

New Insights on the Pathways of Lipid Biohydrogenation in the Rumen with Possible Implications on Animal Performance

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SUMMARY

- Biohydrogenation is a microbial pathway in ruminal contents 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 major intermediates include an array of *trans*-C18:1 and conjugated linoleic acid (CLA) isomers, but most published pathways have traditionally ignored the majority of minor intermediates and present only a superficial view of biohydrogenation.
- Isotopic tracer studies and studies following fatty acid accumulation in ruminal contents have expanded the identity of biohydrogenation intermediates and their regulation.
- Intermediates are now recognized that include *trans*-18:1 positional isomers with double bonds located at all positions between carbons 6 and 16, plus a multitude of CLA isomers.
- The impacts of biohydrogenation on animal performance include protection of ruminal fermentation from antimicrobial effects of unsaturated fatty acids, loss of dietary omega fatty acids needed for optimal reproduction and immune function, and accumulation of CLA intermediates with physiological activity, such as the *trans*-10,*cis*-12 isomer that inhibits mammary lipogenesis and causes milk fat depression.

INTRODUCTION

The major accomplishment of biohydrogenation in ruminal contents is to convert unsaturated fatty acids in plant matter to more saturated endproducts that flow to the intestines. The reason the microbial population developed the capacity for biohydrogenation has been debated. Disposal of H has been considered, but seems unlikely given that lipid biohydrogenation as a H sink in the rumen is small (< 5%) in comparison to methane and propionate production. Considering the sensitivity of many microbial species to cytotoxic effects of unsaturated fatty acids, it seems more likely that biohydrogenation was an evolutionary adaptation to protect the microbial population from the antimicrobial effects of double bonds. The direct addition of hydrogen to a double bond is energetically costly and proceeds only slowly without the presence of a catalyst, as in the case of commercial hydrogenation of oils to raise melting point. The biohydrogenation pathway in the rumen overcame this energetically unfavorable step by first forming *trans* double bonds that lower the energy of activation for subsequent functioning of the reductases. Thus, a variety of *trans*-monoene and conjugated dienes containing one or more *trans* double bonds emerged as intermediates. Over the decades, most published accounts of biohydrogenation ignored early reports of complex intermediates and presented oversimplified pathways that included only predominant *trans* intermediates. Recent work is now being

directed at accounting for all possible *trans*-18:1 and CLA intermediates, including identifying their physiological biopotency.

The impact of biohydrogenation on human health and animal performance is immense. The utility of the pathway for protecting fermentation by converting antimicrobial unsaturated fatty acids to inert saturated fatty acids has already been mentioned. The accumulation of the saturated fatty acids in animal tissue has had tremendous consequences on per capita consumption of meat and milk, following continual scrutiny of saturated fatty acids for their role in etiology of atherosclerosis and other human health risks. Likewise, biohydrogenation greatly depletes the supply of essential fatty acids to ruminant tissues, which recent studies suggests compromises reproductive performance and immune function of the host animal in some circumstances. Notwithstanding are the potent physiological and metabolic functions of certain biohydrogenation intermediates, especially the various CLA isomers produced. Notable effects of CLA include their anti-carcinogenic properties and their ability to inhibit fat synthesis.

This paper will begin with a brief overview of lipid nomenclature as a reminder of the differences between unsaturated and saturated fatty acids. This will be followed by a brief review of the key steps in lipid metabolism in the rumen including lipolysis and biohydrogenation. New information on the identity of intermediates of biohydrogenation will be addressed for each of the three main unsaturated fatty acids consumed by cattle. The paper concludes with a discussion of how biohydrogenation and its intermediates impact animal performance.

FATTY ACID NOMENCLATURE

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 (Figure 1).

Fatty acids, such as stearic acid, are referred to as saturated because all the carbons are holding the maximum number of hydrogens possible, or the fatty acid is “saturated” with hydrogen. Stearic acid is low in plant oils, but present in higher amounts in animal fats, particularly in fats obtained from ruminant species such as beef tallow (Table 1).

Figure 1. The structure of stearic acid; a saturated long-chain fatty acid.

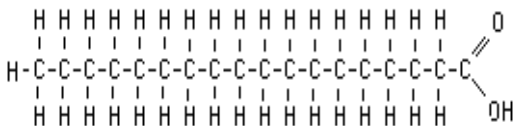


Table 1. Fatty acid composition of several fat supplements used in livestock rations.

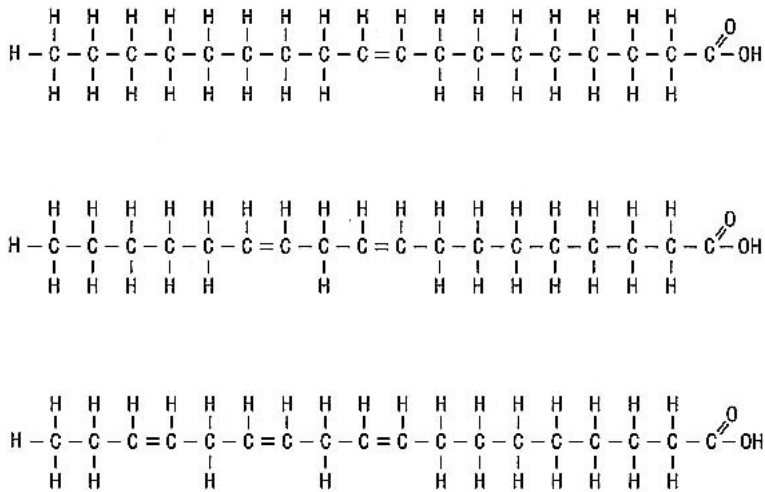
Abbr. ^a	Common	Tallow	Cottonseed	Canola
C14:0	Myristic	3.0	1.0	
C16:0	Palmitic	25.0	23.0	4.0
C18:0	Stearic	21.5	3.0	2.0
C18:1	Oleic	42.018.5	60.0	41.0
C18:2	Linoleic	3.0	52.5	20.0
C18:3	Linolenic			10.0

^aNumber of carbons:number of double bonds.

From Rouse (2003)

Oleic acid and linoleic acid are examples of unsaturated fatty acids containing one or more double bonds (Figure 2). Oleic acid has a single double bond between carbons 9 and 10, and is referred to as a monounsaturated fatty acid. Linoleic acid is a polyunsaturated fatty acid containing two double bonds between carbons 9 and 10, and between carbons 12 and 13. Oleic acid is the predominant fatty acid in animal fats and some plant oils, such as canola oil (Table 1). Linoleic acid is the predominant fatty acid in many plant oils, including cottonseed oil, soybean oil, and corn oil. Linolenic acid, with three double bonds, is the primary fatty acid in most pasture species and in linseed oil from flax.

Figure 2. Structures of the three primary unsaturated fatty acids consumed by cattle, oleic acid (top), linoleic acid (middle), and linolenic acid (bottom).



FAT METABOLISM IN THE RUMEN

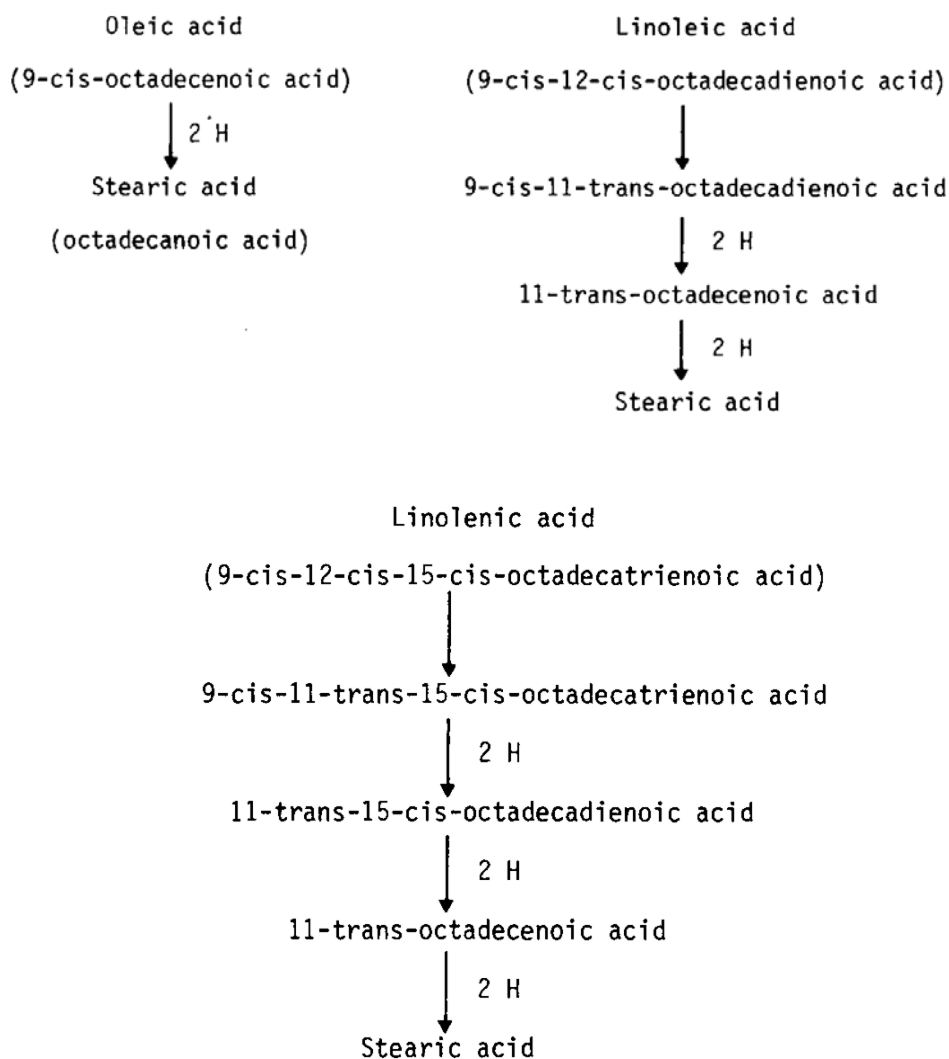
Feed consumed by ruminants first passes through the largest of the four stomach compartments or rumen, which acts like a fermentation vat. Countless numbers of bacteria, protozoa, and fungi

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 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

Figure 3. Common depictions of the pathways of biohydrogenation of oleic, linoleic , and linolenic acids by ruminal microbes. Taken from Kellens et al. (1986).

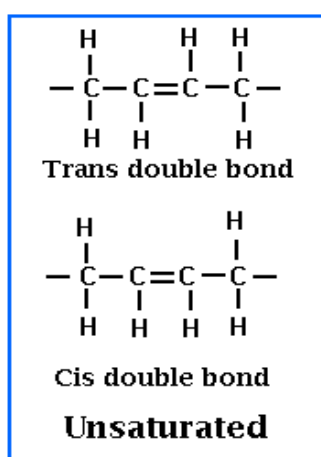


from forages and concentrates in the diet. Rapid hydrolysis of triglycerides occurs by microbial enzymes. Linseed oil incubated with ruminal contents of sheep at 1.0 g/100 ml resulted in greater than 75% of the total lipid recovered in the form of free fatty acids. Phospholipids and galactolipids also 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 ^{14}C -labelled grass to inactivate plant lipolytic enzymes. The grass was then administered intraruminally to a sheep and the galactolipids were rapidly hydrolyzed. Additionally, in an in vitro trial, grass was homogenized with rumen fluid taken from sheep that had been given autoclaved grass for 7 d and the grass galactolipids were rapidly hydrolyzed. 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).

The biohydrogenation of linoleic acid in the rumen (Figure 3) 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 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 biohydrogenation of linoleic acid is *cis*-9, *trans*-11 C18:2.

As biohydrogenation progresses, double bonds in the CLA intermediates are then hydrogenated further to *trans* fatty acids having only one double bond. *Trans* double bonds only differ from

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



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. A final hydrogenation step by the ruminal microbes eliminates the last double bond yielding stearic acid as the final end product. As a result of biohydrogenation, there is extensive loss of unsaturated fatty acids from the mouth to the duodenum of the animal (Table 2).

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 biohydrogenation.

Table 2. Fatty acid intake and duodenal flows in cattle fed control diets with no added fat supplements.

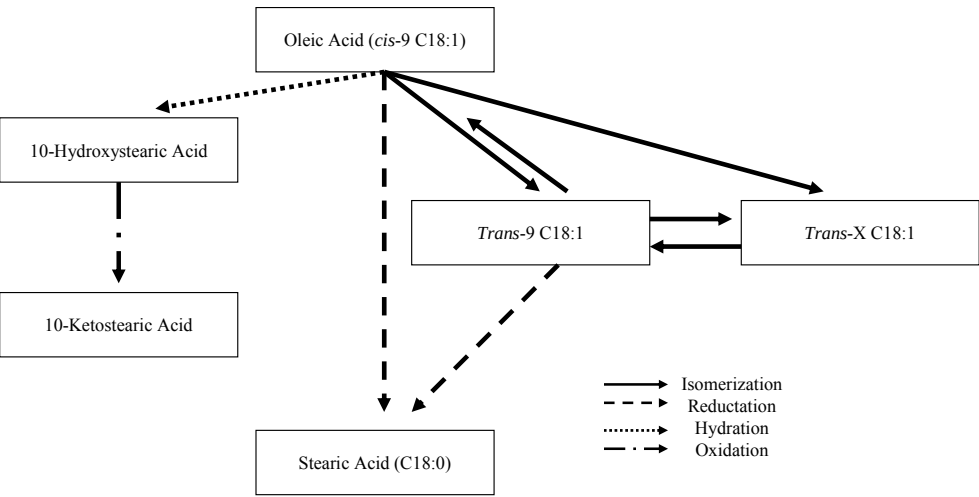
Abbr. ^a	Common	Intake, g/d	Duodenal, g/d
C18:0	Stearic	18	252
C18:1	Oleic	99	45
C18:2	Linoleic	181	38
C18:3	Linolenic	42	5

^aNumber of carbons:number of double bonds.
From Jenkins and Bridges (2007)

Oleic Acid (*cis*-9 C18:1)

Biohydrogenation of oleic acid, a non-essential fatty acid synthesized *de novo*, was initially thought to produce only stearic acid without the formation of *trans* intermediates (Figure 3). However, several bacterial species have reported the capability of *cis* to *trans* isomerization. Mortimer and Niehaus (1972) found that *Pseudomonas* strain NRRL, a non-ruminal bacteria, converted oleic acid to *trans*-10 C18:1 under low pH conditions. Kemp et al. (1975) found that *Fusocillus* T344, a ruminal bacteria, was capable of converting oleic acid to *trans*-11 C18:1. Okuyama et al. (1991) proposed that *cis* to *trans* isomerization could change the plasma membrane fluidity and protect the bacteria itself against growth inhibitors or environmental insults.

Figure 5. Oleic acid transformation by ruminal microbes. From Jenkins et al. (2006).



More recently, Mosley et al. (2002) observed that mixed ruminal microbes rapidly converted ¹³C-labeled oleic acid to several positional isomers of *trans* monomers. They found that all

trans-C18:1 isomers with double bond positions from C₉ to C₁₆ were produced from labeled oleic acid. In a similar experiment using ¹³C-labeled *trans*-9 C18:1, Proell et al. (2002) traced the intermediates of the biohydrogenation pathway of *trans*-9 C18:1 after being incubated with mixed ruminal microbes. After 48 h incubation, they found that labeled stearic acid, *trans*-C18:1 isomers with double bond positions from C₆ to C₁₆, and two *cis* monoenes (*cis*-9, and *cis*-11 C18:1) were produced from the labeled *trans*-9 C18:1.

Mixed ruminal microbes maintained in continuous culture also were found to convert oleic acid to 10-hydroxystearic and 10-ketostearic acids. Up to 30 % of the oleic acid in these cultures was used in the production of these two stearic acid derivatives, while only 6-10 % of oleic acid was used in a similar process *in vitro* with mixed ruminal microorganisms (Jenkins et al., 2006).

A summary of oleic acid biohydrogenation was proposed by Jenkins et al. (2006) that included three competing routes of carbon flow (Figure 5). One route was the direct hydrogenation of oleic acid to form stearic acid. Another route of carbon flow involved *cis* to *trans* isomerization that formed a multitude of positional isomers of *trans*-C18:1 with double bond positions from C₆ through C₁₆. The isomerization route also included the possibility of reverse isomerization with some *trans*-9 C18:1 being converted to oleic acid. The third route of carbon flow was hydration of oleic acid to 10-hydroxystearic acid followed by its oxidation to 10-ketostearic acid.

Linoleic Acid (*cis*-9 *cis*-12 C18:2)

Most research of linoleic acid biohydrogenation (Figure 3) discusses its conversion to *cis*-9 *trans*-11 CLA and *trans*-11 C18:1 before the formation of stearic acid (Garton, 1977). However, Griinari and Bauman (1999) suggested that *trans*-10 *cis*-12 CLA also originated from linoleic acid, followed by the production of *trans*-10 C18:1 in the rumen. This hypothesis was supported by the study conducted by Kim et al. (2002), who found that some *Megasphaera elsdenii* strains produced *trans*-10 *cis*-12 CLA formed by linoleic acid.

A recent review by Jenkins et al. (2008) identified as many as 17 CLA isomers reported in digesta contents from previous *in vitro* and *in vivo* studies. Some of these 17 CLA isomers were verified as end products of linoleic acid transformation by various microbial species. Coakley et al. (2006) showed that linoleic acid was hydrogenated to *cis*-9 *trans*-11 CLA, *trans*-9 *trans*-11 CLA, and *trans*-10 *cis*-12 CLA via the *Bifidobacterium* species of human intestinal origin. Wallace et al. (2007) also demonstrated that *trans*-9 *trans*-11 CLA was produced from linoleic acid by *B. fibrisolvens*, while Ogawa et al. (2001) showed an alternative route of biohydrogenation where 10-hydroxy-*cis*-12-C18:1 and 10-hydroxy-*trans*-12-C18:1 were produced as intermediates of linoleic acid biohydrogenation by *Lactobacillus acidophilus* AKU 1137 under microaerobic conditions. They found that these two intermediates were produced before the formation of *cis*-9 *trans*-11 CLA and *trans*-9 *trans*-11 CLA.

More recently, Lee et al. (2007) identified seven CLA isomers that originated from linoleic acid biohydrogenation. They incubated 1-¹³C-linoleic acid with mixed ruminal microorganisms *in vitro*. All identified CLA carried a double bond at C₉ and C₁₂ with all possible double bond geometries (*cis/cis*, *trans/trans*, *cis/trans*, and *trans/cis*) except for *trans*-9 *cis*-11 CLA.

Linolenic Acid (*cis*-9 *cis*-12 *cis*-15 C18:3)

The biohydrogenation process of linolenic acid in the rumen has not been researched as extensively as oleic or linoleic acids. In most publications, linolenic acid initially was converted to *cis*-9 *trans*-11 *cis*-15 C18:3 by ruminal biohydrogenation (Figure 3). Then, *trans*-11 *cis*-15 C18:2 was produced through the reduction of the *cis*-9 double bond. This was further hydrogenated to *trans*-11 C18:1 to produce stearic acid. *Trans*-11 *cis*-15 C18:2 can also hydrogenate to *trans*-15 or *cis*-15 C18:1.

In the study conducted by Looor et al. (2004), linolenic acid was isomerized into three C18:3 intermediates: *cis*-9 *trans*-12 *cis*-15 C18:3, *cis*-9 *trans*-12 *trans*-15 C18:3, and *trans*-9 *trans*-12 *trans*-15 C18:3. Low concentrate diets (65:35 forage to concentrate) and the addition of linseed oil in the diet increased the duodenal flow of these 3 C18:3 isomers. In addition, Destailats et al. (2005) reported that 0.3 % of milk fat was the *cis*-9 *trans*-11 *cis*-15 C18:3 and the *cis*-9 *trans*-13 *cis*-15 C18:3 isomers, suggesting that these two isomers were the initial intermediates of linolenic acid biohydrogenation. Subsequently, *cis*-9 *trans*-11 *cis*-15 C18:3 isomer was reduced to *cis*-9 *trans*-11 C18:2 and *trans*-11 *cis*-15 C18:2, and *cis*-9 *trans*-13 *cis*-15 C18:3 isomer to *cis*-9 *trans*-13 C18:2 and *trans*-13 *cis*-15 C18:2. Both *cis*-9 *trans*-11 C18:2 and *trans*-11 *cis*-15 C18:2 were able to hydrogenate to *trans*-11 C18:1, reducing to stearic acid. The *cis*-9 *trans*-13 C18:2 and *trans*-13 *cis*-15 C18:2 hydrogenated to *trans*-13 C18:1, which was then subsequently reduced to stearic acid. They suggested that two CLA isomers (*cis*-9 *trans*-11 CLA; *trans*-13 *cis*-15 CLA) were produced from linolenic acid biohydrogenation. However, no evidence supporting this route was given in this study. Wąsowska et al. (2006) reported that *cis*-9 *trans*-11 *cis*-15 C18:3 and *trans*-9 *trans*-11 *cis*-15 C18:3 were accumulated by linolenic acid biohydrogenation in strained rumen fluid. They also found that the *trans*-11 *cis*-15 C18:2 originated from linolenic acid, but *cis*-9 *trans*-11 CLA was not observed in their study.

IMPLICATIONS OF BIOHYDROGENATION ON ANIMAL PERFORMANCE

Protection of Ruminal Fermentation

Biohydrogenation can be argued as an evolutionary adaptation of the microbial population in the rumen as a self-protection device against the toxic effects of unsaturated fatty acids. Elevating fatty acid concentration in ruminal contents may cause a number of changes in ruminal fermentation characteristics and microbial population distribution. Ruminal changes are the result of the antimicrobial nature of unsaturated fatty acids, where fatty acids adsorb onto the cell membrane of selected microbial species, and then penetrate into the membrane causing disorganization of phospholipids and eventual cytological damage (Jenkins, 2002). Because some bacterial species are more susceptible than others, the result is a microbial shift in the rumen. The fatty acid-induced microbial shift can disrupt fermentation of carbohydrate digestion causing a drop in the acetate to propionate ratio and possibly a reduction in fiber digestion (Jenkins, 2002).

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 free fatty acid concentration is low. Both assumptions can be wrong. Fatty acids from the grain and forage can significantly contribute to total rumen fat load, for example when animals are consuming immature pasture. Also, free fatty acid 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).

Meeting Essential Fatty Acid Demands

Omega fatty acids belong to one of three families, the ω -9, ω -6, or ω -3 family. Each family has a parent fatty acid that is converted to other biologically-active acids within the same omega family (Figure 6). The only parent fatty acid that can be made by body tissues is oleic acid. The ω -6 and ω -3 parent compounds (linoleic and linolenic acids) cannot be synthesized by body tissues and, therefore, must be supplied in the diet. Thus, linoleic and linoleic acids are regarded as essential because they are required for normal tissue function but cannot be synthesized by body tissues.

Figure 6. Parent fatty acids and major metabolites within each of the three omega fatty acid families.

Family Designation	Parent Fatty Acid	Major Metabolites
ω -9	C18:1 ω -9; oleic acid	C20:3 ω -9†; eicostrienoic acid
ω -6	C18:2 ω -6; linoleic acid	C20:4 ω -6; arachidonic acid
ω -3	C18:3 ω -3; linolenic acid	C20:5 ω -3; eicosapentaenoic acid C22:6 ω -3; docosahexaenoic acid

From Gorlin (1988)

A typical total mixed ration of grains and forages generally contains adequate essential fatty acids to meet the needs of the animal. However, the majority of the dietary essential fatty acids are destroyed by microorganisms through biohydrogenation.

In humans, there is clear evidence that omega fatty acids are essential for normal development and needed to achieve good health throughout life. Early human development is affected by omega fatty acids through their effects on neural growth and later infant development. In later life, the omega fatty acids, especially those of the omega-3 family, protect against atherosclerosis and its related thrombotic complications (Willis and Smith, 1989). Marine

omega-3 fatty acids have antithrombotic effects, modify platelet aggregation, decrease vascular adhesiveness, and minimize inflammatory responses in vessel walls (Uauy-Dagach and Valenzuela, 1996). The eicosenoid and leukotriene metabolites originating from the parent omega fatty acids also are associated with beneficial effects in inflammation and immunity (Nettleton, 1995).

Part of the interest in omega fatty acids in dairy cattle is to enhance their concentration in milk for value-added opportunities, and part is to enhance their concentration in body tissues of the cow to enhance production and health. Omega fatty acids in milk are increased to improve manufacturing properties and to increase fatty acid nutraceuticals known to enhance human health. Increasing omega fatty acids in tissues of the cow has potential benefits on reproductive performance, immunity and disease resistance, and positive hormonal shifts.

In a few studies, feeding fat to lactating dairy cows has improved reproductive performance implying possible benefits on lifetime production potential. Reported improvements of reproductive performance from added fat include higher conception rates (Schneider et al., 1988; Sklan et al., 1989), increased pregnancy rates (Schneider et al., 1988; Sklan et al., 1991), and reduced open days (Sklan et al., 1991). However, supplemental fat has had little or no benefit on reproductive efficiency in other studies (Carroll et al., 1990).

The mechanism of how fat supplements alter reproductive performance is not clear. Fat may function in one capacity by providing additional energy during early lactation to support improved productive functions, including reproduction. Negative energy balance delays ovulation and the initiation of the first normal luteal phase (Butler et al., 1981). However, recent studies also suggest that the mechanism involves an energy independent response to fat.

When an equal quantity of energy from glucose, saturated animal fat (tallow), or unsaturated fat (yellow grease) were infused into lactating dairy cows via the abomasum, the fat but not carbohydrate decreased plasma estradiol and increased progesterone (Oldick et al., 1997). The study by Oldick et al. (1997) also demonstrated the potential to decrease PGF_{2α} synthesis by supplying elevated concentrations of omega fatty acids. These results were similar to previous reports that intravenous infusion of unsaturated fatty acids from a soy oil emulsion increased plasma F_{2α}, and number and size of follicles (Lucy et al., 1990, 1991). Ovarian follicular growth was also stimulated more in Brahman x Hereford cattle by fat compared to equal energy from carbohydrate, with a greater effect observed for fats with higher PUFA (Thomas et al., 1997). Staples et al. (2000) showed that size of the dominant follicle was greater for Holstein cows fed Ca salts of linoleic acid or fish oil fatty acids compared to those fed calcium salts of oleic acid. Results such as these continue to demonstrate a reproductive advantage from increased absorption of PUFA compared to other fat sources, such as monounsaturated fats.

Immune System

CLA decreased the growth rate in chicks and rats after they were injected with endotoxin (lipopolysaccharide; LPS). This probably was caused by release of cytokines and the prevention of the catabolic effects (Cook et al., 1993). Miller et al. (1994) examined endotoxin-induced growth suppression in mice fed with 0.5 % fish oil and CLA. The fish oil fed-group lost twice as much body weight after the inoculation with endotoxin than the CLA-fed groups. These researchers found that the CLA in the endotoxin injection inhibited anorexia (a decreased

sensation of appetite) and increased splenocyte blastogenesis, concluding that it might inhibit arachidonic acid synthesis, thus preventing the catabolism of tissue by removing eicosanoid precursors. In addition, Bontempo et al. (2004) examined the effects of CLA on the immunological variables of lactating sows and piglets fed with a 0.5 % CLA diet. They found that CLA-fed sows exhibited increased colostrum IgG and serum leptin, and IgG and lysozyme. Nursing piglets of CLA-fed sows also exhibited higher levels of IgG and lysozyme. As these results show, dietary CLA enhanced the effect of immunological variables in lactating sows and piglets.

Effect on Body Weight and Fat

Park et al. (1997) found that CLA contributed to a reduction in body fat mass and an increase in lean body mass. Mice fed a 0.5 % CLA augmented diet exhibited 57-60 % lower body fat and 5-14 % increased lean body mass than the controls. They also found that the total carnitine palmitoyltransferase activity was enhanced with dietary CLA supplementation. Moreover, in cultured 3T3-L1 adipocytes, CLA treatment reduced the intracellular heparin-releasable lipoprotein lipase activity and triacylglyceride and glycerol concentration. In addition, DeLany et al. (1999) suggested that CLA played a key role in reducing body fat content and increasing protein accumulation in mice. In this study, the diets of mice were supplemented with CLA (0.0, 0.25, 0.50, 0.75, and 1.0 % by weight). In the 0.50, 0.75, and 1.0 % CLA feeding groups, body fat was significantly lower than in the control, however, body energy was not depressed by any CLA dose. As these results suggested, dietary CLA reduces fat deposition and increases lipolysis in adipocytes, coupled with enhanced fatty acid oxidation.

In a human trial, Blankson et al. (2000) conducted a double blind study with 60 overweight or obese volunteers (body mass index 25-35 kg/m²). The CLA doses given varied from 1.7, 3.4, 5.1, or 6.8 g/day for 12 weeks. The reduction of body fat mass was significant for the 3.4, 5.1, and 6.8 g CLA groups. No significant differences in lean body mass, body mass index, blood safety variables or blood lipids were observed in the groups. Long-term supplementation of CLA studied by Gaullier et al. (2007) reduced body fat mass and increased lean body mass. They conducted a double blind placebo-controlled study with 118 healthy, overweight, and obese adults. The CLA dose was 3.4 g/day. During the six-month period of study, the CLA significantly decreased the body fat mass (-3.4 %) located primary in the legs.

Two families of transcription factors may be involved in the intracellular signaling mechanisms of CLA according to Tsuboyama-Kasaoka et al., (2000); peroxisome proliferator activated receptors (PPAR) and sterol regulatory element binding-protein (SREBP). However, the mechanisms by which CLA alters lipid metabolism in the human body are still unclear. Initially, CLA, especially *trans*-10 *cis*-12 CLA, enters the cell membrane through unknown transport mechanisms before being shuttled into various regulatory compartments. Brown and McIntosh (2003) suggested that subsequently three mechanisms in adipose tissue depressed the triglyceride. The first esterified CLA into triglyceride-rich oil droplets. This mechanism did not have much effect on the regulation as a whole, even increasing triglyceride stores. The second mechanism esterified CLA into the membrane-bound phospholipids bilayer, where it changed the fluidity of the membranes associated with a signal transduction cascade. The last mechanism modified the activity of a currently unidentified transcription factor (TFX), resulting in less regulation of PPAR and the depression of lipoprotein lipase, an acyl-CoA-binding protein, an adipocyte fatty acid binding protein, a glucose transporter, and leptin. In addition, CLA reduced

the activity of steroyl-CoA desaturase and acetyl-CoA carboxylase by altering the activity of TFX. By inhibiting these factors, CLA attenuated insulin-stimulated glucose uptake, malonyl-CoA synthesis, and oleate synthesis, collectively decreasing de novo fatty acid synthesis. CLA also inhibited the expression of SREBP, by enhancing SREBP transcript decay as well as by inhibiting the proteolytic cleavage, releasing the activated nuclear SREBP fragment.

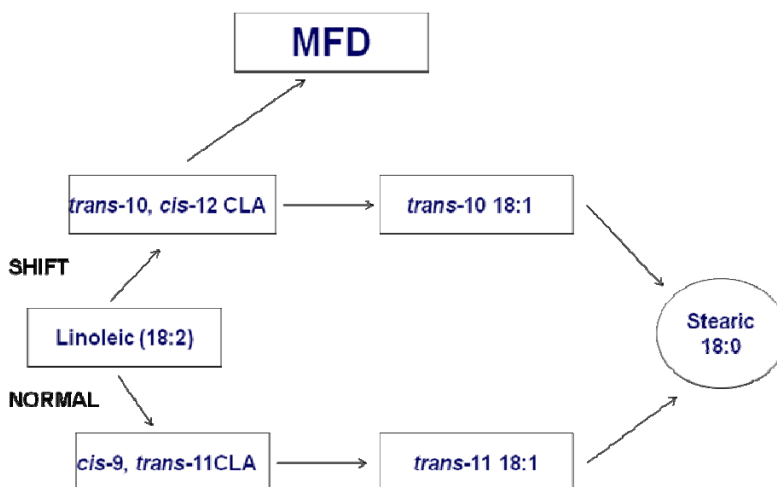
Milk Fat Depression

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 biohydrogenation intermediates in the rumen could have dramatic effects on health and metabolism of both the host animal and the humans consuming animal-food products. Eventually it was discovered that a second CLA, namely the *trans*-10, *cis*-12 isomer, was closely associated with milk fat depression (MFD). This led to the biohydrogenation 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 biohydrogenation theory of MFD. Predicting when fat sources 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 biohydrogenation and its interaction with other feed ingredients. An improved understanding of these events will provide the critical framework with which to better troubleshoot MFD.

The 'biohydrogenation theory' represents a unifying concept to explain the basis for diet-induced MFD where unusual intermediates of ruminal fatty acid biohydrogenation accumulate in the rumen and eventually reduce milk fat synthesis in the mammary gland. Under certain dietary situations the rumen environment is altered and a portion of biohydrogenation occurs via a pathway that produces *trans*-10, *cis*-12 CLA and *trans*-10 18:1 (Figure 7). *Bifidobacterium*, *Propionibacterium*, *Lactococcus*, *Streptococcus*, and *Lactobacillus* isolates from other habitats have been reported to produce *trans*-10, *cis*-12-CLA. As these genera occur in the rumen, although generally at rather low numbers, they may contribute to biohydrogenation 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 biohydrogenation resulting in changes in the specific *trans*-18:1 and CLA isomers available for uptake by the mammary gland and incorporation into milk fat.

As shown in Figure 7, this '*trans*-10 shift' in biohydrogenation pathways, and the associated increase in the *trans*-10 18:1 content of milk fat, is indicative of the complex changes in ruminal biohydrogenation 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 marker for the type of alterations in rumen biohydrogenation that characterize diet-induced MFD.

Figure 7. The shift in intermediates produced from biohydrogenation of linoleic acid in ruminal contents as a result of a diet-induced microbial shift.



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