Lipid Feeding and Milk Fat Depression

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KEYWORDS

• Dietary lipids • Rumen • Biohydrogenation • Milk fat depression
• Conjugated linoleic acid

KEY POINTS

- Diets fed to cattle contain mostly unsaturated fatty acids supplied in grains and forages, by-products, and fat supplements.
- Lipid intake by dairy cattle must be restricted to prevent alterations of microbial populations in the rumen that can negatively affect the yield of milk and components.
- Unsaturated fatty acids consumed by cattle are extensively metabolized by microorganisms in the rumen in a process called biohydrogenation, yielding stearic acid as the end product plus a multitude of bioactive intermediates.
- Intermediates of biohydrogenation include a variety of conjugated linoleic acid (CLA) and *trans*-monoenoic acid isomers. Production of bioactive CLA isomers by rumen microorganisms is controlled by interactions among several dietary risk factors.
- Three specific CLA intermediates of biohydrogenation have been shown to cause milk fat depression in dairy cattle through coordinated suppression of mammary lipogenic genes by a transcription factor that is a central regulator of lipid synthesis.

FEED LIPIDS

Key Definitions and Nomenclature

- Ether extract: The fraction of feed extracted by organic solvents that includes nonlipid contaminants (such as pigments, water, and sugars), non–glycerol-based lipids (such as alkanes and waxes), and glycerol-based lipids (such as triglycerides, glycolipids, and phospholipids).
- Fatty acids: Chains of carbons that end in an acid or carboxyl group. In cereal grains and forages, the predominant fatty acids vary in length from 12 to 18 carbons.

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- Fatty acid abbreviations: Because of the large number of fatty acids found in plant and body tissues, it is often difficult to remember all their names. It is common to simply refer to a fatty acid by the abbreviation "# carbons:# double bonds."
- Saturated fatty acids: Have no double bonds in the fatty acyl chain, such as C16:0 (palmitic acid) or C18:0 (stearic acid).
- Monounsaturated fatty acids: Have a single double bond somewhere in the fatty acyl chain, such as C16:1 (palmitoleic acid) or C18:1 (oleic acid).
- Polyunsaturated fatty acids (PUFA): Have more than 1 double bond in the fatty acyl chain, such as C18:2 (linoleic acid) or C18:3 (linolenic acid).

Lipid Components

Lipids are generally defined as organic compounds that are relatively insoluble in water but soluble in organic solvents.[1] A simple classification divides lipids into glycerol-based and non–glycerol-based components. Nonglycerol lipids include waxes and cutin, which provide an indigestible, impervious barrier on the exterior plant surface to reduce water loss and provide protection against plant pathogens and toxins. Surface lipids also inhibit plant digestion by ruminants because they limit bacterial penetration into the inner plant structures where most of the digestible nutrients are located. Disruption of this barrier by chewing or processing (eg, grinding or chopping) greatly increases bacterial access and rates of nutrient digestion.

The glycerol-based lipids contain fatty acids bound to a glycerol backbone. The value of fats and oils as animal feed ingredients is based on their fatty acid content and fatty acid composition. Content refers to the total concentration (% dry matter [DM]) of fatty acids in a lipid supplement, and composition (% total fatty acids) refers to the mixture of individual fatty acids that make up the lipid supplement. The most important glycerol-based lipids found in animal feed include triglycerides, phospholipids, and galactolipids (**Table 1**).

Fatty Acids

Fatty acids are chains of carbons that end in an acid group, or carboxyl group as it is referred to in biochemistry. An example of a common fatty acid is stearic acid, with 18 carbons and no double bonds. Fatty acids, such as stearic acid, are referred to as saturated because all the carbons are holding the maximum number of hydrogens possible, so the fatty acid is "saturated" with hydrogen. Stearic acid is low in plant oils but is present in higher amounts in animal fats, particularly in fats obtained from ruminant species such as beef tallow.

Oleic acid and linoleic acid are examples of unsaturated fatty acids containing 1 or more double bonds (**Fig. 1**). 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 PUFA

Table 1
Glycerol-based lipids in animal feed ingredients

Lipid	Components	Source	Fatty Acids (g/100 g)[a]
Triglyceride	Glycerol, 3 FA[b]	Cereal seeds	95
Galactolipids	Glycerol, 2 FA, sugar	Forages	56
Phospholipids	Glycerol, 2 FA, P, N	Plant membrane lipids	72[c]

[a] Calculated for a pure compound containing only oleic acid.
[b] Fatty acids.
[c] Assuming the phospholipid consisted only of lecithin or phosphatidylcholine.

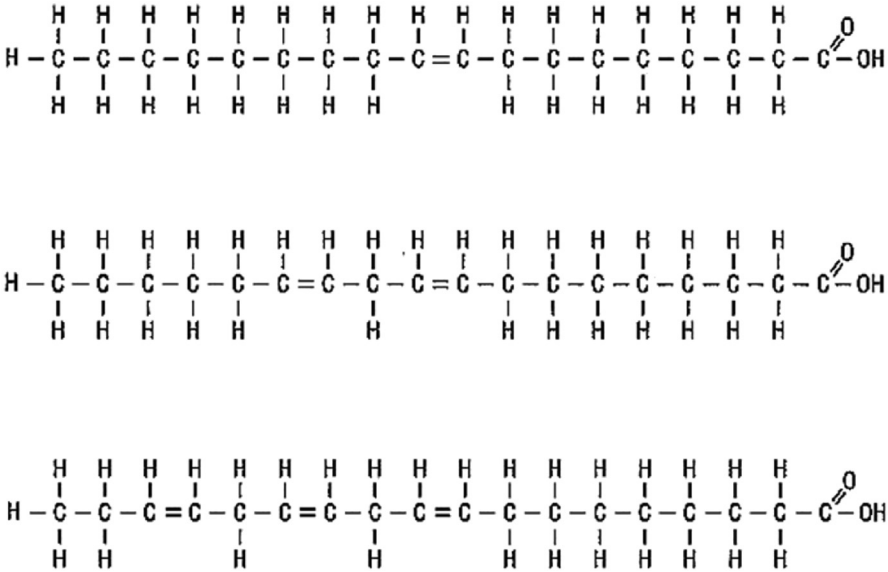

Fig. 1. Structures of the 3 primary unsaturated fatty acids consumed by cattle: oleic acid (*top*), linoleic acid (*middle*), and α-linolenic acid (*bottom*).

containing 2 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. Linolenic acid, with 3 double bonds, is the primary fatty acid in most pasture species and in linseed oil from flax.

Table 2
Nomenclature, structural features, and example sources of common fatty acids found in animal feed

		Double-Bond Geometry/Location		
Abbreviation[a]	**Name**	**Double-Bond Location[b]**	**ω Double-Bond Designation[c]**	**Fat/Oil Sources**
C14:0	Myristic	NA		Animal fats
C16:0	Palmitic	NA		Animal fats
C18:0	Stearic	NA		Animal fats
C18:1	Oleic	c9	ω9	Canola
C18:2	Linoleic	c9c12	ω6	Corn, soybean
C18:3	Linolenic	c9c12c15	ω3	Linseed
C20:5	EPA	c5c8c11c14c17	ω3	Fish
C22:6	DHA	c4c7c10c13c16c19	ω3	Fish

[a] Number of carbons:number of double bonds.
[b] *cis* (c) followed by carbon position of double bond using the acid carbon (carbon 1) as reference. NA, not applicable because no double bonds are present.
[c] Omega (ω) position refers to number of carbons from the methyl end of fatty acyl chain to the first double bond.

Sources of Lipid Intake by Cattle

Grain and forage lipids

The fatty acid content of most cereal seeds and forages typically ranges from 1.0% to 3.0% DM, with most fatty acids classified as unsaturated (predominately oleic, linoleic, and α-linolenic acids). Among the unsaturated fatty acids, linolenic acid is the predominant fatty acid in most forage species, followed by linoleic acid.[2] In the cereal seeds, fatty acids are composed mainly of linoleic acid followed by oleic acid.

Fatty acid concentrations in some pasture can exceed 5.0% of DM, depending on plant species, stage of maturity, environment, and so forth. 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 6.8% DM as total fatty acids.[3] 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, partially owing to loss of plant leaves whereby chloroplast lipid is concentrated but also because of plant metabolism of fat present as stored energy. Considerable variation in total fatty acid content has been reported in grass and corn silage samples (**Table 3**). Although the magnitude of the difference in fatty acid concentration is small in forages and cereal grains, they have a large impact as they make up most of the diet.

Plant maturity has a definite impact on both fatty acid content and fatty acid composition. Fatty acid content (% DM) generally is highest in the spring and fall seasons and lowest in the summer months. For example, fresh perennial ryegrass contained 3.2% DM total fatty acids during primary growth in May, but only 1.2% DM total fatty acids at the beginning of second regrowth.[4] Linolenic acid follows a similar seasonal pattern.[4] As linolenic acid declines over the summer months, the concentration of palmitic and linoleic acid increases.

Fatty acids can be released from glycerol in plant tissue through the action of plant-based lipases. Plant lipases were shown to release free fatty acids from damaged tissue after cutting,[5] and can continue to function in dried forage containing as little as 5% to 10% moisture.[6] Plant matter containing a high content of free fatty acids increases the risk of fermentation problems in the rumen of cattle and sheep.

Fat supplements

A useful way to categorize fat supplements for dairy rations is based on how they affect ruminal fermentation and digestion. One group includes fats that were specifically designed to avoid digestibility problems, such as calcium salts of fatty acids and hydrogenated fats. These fats are available commercially and have the added advantage of being dry fats that are easily transported and mixed with other feed ingredients. This group is best referred to as “rumen-inert” fats, to emphasize that they have little, if any, negative effects on fiber digestion in the rumen. The rumen-inert fats are also often referred to as bypass fats.

Table 3
Range of total fatty acids (FA) in grass and corn silage samples

FA (% Dry Matter)	Grass Silage	Corn Silage
Mean	1.9	2.0
Minimum	0.8	1.2
Maximum	3.3	3.5

Data from Khan NA, Cone JW, Fievez V, et al. Causes of variation in fatty acid content and composition in grass and maize silages. Anim Feed Sci Tech 2012;174:36–45.

The second group of fat supplements includes the unaltered extracts from plant and animal sources that can cause digestion problems in dairy cattle to varying degrees. Included in this group are fats of animal origin (tallow, grease, and so forth), plant oils (soybean oil, canola oil, and so forth), whole oilseeds (cottonseeds, soybeans, and so forth), and high-fat by-products such as residues from food-processing plants. These fats are referred to here as rumen-active to identify their potential to cause significant microbial and fermentation shifts in the rumen.

The distinction between the two groups is not always clear. At normal levels of supplementation some unprotected fats, such as tallow, are fed to dairy cows without evidence of consistent problems with fiber digestion. Even whole oilseeds help to lessen the severity of digestion problems by encapsulation of antimicrobial fatty acids within their hard outer seed coat. Disruption of the outer seed coat exposes the oil to the microbial population, and increases the risk for fermentation problems and microbial shifts. The seed coat can be broken by chewing and rumination, or through a variety of processing techniques such as extrusion or grinding. Roasting of cottonseed was reported to reduce biohydrogenation.[7] Classification according to ruminal digestion is better defined at high levels of supplementation, whereby the frequency of digestibility problems for tallow and oilseeds is much greater than for the rumen-inert fats.

Other rumen-active fats in high use are various corn coproducts from the fermentation industry. 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 distillers grains or dehydrated to produce dried distillers grains. The condensed soluble can be blended with the distillers grains to produce wet distillers grains + soluble (WDGS) or dried distillers grains + soluble (DDGS). The resulting corn-processing procedures leave by-products high in protein and energy, including a high fat content (**Table 4**). Importantly the unsaturated fatty acids are rapidly available in the rumen and are at higher risk for disruption of rumen fermentation. In addition, large variation in the fat concentration can occur between production facilities and runs within some facilities. More recently lowering the oil content has become common, making low-fat distillers products available as animal feed and reducing the risk of disturbance of rumen fermentation.

Uses and Benefits of Fat Supplements

Adding fat to dairy rations can affect the productive efficiency of dairy cows through a combination of caloric and noncaloric effects (**Table 5**). Caloric effects are attributable

Table 4
Nutrient composition of corn coproducts

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

Abbreviations: DDG, distillers dried grains; DDGS, distillers dried grains + soluble; MDGS, modified distillers grains + soluble; WDG, wet distillers grains.

Adapted from Tjardes K, Wright C. Feeding corn distillers coproducts to beef cattle. Extension Extra. Brookings (SD): South Dakota State University; 2002. Available at: http://www.extension.umn.edu/agriculture/beef/components/docs/feeding_corn_distillers_grains_to_beef_cattle_sdsu.pdf.

Table 5
Use and benefits of fat supplements

Fat Use	Benefits
Increase diet energy density	Increase meat and milk production, increase body condition
Reduce dustiness and particle separation of mixed feeds	Improve feed handling and safety
Alter fatty acid profile of meat and milk	Conform to published nutritional guidelines for humans and enhance consumption of animal food products
Enhance tissue delivery of unsaturated fatty acids	Enhance metabolic and physiologic functioning such as improved reproductive performance and immunity
Increase milk fat	Increased milk components

to greater energy content and energetic efficiency of lipid in comparison with carbohydrate or protein, with the overall benefit being increased milk production. Noncaloric effects are benefits of added fat that are not directly attributable to its energy content or increased milk production. Examples of noncaloric effects include improved reproductive performance and altered fatty acid profile of milk.

The noncaloric benefits of fat supplements are directed at maintaining an adequate tissue supply of 2 unsaturated fatty acids: linoleic and linolenic acids. Linoleic and linolenic acids are regarded as essential because they are required for normal cell function but cannot be synthesized in amounts needed by body tissues. A typical total mixed ration (TMR) of grains and forages generally contains adequate essential fatty acids to meet the needs of the animal. However, most of the dietary essential fatty acids are transformed by microorganisms through biohydrogenation. As an example of increasing the rumen output of essential fatty acids, feeding fat to lactating dairy cows has improved reproductive performance in some studies.[8]

FAT METABOLISM IN THE RUMEN

Key Definitions and Nomenclature

- Lipolysis: Hydrolysis of fatty acids from glycerol by lipases produced either by plants or by microbial lipases produced in the rumen.
- Biohydrogenation (BH): Conversion of unsaturated fatty acids to more saturated end products by microorganisms in the rumen, resulting in formation of *trans* fatty acids as intermediates.
- Conjugated linoleic acid (CLA): The first intermediate in BH, whereby microbial isomerases move double bonds in plant unsaturated fatty acids closer together. More than 20 CLA isomers are produced in the rumen, the most recognized being *cis*-9, *trans*-11 18:2.
- CLA_{MFI}: The 3 CLA isomers produced in the rumen shown in research studies to inhibit milk fat synthesis.
- *Trans*-18:1: The intermediate of BH formed following the elimination of a double bond in CLA.

Lipolysis and Biohydrogenation in the Rumen

Food consumed by ruminants first passes through the rumen, where countless numbers of bacteria, protozoa, and fungi ferment the feed, releasing end products that are used 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 glycerol-based plant lipids, and 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 glycerol-based lipids, causing release of fatty acids and glycerol. The glycerol produced is fermented, yielding mostly volatile fatty acids (VFA). Microbes have a high capacity for lipolysis, and the rate of lipolysis predominantly depends on the availability of esterified fatty acids and their release from feed particles.

The BH of linoleic acid in the rumen (Fig. 2) begins with its conversion to 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 2 single bonds, but in CLA the double bonds are only separated by a single bond. Many types of CLA are produced in the rumen of dairy cows,[9] but a common CLA produced from BH of linoleic acid under normal rumen conditions is *cis*-9, *trans*-11 C18:2 (rumenic acid). Under altered conditions a large number of different CLA isomers are synthesized.

As BH progresses, double bonds in the CLA intermediates are then hydrogenated further to *trans* fatty acids having only 1 double bond. A final hydrogenation step by the ruminal microbes eliminates the last double bond, yielding stearic acid as the end product. *Trans* double bonds only differ from *cis* double bonds in the placement of the hydrogens (Fig. 3). 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 to be small, it causes significant differences in their physical and metabolic properties. In cows on a typical forage diet, the major *trans* C18:1 present in ruminal contents is *trans*-11 C18: 1 (vaccenic acid).

The rate of rumen BH and the pathways used depend highly on the microbial population, which is influenced by diet composition and rumen environment. For example,

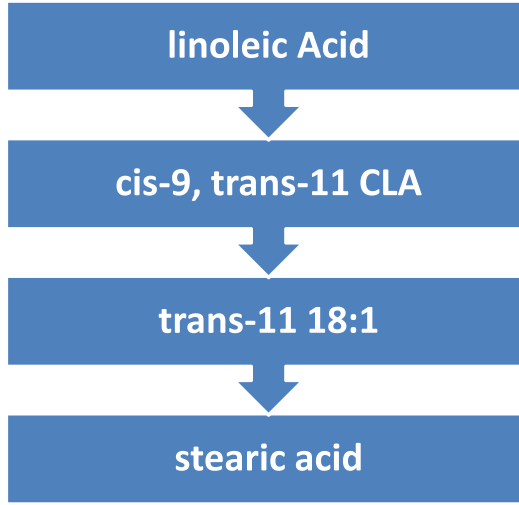

Fig. 2. Major steps in the biohydrogenation of linoleic acid to stearic acid by microorganisms in the rumen.

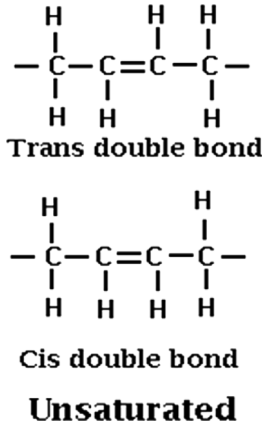

Fig. 3. Structural differences between *cis* and *trans* fatty acids.

increasing unsaturated fatty acids and diet fermentability slow the normal BH pathway and shift BH to alternative pathways.

Key Points About Biohydrogenation in the Rumen

- BH is a microbial pathway designed to reduce unsaturation of lipids found within plant matter, and is likely an evolutionary adaptation to protect the microbial population from antimicrobial effects of unsaturated fatty acids. The resulting *trans* and saturated fatty acids are also preferentially incorporated into microbial phospholipid membranes, as microbial membranes are high in saturated and *trans* fatty acids and low in unsaturated fatty acids.
- The major intermediates of BH include an array of *trans*-C18:1 and conjugated linoleic acid isomers, but most published pathways have traditionally ignored most minor intermediates and present only a superficial view of BH because of the complexity of the analysis.
- The rates and predominant pathways of BH depend on the diet and rumen environment.
- Increasing dietary unsaturated fatty acids challenges the capacity of BH pathways and increases accumulation of bioactive intermediates in the rumen. Fatty acids originate from intake of forages and grains, oilseeds, by-products, and fat supplements.
- The impacts of BH on animal performance include protection of ruminal fermentation from antimicrobial effects of unsaturated fatty acids, loss of dietary ω fatty acids needed for optimal reproduction and immune function, and accumulation of conjugated intermediates with physiologic activity, such as the *trans*-10, *cis*-12 isomer that inhibits mammary lipogenesis and causes milk fat depression (MFD).

MILK FAT DEPRESSION

Previous Theories on Etiology

MFD has been investigated for well over a century and has a rich history of different theories. Identification of the causative factor linking changes in the diet to changes in milk fat synthesis was complicated by complex environmental conditions in the rumen during MFD. One of the earliest theories proposed that a limitation in fatty

acid absorption resulted in MFD,[10] which was quickly disproved, as MFD also occurs when feeding diets high in fat. Recently, it was reported that abomasal fatty acid infusion could not overcome the effects of *trans*-10, *cis*-12 CLA, one of the bioactive CLA isomers that reduces milk fat synthesis.[11] Other theories focused on changes in rumen VFA synthesis and their impact on metabolism. Changes in rumen fermentation during MFD typically decrease the acetate to propionate molar ratio.[12] The low acetate to propionate ratio formed the basis for a widely known theory proposing that inadequate 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,[12,13] and ruminal infusion of acetate to cows during MFD had only a marginal impact on milk fat yield.[14]

Increased absorption of propionate was then proposed as the cause of MFD. The theory was that increased propionate resulted in increased plasma glucose, which stimulated insulin secretion. Increased insulin then would increase adipose tissue lipogenesis and decrease lipolysis (mobilization of body fat stores). However, direct testing by propionate, glucose, or insulin infusion resulted in milk fat reductions of less than 15% in well-fed cows. Infusion of insulin in cows in negative energy balance using hyperinsulinemic-euglycemic clamps resulted in a 35% decrease in milk fat yield, whereas well-fed cows averaged a 5% reduction.[15–17] However, the decrease in milk fat during insulin clamps was due to a decrease in preformed fatty acids originating from the diet and adipose tissue, whereas classic diet-induced MFD is characterized by a substantial decrease in de novo synthesized fatty acids. Lastly, reduced ruminal synthesis of vitamin B_{12} was also proposed as a possible mechanism of MFD. Vitamin B_{12} deficiency may result in increased circulating methylmalonyl-CoA, which is a competitive inhibitor fatty acid synthase, resulting in a reduction in de novo synthesis of fatty acids. However, supplementation of B_{12} failed to alleviate diet-induced MFD. Overall, several decades of research has tested numerous theories based on substrate limitations, and has found little to no evidence in their support.[13,18,19]

The Biohydrogenation Theory of Milk Fat Depression

The BH theory links MFD with the formation of specific CLA isomers produced from the BH of PUFA in the diet. Lipids in feed are metabolized by the rumen microbial population, which leads to the formation of bioactive CLA that affect living cells and tissue. Microorganisms in the rumen produce more than 20 types of known CLA isomers, 3 of which have been shown to cause MFD, subsequently referred to as CLA_{MFI}, because these CLA act as milk fat inhibitors. One of the most studied CLA_{MFI} is *trans*-10, *cis*-12 18:2. The CLA_{MFI} produced in the rumen travel via the blood to the mammary gland, where they inhibit the synthesis of milk fat by impairing the production of several enzymes essential for fat synthesis in the mammary gland. CLA_{MFI} are also present in cows that produce acceptable milk fat levels, but at concentrations too low to cause MFD.

Overproduction of CLA_{MFI} is triggered by nutrition-driven changes in the rumen. Formation of the CLA isomers causing MFD has been associated with several dietary risk factors including excessive unsaturated fat intake, high-grain diets, and low rumen pH. Substrates for BH include both linoleic and linolenic acids[20,21] found in nearly all plant-based components of dairy diets. The bottom line is that the type of feed the cow consumes affects rumen conditions, which in turn affects the amount and type of CLA produced. Because CLA_{MFI} overproduction in the rumen leads to MFD, excess CLA_{MFI} and, therefore, MFD can be controlled by paying close attention to several key nutritional risks.

Changes in the ruminal environment initiated through the diet can lead to a microbial population shift that is accompanied by a change in the type of CLA produced (Fig. 4). For example, low rumen pH can be a key factor contributing to a microbial shift and changes in the type of CLA produced. Dropping pH in continuous cultures of mixed ruminal microorganisms caused an increase in the concentration of *trans*-10, *cis*-12 CLA but no change in *cis*-9, *trans*-11 CLA.[22] Qiu and colleagues[23] reported that reduced ruminal pH can affect microbial populations, especially cellulolytic bacteria. Total cellulolytic bacteria numbers were reduced, accompanied by reduced acetate to propionate ratio and altered BH when pH was low.

The BH theory was directly demonstrated by the abomasal infusion of pure preparations of the normal CLA intermediate (*cis*-9, *trans*-11 CLA) and one of the major alternative (*trans*-10, *cis*-12) intermediates.[24] The *trans*-10, *cis*-12 CLA rapidly decreased milk fat yield over the course of 2 to 4 days, and recapitulates all postabsorptive aspects of the diet-induced MFD phenotype. Furthermore, the response occurs in every cow and the magnitude of the response is very predictable ($R^2 = 0.86$[25]). *Trans*-9, *cis*-11 CLA and *cis*-10, *trans*-12 CLA have also been reported as potential inhibitors of milk fat synthesis,[26,27] although they are less potent than *trans*-10, *cis*-12 CLA. Additional unidentified bioactive isomers likely play a role in MFD, as the known CLA isomers do not account for the entire reduction in milk fat observed during diet-induced MFD.[28]

Cellular and Molecular Mechanisms of Conjugated Linoleic Acid Causing Milk Fat Depression

The fatty acids in milk come from approximately an equal contribution from de novo synthesis within the mammary cell and uptake of preformed fatty acids from circulation (for a review see Ref.[13]). The fatty acids are esterified in the endoplasmic reticulum (ER) and are assembled into lipid droplets that move to the apex of the cell. Several proteins are associated with the milk fat globular membrane that surrounds the lipid droplet, and they are essential for fat secretion.[29,30] The number of essential enzymes and processes required for milk fat synthesis provide the opportunity for regulation at multiple steps and levels. Of note, diet-induced MFD results in up to a 50% reduction in milk fat synthesis, but BH intermediates are not able to reduce milk fat beyond this

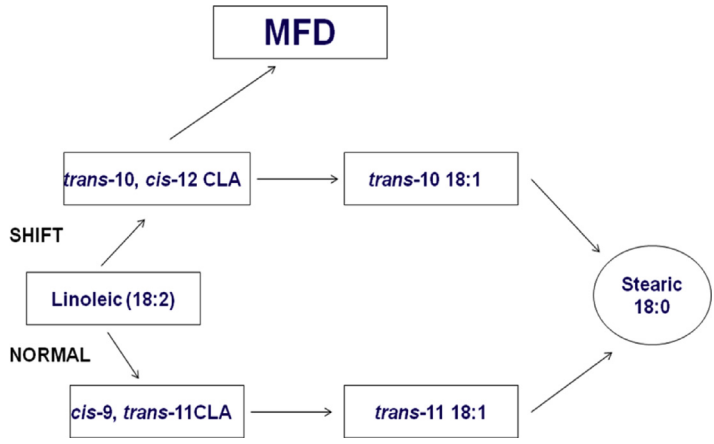

Fig. 4. The shift in intermediates produced from biohydrogenation of linoleic acid in ruminal contents as a result of a diet-induced microbial shift. MFD, milk fat depression.

point. One interpretation of this is that half of the milk fat is under dietary control of and responsive to dietary factors while a basal level of milk fat is constant or regulated by other factors. Milk fat provides a significant portion of the energy for a growing calf, so this may ensure adequate energy for the calf.

During both CLA-induced and diet-induced MFD, the decrease in milk fat includes both de novo and preformed fatty acids, although a larger decrease in the de novo synthesized portion is observed especially with larger reductions in milk fat. This finding suggests a coordinated regulation of the enzymes of lipid synthesis, and that the regulation could occur at the level of gene expression, enzyme abundance, or enzyme activity. Previous work clearly shows a coordinated decrease in mammary expression of many lipid synthesis enzymes during both CLA-induced and diet-induced MFD,[31–34] and regulation of gene expression is considered the predominant level of regulation during MFD. Coordinated suppression of mammary lipogenic genes suggests involvement of a central regulator of lipid synthesis, and a transcription factor called sterol response element binding protein 1 (SREBP1) has become a focus of investigation.[32,33] This transcription factor functions as a global regulator of lipid synthesis, and mammary expression of SREBP1 decreases during CLA-induced and diet-induced MFD. In addition, most lipid synthesis enzymes that are downregulated during CLA-induced and diet-induced MFD contain a SREBP1 response element in their promoter and are regulated by SREBP1c.[32] Other regulators have been investigated and a role for thyroid hormone responsive spot 14 has been proposed,[32] although functional roles of other transcription factors are not supported by current data (eg, LXRs and PPARs).

Investigation of MFD has provided key insights into the mechanisms regulating milk fat synthesis. This understanding of the molecular regulation of milk fat synthesis provides a foundation for the development of methods to increase milk fat. For example, single nucleotide polymorphisms (SNPs) that may explain genetic differences in milk fat yield and fatty acid composition have been identified in SREBP1, SREBP1 regulatory proteins, and S14.[35,36]

Recovery from Milk Fat Depression

The mammary gland is acutely sensitive to absorption of CLA, with reduced milk fat synthesis observed within 12 hours of abomasal infusion.[37] Recent time-course experiments have characterized the timing of induction and recovery of diet-induced MFD.[38] MFD was induced by feeding a diet lower in fiber and high in unsaturated fatty acids (29.5% neutral detergent fiber [NDF] plus 3.7% PUFA) and then recovered by feeding a higher-fiber and low-oil diet (36.9% NDF plus 1.1% PUFA) while sampling milk every other day. Milk fat concentration and yield decreased progressively when the low-fiber and high-oil diet was fed, reaching the maximal decrease by 9 days (**Fig. 5**). When switched to the recovery diet, milk fat yield progressively increased and was fully recovered by 18 days. A key insight from the experiment is the expected lag between making diet adjustments and changes occurring in milk fat synthesis. Addition of a risk factor may cause MFD 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 in identifying what may have caused MFD and knowing how long to wait to determine whether a diet correction has been effective in improving milk fat. In subsequent work focusing on diets that accelerate recovery, it was determined that correction of dietary unsaturated fat was more important than correction of diet fermentability,[39] and that removal of monensin did not change the rate of recovery of normal rumen BH and mammary de novo fatty acid synthesis.[40]

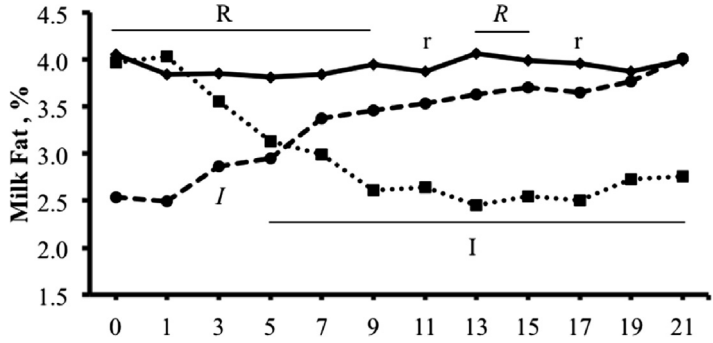

Fig. 5. Time course of induction of milk fat depression by feeding a low-fiber and high-oil diet, and recovery from milk fat depression by feeding a higher-fiber and low-fat diet. Cows were fed a high-fiber, low-oil diet (Control; *diamonds*), a low-fiber. high soy-oil diet to induce milk fat depression (*squares*), or a high-fiber, low-oil diet following high-fiber, low-oil diet that induced milk fat depression (Recovery; *circles*). Significant differences are shown between induction and control (R = $P<.01$, *R* = $P<.05$, and r = $P<.1$) and recovery and control (I = $P<.01$, *I* = $P<.05$, and i = $P<.1$). *From* Rico DE, Harvatine KJ. 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 2013;96(10):6621–30; with permission.

NUTRITIONAL FACTORS THAT CAUSE MILK FAT DEPRESSION

Key Nutritional Factors Targeted for Increased Risk of Milk Fat Depression

- Excessive unsaturated fat
- High rapidly degradable starch
- Amount and type of fiber
- Ionophores
- Feed management
- High yeasts and molds

These 6 independent nutritional factors are currently targeted for influencing rumen production of CLA_{MFI} and the development of MFD. More is known about the influence of forages, starch, and fat in the diet. These factors receive more detailed consideration in this article than yeast and management influences, which have been less tested and documented. Of importance is that MFD is commonly not due to a single factor but to an interaction of risk factors, allowing MFD to occur when more than 1 factor is only marginally high.

Fats

Too much fat in the diet of dairy cows is a classic cause of MFD. Nutritionists are keenly aware that fat must be limited to lower levels than protein or carbohydrate to avoid impaired rumen fermentation, reductions in feed intake, and MFD. It is tempting to push the limit on feeding fat when prices are favorable for high-fat by-products, when grain prices reach record levels making commercial fats more competitive, or when the farm has access to (perceptually inexpensive) high-fat waste products from a nearby food-processing plant. The key to preventing MFD from these high-fat ingredients is to fully understand the nutritional and chemical impact these ingredients have on both the rumen microorganisms and the cow, and to choose a feeding rate that will provide the most benefit with the least risk of detriment to the production of milk and components.

Fat supplements pose different degrees of MFD risk. Low-risk fats are those that cause little disruption of the microbial population in the rumen, and thus maintain

normal fermentation and limited production of CLA_{MFI}. Low-risk fats are generally characterized by high saturated fatty acids or calcium salts of fatty acids. Most commercial bypass fats are based on one or both of these characteristics, so the risk of MFD is low. Calcium salts are most commonly made from palm fatty acids distillate, which is a lesser unsaturated plant oil. More recently calcium salts higher in PUFA have become available. Bypass fat feeding rate is usually limited by cost and availability. In addition, bypass fats are dry solid products rather than liquid fats, and therefore are easier to package, transport, and mix on the farm without specialized equipment. Bypass fats are also called rumen-inert fats to emphasize their lower risk for disrupting the rumen. However, MFD has been reported with inclusion of calcium salts, especially with products with more polyunsaturated fatty acids and when fed in higher-risk situations.[41]

High-risk fat supplements contain more unsaturated fatty acids that are typically found in forages, cereal grains, and oilseeds (cottonseed, soybeans, canola, sunflower, and so forth). A high concentration of unsaturated fatty acids in the rumen from 1 or more of these sources can inhibit some microbial species in the rumen. This change can favor species that produce CLA_{MFI}, the accumulation of which can lead to MFD. In addition, unsaturated fatty acids also increase the amount of substrate that must be biohydrogenated, resulting in increased CLA_{MFI} formation if the capacity of the pathway is limited. These high-risk, unsaturated fat supplements are referred to as rumen-active fats to emphasize their tendency to disrupt rumen conditions.

A convenient tool to monitor risky unsaturated fatty acid intake is called RUFAL or Rumen Unsaturated Fatty Acid Load. RUFAL reflects the total unsaturated fatty acid supply entering the rumen each day from feed. RUFAL accounts for unsaturated fatty acids from all feed ingredients rather than fatty acids only from fat supplements. RUFAL may better indicate potential rumen fermentation disruption than simply calculating the percentage of fat added to the diet. Studies show that increasing RUFAL causes fermentation disruption, which can hinder animal performance. Excessive RUFAL can lead to MFD. The interaction of risk factors makes it difficult to establish a RUFAL cutoff, but values below 3.5% DM are viewed as lower fat intakes, whereas those above 3.5% DM indicate fatty acid intakes that may be at risk of being too high. However, it should be noted that some herds with high milk fat (3.8% or more) have been fed RUFAL in excess of 3.5% DM, most likely because other risk factors were minimal. RUFAL suggests a guideline for identifying diets low or high in fat as a logical assessment of MFD risk.

Of the many strategies to feeding fat to dairy cows, perhaps the most important, yet most elusive, is the proper amount to feed. A proper feeding rate can usually prevent MFD associated with fat supplements. To effectively use the vast array of fat products available, practical guidelines must be developed that match sources of fat with proper supplementation. Many recommendations to limit rumen-active fats suggest a single feeding rate for added fat in dairy rations. These single numbers are easy to remember and calculate, but do not account for fatty acid contributions from the basal diet or adjust fat feeding rates in relation to fat supplement composition. An alternative approach includes the following 2 calculations:

1. Limit the total fat consumed from all sources (basal ingredients plus fat supplements) so that

   lbs total fatty acid intake = lbs milk fat produced

2. Limit rumen-active fats so that

$$\text{lbs rumen} - \text{active fatty acids} = \frac{4 \times \text{NDF} \times \text{DMI}}{\text{UFA} \times 100}$$

where NDF is % neutral detergent fiber (DM basis) of the dairy TMR; DMI is DM intake of cows in lb/d; UFA is % unsaturated fatty acids in the rumen-active fat supplement.

Starch

High-grain diets are also known to cause MFD. Rapid fermentation of starch can cause acid accumulation and lower pH in the rumen. Factors that can result in marked changes in rumen pH through any 24-hour 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. Despite our general understanding of these factors, the degree and duration of low rumen pH required for accumulation of CLA_{MFI} in the rumen is not known. Although data are limited, rumen pH changes are most likely associated with MFD because they alter bacterial populations by favoring those that use the alternative pathways of BH that form CLA_{MFI}.

Studies show that low pH alters the microbial population in the rumen and causes accumulation of CLA_{MFI}. In a study by Fuentes and colleagues,[22] the pH of rumen cultures was lowered from 6.5 to 5.5, causing a shift in CLA production that included increased CLA_{MFI}. Although milk fat percentages often decline as rumen pH values decrease, there is still a lot of variation seen as scatter around the line (**Fig. 6**). This finding indicates that rumen pH is not the only factor controlling CLA_{MFI} and milk fat percentage. Therefore, rumen acidosis should not be viewed as a prerequisite for MFD.

The rate of degradability of the starch fraction in grains also determines risk for MFD. Field observations and inferences from studies indicate that rapid rates of starch fermentability are linked to a greater risk of MFD. Fermented feeds with high grain content, such as corn silage and high-moisture corn, carry the highest risk. Differences in

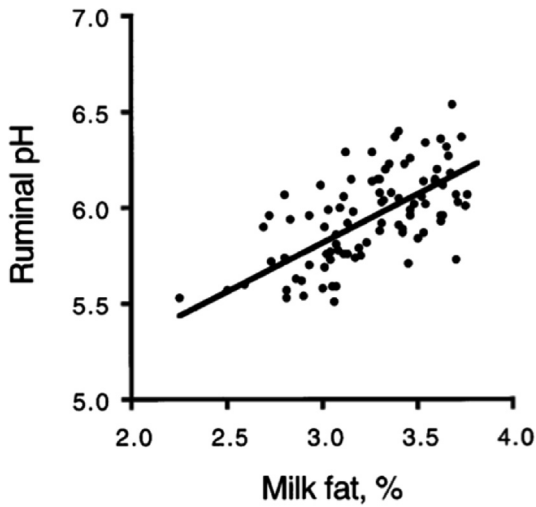

Fig. 6. Relationships between rumen pH and percentage of milk fat as reported by Allen (1997). (*Data from* Allen MS. Relationship between fermentation acid production in the rumen and the requirement for physically effective fiber. J Dairy Sci 1997;80:1447–62.)

corn varieties, silo storage time, and climate conditions for plant growth can all lead to rapid rates of starch degradation in silage and high-moisture corn. Longer storage can lead to higher rates of starch degradability. A study by Newbold and colleagues,[42] using an in vitro test in rumen fluid over 3 hours, found a 30% increase in degradability in corn silage stored for 2 months versus 10 months. If high rates of starch degradability in forages are suspected as a cause of MFD, usually there is little that can remedy the situation. One option is to dilute the forage with less degradable feed, but often this is not available. An alternative option is to focus on other risk factors (such as rumen pH and dietary fat) to minimize CLA_{MFI} production.

Forages/Fiber

Maintaining adequate forage levels in dairy diets decreases the risk of MFD. As explained previously, forage can help maintain rumen pH, slow rumen passage, and limit the synthesis of CLA_{MFI}. This approach emphasizes peNDF to sustain cud-chewing and production of salivary buffers. Nutritionists use specific forage guidelines tailored for specific dairies with individualized forage needs. Within those guidelines, however, maintaining a consistent forage program is the first line of defense against problems with MFD. Again, the rate of starch degradability in forage also affects CLA_{MFI} production. High rates of starch degradability in silage has been associated with an increased risk of MFD, which means that silage NDF alone, as a proxy of forage level and assumed peNDF, is not enough to explain all occurrences of MFD.

A lesser known and often ignored attribute of forages related to MFD is their contribution to the cow's total fat intake. For example, fatty acids in corn silage typically average around 1.5% to 2.0% of DM, but can reach 3.5% or higher. When requesting a forage analysis it is important to remember that fatty acid content is not the same as crude fat content. Fat content has traditionally been determined as the ether-extractable component of the feed. In addition to extracting fat, ether also extracts some carbohydrate, vitamins, and pigments. Therefore, crude fat in cereal grains, forages, and the TMR often contains less than 60% fatty acids. Forage containing 3.5% total fatty acids could contain 5% to 6% crude fat. Given the large quantities of corn silage fed to cows in some operations, this amounts to significant fat intakes just from silages alone.

Yeasts/Management

Yeasts/molds and management factors are both regarded as significant risk factors for MFD, but little is known about exactly how they affect rumen function and the accumulation of CLA_{MFI}. Speculative theories about molds and yeasts suggest they may produce antimicrobial substances as part of their metabolism, which in turn may negatively affect the rumen microbial population; however, much remains to be proved in this regard. High yeast and mold counts in fermented feeds is undesirable not only for the risk of MFD but also because it can reduce feed intake, negatively affect animal health, and decrease overall lactation performance, in addition to incurring additional feed losses through "shrink." In well-preserved silage, yeast counts less than 10,000 CFU/g are common. Counts that affect animal health and performance poorly are not well defined, and likely depend on the specific strain of yeast or mold infecting the plant. As a general rule, yeast counts at or higher than 1 million CFU/g should cause concern.

Several management factors also have been connected with the increased risk of MFD. Among these are bunk space, stocking density, and mixing of the TMR. These factors can all cause sorting and slug-feeding of grain, resulting in low rumen pH and subsequent production of CLA_{MFI} in the rumen. In general, all attempts to maintain

cow comfort and maintain good overall herd management will minimize the risk of MFD.

Summary of Corn Silage Characteristics Often Associated with Increased Risk of Milk Fat Depression

- High fat content (such as fatty acids at 2.5% or more of plant DM)
- High free fatty acids (such as 50% or more of total fatty acids)
- High rates of starch degradability (such as 85% or more in a 7-hour in vitro test)
- High yeasts and molds (such as yeast counts exceeding 1 million CFU/g)

Feeding Strategies

Slug-feeding grain is commonly associated with subclinical rumen acidosis and MFD. Many assume that TMR feeding eliminates this issue because every bite has the same nutrient composition. However, the rate of intake of fermentable organic matter is variable over the day, owing to sorting and variable rates of intake. In general, cows sort for more fermentable feed particle early in the day but also consume feed at approximately a 3 times higher rate after delivery of fresh feed. Feed management may allow a more even distribution of intake across the day. The first consideration is continuous feed availability, as long periods without feed or away from feed is expected to promote high intake once feed becomes available. Increased feeding frequency may also distribute feed across the day, as offering fresh feed is a strong stimulus for feed intake.[43] For example, feeding 4 times per day in equal meals every 6 hours decreased the concentration of alternative BH fatty acids and increased milk fat yield and concentration compared with feeding once per day.[44]

Interactions Among Risk Factors

A single risk factor, such as starch source or feeding ionophores, might not contribute to MFD individually, but when combined interactions could suddenly trigger changes in BH that lead to MFD. As an example, continuous cultures of ruminal microorganisms were fed either a high-corn or high-barley diet along with 2 levels of soybean oil (0% and 5%) and 2 levels of monensin (0 and 25 ppm). *Trans*-10 18:1 was monitored as an indicator of a BH shift. The addition of soybean oil increased *trans*-10 18:1 concentrations in the cultures for both the corn and barley diets.[45] To a lesser extent, monensin also increased *trans*-10 18:1 for both corn and barley. However, an interaction occurred when monensin and soybean oil were combined. Adding monensin with soybean oil did not further elevate *trans*-10 18:1 when the diet was corn-based. When the diet was barley-based, adding monensin with soybean oil elevated *trans*-10 18:1 more than either risk factor alone.

A similar grain $\times$ monensin $\times$ fat interaction was examined in lactating dairy cows.[46] Eighty Holstein cows were assigned either a high (27.7%) or low (20.3%) starch diet for 21 days, followed by the addition of rumensin (13 ppm) or corn oil (1.25%) for an additional 21 days. Thereafter, cows were switched to diets with opposite corn-oil levels for a final 21-day period, giving 8 treatments in a $2 \times 2 \times 2$ factorial design. Oil level was a higher risk factor for MFD in comparison with rumensin, with a decrease from 3.32% to 2.99% for corn oil versus 3.20% to 3.11% for rumensin. Feeding high-starch diets had borderline effects on MFD, causing milk fat to decline from 3.25% to 3.06% ($P = .10$). Starch degradability might also have been a contributing factor to MFD in this study. The diets used in this study contained steam-flaked corn, which has an inherently fast rate of ruminal starch degradation, therefore degradability, compounded by high DMI, may be a more potent MFD risk factor than starch intake alone.

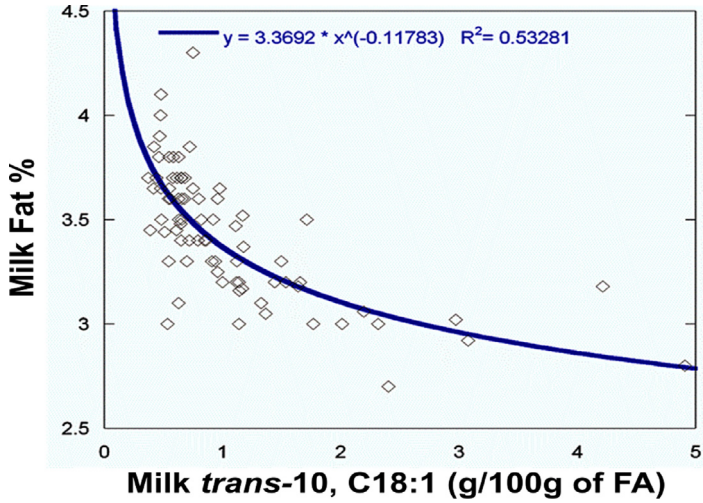

Fig. 7. Relationship between milk *trans*-10 C18:1 content and percentage of milk fat. (*Data from* Nydam DV, Overton TR, Mechor GD, et al. Risk factors for bulk tank milk fat depression in northeast and Midwest US dairy herds feeding monensin. Charlotte (NC): American Association of Bovine Practitioners; 2008.)

An on-farm epidemiologic study was done in 2008[47] to establish risk factors that contribute to MFD in commercial dairy herds feeding rumensin. This extensive study involved 79 commercial dairy herds across 10 states. Cow numbers ranged from 30 to 2800 across herds, with a mean herd size of 474 cows. Milk fat percentage ranged from 2.7% to 4.3% (mean 3.43%) and rumensin dose ranged from 150 to 410 mg/head/d (mean 258 mg/head/d). No significant associations with herd milk fat percentage were seen with stall types, cooling systems, or feeding design. Herds with higher formulated DMI, however, tended to have lower milk fat percentage. No relationships were seen between rumensin dose and herd milk fat percentage. Likewise, TMR concentrations of DM, acid detergent fiber, NDF, nonfiber carbohydrate, and crude fat did not relate to milk fat percentage.

Several significant relationships did surface from the study. Herd milk fat percentage was strongly associated with changes in *trans*-10 C18:1 isomers in milk fat (**Fig. 7**). An identical relationship was reported by Hinrichsen and colleagues[48] between milk fat yield and milk *trans*-10 C18:1 across published research studies. No single TMR characteristic or ration component measured in this study accounted for more than 10 percentage of the variation in herd milk fat percentage. When a multivariate regression was done on all measured TMR variables (such as DM, starch, NDF, and so forth) and herd management factors, followed by a stepwise regression that eliminated nonsignificant variables one at a time, the significant factors remaining in the model were TMR DM% and percentage of particles on the bottom pan of the Penn State particle separator. Together, TMR DM% and bottom-pan particles accounted for 21% of the variation in herd milk fat percentage in the study.

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