MAMMARY GLAND, MILK BIOSYNTHESIS AND SECRETION

Contents

Milk Fat

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This article is a revision of the previous edition article by M. A. McGuire, D. E. Bauman, Volume 3, pp 1828–1834, © 2002, Elsevier Ltd.

Introduction

Fat is the most variable component in the milk of ruminants. The concentration of fat in milk varies among individual cows and is influenced by animal and environmental factors such as breed, diet, stage of lactation, season of year, ambient temperature, and body condition. Over 95% of the fat content of milk is triacylglycerol, with phospholipids, cholesterol, diacylglycerols, monoacylglycerols, and free fatty acids constituting the remainder (**Table 1**).

Biosynthesis of Milk Fat

Ruminants are estimated to have over 400 different fatty acids comprising milk fat, but the majority of the fatty acids have chain lengths between 4 and 18 carbons. Ruminant milk fat is unique among mammals in that it contains a high proportion of short-chain fatty acids (4, 6, 8, and 10 carbons). These fatty acids are not present in typical feedstuffs and are not found in the milk fat of nonruminant species (except in the rabbit) or the body fat of any species. In ruminants, milk fatty acids arise from two sources – *de novo* synthesis in the mammary gland and the mammary uptake of preformed long-chain fatty acids (**Figure 1**). The fatty acid-synthesizing system (*de novo* synthesis) in the mammary gland of the cow produces even-numbered fatty acids that are 4–16 carbons in chain length. *De novo* fatty acid synthesis accounts for approximately 45 and 60% of the total milk fatty acids on a weight and molar basis, respectively. The other fatty acids, which include approximately half of the 16 carbon and all those 18 carbons or greater in length, are taken up preformed from the blood.

De Novo Synthesis

Ruminants primarily use acetate (C_2) and β-hydroxybutyrate (C_4) as the carbon source for milk fat synthesis, and this is in contrast to monogastric animals, which use glucose. Acetate results from carbohydrate fermentation in the rumen. The rumen bacteria also produce butyric acid during fermentation, which is predominantly converted into β-hydroxybutyrate by the rumen wall and liver. Acetate and β-hydroxybutyrate are extracted from the blood by the mammary gland. Once in the mammary cell, acetate and β-hydroxybutyrate are activated to a coenzyme A (CoA) derivative so that they can undergo further metabolism. Acetyl-CoA carboxylase 1 (ACC1) catalyzes the formation of malonyl-CoA from acetyl-CoA, the first committed step in fatty acid synthesis. The activation of β-hydroxybutyrate

Table 1 Lipids in bovine milk

Class of lipid	% of total lipid (g/100 g)
Triacylglycerol	95.80
1,2-Diacylglycerol	2.25
Phospholipids[a]	1.11
Cholesterol	0.46
Free fatty acids	0.28
Monoacylglycerol	0.08

[a]Includes sphingomyelin.
Adapted from Jensen RG and Newburg DS (1995) Bovine milk lipids. In: Jensen RG (ed.) *Handbook of Milk Composition*, pp. 543–575. San Diego, CA: Academic Press.

does not lead to the formation of acetyl-CoA or malonyl-CoA, but instead to β-hydroxybutryl-CoA, which serves only as a 'primer' in the initiation of the synthetic process. Acetate and β-hydroxybutyrate contribute equally to the first four carbons of fatty acids; however, acetate is the source of all other carbons in *de novo*-synthesized fatty acids. Thus, it is estimated that β-hydroxybutyrate contributes only about 8% of the total carbon in milk fatty acids. For acetate to initiate the process, malonyl-CoA is condensed with acetyl-CoA by the enzyme fatty acid synthase (FASN) to produce the first 4-carbon acyl unit. Additional malonyl-CoAs are then condensed with the growing acyl chain to produce longer-chain fatty acids. The mammary gland FASN creates a range of fatty acids with chain lengths of 4–16 carbons. Mechanisms regulating chain length termination are not clearly understood, but an acylthioesterase present in mammary tissue cleaves fatty acids of different lengths from the FASN complex.

Preformed Fatty Acids

Mammary uptake of circulating long-chain fatty acids is the other source of fatty acids for milk fat synthesis. Circulating fatty acids originate from lipids absorbed from the digestive tract and mobilized from body fat reserves. Dietary triacylglycerols are not soluble in water but are packaged in lipoproteins within the blood. The specific lipoproteins that transport dietary triacylglycerols to the mammary gland are the very low-density lipoproteins (VLDLs). Lipoprotein lipase, an enzyme residing within the capillary wall in the mammary gland, cleaves the VLDL triacylglycerols into glycerol and nonesterified fatty acids (NEFAs) that are then taken up by the mammary epithelial cell. Plasma NEFAs also originate from mobilization of body adipose triglycerides (adipocyte hormone-sensitive lipase) and are also taken up by the mammary gland. Movement of NEFAs across the cell membrane and intracellular transport are not well described, but fatty acid transport proteins (FATPs) and fatty acid-binding proteins (FABP) are thought to play key roles. Once in the mammary cell, the preformed fatty acids become activated to CoA esters and glycerol is converted into glycerol phosphate. Although plasma triacylglycerols and NEFAs represent less than 3% of total plasma lipid, their

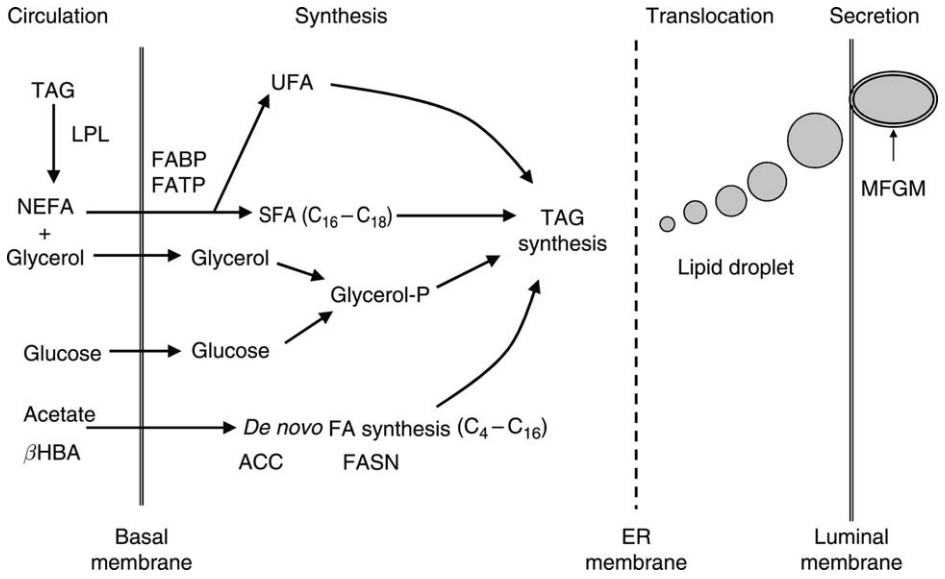

Figure 1 The synthesis of milk fat in the mammary gland of dairy cows includes substrate uptake, *de novo* fatty acid synthesis, desaturation, triacylglyceride synthesis, and milk fat secretion. The Δ^9-desaturase enzyme is capable of inserting a double bond into both saturated and unsaturated fatty acids. A key role of glucose is to provide reducing equivalents (NADPH) for *de novo* fatty acid synthesis (not shown). ACC, acetyl CoA carboxylase; βHBA, β-hydroxybutyrate; ER, endoplasmic reticulum; FABP, fatty acid–binding protein; FASN, fatty acid synthase; FATP, fatty acid transport protein; glycerol-P, glycerol phosphate; LPL, lipoprotein lipase; MFGM, milk fat globule membrane; NEFA, nonesterified fatty acid; SFA, saturated fatty acid; TAG, triacylglycerol; UFA, unsaturated fatty acid.

contribution to total milk fat is approximately 55% by weight and 40% on a molar basis. Mammary uptake of plasma NEFA is proportional to its plasma concentration and the plasma concentration varies by energy state. Thus, the contribution of plasma NEFA to milk fat varies according to physiological state and energy balance. For example, early lactation cows in negative energy balance mobilize fatty acid reserves at high rates, resulting in increased plasma NEFA concentrations and an increased contribution of preformed fatty acids to milk fat.

Triacylglycerol Synthesis

Milk fat is composed mostly of triacylglycerols, and thus esterification of the fatty acids is also an important step in the synthesis of milk fat. The short- and long-chain fatty acids are attached to the glycerol molecule in an orderly and systematic fashion. There are three sites of attachment to the glycerol molecule. Some fatty acids are positioned at random onto glycerol, whereas others occupy a specific position. For example, lauric acid ($C_{12:0}$) is randomly assigned, whereas unsaturated fatty acids are selectively esterified at the second carbon (*sn*-2) and butyric acid (C_4) is positioned primarily on the third carbon (*sn*-3) of the glycerol structure. Once the triacylglycerols are formed, they coalesce into lipid droplets, which move through the epithelial cell toward the luminal side. The droplets are engulfed by a portion of the cell membrane and pinched off into the lumen. Thus, a membrane referred to as the 'milk fat globule membrane' surrounds the fat droplets present in milk. The proteins of the milk fat globule membrane have been well studied and include an enrichment of approximately six proteins, some of which have been demonstrated to be essential to secretion of the milk fat in genomic studies with mouse models.

Milk Fatty Acids

As stated earlier, milk fat contains a multitude of fatty acids (**Table 2**). Saturated, monounsaturated, and polyunsaturated fatty acids are all present in bovine milk fat. The variety of fatty acids allows the mammary gland to produce triacylglycerols with a range of fluidity so that the mammary cell can secrete the milk fat. The fluidity of the triacylglycerol is increased by use of short- and medium-chain fatty acids that arise from *de novo* synthesis as well as long-chain unsaturated fatty acids. The mammary gland also has an additional means to regulate the fluidity of the milk fat via the enzyme Δ^9-desaturase (stearoyl-CoA desaturase). This enzyme is very active in cow mammary cells and inserts a double bond into a variety of saturated and monounsaturated fatty acids. The increased unsaturation of the resulting fatty acids decreases the melting point of the fatty acids present in milk. This is critical for the maintenance of the fluidity of both milk fat and cellular membranes. The main action of the Δ^9-desaturase enzyme is to convert $C_{18:0}$ into $C_{18:1n\text{-}9}$. Oleic acid constitutes over 20% of total milk fatty acids, and estimates are that 60% of milk fat oleic acid is derived from stearic acid via Δ^9-desaturase. However, Δ^9-desaturase is also important in the production of *cis*-9, *trans*-11 $C_{18:2}$ (conjugated linoleic acid; CLA), and this is discussed later.

Table 2 Fatty acids in bovine milk fat as determined by gas–liquid chromatography with capillary columns

Saturated fatty acids		*Monounsaturated fatty acids*		*Polyunsaturated fatty acids*	
Fatty acid	*g/100 g total fatty acids*	*Fatty acid*	*g/100 g total fatty acids*	*Fatty acid*	*g/100 g total fatty acids*
4:0	4.5	14:1*n*-5	0.9	18:2*t*	0.4
6:0	2.3	15:1	0.3	18:2*n*-6	2.9
8:0	1.3	16:1*n*-7	1.8	18:3*n*-3	0.3
10:0	2.7	17:1	0.4	20:4*n*-6	0.2
11:0	0.3	18:1*t*	1.7		
12:0	3.0	18:1*n*-9	21.4		
13:0	0.2	20:1*n*-9	0.6		
14:0*i*	0.1				
14:0	10.6				
15:0*i*	0.7				
15:0	1.0				
16:0	28.2				
17:0*i*	0.7				
17:0	0.6				
18:0	12.6				
20:0	0.2				

Adapted from Jensen RG and Newburg DS (1995) Bovine milk lipids. In: Jensen RG (ed.) *Handbook of Milk Composition*, pp. 543–575. San Diego, CA: Academic Press.

Environmental Effects Including Diet

The high genetic correlation between fat yield and the yield of milk and other milk components makes it difficult to use genetic selection to alter milk fat independent of other milk components. However, milk fat is affected markedly by physiological and environmental factors. Physiological factors generally involve changes in energy balance (i.e., stage of lactation) and offer little potential as a practical means of manipulating milk fat. However, nutrition is the predominant environmental factor affecting milk fat and represents a practical tool to alter its yield and composition.

Effect of Diet on Milk Fat Percentage and Composition

Nutrition is the predominant factor affecting milk fat and provides a practical tool to alter the yield and composition of milk fat. However, in contrast to nonruminants, the composition of dietary fat has only a minor effect on the milk fatty acid composition in ruminants. In ruminants, dietary fat undergoes two important processes in the rumen. First, the esterified fatty acids are hydrolyzed by the rumen bacteria to yield free fatty acids. Second, the free unsaturated fatty acids are biohydrogenated because they are toxic to many rumen bacteria and would adversely affect rates of fermentation. The major fatty acids in typical ruminant feedstuffs are linolenic acid ($C_{18:3}$) predominately from the forage components and linoleic acid ($C_{18:2}$) from concentrates and seed oils. Rumen hydrolysis and biohydrogenation are extensive so rumen outflow of lipids is mainly saturated free fatty acids, with the largest portion being stearic acid. The major rumen pathways for the biohydrogenation of linolenic and linoleic acids to stearic acid ($C_{18:0}$) are shown in Figure 2. Although most linoleic and linolenic acids are completely hydrogenated to stearic acid, rumen outflow also contains small quantities of biohydrogenation intermediates, and these are also absorbed and incorporated into body fat and milk fat. Recent studies with labeled substrates have shown that the pathways of rumen biohydrogenation are much more complex than the simple depiction in Figure 2, so trace quantities of many *trans*-18:1 and conjugated linoleic acid (CLA) isomers are found in rumen outflow and ruminant fat.

Various methods of protecting lipid supplements have been developed in an attempt to bypass rumen fermentation. Examples of these technologies include the formation of Ca salts or amides of unsaturated fatty acids and the use of encapsulation methods. These formulation methods reduce the amount of unsaturated fatty acids available in the rumen and decrease the adverse effects of unsaturated fatty acids on rumen fermentation, especially fiber digestion. To the extent these formulations also protect from rumen biohydrogenation, they offer a means to supply unsaturated fatty acids that could be used for milk fat synthesis. Indeed, rumen-protected fatty acid supplements have been used to modestly enhance the milk content of oleic acid, linoleic acid, CLA, and omega-3 fatty acids. However, rumen protection methods differ in their efficacy, and, to date, these approaches have had only limited commercial use for modification of milk fatty acid profile.

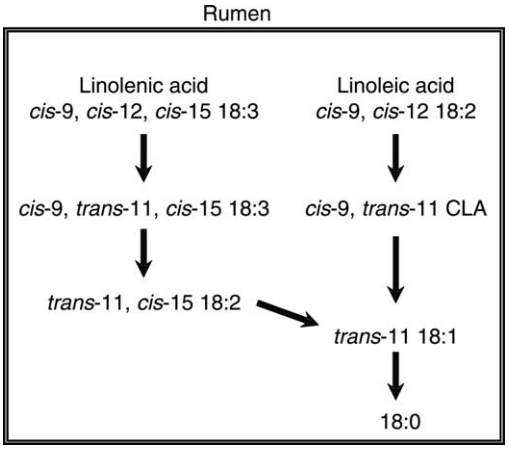

Figure 2 Pathways of microbial biohydrogenation of linoleic and linolenic acids in the rumen. Note CLA and *trans* fatty acids as intermediates. CLA, conjugated linoleic acid. Adapted from Bauman DE and Lock AL (2006) Conjugated linoleic acid: Biosynthesis and nutritional significant. In: Fox PF and Sweeney PLH (eds.) *Advanced Dairy Chemistry, Vol. 2: Lipids*, pp. 93–136. New York: Springer.

The most dramatic example of nutritional effects on milk fat is the low-fat milk syndrome, typically referred to as milk fat depression (MFD). First observed over 150 years ago, diet-induced MFD remains a challenge in modern dairy production, and the decrease in milk fat concentration and yield can be substantial. In the first half of the twentieth century when the feeding of dairy cows began to follow 'scientific principles', reductions in milk fat yield were observed for a range of common diets, including those supplemented with fish or plant oils, diets high in concentrates and low in fiber, and diets low in 'effective fiber' (e.g., grinding or pelleting of the roughage). The investigation of diet-induced MFD has a rich history that has included many theories to explain reduced milk fat synthesis. Most of these theories postulated that limitations in substrate supply for milk fat synthesis caused MFD, generally based on changes in absorbed metabolites as a consequence of alterations in ruminal fermentation. Over several decades, researchers have tested theories based on substrate limitations and found little to no evidence in their support. However, from these investigations, several general characteristics

were recognized. First, milk fat was specifically reduced by up to 50% with no change in the yield of milk or other milk components. Second, the yield of all individual fatty acids was reduced during MFD, but the decline was greatest for short- and medium-chain fatty acids that are synthesized in the mammary gland. Third, when studies were examined more broadly, it became apparent that two conditions are needed for MFD: (1) the diet must alter the rumen environment, thereby cause changes in ruminal microbial processes, and (2) the diet must contain at least a modest level of unsaturated fatty acids. MFD did not occur if either of these conditions was absent. Thus, the etiology of diet-induced MFD involves products of rumen bacteria that are produced as a consequence of the diet-induced shifts in rumen microbial processes and the presence of unsaturated fatty acids.

The cause of MFD perplexed scientists and producers for over a century, but key insight was provided by recognition that increases in milk *trans*-18:1 fatty acids (TFAs) concentration was associated with MFD. TFAs are formed as intermediates in rumen biohydrogenation and *trans*-11 18:1 (vaccenic acid) is the predominant isomer produced, as illustrated by the pathway for the biohydrogenation of linoleic acid (**Figure 2**). However, in some studies, the increases were poorly correlated to milk fat yield. Thus, the basis by which certain diets cause a reduction in milk fat yield had to be more complex than a simple relationship to the rumen production of TFA. As analytical techniques improved, it was discovered that it was a shift in the pattern of TFA isomers rather than total TFA that was correlated with MFD. The net effect was that under certain dietary situations a portion of the linoleic acid undergoes biohydrogenation via different pathways that produce unusual TFA isomers (**Figure 3**). On the basis of these results,

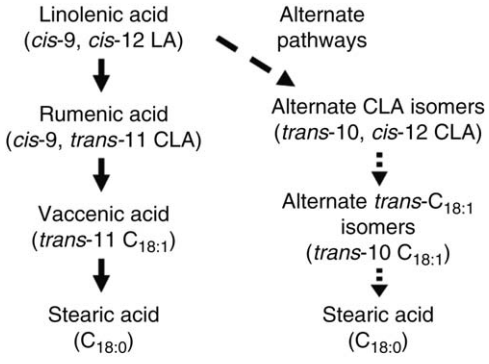

Figure 3 Pathways of ruminal biohydrogenation of linoleic acid and CLA under normal and altered ruminal fermentation. CLA, conjugated linoleic acid. Adapted from Harvatine KJ, Boisclair YR, and Bauman DE (2009) Recent advances in the regulation of milk fat synthesis. *Animal* 3: 40–54.

the 'biohydrogenation theory' was proposed as a unifying concept to explain diet-induced MFD; this theory hypothesized that under certain dietary conditions the pathways of rumen biohydrogenation are altered to produce unique fatty acid intermediates that are potent inhibitors of milk fat synthesis. Subsequent studies have validated this theory and established that diet-induced MFD coincides with a marked shift in the milk fat concentration of many biohydrogenation intermediates. The first of these to be identified as regulating milk fat synthesis was *trans*-10, *cis*-12 CLA. Treatment with purified *trans*-10, *cis*-12 CLA induces MFD with the same phenotype as diet-induced MFD. Thus, *trans*-10, *cis*-12 CLA provided a clear demonstration of the interrelationship between digestive processes in the rumen and metabolism in the mammary gland, where a specific fatty acid produced naturally by rumen bacteria affects mammary gene expression, thereby regulating rates of milk fat synthesis.

Trans-10, *cis*-12 CLA is a potent inhibitor of milk fat synthesis (**Figure 4**); effects are dose dependent and as little as 2.5 g day^{-1} leaving the rumen is sufficient to cause a 25% reduction in milk fat production. The mechanism by which *trans*-10, *cis*-12 CLA causes a reduction in milk fat synthesis has been investigated, and it involves regulation of gene expression in the mammary epithelial cells that results in a coordinated reduction in key enzymes involved in pathways of milk fat synthesis. Consistent with the biohydrogenation theory, several lines of evidence suggested that there must be additional fatty acid intermediates that reduce the synthesis of milk fat. This is an active area of research, and, to date, two additional CLA isomers that regulate milk fat synthesis have been identified, *trans*-9, *cis*-11 and *cis*-10, *trans*-12. The predominant CLA isomer in milk fat is *cis*-9, *trans*-11 CLA (trivial name: rumenic acid (RA)), typically constituting about 75–90% of total CLA isomers in milk fat. It is interesting that RA has no effect on milk fat synthesis, but *trans*-9, *cis*-11 CLA is a potent inhibitor. Although the double bonds are in the same position in both of these isomers (carbons 9 and 11), their orientation has been reversed (*trans*/*cis* vs. *cis*/*trans*). This emphasizes the critical importance of bond position and orientation in determining the biological activity of a fatty acid.

Rumenic acid is only a minor component of milk fatty acids, but there is widespread interest in RA because of its potential benefits to human health and the prevention of chronic diseases. Biomedical studies with animal models have demonstrated that RA has both anticarcinogenic and antiatherogenic activity, and over 90% of the natural CLA in human diets comes from ruminant-source foods. Although RA is an intermediate in the rumen biohydrogenation of linoleic acid (**Figure 2**), most RA in milk and meat fat is derived

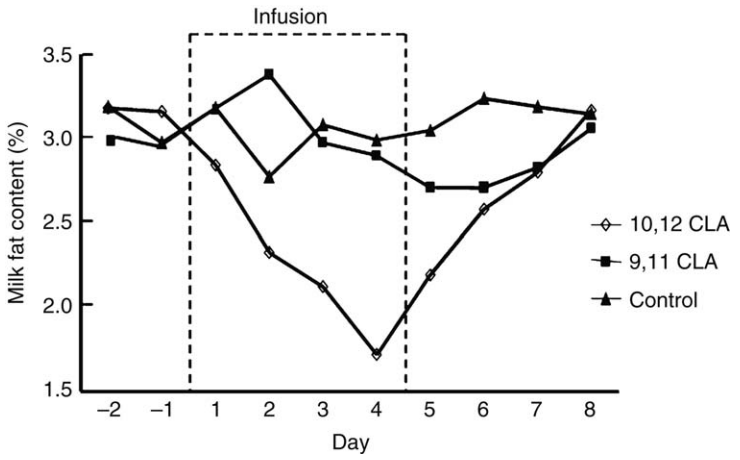

Figure 4 Effect of abomasal infusion of CLA supplements of milk fat synthesis in dairy cows. Abomasal infusion is a convenient experimental approach to bypass possible alterations by rumen microbial fermentation and treatments were control or CLA isomers (10 g day^{-1}) *cis*-9, *trans*-11 and *trans*-10, *cis*-12. CLA, conjugated linoleic acid. From Baumgard LH, Corl BA, Dwyer DA, Saebo A, and Bauman DE (2000) Identification of the conjugated linoleic acid isomer that inhibits milk fat synthesis. *The American Journal of Physiology* 278: R179–R184, used with permission.

from endogenous synthesis involving the enzyme Δ^9-desaturase, with the substrate being vaccenic acid, rumen biohydrogenation intermediate produced from both linoleic and linolenic acids. Of special importance, research has established that the level of RA in milk fat can be markedly enhanced by controlling the nutrition of the cow. Diets formulated with oil seeds or plant and fish oils high in polyunsaturated fatty acids are especially effective. Thus, this is an active area of research by animal scientists and biomedical scientists.

Insights Gained from Milk Fat Depression

Research in the regulation of milk fat synthesis has focused on investigations of MFD rather than on situations or models where milk fat synthesis is enhanced. Nevertheless, MFD represents a biologically significant and physiologically relevant example in which a metabolite(s) produced in digestive processes regulates metabolism, and the basis for this regulation can be explained at the molecular level. Knowledge of the basis for MFD allows the development of feeding strategies and provides the opportunity to troubleshoot commercial problems in low milk fat production. Milk fat depression continues to be a real-world condition that reduces the efficiency and productivity of dairy cows, but understanding its fundamental basis allows for effective management and intervention strategies.

Under certain marketing systems and management schemes, it may be advantageous to reduce milk fat yield, and in some feeding and management systems, the reduction in milk fat yield has allowed for a repartitioning of nutrients to support increased milk and milk protein yield. Producers may also find it advantageous to induce MFD during periods of limited feedstuff availability such as inadequate rainfall in pasture-based systems or for a short period while breeding. Inducing MFD during breeding periods may also be a useful management practice to improve short-term energy balance and subsequently reproductive efficiency, although caution is important in application of classical MFD diets.

Climate Considerations

Milk fat percentage is typically higher in the winter than in the summer for the northern hemisphere. One could attribute the changes in milk fat percentage to differences in nutrient intake or specific effects of climate (i.e., environmental temperature). Certainly changes in carbohydrate and polyunsaturated fatty acid intake may be the driving force behind some changes associated with seasonal effects as pasture available in the summer is replaced with preserved forages and grains in the winter. However, milk fat percentage still varies similarly in pasture-fed herds compared with commercial herds without access to pasture. Concentrations of unsaturated fatty acids in milk including vaccenic acid, oleic acid, CLA, and linolenic acid are greatest in the summer. The coordinate increase in vaccenic acid and CLA suggests an enhancement of Δ^9-desaturase activity. The changes in saturated fatty acids are inverse to those of unsaturated fatty acids, suggesting a reduction in *de novo* synthesis during the summer months. The factors causing these changes in milk fat percentage and fatty acids beyond nutrition are unknown.

The specific effects of temperature (i.e., heat or cold stress) on milk fat, however, are not clear. Some have

speculated that heat stress would cause a condition similar to ruminal acidosis that should lead to a reduction in milk fat percentage. Direct assessment of the effect of heat stress through the use of environmental chambers failed to detect an effect on milk fat percentage even though obvious effects of heat stress (i.e., increased body temperature and respiration rate and decreased feed intake) were apparent. Thus, it is likely that the changes during the summer are not attributable to warmer environmental temperatures. The impact of cold stress may again not be a direct effect of climate on milk fat synthesis but more an indirect effect on nutrient utilization. Milk production is reduced in cows exposed to temperatures below −5 °C. As milk yield declines, milk fat percentage increases. The changes are probably related more to the utilization of energy for maintenance of body temperature, leading to reduced energy available for milk synthesis. However, milk fat synthesis is maintained or declines less than milk yield, and thus, milk fat percentage increases. Few data are available regarding the effects of cold stress on fatty acid composition of milk.

See also: **Feed Ingredients**: Feed Supplements: Fats and Protected Fats. **Milk Lipids**: Conjugated Linoleic Acid; Fatty Acids; General Characteristics; Nutritional Significance. **Stress in Dairy Animals**: Heat Stress: Effects on Milk Production and Composition.

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