Factors that Modify Rumen Fatty Acid Flow Versus Feed Input

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Introduction

Dietary fatty acids undergo significant structural changes via a process called biohydrogenation as they pass through ruminal contents and are delivered to the intestines for absorption. A significant portion of milk fat yield, which is a primary driver of milk income, is guided by the direction of these biohydrogenation pathways. Changes in nutritional composition of the feed, brought about either by design or inadvertently because of nutritional variation in feed ingredients, can shift biohydrogenation pathways causing changes in rumen fatty acid outflow of bioactive lipids that adversely affect milk fat synthesis. Therefore, identifying the trigger that shifts fatty acid biohydrogenation in the rumen from “milkfat friendly” to “milkfat unfriendly” is of upmost importance. \

The intention of this paper is to offer a possible trigger mechanism that initiates the rumen microbial population to shift its pathways of biohydrogenation toward a direction unfavorable for milk fat synthesis. Much of the direct evidence for the trigger is revealed from recent studies of isolated ruminal bacteria, in vitro rumen cultures, and cow data. Data across these studies suggests that when dietary fatty acids, coming from both the basal diet and from added fat, reaches a level sufficient to cause antibacterial effects in the rumen the result is a shift from normal biohydrogenation to an alternate pathway. The alternate pathway produces lipid bioactive intermediates that lower milk fat. The data summarized below also shows that the type and concentration of fatty acid required to reach antibacterial effects is subject to modification by other dietary nutrient considerations.

What Are Fatty Acids?

Before beginning a discussion about the fate of fatty acids as they pass through the rumen, it seems appropriate to start with a brief refresher on defining fatty acids. Put simply, fatty acids are the basic building blocks of fats just as amino acids are the building blocks of protein. Amino acids are chained together with peptide bonds in different lengths to form everything from dipeptides (2 amino acids) to polypeptides (> 10 amino acids). Fats, unlike protein, consist of no more than three fatty acids grouped together as attachments on a glycerol backbone. Fats and oils primarily consist of three fatty acids attached to glycerol referred to as triglycerides (or more correctly triacylglycerols). Forage lipids contain primarily galactolipids, where the glycerol backbone has two bound fatty acids along with a bound sugar molecule.

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Fatty acids, and not the glycerol backbone, provide the benefits to animal performance, including high energy, tissue benefits, and rumen effects. For this reason, it is important to have a basic understanding of the differences among 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 (**Figure 1**). 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.

Oleic, linoleic, and linolenic acids 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. 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.

Fatty Acid Input into The Rumen

Typical daily intakes of unsaturated fatty acids for diets with and without added fat are shown in **Table 1**. Total fatty acid concentration in feed mixes can range from less than 20 mg/g of dry matter (**DM**) for basal ingredients to more than 80 mg/g DM when fat is added. Linoleic acid is the predominant unsaturated fatty acid consumed in most cases with upper intakes exceeding 700 g/day in published studies (**Table 1**), or even exceeding 1000 g/day under field conditions (Chase, 2019).

Fatty acid concentration in ruminal contents reflects their concentration and variability in feed. Using results from three published studies as an example (**Table 2**), fatty acids varied in ruminal contents from < 10 mg/g DM for a basal diet containing 50% bermudagrass hay (Bateman and Jenkins, 1998) to 29 mg/g DM for an alfalfa/corn silage diet (Loor et al., 2002), and to just under 50 mg/g DM for diets with added plant oils. Cows grazing high quality ryegrass and clover pasture (Sun and Gibbs, 2012), interestingly, had ruminal fatty acid concentrations approaching levels observed for totally mixed rations (**TMR**) with added fat. The implication of maintaining fatty acids in ruminal contents is that many microbial species are sensitive to high fatty acid concentrations and respond with reduced growth and metabolic activity. More specifically, antibacterial activity is greatest for unsaturated fatty acids and is not a characteristic of saturated fatty acids.

Select ruminal bacteria have an inherent protective mechanism in place designed to reduce unsaturated fatty acid concentration in the rumen and lessen the chances of antibacterial activity. This protective mechanism is referred to as biohydrogenation, where unsaturated fatty acids are enzymatically converted to saturated fatty acids (Jenkins et al., 2008). The efficiency of biohydrogenation can be seen from the results

of the three studies in **Table 2** where ruminal unsaturated fatty acid concentrations are much lower and less variable compared with feed or ruminal total fatty acid concentrations. Biohydrogenation, while assisting the microbial population in controlling antibacterial effects of unsaturated fatty acids, also greatly transforms the nature of fatty acid outflow from the rumen compared to its inflow from feed. This fatty acid transformation process has both positive and negative consequences on animal production and acceptability of animal based food products.

Changes in Rumen Fatty Acid Concentration Over Time

The pathways of biohydrogenation are highly complex and yield a wide variety of intermediates. The three main unsaturated fatty acids consumed (oleic, linoleic, and linolenic acids) are all subjected to enzymatic transformations that yield a multitude of unique intermediates. As an example, the pathways and intermediates of linoleic and linolenic acid biohydrogenation to stearic acid are shown in **Figure 3** (Ferlay et al., 2017). As knowledge increases about the pathways of biohydrogenation the identity of intermediates expands. Input into the rumen of just three unsaturated fatty acids (oleic, linoleic, and linolenic acids) as raw materials leads to the production of dozens, if not hundreds, of complex fatty acid isomers in rumen outflow. Yet, this complexity of biohydrogenation is largely ignored in most discussions of biohydrogenation. Most of the attention is directed at a more simplistic version of linoleic acid biohydrogenation (**Figure 4**) that emphasizes only the few intermediates that were shown previously to inhibit fat synthesis in the mammary gland.

Very briefly, biohydrogenation of linoleic acid in the rumen begins with its conversion to conjugated linoleic acid (**CLA**). In this initial step, the number of double bonds remains the same but one of the double bonds is shifted to a new position by microbial enzymes. Normally, the double bonds in linoleic acid are separated by two single bonds, but in CLA, the double bonds are only separated by one single bond. Many types of CLA are produced in the rumen of dairy cows, 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. A final hydrogenation step by the ruminal microbes eliminates the last double bond yielding stearic acid as the final end product. *Trans* double bonds only differ from *cis* double bonds in the placement of the hydrogens. The hydrogens are shown 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. The *trans*-11 route (abbreviated as t11) of linoleic acid biohydrogenation in **Figure 4** involves intermediates, including *cis*-9, *trans*-11 CLA, proven to have little effect on milk fat. The *trans*-10 route (abbreviated as *t*10) involves intermediates, including *trans*-10, *cis*-12 CLA, proven to reduce milk fat synthesis (Baumgard et al., 2000).

With biohydrogenation in place, it might be argued that unsaturated fatty acid concentration remains low enough to avoid antibacterial effects. However, when the time course of biohydrogenation is examined immediately after feeding, it is common to see a large spike in unsaturated fatty acids that quickly declines over time. An example of the spike in unsaturated fatty acids immediately after feeding is shown in **Figure 5**. In a continuous culture study done at Clemson University, suddenly switching from a basal diet to a 3% soybean oil diet after 5 days of fermentation increased linoleic acid concentration from < 1 mg/10 ml culture contents to over 8 mg/10 culture contents by 1 hour after feeding. Sampling times earlier than 1 hour after feeding might have revealed a linoleic acid spike that was even higher. Declines in linoleic acid concentration occurred by hour 2 and then steadily declined with each advancing hour after feeding. The spike in unsaturated fatty acids immediately after feeding might induce antibacterial effects, even though biohydrogenation maintains much lower concentrations of unsaturated fatty acids at most other times.

An in vivo example of this unsaturated fatty acid spike in ruminal contents was seen in the data of Baldin et al. (2018). They reported the highest concentration of unsaturated fatty acids in ruminal contents of cows within 5 minutes of an intraruminal dose of 200 g unsaturated oil. Ruminal concentrations of unsaturated fatty acids then returned to basal values by 4 hours after dosing. This suggests that cows fed under field conditions experience a spike in ruminal unsaturated fatty acid concentration immediately after feeding that might be sufficient to cause antibacterial effects in the rumen. The amplitude of the spike would likely be a function of fatty acid input from the diet, percentage unsaturation of diet fatty acids, feeding frequency, and rate of feed consumption.

Antibacterial Effects of Unsaturated Fatty Acids

There is an extensive literature describing the antibacterial activity of various fatty acids (Desbois and Smith, 2010). 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 (Desbois and Smith, 2010). 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 acids (**FFA**) concentration is low. Both assumptions can be wrong. Fatty acids from the TMR and forage can significantly contribute to total rumen fat load, for example when animals are consuming immature pasture. Also, FFA concentration may be elevated in some feed ingredients such as whole cottonseed stored in warm, humid conditions (Cooke et al., 2007), or in forages resulting from hydrolytic cleavage of esterified lipids during hay-making (Yang and Fujita, 1997).

The mechanisms of fatty acid antibacterial effects are primarily directed at their intrusion into the bacterial cell membrane. The details of the mechanistic processes

(**Figure 6**) can be classified based on the relationship between the following three aspects: (i) increased membrane permeability and cell lysis, (ii) disruption of electron transport chain and uncoupling oxidative phosphorylation, and (iii) inhibition of membrane enzymatic activities and nutrient uptake (Yoon et al., 2018). For anaerobes that inhabit the rumen, fatty acids exert antibacterial properties through disorganization of the cytoplasmic outer membrane that can lead to increased membrane permeability and even cell lysis, inhibition of membrane enzymatic activity, and impaired nutrient uptake.

Several properties of antibacterial effects in the rumen directly impact the *t*10 versus *t*11 pathways of biohydrogenation and the eventual impact on milk fat.

I. One important factor is that fatty acids appear to exhibit antibacterial effects quickly. Maia et al. (2010) reported a > 96% reduction in metabolic activity in the ruminal bacterium *B. fibrisolvens* within 20 minutes following the addition of 0.2 mg/mL of linoleic acid to cultures. A recent continuous culture trial done at Clemson University examined how quickly soybean oil shifted biohydrogenation pathways from the normally predominant *t*11 pathway to the minor *t*10 pathway. Cultures were maintained on a basal diet for 4 days with *t*10-18:1 and *t*11-18:1intermediates analyzed just before the morning feeding (8 am) and then again at 2 and 4 hours after the morning feeding. On day 5 the cultures were suddenly switched to a diet containing 3% added soybean oil with samples analyzed every hour for 12 hours. The results (**Figure 5**) revealed an escalation of the *t*10 pathway within a few hours after introducing soybean oil into the cultures. This could mean that unsaturated fatty acid concentration may not need to be sustained at high levels at all times to cause antibacterial effects. Perhaps just the transient peak in ruminal unsaturated fatty acid concentration that occurs immediately after feeding is sufficient to trigger antibacterial effects.

II. A second critical point of antibacterial activity in the rumen is that not all bacterial species are equally susceptible. Disruption of membrane integrity following the addition of linoleic acid to cultures ranged widely across 17 species of ruminal bacteria (Maia et al., 2007) monitored by fluorescence techniques. Generally, the bacterial species following the *t*11 route of biohydrogenation showed greater disruption of membrane function, including > 50% disruption for *Butyrivibrio spp.* and > 90% disruption for *Pseudobutyrivibrio*. Membranes of *M. elsdenii* that follows the *t*10 route of linoleic acid biohydrogenation were < 5% disrupted by the same dosage of linoleic acid. Thus, fatty acid concentrations above the antibacterial threshold cause selective damage in the rumen depending on bacterial species, with less damage seen for *t*10 microorganisms.

III. Third, not all unsaturated fatty acids have equal propensity to cause antibacterial effects at the same concentration. For instance, relative inhibitory effects of individual fatty acids on growth of *B. fibrisolvens* was linolenic>linoleic>oleic>stearic according to Maia et al. (2010). The general trend was greater inhibition with increasing number of double bonds in the acyl chain. Similar trends have been

reported in vivo. Dorea and Armentano (2017) reported feeding saturated fatty acids to cows, such as palmitic acid, increased total milk fatty acids, mainly by increasing milk C16 yield. However, feeding unsaturated fatty acids decreased total milk fatty acid by inhibiting secretion of milk fatty acids shorter than C18, with linoleic acid being more inhibitory than oleic.

IV. A fourth and perhaps most significant property of antibacterial effects is that the threshold to cause a shift in the pathway of biohydrogenation is modified by environmental and chemical conditions in the rumen. If the threshold was a constant concentration of unsaturated fatty acids in ruminal contents, then feeding recommendations for fat could be modelled much easier. Instead, low pH and lactic acid accumulation were both shown to accentuate antibacterial effects of unsaturated fatty acids, specifically targeting *t*11 microorganisms (Maia, personal communication and Maia et al., 2010). Both of these conditions implicate high starch levels as a negative predictor of milk fat synthesis.

Conclusions

Using the antibacterial switch described in this paper, a sequence of events can be suggested whereby the pathways of biohydrogenation change course moving the rumen from milkfat “friendly” to milkfat “unfriendly”. The initial step is for unsaturated fatty acid concentration in the rumen to exceed the threshold for antibacterial effects. This could happen in one of two ways; 1) increase dietary concentration of unsaturated fatty acids that could arise from variation in basal fatty acids or from added fat to the diet, or 2) lower the antimicrobial threshold. The threshold that ruminal microorganisms can tolerate is lowered by increasing starch, lowering rumen pH, increasing lactate, or a combination of these. Once the antibacterial threshold is reached, *t*11 microorganisms respond within hours by shutting down metabolic activity including rates of biohydrogenation. This reduces the flux of linoleic acid flow through the normal *t*11 pathway of biohydrogenation. Consequently, because *t*10 microorganisms are less sensitive to antibacterial effects, more linoleic acid is now available for biohydrogenation through the alternate *t*10 pathway. With each subsequent feeding of the same diet the accumulation of CLA (specifically *trans*-10, *cis*-12) in the rumen continues providing a steady CLA flow to the mammary gland where *de novo* fatty acid synthesis is inhibited.

Some high producing herds consume in excess of 1000 g unsaturated fatty acids per day but still maintain milk fat around 4% (Chase, L. E., 2019). Other herds experience milk fat depression with <500 g of unsaturated fatty acids. McCarthy et al. (2018) failed to detect a significant relationship between milk fat yield and intake of unsaturated fatty acids across 79 herds in the northeast and upper Midwest. This variation clearly shows that unsaturated fatty acid intake alone is not a good predictor of milk fat. Models predicting milk fat should include all factors known to affect their antibacterial effects including amount and type of starch, rumen pH (effective fiber, type and amount of buffers, TMR mixing, etc), and fatty acid release rates from plant structure.

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Table 1. Average intakes of major unsaturated fatty acids by dairy cattle fed a TMR without and with added fat averaged across five published studies[1]

	DMI[2] kg/d	Diet FA[3] mg/g DM	18:1 g/d	18:2 g/d	18:3 g/d	RUFAL[4] g/d
Control (n=5)						
Mean	19.4	37.3	139	299	44	473
Min	12.0	18.6	53	133	26	220
Max	27.3	55.4	242	690	74	973
Fat Diets (n=21)						
Mean	19.4	59.5	280	371	56	696
Min	12.3	28.2	111	164	26	362
Max	25.7	83.5	571	710	88	1118

[1] Taken from Jenkins and Bridges (2007).
[2] DMI = Dry matter intake.
[3] FA = Fatty acid
[4] RUFAL = rumen unsaturated fatty acid load (g/day) = C18:1 + C18:2 + C18:3.

Table 2. Fatty acid concentrations (mg/g DM) reported in feed and rumen samples from three studies when cows were fed a basal diet with and without added oil

Reference/Diet	Feed	Rumen total	Rumen unsaturated
Loor et al. (2002)			
Basal (61% alfalfa/corn silage)	35.2	28.8	1.33
3.5% canola oil	61.4	48.2	2.42
3.5% soybean oil	63.8	48.9	1.69
Bateman and Jenkins (1998) nonlactating cows			
Basal (50% bermudagrass hay)	14.7	8.1	1.40
4% soybean oil	49.7	25.0	1.75
8% soybean oil	83.5	32.4	2.14
Sun and Gibbs (2012)			
High quality pasture	42.4	46.9	20.5

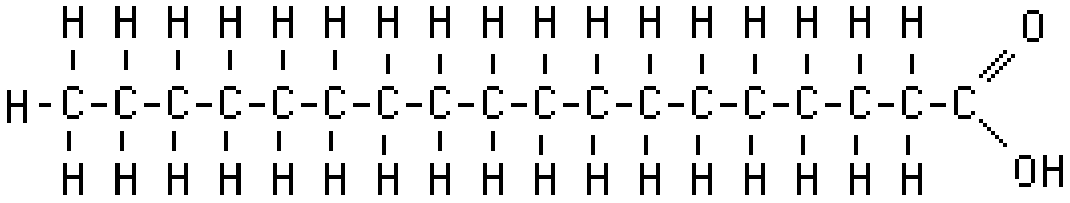

Figure 1. The structure of stearic acid; a saturated long-chain fatty acid.

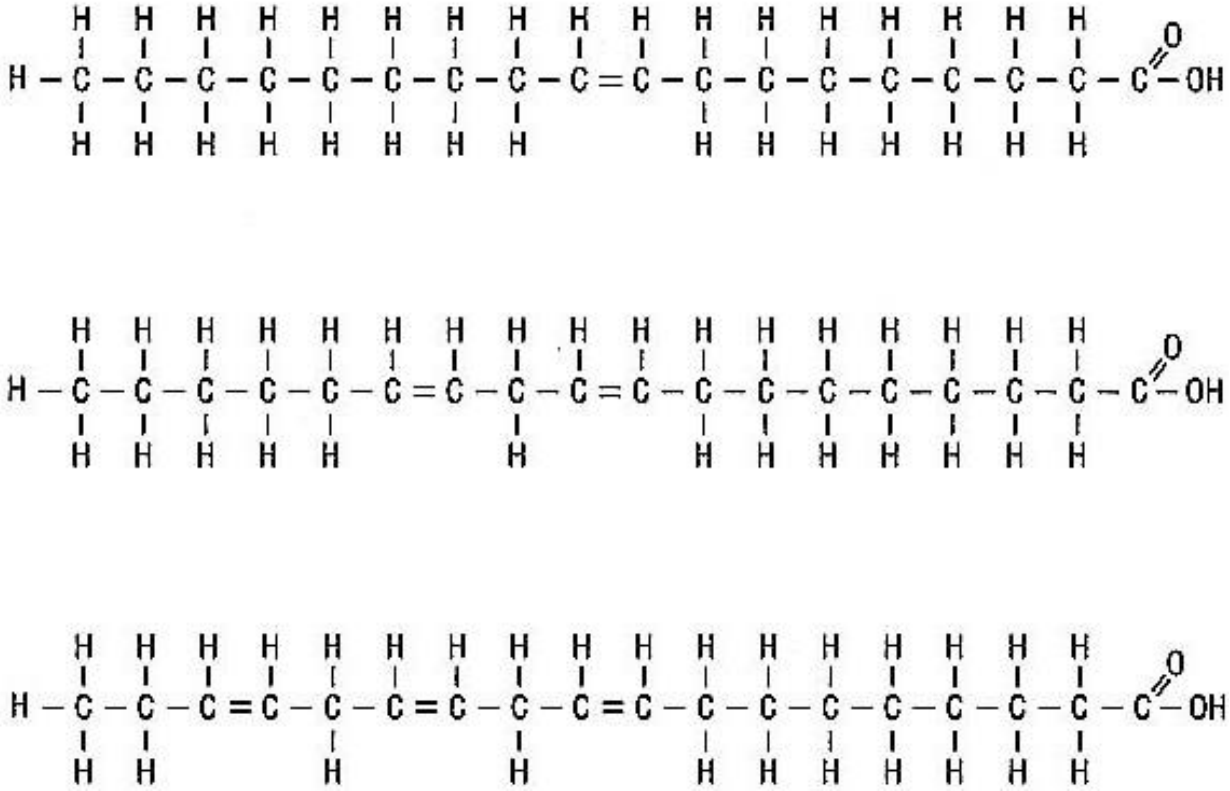

Figure 2. Structures of the three primary unsaturated fatty acids consumed by cattle, oleic acid (top), linoleic acid (middle), and α-linolenic acid (bottom).

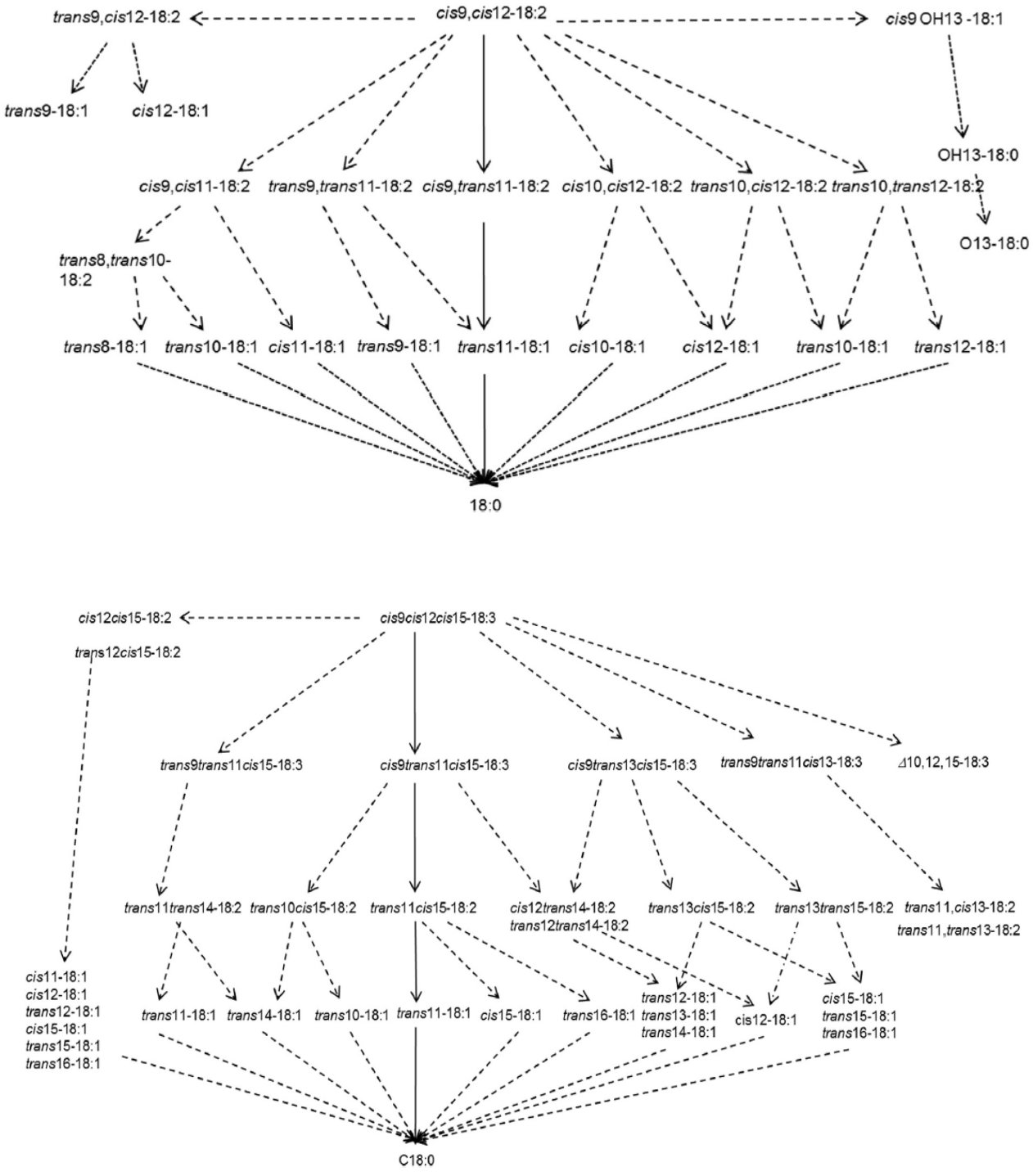

Figure 3. Proposed pathways of linoleic (top) and α-linolenic acid (bottom) biohydrogenation to stearic acid in ruminal contents proposed by Ferlay et al. (2017) illustrating the complexity and abundance of intermediates.

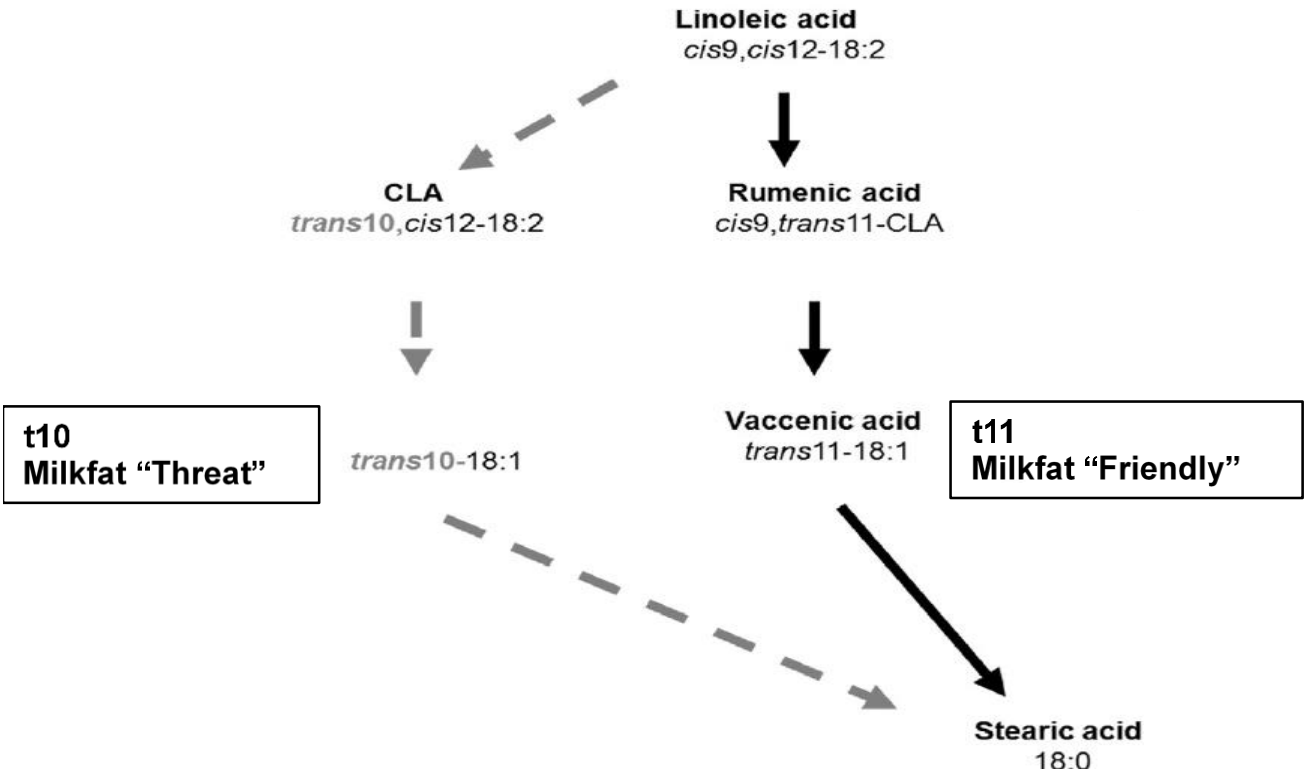

Figure 4. Simplified pathways of linoleic acid biohydrogenation emphasizing the major ***t*11 route** involving milkfat friendly intermediates and the minor ***t*10 route** involving intermediates known to inhibit milk fat synthesis by the mammary gland. Adapted from Ferlay et al. (2017).

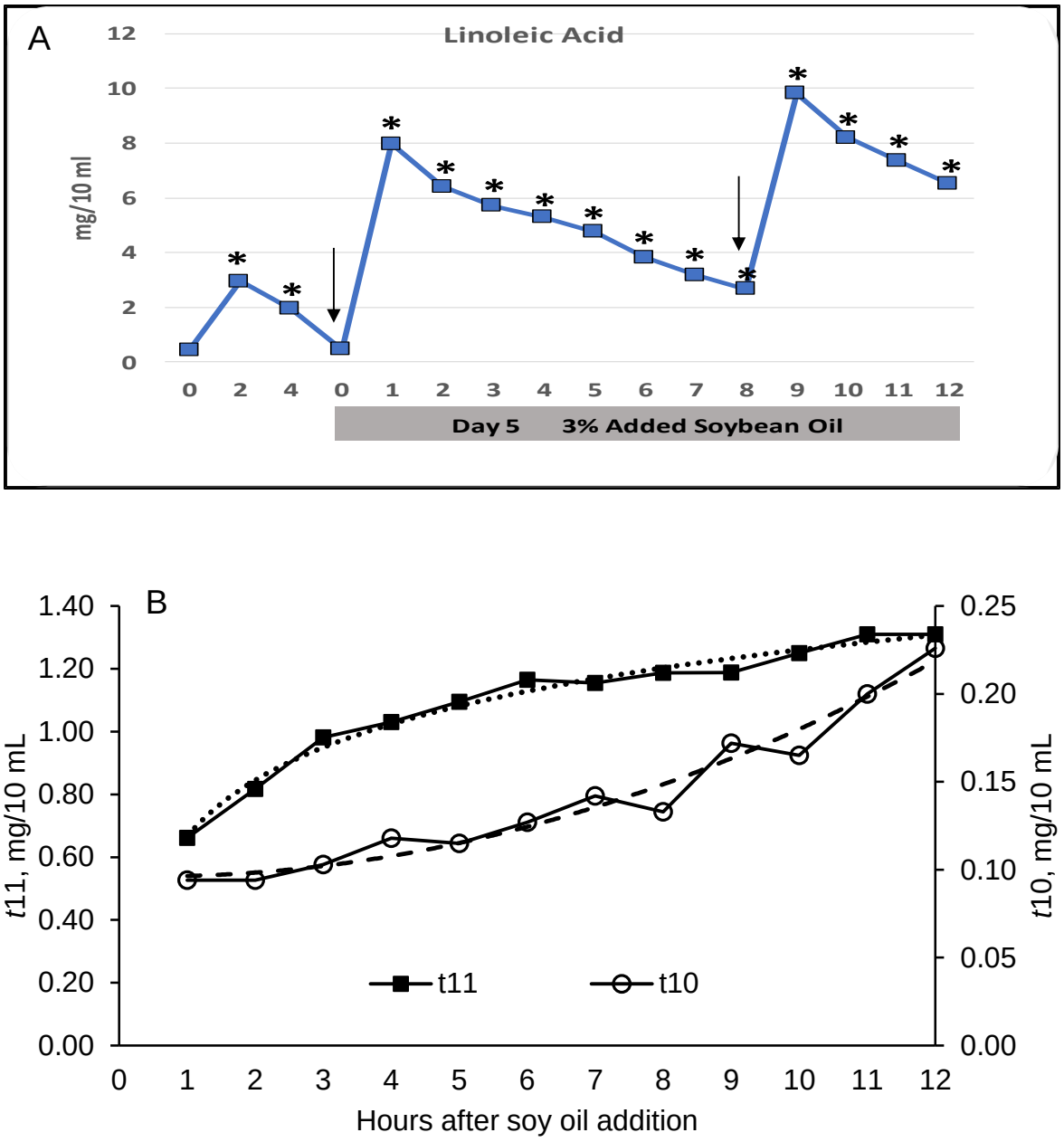

Figure 5. Concentrations of linoleic acid (A, mg/10 mL) and *trans* 18:1 isomers (B) in contents taken from continuous cultures of mixed ruminal bacteria. Cultures were fed a basal diet without soybean oil for 4 days then immediately switched to a diet containing 3% soybean oil on day 5. Top graph (A) shows linoleic acid concentrations on day 4 taken at 0, 2, and 4 hours after the morning feeding, and on day 5 taken hourly after soybean oil addition. Arrows on top graph (A) indicate when the diet containing soybean oil was introduced into cultures. Bottom graph (B) shows *t*11-18:1 and *t*10-18:1 concentrations taken hourly on day 5 after feeding soybean oil. Unpublished results from Clemson University.

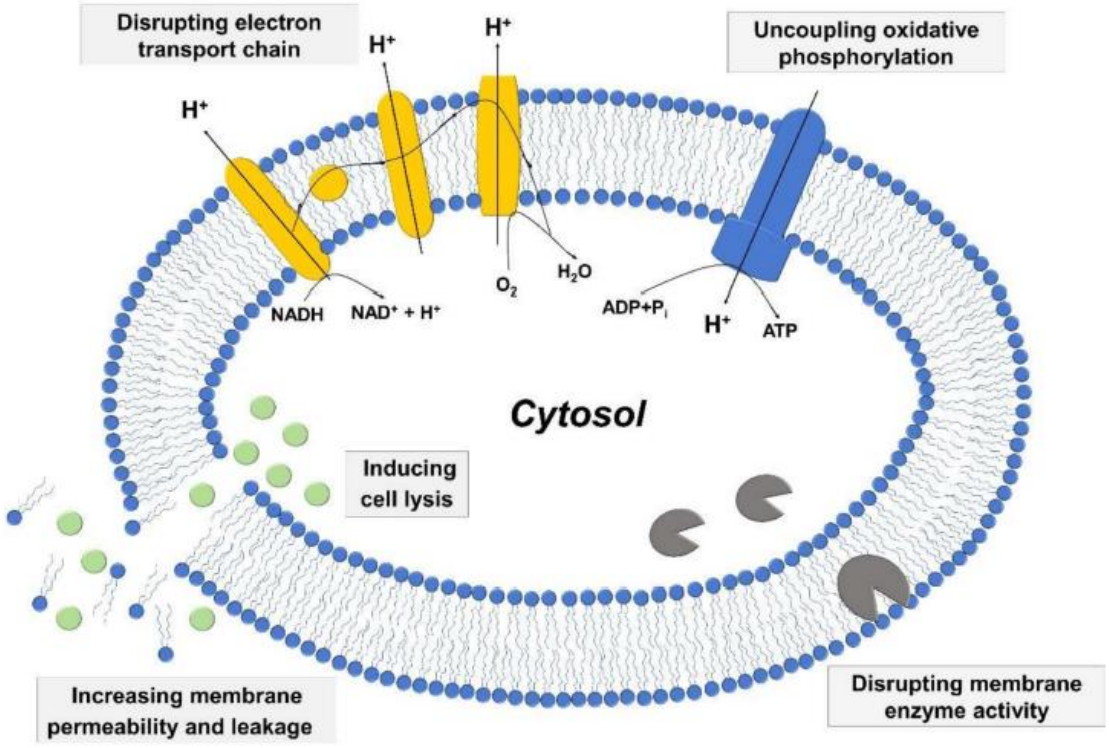

Figure 6. Mechanisms of antibacterial activity of fatty acids (Yoon et al., 2018). For ruminal microorganisms, fatty acids exert antibacterial properties through disorganization of the cytoplasmic outer membrane that can lead to increased membrane permeability and even cell lysis, inhibition of membrane enzymatic activity, and impaired nutrient uptake.

SESSION NOTES