLA, *trans*-9, *trans*-11 CLA, *trans*-10, *trans*-12 CLA and *cis*-11, *trans*-13 CLA (Perfield et al., 2004; 2005; 2006). Therefore, to date a total of 3 CLA isomers have been shown to regulate (inhibit) milk fat synthesis (Table 1); the production of these is increased in different types of diet-induced MFD, but analyzing for them remains problematic.

Table 1. Range of CLA isomers (% of total CLA) in milk fat and their ability to cause milk fat depression.

CLA Isomer	Percent of Total CLA ¹	Inhibits Milk Fat Synthesis ²
<i>trans</i> -7, <i>cis</i> -9	1.2 - 8.9	
<i>trans</i> -7, <i>trans</i> -9	<0.1 - 2.4	
<i>trans</i> -8, <i>cis</i> -10	<0.1 - 1.5	-
<i>trans</i> -8, <i>trans</i> -10	0.2 - 0.4	
<i>trans</i> -9, <i>cis</i> -11	<0.1 - 1.5	+
<i>cis</i> -9, <i>trans</i> -11	72.6 - 91.2	-
<i>trans</i> -9, <i>trans</i> -11	0.8 - 2.9	-
<i>trans</i> -10, <i>cis</i> -12	<0.1 - 1.5	+
<i>cis</i> -10, <i>trans</i> -12	<0.1 - 1.5	+
<i>trans</i> -10, <i>trans</i> -12	0.3 - 1.3	-
<i>cis</i> -11, <i>trans</i> -13	0.2 - 4.7	-
<i>trans</i> -11, <i>cis</i> -13	0.1 - 8.0	
<i>trans</i> -11, <i>trans</i> -13	0.3 - 4.2	
<i>cis</i> -12, <i>trans</i> -14	<0.1 - 0.8	
<i>trans</i> -12, <i>trans</i> -14	0.3 - 2.8	
<i>cis-cis</i> isomers	0.1 - 4.8	

¹ Adapted from Lock and Bauman, 2004.

² When abomasally infused at a dose comparable to *trans*-10, *cis*-12 CLA. The CLA isomer either had no effect (-) or inhibited (+) milk fat synthesis. Effects on milk fat synthesis derived from data by: Baumgard et al., 2000; Perfield et al., 2004; 2005; 2006; Sæbø et al., 2005.

Trans 18:1 Fatty Acids

The milk fat content of many TFA isomers also increases when diets causing MFD are fed and their possible role in regulating milk fat synthesis is of interest. This interest comes in part from results with partially hydrogenated vegetable oils (PHVO). PHVO generally contain about 40-50% TFA and when abomasally infused or fed as a rumen-protected formulation, MFD occurs. First shown by Selner and Schultz (1980), subsequent studies concurred and attributed the reduction in milk fat to the TFA in PHVO (Gaynor et al., 1994; Romo et al., 1996; Piperova et al., 2004; Selberg et al., 2004). However, PHVO contains a number of conjugated, partially conjugated, and other unique fatty acids formed during the chemical hydrogenation process, and effects

of these cannot be excluded as the cause of observed MFD (see discussion in Bauman and Griinari, 2003).

To date, the TFA isomers that have been directly examined have not affected milk fat yield at the doses tested. These include *trans*-9 18:1 (25 g/d), *trans*-11 18:1 (12.5 g/d), and *trans*-12 18:1 (12.5 g/d) (Rindsig and Schultz, 1974; Griinari et al., 2000). Consistent with this research, we recently re-examined *trans*-9 18:1 and *trans*-11 18:1 at a higher dose (~ 45 g/d) and observed no effect on milk fat production (unpublished data).

One of the TFA isomers that increases during diet-induced MFD is *trans*-10 18:1 and articles have often implied that this isomer causes milk fat depression. We have recently conducted an extensive summary of published data (35 publications involving 109 treatments) to highlight the curvilinear relationship that exists between increases in milk fat content of *trans*-10 18:1 and reductions in milk fat percent (Figure 1; Hinrichsen et al., 2006). *Trans*-10 18:1 has been unavailable for direct testing and its increase in milk fat could simply be a marker for altered rumen biohydrogenation rather than reflecting an inhibitory effect per se. We recently carried out the first direct examination of the effects of *trans*-10 18:1 on milk fat synthesis (unpublished data). In collaboration with scientists from Nestle Research Center, Switzerland, we synthesized pure *trans*-10 18:1 and examined its effects at a physiological dose that would be expected to cause MFD based on reports of rumen TFA outflow and the studies cited above using PHVO supplements. We provided *trans*-10 18:1 by abomasal infusion (43 g/d) and observed that it was taken up by the mammary gland and transferred to milk fat. However, *trans*-10 18:1 had no effect on milk fat synthesis even when provided at a dose 10-fold greater than the effective dose of *trans*-10, *cis*-12 CLA; the yield of milk fat was not significantly different between Control and *trans*-10 18:1 treatments ($P = 0.68$) whereas the *trans*-10, *cis*-12 CLA treatment reduced milk fat yield by 25% ($P = 0.002$). Thus, our results offer no support for *trans*-10 18:1 as a cause of diet-induced MFD, and highlight the problem of implying cause-effect relationships based on correlations between specific milk fatty acids and MFD. However, *trans*-10 18:1 is relatively easy to analyze compared to many of the CLA isomers and the results in Figure 1 raise the possibility that this fatty acid could serve as a surrogate marker for the type of alterations in rumen biohydrogenation that characterize diet-induced MFD.

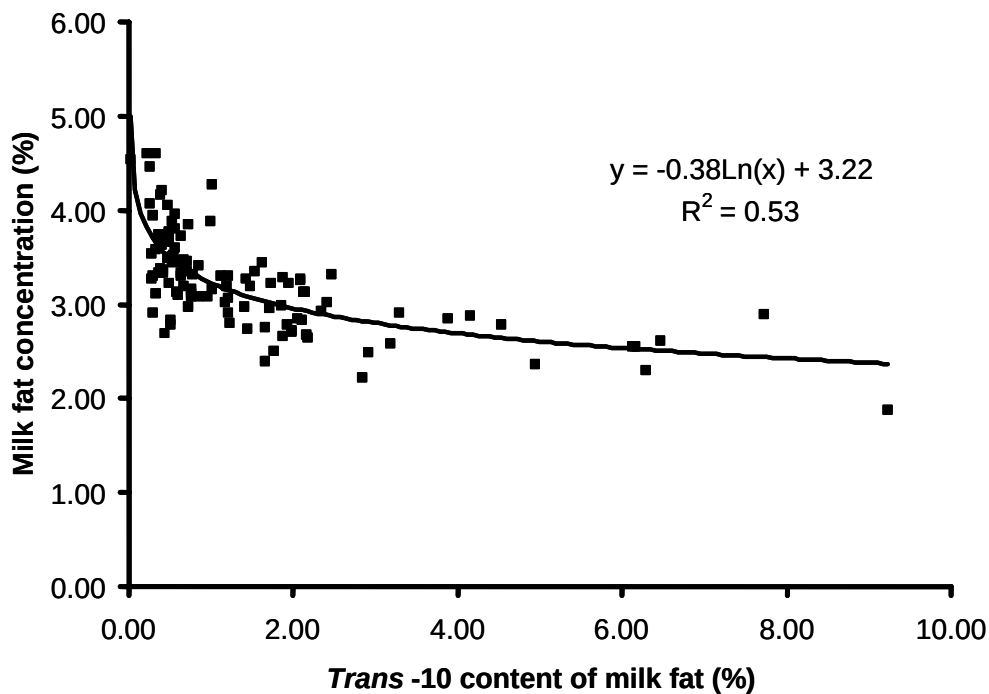


Figure 1. The relationship between the content of *trans*-10 18:1 in milk fat and milk fat percent (adapted from Hinrichsen et al., 2006).

CONCLUSIONS

The problem of diet-induced MFD has challenged producers and scientists for over a century. The biohydrogenation theory provides a unifying concept to explain diet-induced MFD. While investigations to date offer strong scientific support for this theory, rigorous evaluation continues to determine if it is applicable to all situations of diet-induced MFD. The alterations in rumen biohydrogenation that occur during diet-induced MFD result in marked changes in TFA and CLA isomers. Individual fatty acids are very different in their biological effects and thus generalizations that associate MFD with TFA and/or CLA isomers can be misleading and inaccurate. The challenge is to identify those specific biohydrogenation intermediates that inhibit mammary synthesis of milk fat. Thus far, three CLA isomers have been identified as potent inhibitors of milk fat production and these are increased in situations of MFD. However, investigations to date with specific TFA isomers have not demonstrated affects on milk fat production, although the change in milk fat content of these, especially *trans*-10 18:1, is correlated with MFD. Further characterization of fatty acids known to regulate milk fat synthesis and identification of additional fatty acid regulators are essential to be able to effectively troubleshoot milk fat problems on commercial dairy farms.

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