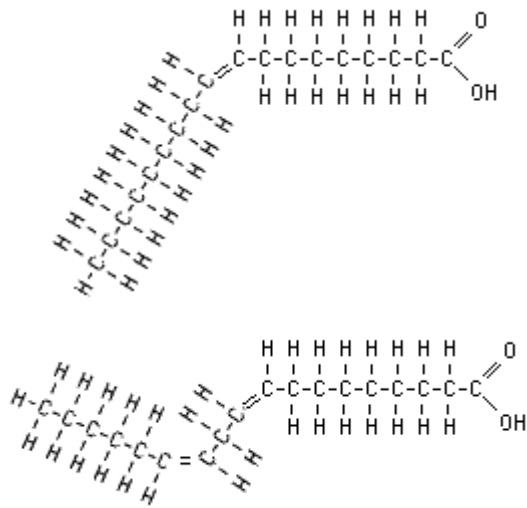


Linoleic acid is the predominant fatty acid in many plant oils, including cottonseed oil, soybean oil, and corn oil.

Figure 2. The structures of oleic and linoleic acids.



The energy value of fat supplements is determined almost exclusively by the type and amount of fatty acid present in the supplement. Most fat supplements are comprised of different proportions of 5-8 common fatty acids all of which have similar energy values (approximately 9.4 kcal/g). Therefore, fatty acid content (g fatty acid/100 g fat supplement) is more important than fatty acid composition (g fatty acid/100 g total fatty

acids) in determining the total energy value of the supplement.

Fat Metabolism in the Rumen

Food consumed by ruminants first passes through the largest of the four stomach compartments or rumen, which acts like a fermentation vat. Countless numbers of bacteria, protozoa, and fungi in the rumen ferment the feed releasing end products that are utilized by the host animal for maintenance and growth of body tissues. The microbial population in the rumen also is responsible for extensive transformation of dietary lipid. Lipid transformations include lipolysis to release free fatty acids from complex plant lipids, and biohydrogenation (BH) to convert unsaturated fatty acids in plant matter to more saturated lipid end products.

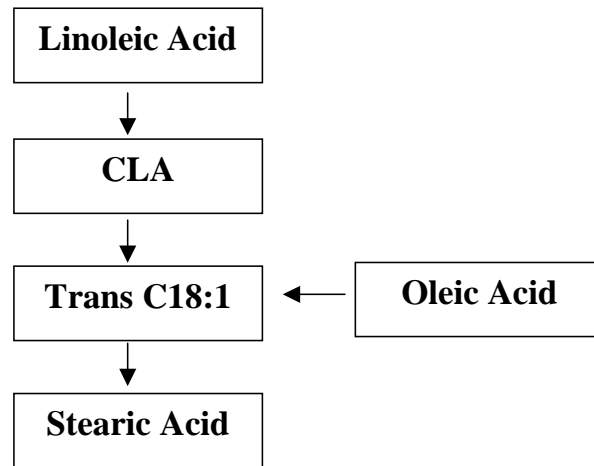
Lipids entering the rumen are first transformed by microbial lipases in a process called lipolysis. The microbial lipases hydrolyze the ester linkages in complex lipids causing release of fatty acids and glycerol. The glycerol produced is fermented yielding mostly volatile fatty acids.

The main types of lipids entering the rumen are triglycerides, phospholipids, and galactolipids from forages and concentrates in the diet. Rapid hydrolysis of triglycerides occurs by microbial enzymes. Linseed oil incubated with ruminal contents of sheep at 1.0 g/100 ml resulted in greater than 75% of the total lipid recovered in the form of free fatty acids. Phospholipids and galactolipids also undergo rapid and extensive breakdown in the rumen as a result of the enzyme activity of ruminal microorganisms. Some evidence suggests hydrolysis of triglycerides and galactolipids from pasture grass was due primarily to plant enzyme activity. Dawson et al. (1977) autoclaved ¹⁴C-labelled grass to inactivate plant lipolytic enzymes. The grass was then administered intraruminally to a sheep and the galactolipids were rapidly hydrolyzed. In an in vitro trial, ruminal contents were taken from sheep that had been given

autoclaved grass for 7 days and were homogenized with grass that had been heated to 100°C for 8 minutes. It was assumed that the ruminal contents and grass were devoid of plant lipases. In the absence of plant lipases the grass galactolipids were rapidly hydrolyzed. Grass was also homogenized with boiled ruminal fluid. In the absence of microbial lipases the galactolipids were not metabolized. Therefore, it was concluded that lipases produced by ruminal microorganisms are mainly responsible for the breakdown of ingested plant lipids (Dawson et al., 1977).

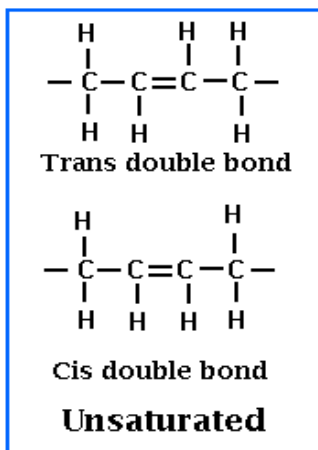
Biohydrogenation of linoleic acid in the rumen begins with its conversion to conjugated linoleic acid (CLA). In this initial step, the number of double bonds remains the same but one of the double bonds is shifted to a new position by microbial enzymes. Normally, the double bonds in linoleic acid are separated by two single bonds, but in CLA, the double bonds are only separated by one single bond. Many types of CLA are produced in the rumen of dairy cows (Bauman and Lock, 2006), but a common CLA produced from BH of linoleic acid is *cis*-9, *trans*-11 C18:2.

Figure 3. Major steps in the biohydrogenation of linoleic and oleic acids by ruminal microbes.



As BH progresses, double bonds in the CLA intermediates are then hydrogenated further to *trans* fatty acids having only one double bond. A final hydrogenation step by the ruminal microbes eliminates the last double bond yielding stearic acid as the final end product. *Trans* double bonds only differ from *cis* double bonds in the placement of the hydrogens (Figure 4). The hydrogens are located on opposite sides of the double bond for *trans* fatty acids, but on the same side of the double bond for *cis* fatty acids. Although the difference in structure between *trans* and *cis* fatty acids appears small, it causes significant differences in their physical and metabolic properties.

Figure 4. Structural differences between *cis* and *trans* fatty acids.



In cows on a typical forage diet, the major *trans* C18:1 present in ruminal contents is *trans*-11 C18:1. Most of the remaining isomers have double bonds distributed equally among carbons 12 through 16 (Bickerstaffe et al., 1972). The exact pathways for the production of these positional isomers

are not known. Linoleic and linolenic acids are converted to several *trans* C18:1 and C18:2 intermediates during BH. Mosley et al. (2002) recently showed that the BH of oleic acid by mixed ruminal microorganisms involves the formation of several positional isomers of *trans* C18:1 rather than only direct BH to form stearic acid as previously described.

Distillers Grains

Fermentation of corn mash produces ethanol, which is distilled to remove the ethanol and centrifuged to remove as much excess liquid as possible. The liquid fraction can be dehydrated to produce condensed solubles and the solid fraction may be sold directly as wet distiller's grains or dehydrated to produce dried distiller's grain. The condensed soluble can be blended with the distillers grains to produce wet distiller's grains + soluble (WDGS) or dried distiller's grains + soluble (DDGS).

Table 2. Nutrient composition of corn co-products.

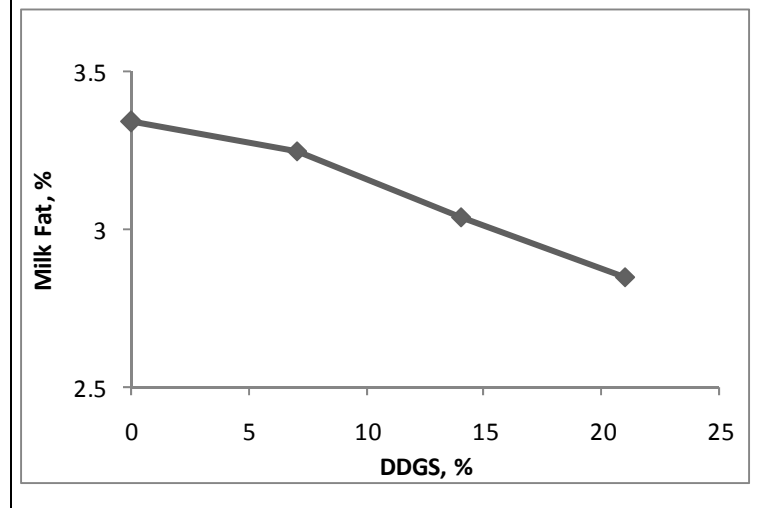
Nutrient	WDG	MDGS	DDG	DDGS
DM, %	25-35	50	88-90	88-90
CP, %	30-35	30-35	25-35	25-32
Fat, %	8-12	8-12	8-10	8-10
NDF, %	30-50	30-50	40-45	39-45
TDN, %	70-110	70-110	77-88	85-90

^aWet distillers grain (WDG); Modified distillers grains + soluble (MDGS); Distillers dried grains (DDG); Distillers dried grains + soluble (DDGS)

Adapted from Tjardes and Wright. (2002).

Large quantities of DDGS are now available throughout the United States as a dairy feed ingredient due to the rapid growth of ethanol plants primarily in the Midwest. Maximum feeding

Figure 5. Effect of increasing DDGS on milk fat % in dairy cows. From Cyriac et al., 2006.



levels of DDGS in dairy diets can approach 20% or more of the feed dry matter (Schingoethe et al., 2002). With DDGS containing 25-35% crude protein and 8-10% fat (Table 2), its inclusion in the diet can substantially replace other protein supplements and elevate total ration fat content. Modified distiller's grains + solubles (MDGS) contain even higher protein (30-35%) and fat (8-12%) concentrations. Fat concentrations in MDGS can reach 15% or higher. Variability in the fat content of DDGS both within and across production plants is an important

consideration that should be taken into account when formulating diets.

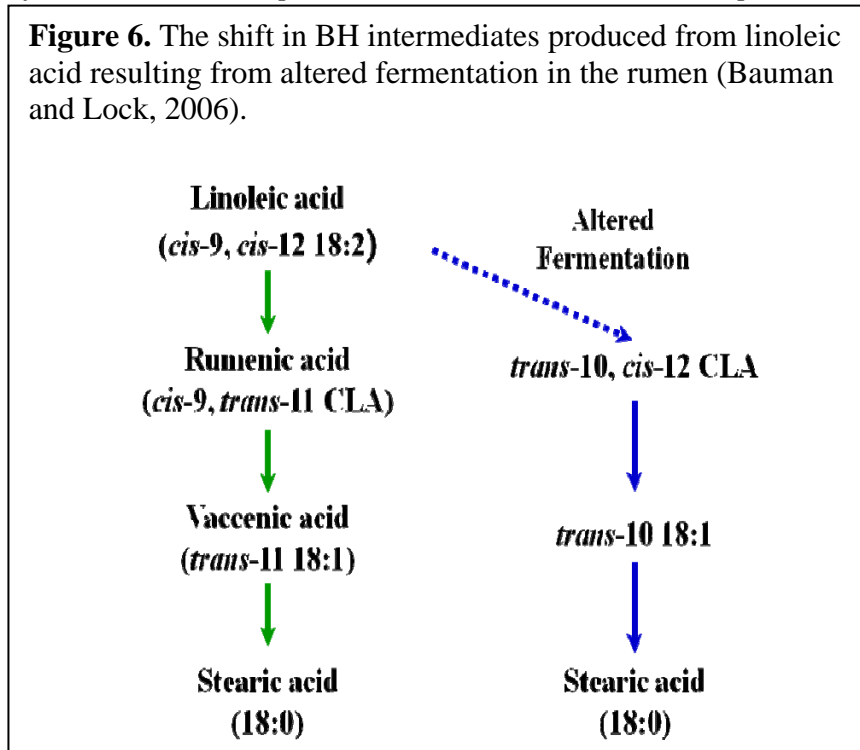
Changes in milk components from feeding distillers grains, if they occur, are most often directed at milk fat percentage. Milk fat content is the most sensitive component of milk to dietary manipulation, which can be changed over a range of 3 percentage units. Lactose content is extremely difficult to manipulate by dietary changes, except under extreme and unusual feeding situations. Milk protein is more responsive to diet (over a 0.5 percentage unit range) than lactose, but less responsive than fat. Thus, the discussion on milk components will focus on the extent that DDGS may affect milk fat percentage.

The addition of distiller's grains to dairy diets has caused MFD in some studies but not in others. When DDGS increased from 0 to 20% of the diet DM in place of corn silage, Cyriac et al. (2006), reported a steady decline in milk fat from 3.34 to 2.85% (Figure 5). Milk fat percentage, however, was not affected by either WDGS or DDGS when added to a dairy ration at 10 or 20% (Anderson et al., 2006). Also, milk fat percentage declined only slightly when DDGS from three different sources were fed to dairy cows at 20% of dry matter intake (Kleinschmit et al., 2006). The variability in MFD response from feeding DGGS mimics other fat sources, and can be attributed to the complex interactions in the rumen that affect microbial lipid metabolism.

Distillers grains and the BH theory

The 'BH theory' represents a unifying concept to explain the basis for diet-induced MFD where intermediates of ruminal fatty acid 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. 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 6). *Bifidobacterium*, *Propionibacterium*, *Lactococcus*, *Streptococcus*, and *Lactobacillus* isolates

Figure 6. The shift in BH intermediates produced from linoleic acid resulting from altered fermentation in the rumen (Bauman and Lock, 2006).



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.

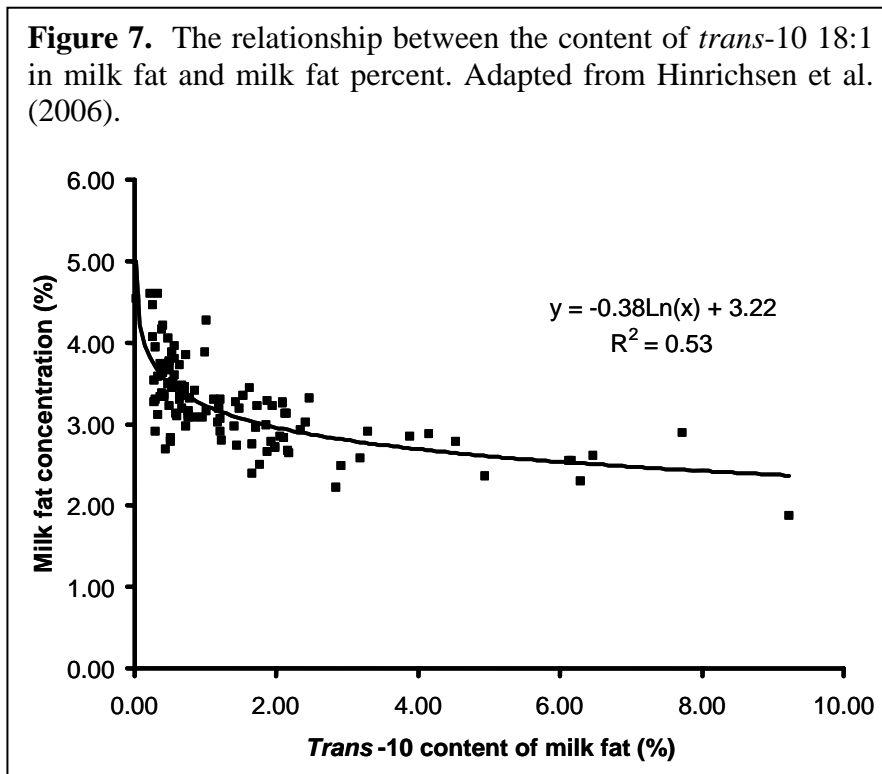
Propionibacterium, *Streptococcus*, and *Lactobacillus* are also more numerous in the rumen with concentrate diets (Jenkins et al., 2008), which would again be consistent with greater *trans*-10, *cis*-12 CLA

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.

The first rumen BH intermediate 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 (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 altered rumen fermentation and the presence of PUFA in the rumen.

In vivo studies have revealed a vast array of *trans*-18:1 and CLA isomers present in digesta

Figure 7. The relationship between the content of *trans*-10 18:1 in milk fat and milk fat percent. Adapted from Hinrichsen et al. (2006).



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 16 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 acid BH 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.

As shown in Figure 6, 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 7, which shows the relationship between the content of *trans-10* 18:1 in milk fat and milk fat percent (Hinrichsen et al., 2006).

Contributions of DDGS to Rumen Fat Load

Elevating fatty acid concentration in ruminal contents may cause a number of changes in ruminal fermentation characteristics and microbial population distribution. Ruminal changes are

Table 3. Average intakes of major unsaturated fatty acids by cattle fed a TMR without and with added fat averaged across published studies. Taken from Jenkins and Bridges (2007).

	<u>TMR - fat</u>	<u>TMR + fat</u>
n	21	37
Oleic (18:1)	99	230
Linoleic (18:2)	181	234
Linolenic (18:3)	42	55
<u>TOTAL</u>	<u>322</u>	<u>519</u>

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 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).

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. Total unsaturated fatty acid intakes taken from published studies exceeded 500 g/d with linoleic acid comprising nearly half of all three major unsaturated fatty acids (Table 3). Estimates of linoleic acid intake using CPM-Dairy indicates that when corn silage comprises the majority of the forage base in the ration and oilseeds are the major source of added dietary fat linoleic acid intake can approach and even exceed 400 to 500 g/d (Lock et al., 2006). 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. DDGS) 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 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 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.

Table 4. 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
<i>trans</i> -10 18:1, %	0.75	2.15	0.78

¹Adapted from Onetti et al. (2004).

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

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 4). 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 4).

It may be appropriate to more broadly consider overall ‘unsaturated load’ in the rumen when troubleshooting MFD (Lock et al., 2006). Increasing the 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 carried out at Clemson University 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., 2006).

Summary

The discovery of major events in rumen lipid metabolism occurred decades ago, with very little new information available on the details of its biochemistry and regulation until the discovery of the anticarcinogenic properties of *cis*-9, *trans*-11 CLA. This discovery made it apparent that even minute quantities of BH intermediates in the rumen could have dramatic effects on health and metabolism of the host animal and humans consuming animal-food products. Eventually it was discovered that a second CLA, namely the *trans*-10, *cis*-12 isomer, was closely associated with MFD. This led to the BH theory of MFD that suggested feeding management was linked to an abnormal ruminal fermentation causing accumulation of the *trans*-10, *cis*-12 isomer. Feed ingredients containing appreciable concentrations of fat often cause MFD, an effect that can be explained by the BH theory of MFD. The rapid and extensive availability of distiller's grains in recent years from ethanol production has created opportunity for the inclusion of low-cost nutrients into dairy rations, but also led to production problems including MFD. The variable lipid concentration in distillers is the likely cause of the MFD according to what is now known about the BH theory. Predicting when DDGS will lead to MFD is complex and is a function of total unsaturated fatty acid supply to the rumen, how fatty acid supply will impact the pathways of lipid BH and its interaction with other feed ingredients. Further research is required to better understand the ruminal conditions that promote the formation of BH intermediates that may trigger MFD from distiller's grains. An improved understanding of these events will provide the critical framework with which to better troubleshoot MFD.

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