

UNDERSTANDING THE BIOLOGY OF MILK FAT DEPRESSION: FROM BASIC CONCEPTS TO PRACTICAL APPLICATION

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INTRODUCTION

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 and our understanding of the interrelationship between rumen lipid metabolism and milk fat synthesis has progressed significantly over the last decade. The ‘biohydrogenation theory’ represents a unifying concept to explain the basis for diet-induced MFD where intermediates of ruminal fatty acid biohydrogenation (BH) escape the rumen, are absorbed, and signal a decreased expression of lipogenic enzymes and a reduction in milk fat synthesis in the mammary gland. The first rumen BH intermediate shown to effect milk fat synthesis was trans-10, cis-12 conjugated linoleic acid (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 (de Veth et al., 2004), with as little as 2.0 g/d being sufficient to cause a 20% reduction in milk fat production. Recently, two additional BH intermediates that regulate milk fat synthesis have been identified, trans-9, cis-11 CLA (Perfield II et al., 2007) and cis-10, trans-12 CLA (Saebo et al., 2005). MFD has been observed over a wide range of feeding situations including diets high in concentrates and low in fiber, and diets supplemented with plant or fish oils. Although the cause of all types of diet-induced MFD involves inhibition of milk fat synthesis by unique BH intermediates, troubleshooting milk fat issues on dairy farms remains one of the more challenging tasks within overall nutritional management of dairy cows. Clearly, small quantities of specific BH intermediates produced in the rumen and subsequently taken up by the mammary gland are sufficient to induce substantial decreases in milk fat content and yield. Escape of these intermediates from the rumen is influenced by ruminal passage rate, bacterial BH capacity and dietary polyunsaturated fatty acid (PUFA) concentration and profile. Bacterial BH capacity is intrinsic to the bacterial population, and numerous factors are known to cause altered ruminal fermentation with a propensity towards production of BH intermediates that are associated with MFD. Therefore, the induction of MFD requires both an altered rumen fermentation and the presence of PUFA in the rumen, and within each of these categories there are a number of potential risk factors and areas to address when developing nutritional strategies designed to minimize effects on milk fat production (Table 1).

The focus of the following sections will be to discuss the impact of dietary components and rumen environment interactions on milk fat. Experience indicates that MFD occurs as a result of several concurrent diet or management factors rather than as a result of a single factor. It will always be a challenge to troubleshoot MFD when the magnitude of decrease in milk fat (e.g. 3.8 to 3.2%) may be caused by 1 to 2 g/d or less of trans-10, cis-12 CLA or a related intermediate passing to the small intestine. Although we do not fully understand all of the ruminal conditions that may trigger MFD, an improved understanding of the impact of dietary components and their interaction during rumen fermentation will provide the critical framework with which to better troubleshoot this issue.

RUMEN BIOHYDROGENATION

Since unsaturated fatty acids are toxic to many rumen bacteria, the majority of dietary lipids are biohydrogenated through a series of fatty acid intermediates that ultimately results in saturated fatty acids being produced (Palmquist et al., 2005). Accordingly, there is an extensive metabolism of lipids in the rumen and this has a major impact on the profile of fatty acids available to the dairy cow (Lock et al., 2006a).

One area that has received renewed attention is attempting to establish the pathways of BH and the identity of intermediates. In vivo studies have revealed a vast array of trans-18:1 and CLA isomers present in digesta contents of cattle and sheep (Bauman and Lock, 2006). These in vivo studies were important in showing that many more intermediates exist in ruminal contents than can be accounted for by most accepted pathways of BH. As many as 14 CLA isomers have been identified in ruminal contents taken from cattle (Jenkins et al., 2008). Yet, most published pathways of biohydrogenation account for the synthesis of only one or two CLA isomers. Much of the attention has been directed at intermediates of linoleic

Table 1. Partial list of potential risk factors for reduced milk fat and areas to address when developing nutritional strategies designed to avoid diet-induced MFD.¹

Altered Rumen Environment	Supply of PUFA
Low rumen pH/low peNDF	Amount (esp. linoleic acid intake)
Feed particle size	Availability
Fiber	PUFA:SFA
Starch (NSC)	Feeding pattern
Rumensin	Variation in fat content and FA composition of feed ingredients
Feeding pattern	

¹ Adapted from Bauman and Lock (2006) and Overton et al. (2006).

acid biohydrogenation because it is believed to be the parent compound for most of the CLA isomers found in digesta contents of cattle. A recent study completed at Clemson University incubated a stable isotope of linoleic acid with ruminal contents for 48 h. The results revealed the formation of seven CLA isomers from linoleic acid, including *cis*-9 *trans*-11 and *trans*-10 *cis*-12 CLA. The other isomers labeled with the isotope were *trans*-9, *trans*-11 CLA, *cis*-10 *trans*-12 CLA, *cis*-9 *cis*-11 CLA, *cis*-10 *cis*-12 CLA, and *trans*-10 *trans*-12 CLA.

Under certain dietary situations the rumen environment is altered and a portion of BH occurs via a pathway that produces *trans*-10, *cis*-12 CLA and *trans*-10 18:1 (Figure 1). *Bifidobacterium*, *Propionibacterium*, *Lactococcus*, *Streptococcus*, and *Lactobacillus* isolates from other habitats have been reported to produce *trans*-10, *cis*-12-CLA. As these genera occur in the rumen, although generally at rather low numbers, they may contribute to BH and specifically to *trans*-10, *cis*-12-CLA formation in the rumen. *Propionibacterium*, *Streptococcus*, and *Lactobacillus* are also more numerous in the rumen with concentrate diets (Jenkins et al., 2008), which would again be consistent with greater *trans*-10, *cis*-12 CLA production with concentrate diets. Therefore, dietary situations causing MFD alter the pathways of rumen BH resulting in changes in the specific *trans*-18:1 and CLA isomers available for uptake by the mammary gland and incorporation into milk fat. As shown in

Figure 1. Generalized scheme of ruminal biohydrogenation of linoleic acid under normal conditions (left side) and during diet-induced milk fat depression (dotted lines, right side). Adapted from Bauman and Griinari (2003). The grey boxes highlight three potential means by which dietary components can increase the risk of milk fat depression.

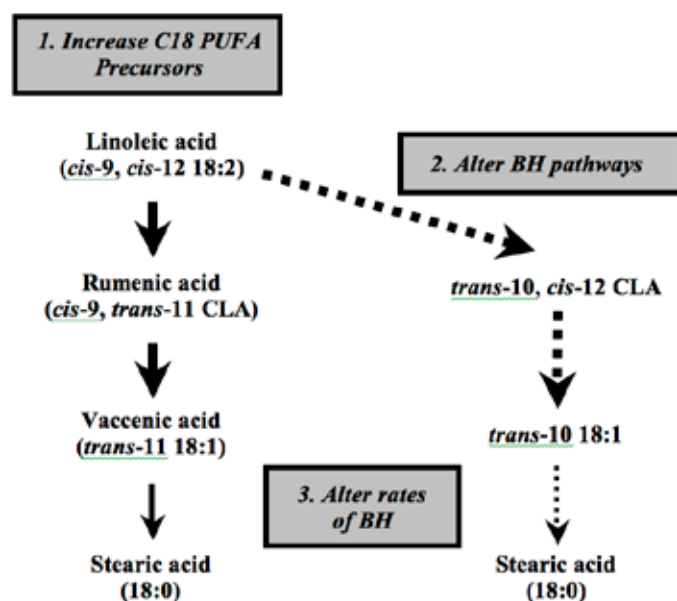


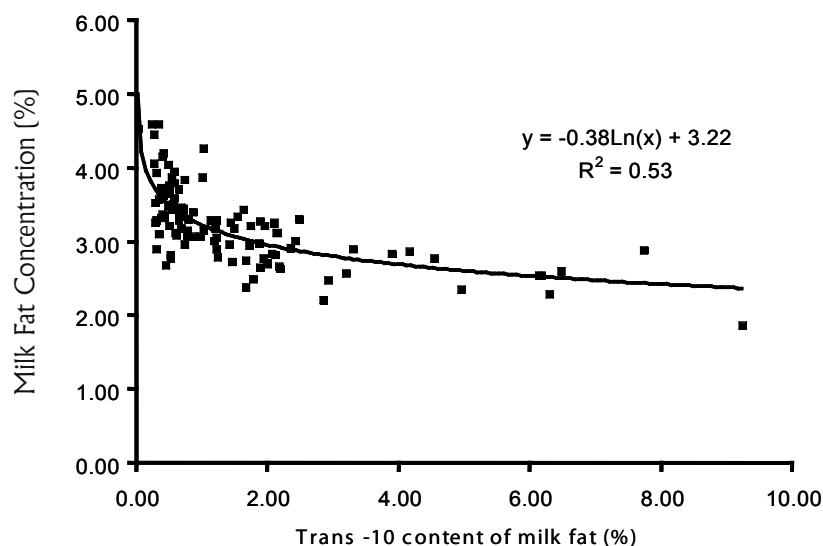
Figure 1, this ‘trans-10 shift’ in BH pathways, and the associated increase in the trans-10 18:1 content of milk fat, is indicative of the complex changes in ruminal BH pathways characteristic of MFD. Although trans-10 18:1 does not directly inhibit mammary synthesis of milk fat (Lock et al., 2007), it is relatively easy to analyze compared to trans 10, cis-12 CLA and other CLA isomers. Therefore, in general, this fatty acid can serve as a surrogate marker for the type of alterations in rumen BH that characterize diet-induced MFD. This is highlighted in Figure 2, which shows the relationship between the content of trans-10 18:1 in milk fat and milk fat percent (Hinrichsen et al., 2006). Also shown in Figure 1 are the three predominant ways in which dietary components can impact the risk of milk fat depression: 1) through increasing substrate supply of 18-carbon unsaturated fatty acids, 2) by altering the rumen environment and BH pathways, and 3) via changes in the rate of BH at various steps in the BH process. These three areas are discussed in the following sections.

SUPPLY OF UNSATURATED FATTY ACIDS

Grain and Forage Lipids

The fatty acid content of most cereal seeds and forages typically ranges from 10 to 30 g/kg DM, with the majority of the fatty acids classified as unsaturated (predominately oleic, linoleic, and linolenic acids). Among the unsaturated fatty acids, linolenic acid is the

Figure 2. The relationship between the content of trans-10 18:1 in milk fat and milk fat percent. Adapted from Hinrichsen et al. [2006].



predominant fatty acid in most forage species followed by linoleic acid (Hatfield et al., 2007). In the cereal seeds, fatty acids are comprised mainly of linoleic acid followed by oleic acid.

Fatty acid concentrations in some pasture can exceed 50 g/kg DM, depending on plant species, stage of maturity, environment, etc. Fatty acid content of annual ryegrass pasture that was clipped in the field, immediately immersed in liquid nitrogen, and then freeze dried contained as much as 68 g/kg DM total fatty acids (Freeman et al., 2002). CPM-Dairy lists 116 g/kg DM fatty acids in perennial ryegrass pasture. Cattle grazing some species of immature pasture, in effect, may be consuming a high fat diet. Much lower concentrations are usually seen in hay and silage prepared from the same plant species. This is partially due to loss of plant leaves where chloroplast lipid is concentrated, but also due to plant metabolism of stored energy sources. Plant enzymes can continue to function in dried forage containing as little as 5 to 10% moisture. Plant maturity has a definite impact on both fatty acid content and fatty acid composition. Fatty acid content (g/kg DM) generally is highest in the spring and fall seasons and lowest in the summer months. For example, fresh perennial ryegrass contained 32 g/kg DM total fatty acids during primary growth in May, but only 12 g/kg DM at the beginning of second regrowth (Bauchart et al., 1984). Linolenic acid follows a similar seasonal pattern (Bauchart et al., 1984). As linolenic acid declines over the summer months, percentages of palmitic and linoleic acid increases.

Fat Supplements

Most plant oils contain 100% ether extract with a high percentage of fatty acids. The impurities extracted, such as water and pigments, are removed during refining leaving the commercial plant (soybean oil, canola oil, corn oil, etc) and animal (tallow, grease, etc) fats with mainly triglycerides consisting of 90-93% fatty acids. The remaining 7-10% is mainly glycerol. Glycerol is readily utilized as an energy source, but only contains the energy of carbohydrates. Caution is advised when obtaining fats from unknown vendors to be sure that considerable impurities do not still remain in the product that lower the fatty acid and energy content. Rather than guessing, it pays to have a sample of the fat analyzed for fatty acid content and profile. The ether extract values listed in NRC (2001) for dairy cattle exceed 99% for all fat categories (vegetable oil, tallow, hydrolyzed tallow fatty acids, and partially hydrogenated tallow) except for calcium soaps. Ether extract for the vegetable oil, tallow, and partially hydrogenated tallow are assumed to contain 90% fatty acid and 10% glycerol. Ether extract for the hydrolyzed tallow fatty acids is assumed to contain 100% fatty acids. Ether extract for calcium soaps is listed as 84.5% in NRC (2001) for dairy cattle, which is assumed to be all fatty acid.

Fatty acids, such as stearic acid, are referred to as saturated because all the carbons are holding the maximum number of hydrogens possible, or the fatty acid is “saturated” with hydrogen. Stearic acid is low in plant oils, but present in higher amounts in animal fats, particularly in fats obtained from ruminant species such as beef tallow (Table 2). Oleic acid and linoleic acid are examples of unsaturated fatty acids containing one or more double

bonds. Oleic acid has a single double bond between carbons 9 and 10, and is referred to as a monounsaturated fatty acid. Linoleic acid is a polyunsaturated fatty acid containing two double bonds between carbons 9 and 10, and between carbons 12 and 13. Oleic acid is the predominant fatty acid in animal fats and some plant oils, such as canola oil (Table 2). Linoleic acid is the predominant fatty acid in many plant oils, including cottonseed oil, soybean oil, and corn oil.

RUMEN FAT LOAD

Implications on Microbial Shifts

Elevating fatty acid concentration in ruminal contents may cause a number of changes in ruminal fermentation characteristics and microbial population distribution. Ruminal changes are the result of the antimicrobial nature of unsaturated fatty acids, where fatty acids adsorb onto the cell membrane of selected microbial species, and then penetrate into the membrane causing disorganization of phospholipids and eventual cytological damage (Jenkins, 2002). Because some bacterial species are more susceptible than others, the result is a microbial shift in the rumen. The fatty acid-induced microbial shift can disrupt fermentation of carbohydrate digestion causing a drop in the acetate to propionate ratio and possibly a reduction in fiber digestion (Jenkins, 2002). The microbial shift also can redirect the pathways of fatty acid BH causing accumulation of CLA isomers linked to milk fat depression (Bauman and Lock, 2006).

Two factors that affect the antibacterial activity of lipids are fatty acid structure and concentration. Free fatty acids generally disrupt fermentation more than triglycerides and antibacterial activity of free fatty acids can be enhanced by increasing the number of

Table 2. Fatty acid composition of several fat supplements used in livestock rations [adapted from Rouse, 1996].

Abbreviation ^a	Common Name	Tallow	Cottonseed	Canola	Poultry
C14:0	Myristic	3.0	1.0		1.0
C16:0	Palmitic	25.0	23.0	4.0	21.0
C18:0	Stearic	21.5	3.0	2.0	8.0
C18:1	Oleic	42.0	18.5	60.0	41.0
C18:2	Linoleic	3.0	52.5	20.0	19.0
C18:3	Linolenic			10.0	1.0

^aNumber of carbons: number of double bonds.

double bonds (Chalupa et al., 1984). Growth of some bacterial species is stimulated by low concentrations of fatty acids, but inhibited at higher concentrations (Maczulak et al., 1981). In attempting to predict ruminal fermentation changes caused by dietary lipid, it is often assumed that the fat load is contributed only by the fat supplement and that FFA concentration is low. Both assumptions can be wrong. Fatty acids from the TMR and forage can significantly contribute to total rumen fat load, for example when animals are consuming immature pasture. Also, FFA concentration may be elevated in some feed ingredients such as whole cottonseed stored in warm, humid conditions (Cooke et al., 2007), or in forages resulting from hydrolytic cleavage of esterified lipids during hay-making (Yang and Fujita, 1997).

Implications on MFD

Given that the specific fatty acids that cause MFD are intermediates produced during ruminal BH of PUFA, it is logical that the amount of initial substrate (linoleic acid and perhaps linolenic acid) may be related to the amount of the key BH intermediates that are produced. Linoleic and linolenic acids represent a large percentage of the fatty acids found in most forages and other plant-based feedstuffs fed to dairy cattle, with linoleic acid representing the predominant PUFA in corn and corn byproducts. As a result, under typical US situations linoleic acid is the major dietary fatty acid, particularly when corn silage comprises the majority of the forage base in the ration and oilseeds are the major source of added dietary fat. Estimates of linoleic acid intake using CPM-Dairy indicates that in these situations linoleic acid intake can approach and even exceed 400 to 500 g/d (Table 3). Therefore, it would appear that typical rations have more than enough substrate as linoleic acid to meet the required presence of PUFA for MFD to occur if rumen fermentation is altered. Nevertheless, this is a moving threshold which depends on the rate at which the PUFA become available to the rumen bacteria and the extent to which perturbations in rumen fermentation occur. With the increased availability of corn byproducts (e.g. distillers' grains) an additional important consideration is their fat content because they can contain a considerable amount of lipid which is predominately linoleic acid. In particular, the fat content of corn distillers' grains is highly variable (e.g. ~5 to 15% of DM), and this degree of variation can significantly alter the dietary supply of unsaturated fatty acids to the dairy cow, thereby increasing the risk of MFD.

The feeding of supplement fat can be challenging since various lipids and fatty acids can trigger a number of changes in rumen metabolism. Space does not permit a detailed discussion of specific fat sources, but readers are directed to a recent review by Staples (2006) which discusses the influence of different fat supplements on milk fat. In general, as you increase the degree of unsaturation of supplemental fat and/or the availability of the fatty acids present (e.g. extruded vs. roasted oilseeds), the chances of MFD occurring will increase. Recently, Relling and Reynolds (2007) examined the impact of feeding rumen-inert fats differing in their degree of saturation on performance of lactating dairy cows. Cows

Table 3. Modified CPM Dairy lipid submodel output showing the sources of dietary fatty acids from a diet formulated for a cow producing 100 lbs milk/d.

	----- Fatty Acid (g/d) -----					
	C16:0	C18:0	C18:1c	C18:2	C18:3	Total
Alfalfa Silage	15	3	2	13	31	80
Corn Silage	30	4	32	79	14	166
Soybean Hulls	3	1	3	8	3	20
Corn Grain Ground	28	4	51	117	3	211
Soybean Meal	14	3	10	42	7	78
Blood Meal	2	2	2	1	0	7
Cottonseed Whole	92	9	58	217	1	384
Megalac	107	9	75	15	0	210
Ration	290	35	234	496	59	1161

were fed a Control mixed ration ad libitum, and treatments were the dietary addition (3.5% of ration dry matter) of 3 rumen-inert fat sources differing in fatty acid profile. As shown in Table 4, as the unsaturation of the supplemental fat increased, this was associated with reduced milk fat content and yield.

It is also clear that cows consuming diets that contain corn silage as the only or major forage source appear to be more susceptible to MFD when unsaturated fats are supplemented. Partial substitution of corn silage with another forage such as alfalfa may alleviate this negative effect. For example, Ruppert et al. (2003) showed that changing the forage in the diet from predominantly corn silage to alfalfa silage offset the depressing effect that tallow can have on milk fat. The concentration of trans 18:1 BH intermediates in milk fat tended to increase to a greater extent when tallow was fed in the corn silage-based diets than in the alfalfa silage-based diets. Although not reported in this study it is most likely that the profile of trans 18:1 fatty acids also shifted to favor trans-10 18:1 with the corn silage-based diets. This is supported by a study by Onetti et al. (2004) which observed that replacing half the dietary corn-silage with alfalfa silage negated the negative effect of tallow on milk fat yield (Table 5). Furthermore, the addition of alfalfa silage to the diet attenuated the tallow-induced increase in trans-10 18:1 formation in the rumen and subsequent incorporation into milk fat (Table 5).

The example shown in Table 5 raises a number of interesting questions relating to substrate supply of unsaturated fatty acids. Since it appears to be 18:2 BH intermediates that are responsible for MFD, we have typically only looked at PUFA when considering substrate supply. These data, however, suggest that it may be appropriate to more broadly consider overall ‘unsaturated load’ in the rumen when troubleshooting MFD. Increasing the

Table 4. The effect of rumen-inert fats containing mostly saturated fatty acids (SFA), mostly monounsaturated fatty acids (MUFA), or mostly polyunsaturated fatty acids (PUFA) on dry matter intake (DMI), milk yield and milk fat synthesis in midlactation dairy cows.¹

	----- Diet -----				
	Control	SFA	MUFA	PUFA	P ¹
DMI, kg/d	23.8	23.1	22.1	22.0	0.12
Milk, kg/d	36.9	37.3	35.8	34.8	0.44
Fat, %	3.37	3.86	3.32	2.61	0.03
Fat, g/d	1,249	1,436	1,184	911	0.02

¹ Adapted from Relling and Reynolds (2007).

² Probability comparing the difference between saturated and unsaturated fat supplements (SFA vs. MUFA and PUFA).

Table 5. Effect of feeding tallow on rumen fermentation and milk fat synthesis in dairy cows fed diets based upon corn silage (CS) or alfalfa silage (AS) with, or without tallow supplementation.¹

	----- Treatment ² -----		
	CS	CST	AST
DMI, kg/d	27.6	25.9	26.5
Milk, kg/d	44.9	44.3	43.6
Fat, %	3.12	2.68	3.32
Fat, kg/d	1.38	1.17	1.45
trans-10 18:1, %	0.75	2.15	0.78

¹ Adapted from Onetti et al. (2004).

² CS = 50% corn silage + 50% conc; CST = 50% corn silage + 50% conc + 2% tallow;

AST = 25% corn silage + 25% alfalfa silage + 50% conc + 2% tallow.

dietary supply of oleic acid (cis-9 18:1) from tallow or other sources (e.g. palm fatty acid distillate), will not directly increase the rumen outflow of 18:2 BH intermediates because these fat supplements supply very little PUFA and, as we showed previously, under some circumstances we can feed high levels of oleic acid without inducing MFD (Hinrichsen et al., 2006). In some circumstances, however, it would appear that the increase in unsaturated load from increasing oleic acid supply is sufficient to alter BH pathways to favor the production of trans-10, cis-12 CLA and related intermediates from the PUFA already in the diet. This hypothesis is supported by a recent study using continuous cultures and ¹³carbon-labeled oleic acid. As expected, lowering culture pH to 5.5 reduced the concentration of trans-11

18:1 and increased trans-10 18:1 concentration. The ¹³carbon enrichment of trans-10 18:1, however, was lower at pH 5.5 compared with pH 6.5 indicating that more of the trans-10 at low pH originated from sources other than oleic acid (AbuGhazaleh et al., 2005). This must come from PUFA sources and will presumably be driven through BH pathways that also promote the formation of trans-10, cis-12 CLA or related intermediates, thereby increasing MFD risk (Lock et al., 2006b).

ALTERATION OF THE RUMINAL ENVIRONMENT

Factors that alter rumen environment are traditionally first considered when troubleshooting MFD on dairy farms. One major change in the rumen environment that leads to flux of fatty acids through alternate pathways of ruminal BH is low ruminal pH. Factors that can result in marked changes in ruminal pH through any 24-h period include: dietary carbohydrate profile and rates of degradation of the carbohydrate fractions as affected by source, processing, and moisture; physically effective NDF (peNDF) supply as affected by source and particle size; and production of salivary buffers as a function of peNDF supply and source (Shaver, 2005). Despite our general understanding of these factors, the degree and duration of low ruminal pH required to cause sufficient flux of PUFA through alternative pathways of ruminal BH is not known. Although data are limited, changes in rumen pH are most likely associated with MFD because they cause a change in the bacterial population favoring those that have alternative BH pathways. A common misconception, however, is that acidosis is a prerequisite for MFD to occur. This is not the case and in most situations rumen health appears excellent and there are no overt signs of ruminal acidosis (Overton et al., 2006). For example, Harvatine and Allen (2006a) reported increased duodenal flow of BH intermediates and MFD with no change in ruminal pH measured every 5 seconds over 4 d. Again, this highlights the fact that only small changes in the rumen environment can lead to increased risk of MFD.

A cursory review of the literature highlights the impact of different dietary carbohydrates on the risk of MFD as affected by source, processing, and moisture, presumably as a result of differences in the rate of rumen fermentation. A somewhat extreme example was reported by Jurjanz et al. (2004) which compared the effect of different starch sources (potato vs. wheat) on rates of milk fat synthesis; although there were no significant differences in milk yield, the wheat diet significantly reduced milk fat yield by 11%. Of greater relevance, a number of studies have reported an effect of corn processing method on risk of MFD. For example, Guyton et al. (2003) reported a 10% reduction in milk fat yield when steam-flaked corn replaced dry-ground corn. Clearly, careful consideration should be given to the fermentation rate of starch sources when troubleshooting MFD issues. As we have highlighted previously, however, no single factor tends to result in low milk fat and an example of the impact of some of these dietary interactions is highlighted in Table 6. Oba and Allen (2003) fed diets containing high moisture and dry ground corn at either a high or low starch level. At the low starch level there was no significant effect of grain processing on

milk fat parameters, whereas at the high starch level high moisture corn significantly reduced milk fat yield by 15% compared to dry ground corn.

The preceding paragraphs have discussed situations in which changes in dietary components and their interactions have resulted in alterations in the rumen environment and BH pathways. It is worth noting, however, that risk of MFD can also be increased not only by changes in dietary components, but also via changes in how the diet is presented to the cow. An example of this is shown in Table 7 in which the effect of forage particle size on risk of MFD is reported (Grant et al., 1990). Cows were fed total mixed rations containing either fine (2.0 mm), medium (2.6 mm), or coarse (3.1 mm) ground alfalfa silage as 55% of

Table 6. Effect of corn grain processing method and starch intake on milk fat synthesis.¹

	----- High Starch -----		----- Low Starch -----	
	High Moisture Corn	Dry Ground Corn	High Moisture Corn	Dry Ground Corn
Milk yield (kg)	38.8	38.4	33.4	34.3
Milk fat %	3.05 ^b	3.59 ^a	3.95 ^a	3.73 ^a
Milk fat yield (kg)	1.17 ^b	1.35 ^a	1.33 ^{ab}	1.27 ^{ab}

¹ Adapted from Oba and Allen (2003). Treatment significance (P < 0.05) indicated by differences in superscript letters.

Table 7. Influence of ration particle size on rumen fermentation and milk fat synthesis.^{1,2}

	----- Treatment ³ -----			
	Fine	Medium	Coarse	P
Dry Matter Intake, kg/d	22.4	22.0	22.2	0.88
Milk Yield, kg/d	31.5	32.1	31.1	0.56
Milk Fat, %	3.0	3.6	3.8	0.001
Milk Fat Yield, g/d ⁴	945	1156	1182	—
Rumen pH	5.3	5.9	6.0	0.1
Rumination Time, min/24 h	374	466	531	0.001
Total Chewing Time, min/24 h	570	671	735	0.001

¹ Adapted from Grant et al. (1990).

² Arithmetic mean particle size of the fine and coarse silages used in the study were 2.0 and 3.1, respectively.

³ Rations formulated on 55:45 silage:concentrate basis.

⁴ Calculated from reported values.

dietary DM. Intake of DM and NDF was not influenced by particle size of the ration. Milk production also was unaffected, but milk fat decreased from 3.8% for cows fed the coarse ration to 3.0% for cows fed the fine ration. The decrease in milk fat secretion with reduced size of silage particles was also associated with reduced rumination and total chewing times and a lower rumen pH (Table 7).

Although the implications of low ruminal pH for production of the MFD-causing intermediates have been considered, it is probable that other factors can also cause changes in the rumen bacteria population resulting in an increased flow of fatty acids through alternate pathways of ruminal BH (Palmquist, et al., 2005). Overton et al. (2006) hypothesized that factors such as ensiled feeds with abnormal fermentation profiles (particularly high acetic acid corn silages) or moldy feeds may also cause the changes in BH required to cause MFD, however, these factors remain unstudied in a controlled manner. Additional issues that warrant further attention include environmental factors such as heat stress as well as management factors such as stocking density. In addition, when considering factors related to rumen environment, the impact of changes in rate of passage out of the rumen should also be considered; cows with higher DMI have higher rates of passage which potentially will ‘flush’ more BH intermediates out of the rumen. Cows with slower rates of fermentation, potentially increasing DMI and passage rate to compensate, and cows with higher DMI in general (e.g. higher producing cows) are, therefore, more likely at risk of MFD, and thus the margin of error is less in these animals (Overton, et al., 2006). Finally, an area that merits further research is the potential role of specific feed ingredients in maintaining normal BH pathways. For example, antioxidants have been shown to play a role in maintaining normal BH pathways; the addition of vitamin E (~10,000 IU/d) to a diet supplemented with plant oil efficiently limited the shift in BH pathways (as measured by the trans-10 18:1 content of milk fat) resulting in an increase in milk fat content and yield (Pottier et al., 2006). Furthermore, in recent work, the supplementation of a combination of synthetic antioxidants (Agrado Plus®) to late lactation dairy cows was reported to result in a 5.3% increase in milk fat yield compared with an unsupplemented group (Vázquez-Añón et al., 2007).

ALTERATION OF RATES OF BIOHYDROGENATION

Under some circumstances specific feed components can alter rumen fermentation in a manner that results in changes in BH rates of fatty acids. Altering these rates can potentially increase the rumen outflow of trans-10, cis-12 CLA and related intermediates responsible for MFD, thereby increasing risk of MFD. This is a facet of troubleshooting MFD which is not typically considered when thinking about the traditional ‘supply of PUFA’ or ‘altered fermentation’ groupings, even though these changes are a result of changes in the rumen environment. Monensin is an example of a feed ingredient that can affect BH rates through altering rumen fermentation and the bacterial species present. In some cases during established lactation monensin supplementation can result in decreased milk fat percentage and yield (Duffield and Bagg, 2000). These effects are likely the result of interactions with

other dietary or management factors that predispose cows to experience MFD. Monensin increases maintenance requirements of gram positive bacteria in the rumen which renders these bacteria less competitive in the ruminal environment (Duffield and Bagg, 2000). The net result is changes in the ruminal bacterial population that appear to decrease rates of BH of PUFA in the rumen. Very few species of bacteria have been identified that can convert trans-18:1 fatty acids to stearic acid (18:0), and most of these have been identified as being gram positive. Thus the final step in BH is already the 'rate-limiting' step; therefore decreasing the number of bacteria that can carry out this process can potentially lead to a 'build-up' of BH intermediates in the rumen thereby increasing their passage to the small intestine. This was highlighted by Fellner et al. (1997) when they examined the effect of monensin on the formation of BH products when linoleic acid was infused continuously into rumen fermentors. With an unsupplemented diet the rate of 18:0 formation was 7.5 mg/L/hr whereas this decreased to only 2.7 mg/L/hr when monensin was supplemented (Fellner et al., 1997). It is important to remember, however, that an increased rumen outflow of BH intermediates will not be a problem if typical BH pathways are present. However, even if a small proportion of dietary PUFA are being biohydrogenated through pathways that produce trans-10, cis-12 CLA and related intermediates, Monensin can potentially increase the passage of these to the small intestine and increase the risk of MFD.

Dietary fatty acids can also modify ruminal fermentation and may shift BH towards the production of intermediates that cause MFD. For example, Harvatine and Allen (2006b) reported that fat supplements affected fractional rates of ruminal fatty acid BH and passage in dairy cows; increasing the unsaturation of the fat supplement slowed down the BH of 18:1 to 18:0 while causing a significant reduction in milk fat yield. It is also well known from experimental diets that the addition of fish oil to the diet alters ruminal fermentation towards increased production of BH intermediates. Long chain n-3 PUFA present in fish oils appear to affect rumen bacteria catalyzing the terminal step in BH, thereby increasing the rumen outflow of these intermediates. In vitro studies with mixed cultures of rumen bacteria have established that docosahexaenoic acid is a specific n-3 PUFA responsible for this effect (AbuGhazaleh and Jenkins, 2004), though it is likely that other fatty acids may have similar effects. We have previously taken advantage of the effects of fish oil on rumen lipid metabolism as a method to facilitate the production of cis-9, trans-11 CLA-enriched milk (e.g. Lynch et al., 2005). Interactions are once again key; if normal BH pathways are maintained then the rumen outflow of trans-11 18:1 and cis-9, trans-11 CLA will increase. Small changes, however, in rumen fermentation as a result of fish oil feeding can alter these pathways thereby increasing the rumen outflow of intermediates that cause MFD. This is highlighted by recent studies by our group that emphasize the impact of feeding pattern of fish oil on MFD risk. In our first study we infused fish oil into the rumen 4X / day and observed a 24% decrease in milk fat yield. However, a follow up study which utilized a similar basal diet but infused the fish oil 6X / day resulted in no MFD (McConnell, Lock and Bauman, unpublished). Due to these multifaceted interactions it has proven difficult to experimentally distinguish the effect of PUFA as increased substrate vs. its potential role as a modifier of rumen fermentation.

CONCLUSIONS

Low milk fat percentage and yield is an important economic issue to dairy farms across North America. The available evidence indicates that all situations of MFD are due to changes in rumen BH of unsaturated FA and the passage of specific intermediates out of the rumen that subsequently reduce milk fat synthesis in the mammary gland. These changes in ruminal microbial processes are an essential component for the development of MFD and are centered on both an altered rumen environment and an alteration in the rumen pathways of PUFA BH. In general, no single dietary factor is responsible for MFD, and this paper has highlighted the interactions between various dietary components that can increase the rumen outflow of BH intermediates associated with MFD. Dietary components can increase the risk of MFD by increasing substrate supply, altering rumen BH pathways, and altering rates of BH. With the latter, it is important to consider factors that alter rates of BH (e.g. monensin) as not being causative for MFD per se; rather they interact with a predisposing condition (e.g., altered ruminal BH pathways) to accentuate the effects on milk fat. Finally, our understanding of the effect of specific BH intermediates on milk fat synthesis in the mammary gland has advanced at a much greater rate than our knowledge of their production in the rumen. Therefore, further research is required to better understand the ruminal conditions that promote the formation of BH intermediates that may trigger MFD. An improved understanding of these events will provide the critical framework with which to better troubleshoot MFD.

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