Evaluation of lipid metabolism in the rumen

by

Lauren Dock

B.S., Colorado State University, 2020

A REPORT

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Animal Sciences and Industry
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2022

Approved by:

Major Professor
Evan Titgemeyer

Copyright

Abstract

Lipids as nutrient sources have been researched for many years. Dietary lipids can increase the efficiency of ruminant production and create a better quality of meat that has positive human health benefits. Lipid inclusion in rations increases dietary energy content. This review discusses biohydrogenation as a major pathway for lipids. Whereas unsaturated fatty acids are good for cattle, they are more toxic than saturated fatty acids to the bacteria in the rumen, and longer chain fatty acids have a higher toxicity; therefore biohydrogenation must take place (Lock et al., 2006). The process of biohydrogenation of fatty acids is to reduce the unsaturated fatty acids into saturated fatty acids using hydrogenation (Byers and Schelling, 1988). Biohydrogenation, which usually is greater than 80% of dietary unsaturated fatty acids, occurs in relation to fine food or feed particles, which allows for extracellular enzymes of bacteria to either associate with the feed or be free within the rumen (Harfoot and Hazlewood, 1997). The first step of biohydrogenation is isomerization to convert the *cis* configuration to the *trans* configuration. Conjugated linoleic acids are isomers of linoleic acid with conjugated double bonds separated by one single carbon-carbon bond, and the double bonds can be of cis or trans configurations (Lehnen et al., 2015). In dairy cattle, dietary lipids can improve efficiency of energy utilization for milk production, and in feedlot cattle dietary lipids can improve feed efficiency. This review of lipids in ruminants provides insight into how digestion, fatty acid metabolism, and microbial activity are affected when lipids are added to the diet.

Keywords: Biohydrogenation, fermentation, benefits, ruminant

Table of Contents

List of Abbreviations v
Acknowledgments vi
Dedication vii
Introduction 1
Background of Lipids 2
    Definition 2
    Functions 2
    Structure 2
    Fatty acid compositions 3
    Types of lipids 4
        Triglycerides 4
        Phospholipids 5
        Omega-3 fatty acids 6
        Bile salts 7
        Lipoproteins 8
Ruminal Fermentation 9
    Lipolysis 9
    Biohydrogenation 10
    Organisms 15
    Disruption of fermentation 16
    Hindgut fermentation 16
    Lipid fermentation 17
    Effects on rumen fermentation 17
    Recommendations 19
Composition of Lipids in Feed 20
Fatty Acid Absorption 23
Health Benefits of CLA 27
Conclusion 30
References 31

List of Abbreviations

Abbreviation 1: Long chain fatty acid (LCFA)

Abbreviation 2: Polyunsaturated fatty acid (PUFA)

Abbreviation 3: Triglyceride (TAG)

Abbreviation 4: Volatile fatty acid (VFA)

Abbreviation 5: Eicosapentaenoic **(**EPA)

Abbreviation 6: Docosahexaenoic acid (DHA)

Abbreviation 7: Alpha-linolenic acid (ALA)

Abbreviation 8: Conjugated linoleic acid (CLA)

Abbreviation 9: Free fatty acid (FFA)

Abbreviation 10: *Trans* fatty acid (TFA)

Acknowledgments

First, I would like to acknowledge my committee members, Dr. Micheal Brouk and Dr. A.J. Tarpoff. These two professors were there to support me and guide me through my time at K-State. Without their encouragement, support, and mentorship, I would not be where I am today. I would also like to thank my advisor, Dr. Evan Titgemeyer, for helping me and supporting me when I needed it the most. You had faith in me that I could move forward and conquer anything. I have learned valuable life and career skills through this journey. I want to thank my friends who I have made here that have guided, taught, and accepted me through this journey. I would also like to thank my boyfriend, Ross, for standing by my side through all the ups and downs and coming to my rescue when I needed him the most; I am thankful Kansas brought us together.

Lastly, I would like to thank my family who have been supportive and continued to show me love throughout this journey and in life. My mother, Kathy, my father, Scott, and my sister, Kallie, have pushed me to be the best person I can be, and I am thankful I have them to always call when I need a good laugh or advice. You believed in me when at times it was hard to believe in myself and pushed me to be the best student and person I could be. Thank you for encouraging me to follow my dreams and to keep my head high.

Dedication

I would like to dedicate this thesis to Mom, Dad, and Kallie, as well as my grandparents who are no longer with me: Grandma Trudy, Grandpa Stan, Papa Kenny, and Grumpy Al, as I know they are looking down on me and cheering me on. Finally, I would like to dedicate this thesis to my Grammy, Aunt Dona, and Uncle Myron. To you all, thank you for helping me to just keep swimming

Introduction

Through years of research, lipids have been a main topic for the cattle industry. Lipids, or fats, are needed for cattle to grow, reproduce, and maintain body weight. Without lipids, cattle cannot grow. Types of lipids, which will be discussed through this report, include triglycerides (TAG), which are the most predominant of fats. A common lipid is Omega-3 fatty acids, which are named after where the double bond is located. This is an unsaturated fatty acid, which can be toxic to the ruminal microbes if not hydrogenated. Omega-3 and omega-6 unsaturated fatty acids are not made by the body and must be provided through animals' diets. In this report, the processes of biohydrogenation and lipolysis will be discussed to describe how fats are metabolized. I will also discuss how digestibility and end-products are affected by lipid metabolism. There are advantages and disadvantages of including lipids in the diet, including post-absorptive effects on human health. This can include anti-obesity effects and decreases in fat accumulation, among more health benefits. The importance of conjugated linoleic acids (CLA) will be discussed as well. The effects of the type of diet and how fat can affect fermentation will also be covered. Finally, intestinal fatty acid absorption will be discussed, which will tie to the health benefits to humans when consuming meat.

Background of Lipids

Definition

Lipids are water-insoluble biochemicals that have many important biological roles including energy storage, function as signal molecules, structural components of cell membranes, and storage in different ways (Jarvis and Moore, 2010). The word lipid is derived from the Greek word *lipos*, which means fat (Voet et al., 2016). Lipids are soluble in organic solvents, such as chloroform and methanol, and are easily separated from other biological materials by extraction into organic solvents (Voet et al., 2016). Lipids are the fourth major group of molecules found in all cells (Voet et al., 2016). Fatty acids rarely exist naturally as a free form, but are linked to different molecules, mainly attaching to glycerol (Voet et al., 2016).

Functions

Lipids cluster and perform central functions as a structural matrix of biological membranes (Voet et al., 2016). There are three major biological functions of lipids (Voet et al., 2016). The first is they can form lipid bilayers that are essential components of biological membranes. The second is lipids contain hydrocarbon chains that serve as energy stores. And finally, in many situations, there are intracellular and intercellular signaling that involves lipid molecules.

Structure

The structure of fatty acids is a long chain hydrocarbon side group on a carboxylic acid, and the esterified form is a major component of various lipids (Voet et al., 2016).

Fatty acids are described by the number of carbon atoms and whether the chain is straight or branched and saturated (anoic acid) or unsaturated (enoic acid), and most chains are straight with an even number of carbons (Voet at al., 2016). For plants and animals, the most common

chain length is between 12 and 24, but they can span from two to 80 carbons long (Voet et al., 2016). Two to six carbons are considered short-chain fatty acids, eight to 12 are medium chain fatty acids, and anything over 12 carbons are long chain fatty acids (LCFA) (Voet et al., 2016). Plants and animals predominantly have 16- or 18-carbon fatty acids, which include palmitic, oleic, linoleic, and stearic acids (Voet et al., 2016). Unsaturated fatty acids contain double bonds within the configuration, and polyunsaturated fatty acids (PUFA) contain two or more double bonds and are a type of lipids plants and animals have. Saturated fatty acids are fully reduced and are highly flexible molecules that have a large range of conformations and free rotation around each of the C-C bonds (Voet et al., 2016). The lowest energy conformation is a fully extended conformation with the least amount of steric interference between methylene groups (Voet et al., 2016). The melting point of each carbon chain increases with the molecular mass (Voet et al., 2016). The double bonds in fatty acids can have a *trans* configuration or a *cis* configuration, which is the natural form, which creates an angled bend in the hydrocarbon chain making the unsaturated fatty acids pack together less efficiently than the saturated fatty acids due to the bond properties (Voet et al., 2016). The number of double bonds and their positions determine their biological activity (Voet et al., 2016). There is a reduced van der Waals interaction with unsaturated fatty acids, which results in their melting points decreasing with the degree of unsaturation (Voet et al., 2016). The increased fluidity of lipids with degree of unsaturation improves functionality of biological membranes (Voet et al., 2016).

Fatty acid compositions

Data on lipid digestion from 20 trials where total and individual fatty acid intakes and duodenal or omasal flows were measured, and 14 had calculations of intestinal digestibility of individual fatty acids (Lock et al., 2006). Lactating dairy cows were used in all of the studies,

and digestibilities were based on measurements between the duodenum and ileum or the duodenum and feces (Lock et al., 2006). Over the years of analytical procedures there has been advancement in understanding fatty acid compositions. There are complex patterns of fatty acids produced in the rumen and then incorporated into meat and milk fat (Lock et al., 2006). Lock et al. (2006) evaluated specific fatty acids, and they grouped 18:1 fatty acids together. Intake fatty acids were primarily oleic acid (*cis*-9 18:1), whereas rumen metabolism had mostly *trans* 18:1 fatty acid and a smaller amount of *cis* 18:1 fatty acids. The fatty acid composition of alfalfa hay has roughly 34% of the fatty acids as 16 carbons, 3.8% as 18:0, 3% as 18:1, 24% as 18:2, and 24% as 18:3 (Katz and Keeny, 1966). The ruminal contents one hour post feeding of hay indicated that stearic acid (18:0 fatty acid), increased to 41%, whereas 18:1 and 18:3 decreased to around 7%, 18:2 decreased to about 4%, but 16-carbon fatty acids stayed at 33% (Katz and Keeny, 1966).

Types of lipids

There are multiple types of lipids. They include polar and neutral lipids, TAG, sterols, and free fatty acids (FFA) (Jarvis and Moore, 2010). Vertebrates can synthesize most of the PUFA, except for linoleic acid and alpha-linolenic acid (ALA), which must be obtained through the diet and are the pathway precursors for biosynthesis of n-3 and n-6 PUFA (Jarvis and Moore, 2010). The structural lipids are important for maintaining structure and serving as cell membrane components. These include polar lipids, phospholipids, and glycolipids (Jarvis and Moore, 2010).

Triglycerides

TAG, which are largely composed of fatty acids, are lipids that often have high a saturated fatty acid content and some unsaturated fatty acids (Voet et al., 2016). TAG are simple

lipids containing fatty acids that are linked with an ester bond to a glycerol, which is a 3-carbon compound (Voet et al., 2016). They are nonpolar, water insoluble fatty acid triesters of glycerol (Voet et al., 2016). They function as energy storage in animals and are the most abundant class of lipids (Voet et al., 2016). Their fatty acid compositions are different in each organism that produces them (Voet et al., 2016). Plant oils are usually more abundant in unsaturated fatty acid than animal fats, with a lower melting point of oil (Voet et al., 2016). Fats are very efficient in storing metabolic energy and acting as an energy reserve (Voet et al., 2016). TAG are more reduced than carbohydrates or proteins, and they yield more energy per unit mass upon complete oxidation. In animals, the fat cells, also referred to as adipocytes, are specialized for the synthesis and storage of TAG, and can be almost entirely filled with fat globules (Voet et al., 2016). Adipose tissue is mostly in the subcutaneous layer and in the abdominal cavity (Voet et al., 2016). In humans, approximately 90% of the dietary lipids are made up of TAG and are a major form of metabolic energy storage (Voet et al., 2016). Most fats consumed by ruminants in diets are TAG, and lipolysis in the rumen releases FFA, which can affect bacteria; this will be further discussed in another chapter.

Phospholipids

Phospholipids are membrane lipids that are amphipathic molecules made of 1,2-diacylglycerol or N-acylsphingosine (ceramide) linked to a polar head group that is a carbohydrate or a phosphate ester (Voet et al., 2016). Phospholipids are important structural components of brain and nervous tissue, membranes throughout body tissues, and lipoproteins (National Research Council, 1989). Phospholipids can be synthesized in the body and are not normally required in the diet (National Research Council, 1989).

The polar end of the phospholipid molecule is the phosphate and an alcohol and is attracted to water (i.e., hydrophilic) (Britannica, 2022). The other end consists of the fatty acids, which are neutral, hydrophobic, water insoluble, and fat soluble (Britannica, 2022). Phospholipids form two-layer structured membranes that make up a lipid bilayer (Britannica, 2022). The polar head faces out to interact with water, while the fatty acid tails are inward and face each other (Britannica, 2022). All cell membranes have this lipid bilayer, which is impermeable to ions and most polar molecules due to its properties (Britannica, 2022).

Omega-3 fatty acids

Omega-3 fatty acids are a type of LCFA that are metabolized and utilized in the rumen. Omega-3 fatty acids are characterized by their carbon length and where the double bond is placed, as they are PUFA. The last carbon, which is a methyl group, is the omega carbon (Gammone et al., 2019). In the omega nomenclature system, double bonds are counted from the methyl group, so the omega-3 fatty acids have a double bond on the third carbon (Gammone et al., 2019).

There are two important "omega fatty acids" for ruminants. ALA is an omega-3 fatty acid, whereas linoleic acid is an omega-6 fatty acid (Voet et al., 2016). ALA is the parent fatty acid to n-3 PUFA (Sinclair et al., 2002). ALA is an essential fatty acid for mammals because they cannot insert double bonds in 18 carbon PUFA between the omega end and the middle of the molecule (Sinclair et al., 2002). The position of the first double from the methyl end of the molecule has led to ALA being characterized as an n-3 PUFA (Sinclair et al., 2002).

Beta oxidation is the major catabolic route of metabolism for ALA in the body, with the higher proportion of PUFA in the diet creating a higher rate of beta-oxidation of ALA (Sinclair et al., 2002). Another major pathway of metabolism is carbon recycling, which involves

lipogenesis in the brain and other tissues (Sinclair et al., 2002). When ALA is fed to animals, much of it ends up in the brain as saturated and unsaturated fatty acids and cholesterol (Sinclair et al., 2002). The third and main metabolic pathway for ALA is its role as a precursor to eicasopentanoic acid (C20:5; EPA) and docosahexanoic acids (C22:6; DHA) (Sinclair et al., 2002).

Bile salts

Bile acids or salts are amphipathic detergent-like molecules that solubilize fat globules by dispersing them into micelles (Voet et al., 2016). They are derived from cholesterol and synthesized by the liver, where they are secreted as glycine or taurine conjugates into the gallbladder for storage or secreted into the small intestine as an aid to lipid digestion and absorption (Voet et al., 2016). Pancreatic lipase catalyzes the hydrolysis of triacyclglycerols, and its activity is at the lipid-water junction. It requires phosphatidylcholine, bile acids, and pancreatic colipase protein that forms a complex with lipase (Voet et al., 2016). Bile salts and fatty acid binding proteins facilitate intestinal absorption of lipids, where the digestion of lipids with the help of bile acids is important for absorption of digestion products (Voet et al., 2016). A mixture of fatty acids, monoacylglycerols, and diacylglycerols are produced through lipid digestion, and fatty acids and monoglycerides are absorbed by the cells lining the small intestinal mucosa (Voet et al., 2016). Micelles formed by bile salts can incorporate nonpolar lipid degradation products to allow the transport across the aqueous boundary layer of the intestinal wall (Voet at el., 2016). When bile ducts are obstructed, the intestine absorbs little ingested lipids, but fatty acids are eliminated in the hydrolyzed form in feces (Voet et al., 2016). The fatty acid products of lipid digestion are absorbed by intestinal mucosa and transported to other tissues

for catabolism or storage; they are predominantly transported as lipoproteins such as chylomicrons and very-low density lipoproteins (Voet et al., 2016).

Lipoproteins

Lipoproteins are globular micelle-like particles that have a nonpolar core of TAG and cholesteryl esters surrounded by an amphiphilic coating of protein, phospholipid, and cholesterol (Voet et al., 2016). Intestinal mucosal cells convert fatty acids to TAG and package them with cholesterol into lipoproteins called chylomicrons (Voet et al., 2016). The chylomicrons are released into intestinal lymph and transported through the lymphatic vessels before draining into large veins (Voet et al., 2016). Very low-density lipoproteins, intermediate density lipoproteins, and low-density lipoproteins are synthesized by the liver to transport internally produced TAG and cholesterol from the liver to tissues (Voet et al., 2016). High-density lipoproteins transport cholesterol and other lipids from the tissues back to the liver, which is the only organ in the body that can dispose of significant amounts of cholesterol (Voet et al., 2016). FFA are released from adipocytes into blood stream and attach to serum albumin, which transports nonpolar substances including hormones and drugs (Voet et al., 2016).

Ruminal Fermentation

Lipolysis

Lipolysis is a metabolic process that breaks TAG into glycerol and FFA from esterified plant lipids (Jenkins, 1993). Lipolysis occurs very quickly with intermediates of mono- and digalactosylglycerol, which are not detectable in significant amounts in the rumen (Jenkins, 1993). There is minimal loss of fatty acids from the rumen from either absorption across the ruminal epithelium or catabolism to volatile fatty acids (VFA) or carbon dioxide (Jenkins, 1993). Lipolysis releases glycerol and fatty acids in both the unsaturated and saturated forms. Saturated fatty acids can be incorporated into the microbial cells for biosynthetic processing, whereas unsaturated fatty acids can go through further transformation through biohydrogenation (Jarvis and Moore, 2010). Glycerol derived from the breakdown of TAG is rapidly fermented and the free glycerol concentration is low, meaning there is a large population of glycerol-dissimilating bacteria present in the rumen (Harfoot and Hazelwood, 1997).

Microbes synthesize fatty acids de novo from carbohydrate precursors (Jenkins, 1993). Microbial lipases hydrolyze esterified plant lipids after consumption, thus causing the release of constituent fatty acids (Jenkins, 1993). Lipolysis is followed by biohydrogenation, which reduces double bonds (Jenkins, 1993). The lipid enzymes (lipases) used throughout lipolysis are from bacteria and protozoa which are the most important microbes for biohydrogenation as well, with protozoa accounting for about 30% of total lipolysis; the role of fungi is not known and ciliated protozoa plays minor roles (Lourenço et al., 2010).

There are two major lipolytic organisms in rumen, *Butyrivbrio fibrisolvens* and *Anaerovibrio lipolytica* (Jenkins, 1993). *Anaerovibrio lipolytica* is known for its lipase activity and produces a cell-bound esterase and a lipase (Jenkins, 1993). The lipase is an extracellular

enzyme packed into membranous particles made up of protein, lipid, and nucleic acid (Jenkins, 1993). It hydrolyzes acylglycerol completely to FFA and glycerol with small amounts of mono- or diglycerides (Jenkins, 1993). Glycerol is fermented rapidly, creating propionic acid as a major end-product (Jenkins, 1993). Fatty acids are released from plant galactolipids and phospholipids when lipases are incubated in ruminal contents (Jenkins, 1993). Hydrolysis of these esterified lipids is connected to a variety of galactosidases and phospholipases, including phospholipase A, phospholipase C, lysophospholipase, and phosphodiesterase, which are produced by ruminal microbes (Jenkins, 1993).

A reduction in lipolysis has been recorded with diets low in protein and high in starch, and this response is possibly pH mediated (Gerson et al., 1983). Detrimental properties of lipids can negatively impact fermentation. Unsaturated fatty acids inhibit fermentation more than saturated fatty acids (Jenkins, 1993). More double bonds within a structure creates more angles therefore leading to less flexibility and different melting points. There is an impact of free carboxyl groups that inhibit fermentation, such that fatty acid derivatives such as calcium salts of LCFA, fatty alcohols, fatty acyl amides, and TAG inhibit fermentation less than FFA (Jenkins, 1993). Commercially produced fats from the industry can reduce or eliminate problems with fermentation and digestion (Jenkins, 1993). These ruminally inert fats include calcium salts of LCFA and fats protected by encapsulation (Jenkins, 1993). Calcium salts of LCFA have no toxicity because formation of insoluble complexes from cations and LCFA eliminates free carboxyl groups (Devendra and Lewis, 1974).

Biohydrogenation

Unsaturated fatty acids are toxic to many rumen bacteria, therefore biohydrogenation must take place. Unsaturated fatty acids are more toxic than saturated fatty acids, and longer

chain fatty acids have a higher toxicity (Lock et al., 2006). The toxicity of LCFA requires a free carboxyl group (Lock et al., 2006). Biohydrogenation requires a FFA, with the result that rates of biohydrogenation are always less than rates of hydrolysis, and the factors that affect hydrolysis also affect biohydrogenation (Lock et al., 2006). For biohydrogenation to occur, there also needs to be hydrogen to reduce cofactors, including NAD(P)H, FADH, and FDH (Shingfield and Wallace, 2014). The process of biohydrogenation of fatty acids is to reduce the unsaturated fatty acids, which contain double bonds, into saturated fatty acids that do not have double bonds, using hydrogenation (Byers and Schelling, 1988).

The goal of biohydrogenation is to use hydrogen to keep the reaction going and dispose of the hydrogen because there are no water molecules present to take up the hydrogen atoms (Jarvis and Moore, 2010). The second goal is to detoxify because unsaturated fatty acids are toxic to bacteria (Jarvis and Moore, 2010). Unsaturated FFA have a short half-life in ruminal contents because they are quickly hydrogenated by microbes to become saturated end-products (Jenkins, 1993). The role of the short half-life and biohydrogenation is to protect microbes from the potential toxic effects of unsaturated fatty acids (Jenkins, 1993). Biohydrogenation, which usually is greater than 80% of dietary unsaturated fatty acids, occurs in relation to fine food particles, which allows for extracellular enzymes of bacteria to either associate with the feed or be free within the rumen (Harfoot and Hazlewood, 1997).

The first step of biohydrogenation is isomerization to convert the cis configuration to the *trans* configuration. The *cis*-Δ12 double bond in unsaturated fatty acids is converted to the *trans*-Δ11 isomer by the action of an isomerase, and hydrogenation of the *cis*-Δ9 bond in C18:2 occurs by microbial reductase (Jenkins, 1993). This all depends on conditions and how much is hydrogenated (Jenkins, 1993). The extent of hydrogenation of C18:2 or 18:3 is high at 60 to 95%

of for linoleic acid and 80 to 100% for ALA acid (Doreau and Ferlay, 1994). Hydrogenation is lower in grain-based diets, and it could occur at lower percentages because of reduced lipolysis in animals fed high-grain diets (Doreau and Ferlay, 1994). When fed low-fiber diets, *trans*-10 C18:1 is the main *trans* fatty acid (TFA) (Dewanckele et al., 2020). The higher grain diets lead to lower ruminal pH, and the lower pH leads to a lower percentage of fatty acids being hydrogenated (Doreau and Ferlay, 1994). As pH influences hydrogenation of fats, the intermediate products accumulate more when ruminal pH is more acidic (Van Nevel and DeMeyer, 1996). Many of these accumulated products are positional and stereoisomers of monoenoic and dienoic acids, mainly with diets high in fat (Van Nevel and DeMeyer, 1996). Important intermediates are cis-9, *trans*-11 CLA and TFA (Van Nevel and DeMeyer, 1996). *Trans* C18:1 is one of the most important quantitatively and has an important role in milk fat depression syndrome (Van Nevel and DeMeyer, 1996). Although there has been extensive research on other aspects of fatty acids and their length, research on C20 and C22 fatty acids is limited and has yielded conflicting outcomes (Van Nevel and DeMeyer, 1996). Bacteria and protozoa can synthesize LCFA, but they normally synthesize LCFA in the 16 to 18 carbon length (Abedi and. Sahari, 2014). Diets rich in fat allow bacteria to store fatty acids in large amounts, which includes mostly linoleic acid, as free acids (Abedi and. Sahari, 2014).

During lipolysis, lipids, including TAG, phospholipids, and galactolipids, generate ALA, linoleic acid, and gamma-linolenic acid (Jenkins, 1993). This occurs with the actions of lipase, phospholipase, and galactosidase enzymes. Byproducts of lipolysis include phosphate, glycerol, and galactose (Jenkins, 1993). There are two pathways to create stearic acid, one that makes vaccinic acid and the other that makes *trans*-10 C18:1. Vaccenic acid is a common intermediate for both linoleic and ALA (Jenkins, 1993). It is created from ALA when it is converted to

conjugated triene with linolenic isomerase and metabolized with hydrogen and hydrogenase to *trans*-11, *cis*-15 C18:2 (Salsinha et al., 2018). The other way vaccenic acid is made is when gamma-linolenic acid is converted to conjugated triene with linoleic isomerase and then hydrogenated to *cis*-6, *trans*-11 C18:2 (Salsinha et al., 2018). These reactions use hydrogenase and hydrogen to make vaccenic acid and then further hydrogenation converts it to stearic acid (Salsinha et al., 2018). There is another pathway that follows the same pattern, but uses *cis*-9, *trans*-10 isomerase for the second step, and this produces *trans*-10 C18:1 instead of vaccenic acid; this pathway also can yield stearic acid (Salsinha et al., 2018). As described in the two previous pathways, stearic acid, C18:0, is the major end-product of hydrogenation. The rates of biohydrogenation of fatty acids are typically faster with increasing unsaturation, and for most diets linoleic acid and ALA are hydrogenated to the extent of 70 to 100% (Lock et al., 2006). Thus, ruminal microbial metabolism of dietary unsaturated fatty acids in the rumen results in stearic acid being the major fatty acid entering the duodenum (Lock et al., 2006).

Linoleic acid is converted to conjugated linoleic acid (CLA) with linoleic isomerase (Salsinha et al., 2018). Bacterial *cis*-9, *trans*-10 isomerase forms trans-10, *cis*-12 CLA as the first step in the process leading to formation of *trans*-10 C18:1; this enzyme was discovered through the presence of TFA in low-fiber diets (Dewanckele et al., 2020). Only linoleic acid can form CLA (Dewanckele et al., 2020). Linoleic acid is one of the most common fatty acids present in diets of dairy cows, though the intake varies. A fraction of dietary linoleic acid is available for absorption (Lock et al., 2006). Very little stearic acid (18:0) is typically consumed, but there is an increase in stearic acid flow to the duodenum because it is the end-product of biohydrogenation of all 18-carbon PUFA (Lock et al., 2006). The formation of *trans*-11 18:1 and

cis- 9, *trans*-11 CLA comes from the pathways of biohydrogenation, but does not explain the other fatty acid intermediates identified in rumen digesta and milk fat (Lock et al., 2006). CLA is the intermediate product of linoleic acid biohydrogenation in rumen and is produced in the rumen as a product of incomplete hydrogenation (Bauman et al. 2003). CLA is a mixture of positional and geometric isomers of linoleic acid with conjugated double bonds separated by one single carbon-carbon bond, and the double bonds can be of cis or trans configurations (Lehnen et al., 2015). CLA isomers have been demonstrated to inhibit chemically induced tumorigenesis *in vitro*, reduce arthrosclerosis risk, and enhance immune response (Jarvis and Moore, 2010). *Cis*-9, *trans*-11 CLA, rumenic acid, is an isomer that is an intermediate product of bacterial biohydrogenation (Jarvis and Moore, 2010). *Cis*-9, *trans*-11 CLA is the major form of CLA in the rumen (Wallace et al., 2007). TFA are intermediates formed in the rumen during biohydrogenation of C18 unsaturated fatty acids (Wallace et al., 2007). *Trans*-C18:1 is higher in milk fat of cows with milk fat depression syndrome (Gaynor et al., 1995). Milk fat depression syndrome is a decrease in the fat content in milk in healthy cows, with the reduction of milk fat concentration and fat yield being almost 50% or more with no change in lactose or protein content of milk (Ronning and Laben, 1966). TFA inhibit fat synthesis by influencing regulation of key lipogenic enzymes in the mammary gland (Gaynor et al., 1995). Microbial lipid metabolism plays a major role in regulating the overall lipid composition, including milk and tissues, of ruminant animals (Harfoot and Hazelwood, 1997).

The use of antimicrobials may allow more fat to be fed to ruminants without disruption of ruminal fermentation and digestion (Jenkins, 1993). Ionophores slow down lipolysis *in vitro* (Van Nevel and DeMeyer, 1995). Also, it is important to regulate microbial biohydrogenation to

allow absorption of selected fatty acids that could increase performance or improve nutritional qualities of animal food products (Jenkins, 1993).

Organisms

Kemp and Lander (1984) classified the bacteria involved in biohydrogenation into two groups, A and B, based on the metabolic pathways with which they are involved. For complete biohydrogenation of PUFA, bacteria from both groups are required. Group A contains bacteria that can hydrogenate PUFA to *trans* 18:1 fatty acids, and very few species in group B can hydrogenate a *trans* 18:1 fatty acid to stearic acid (Harfoot and Hazlewood, 1997). Increased feeding of PUFA could cause an increase in rumen concentration of monounsaturated fatty acids and a decrease in saturated fatty acids (Lock et al., 2006). Most bacteria isolated are in group A, which cannot hydrogenate octadecenoic acids, but hydrogenate linoleic acid and ALA to trans-octadec-11-enoic acid and related isomers (Kemp and Lander, 1984). In this group, there are *Ruminococcus albus* and *Butyrivibrio* species. Only three bacteria are in the group B and are characterized by their ability to hydrogenate many octadecenoic acids, including oleic acid, trans-octadec-11-enoic acid and linoleic acid, to stearic acid (Kemp and Lander, 1984). The products from ALA are predominantly cis- and trans-octadec-15-enoic acids which are not further hydrogenated even by mixed rumen micro-organisms (Kemp and Lander, 1984). Group B contains *Fusocillus babrahamensis, Fusocillus* species and Gram-negative rods (Kemp and Lander, 1984).

There are possible mechanisms through which lipids inhibit microbial activity. There is a physical coating made of lipids on feeds, which prevent microbial attack (Devendra and Lewis, 1974). A physical coating on the microbial cells can also inhibit microbial activity, with a surface-active effect of lipids on cell membranes that can affect cell permeability for nutrient

transport (Devendra and Lewis, 1974). Another inhibition mechanism is formation of insoluble complexes from cations and LCFA, which reduces cations availability for microbes (Devendra and Lewis, 1974).

Disruption of fermentation

The relationship between ruminants and lipids can influence ruminal fermentation, either negatively or positively. Because lipids are water insoluble, digestion takes place at lipid-water interfaces, where the rate depends on surface area (Voet et al., 2016). Churning peristaltic movements of the intestine combined with the emulsifying action of bile salts increase digestion rate (Voet et al., 2016). The effects on digestion from dietary lipids can include disruption of fermentation, which leads to reduced digestibility of nonlipid energy sources and reduced production of methane, hydrogen, and VFA, and a lower acetate:propionate ratio (Jenkins, 1993). One possible mechanism is a reduction in the rate of fermentation of carbohydrates, particularly of fiber because of shifting the site of digestion from rumen to post-rumen (Palmquist, 1984). The second mechanism is a reduction in proteolysis. There also is an increase in bacterial biomass because of reduced ciliated protozoa (Ikwuegbu and Sutton 1982). There is an increased efficiency of bacterial protein synthesis because of the reduced ciliated protozoal and methanogen populations (Ikwuegbu and Sutton 1982).

Hindgut fermentation

Fat supplements inhibit ruminal fermentation which affects hindgut fermentation (Jenkins, 1993). There is limited hindgut fermentation that may decrease fiber digestibility overall in the digestive tract, while an increase in fecal fiber excretion occurs (Jenkins, 1993). Dietary fat can be less detrimental to digestibility of nonstructural carbohydrates than of structural carbohydrates. Structural carbohydrate digestion can be reduced 50% or more by less

than 10% added fat (Jenkins, 1993). Protein metabolism also is affected by fat supplements (Jenkins, 1993). Linseed oil in the rumen of sheep decreased protein digestion in the rumen and was paired with a decrease in ammonia concentration and increased nitrogen flow to the duodenum, which was similar to addition of lipids such as corn oil or lecithin to the diet (Ikwuegbu and Sutton, 1982). Blended fat sources have the potential to improve fermentation compared to single fat sources (Jenkins, 1993). Unsaturated FFA concentration in the rumen is regulated by the amount and type of fats fed, as well as the rates of reactions, such as lipolysis, biohydrogenation, and formation of carboxylate salts (Jenkins, 1993).

Lipid fermentation

Lipid fermentation goes through pathways in the rumen that make glycerol from TAG, galactolipids, and phospholipids.

High concentrations of TAG in the diet can increase the total lipid content in the rumen, but the increase in the ruminal pool of unsaturated FFA may be lower if lipolysis and biohydrogenation declines or if formation of carboxylate salts is high (Jenkins, 1993). Lipolytic rates are usually sufficient to convert most TAG in the diet to FFA within a short time. The rate of lipolysis and biohydrogenation are altered substantially by forage maturity and nitrogen contents and by feed particle size in the rumen (Jenkins, 1993). Rates of biohydrogenation *in vitro* depend on the concentration of substrates, age and type of microbial inoculum, and cofactors in ruminal fluid (Jenkins, 1993).

Effects on rumen fermentation

There are effects on ruminal fermentation from fat metabolism. In the rumen, there are changes and modifications of microbial populations that occur during fermentation of fats. Lipid metabolism has a major impact on the profile of fatty acids available for absorption and tissue

usage (Lock et al., 2006). In the rumen, the two vital processes are hydrolysis of ester linkages in lipids and biohydrogenation of unsaturated fatty acids (Lock et al., 2006). Hydrolysis of dietary lipids is mainly due to rumen bacteria as well as salivary and plant lipases, with very little by rumen protozoa and fungi (Lock et al., 2006). The rate and extent of hydrolysis can be altered, even though hydrolysis is usually greater than 85% (Lock et al., 2006). For examples, the scope of hydrolysis is reduced as the dietary level of fat is increased or when factors, such as low rumen pH, inhibit the activity and growth of bacteria (Lock et al., 2006).

Lipids influence the fermentation products produced as well. In relation to the bacteria that is present in the rumen, gram-positive bacteria are more sensitive to the changes and breakdown of fatty acids than gram-negative bacteria (Henderson, 1973). Fiber digesters and methane producers are also sensitive to ruminal fatty acids (Henderson, 1973). There is a reduction in methane production due to fatty acids 1) inhibiting methanogenic bacteria, 2) decreasing ciliated protozoa numbers, which will also lead to a decrease in methanogens, and 3) decreasing hydrogen availability because of the competition with biohydrogenation (DeMeyer et al., 1996). Dietary fat also reduces total VFA concentrations and sometimes reduces lactate and ammonia concentrations and the acetate:propionate ratio. On the other hand, dietary fat additions increase efficiency of microbial protein synthesis (DeMeyer et al., 1996).

There is manipulation of lipid fermentation that occurs in the rumen. It starts with TAG, including cereal grains, oil seeds, and fat supplements, which are broken down to glycerol and saturated fatty acids and cis unsaturated fatty acids by the enzyme lipase (Nagaraja et al.,1997). Glycerol is metabolized further to acetate, propionate, and butyrate (Wright, 1969). Saturated and unsaturated fatty acids can be used for microbial cell lipid synthesis or be used in separate paths (Nagaraja et al.,1997). Saturated fatty acids are largely an end-product, whereas

unsaturated fatty acids can be converted to conjugated linoleic acids and subsequently to TFA, or just directly converted to TFA (Nagaraja et al.,1997). Reductase and hydrogen work together to saturate fatty acids (Nagaraja et al.,1997).

Glycolipids are present in forages (Nagaraja et al.,1997) and are broken down by lipase and galactosidase to glycerol, galactose, cis unsaturated fatty acids, and saturated fatty acids (Nagaraja et al.,1997). The glycerol and galactose can be fermented to yield acetate, propionate, and butyrate. The way glycolipids are metabolized follows the pathways of TAG (Nagaraja et al.,1997).

Recommendations

There are recommendations to improve utilization of fats by ruminants. The use of fats with slow rumen release allows lipid hydrolysis to be slow, such that unsaturated fatty acids are generated at rates that allow them to be biohydrogenated to saturated fatty acids, which are less toxic to bacteria (Palmquist and Chalupa, 1986). An increase of dietary forage concentration improves feed lipids utilization. Increasing dietary calcium content to 0.9-1.0% and magnesium to 0.3% of diet dry matter for dairy cattle allows these cations to combine with the free carboxyl group of fatty acids to make them less toxic. The use of buffer to maintain normal ruminal pH can benefit fat utilization by allowing for the rumen environment to overcome effects of higher acid being produced and help resist changes in the rumen (Palmquist and Chalupa, 1986).

Composition of Lipids in Feed

Dietary lipids enter the rumen in many forms which depend on the diets provided to the animals. Concentrate-based diets have dietary lipids mainly in the form of TAG, whereas in forage-based diets the lipids are in the form of glycolipids and phospholipids (Harfoot and Hazelwood, 1997). Roughan and Bratt (1969) noted that the lipid content in forages is between 2 and 3% and grains are between 2 and 4%. For ruminants, each kilogram of dry matter consumed contains approximately 40 grams of ether-soluble material, which contains 40% fatty acids in forages and 70% fatty acids in grains, which yields about 16 to 28 g fatty acids/kg feedstuff (Jenkins, 1993). Roughan and Batt (1969) determined that overall forage contained 2-7% lipids, with less than 50% as fatty acids. Grass preservation, including hay making, decreases fat content because hay generally has less than 3% lipids (Roughan and Batt, 1969). Concentrates contained less than 5% lipids, except oil seeds, which contain 20-50% lipids on a dry matter basis (Roughan and Batt, 1969). Doreau et al. (1997) concluded that almost all fat sources contained mainly C16 and C18 fatty acids, but there was not a consistency between double bonds in the cis configurations. Fish oil is the only fat source that is rich in C20 to C22 unsaturated fatty acids, at about 30-53%, and the most important ones noted are EPA and DHA (Doreau et al., 1997). Glycerol, a gluconeogenic compound that can be converted into glucose in the liver, is a feed supplement has been researched over the years. When converted, glycerol could be as an important feed supplement to prevent ketosis in lactating dairy cows (Wright, 1969). Glycerol, also known as glycerine or glycerin, is the major byproduct of biodiesel from vegetable oil or animal fat (Wright, 1969). Biodiesel is created through the reaction of TAG with methanol or ethanol and a catalyst, producing fatty acid methyl or ethyl esters and glycerol as the byproduct (Wright, 1969). Glycerol byproduct is available as a feed supplement is an unprocessed

preparation that contains water, methanol, and phosphorus (Wright, 1969). Glycerol is quickly fermented in the rumen at about 0.07 μmole/mL/min (Wright, 1969). In the rumen, glycerol is fermented to acetate, propionate, butyrate, and carbon dioxide (Wright, 1969). Addition of glycerol *in vitro* decreased the FFA concentration due to reduced lipolysis and end-product inhibition or increased esterification of glycerol (Krueger et al., 2010).

The important marine sources of omega-3 fatty acids include algae and microalgae (Gammone et al., 2019). Omega-3 fatty acids are found in fish oil and have been proven to have human health benefits through EPA and DHA (Gammone et al., 2019). ALA can be found in many feed products, including flaxseeds, canola oil, soybeans, and pumpkin seeds (Gammone et al., 2019). ALA is found in plants, animals, zooplankton, phytoplankton, and marine species (Sinclair et al., 2002). It is found in the chloroplasts membranes where it is synthesized from linoleic acid, which can be derived from acetate (Sinclair et al., 2002).

Omega-3 fatty acids are stored in membrane phospholipids, are responsible for cellular functions, such as maintenance of the cell membrane structure, fluidity, signaling, and cell-to-cell interaction (Gammone et al., 2019). This includes regulation of ion flux in cardiac cells and regulation of gene expression through proliferation systems. Research indicates that omega-3 fatty acids help prevent myocardial infarction (Sinclair et al., 2002).

Composition of the basal diet impacts how fat source affects ruminal fermentation (Jenkins, 1993). Fats that normally inhibit fermentation and digestion can cause less inhibition when hay content is high in the basal diet (Jenkins, 1993). Changing nutrient composition of diets for animal production could alter the fatty acid profile of meat, milk, and eggs (Kris-Etherton et al., 2000). Animal feed supplemented with algae, fishmeal, or fish oil increases EPA and DHA concentrations in tissues (Kris-Etherton et al., 2000). Flaxseed and flax oil are good

sources of ALA (Kris-Etherton et al., 2000). ALA is a precursor for longer-chain PUFA as mentioned above. When feeding animals diets with elevated concentrations of omega-3 fatty acids, there could be increased amounts of ALA in eggs, milk, pork, chicken, and beef (Kris-Etherton et al., 2000). An obstacle can include the tendency of these fatty acids to oxidize, which leads to off flavors in food products (Kris-Etherton et al., 2000). There is the additional expense of supplementing animal feed with n-3 sources (Kris-Etherton et al., 2000). As of 2000, eggs were the only product on the market to have elevated omega-3 fatty acid concentration as a result of n-3 sources supplemented into the animal feed. Eggs were a source of EPA and DHA (Kris-Etherton et al., 2000). Eggs were targeted first because a large percentage of the n-3 content of the hen's diet is transferred to the egg yolk (Kris-Etherton et al., 2000). It is important to know how these fats can be incorporated into the diet for the animals and humans (Kris-Etherton et al., 2000). The extent to which n-3 fatty acids will be incorporated into meat depends on the amounts fed to animals raised for meat and the rate of lipid deposition in meat, which affects the amounts of both intermuscular and intramuscular fat (Kris-Etherton et al., 2000). Milk enriched with n-3 fatty acids is high in fat which is an undesirable trait for consumer trends as buying lower-fat milk is more desirable (Kris-Etherton et al., 2000). The best opportunity for n-3 fatty acid–enriched milk to enter the marketplace may be as butter and cheeses high in n-3 fatty acids (Kris-Etherton et al., 2000).

Fatty Acid Absorption

Fatty acid absorption occurs mainly in the jejunal region of the small intestine (Lock et al., 2006). Before fatty acid absorption can occur, lipid material is adsorbed onto feed particles to be solubilized into the aqueous environment (Lock et al., 2006). Bile and pancreatic secretions are added to the digesta in the duodenum (Lock et al., 2006). In ruminants, micelle formation must occur and is important for the efficiency of fatty acid absorption (Lock et al., 2006). Micellar solutions dissolve and solubilize water-insoluble fatty acids by placing shaped and charged molecules in the core or outer sheath of the micellar matrix (Freeman, 1984). Bile supplies bile salts and lecithin, and pancreatic juice provides phospholipase A2, the enzyme needed to convert lecithin to lysolecithin, and bicarbonate to raise the pH (Lock et al., 2006). Lysolecithin and bile salts remove fatty acids from feed particles and bacteria to form the micelle (Lock et al., 2006). Ruminant bile is predominantly taurine-conjugated bile acids, whereas in most other herbivores glycine-conjugated bile acids dominate (Noble, 1981). Due to the acidic conditions of the ruminant upper-small intestine, taurine-conjugated bile acids are partially ionized, and in the micelle, they can effect the solubilization of fatty acids (Noble, 1981). At low pH, taurine-conjugated bile acids are soluble and partly ionized, whereas glycine-conjugated bile acids are insoluble and unable to effect solubilization (Moore and Christie, 1984). The importance of the role of lysolecithin and bile salts has been noted in sheep where fatty acid absorption did not occur when bile secretion into the duodenum was blocked (Moore and Christie, 1984).

When micelles are formed, they transfer the water-insoluble lipids to the unstirred water layer of the intestinal epithelial cells of the jejunum, which is where fatty acids and lysolecithin are then absorbed (Lock et al., 2006). Amphiphiles or ‘swelling agents’ promote micelle

formation (Lock et al., 2006). In ruminants lysolecithin is the amphiphile involved in micelle formation, whereas in nonruminants the formation of the micelle comes from the interaction of monoglycerides and bile salts with fatty acids (Lock et al., 2006). Monoglycerides, short chain saturated fatty acids, unsaturated fatty acids, and phospholipids can invade and increase the size of the micelle, which is the swelling mechanism, and increase the capacity of the hydrophobic core (Loc et al., 2006). Non-polar solutes, such as long-chain saturated fatty acids, have non-amphiphilic characteristics and must be solubilized in the hydrophobic interior of the micelle (Freeman, 1984). This then increases the size of the micelle's hydrophobic core and increases the solubility of long-chain saturated fatty acids with a consequent improvement in their absorption (Freeman, 1984). Lysolecithin influences the micellar solubility of stearic acid and due to its ability to increase the solubility of stearic acid, it is more effective than other amphiphiles (Lock et al., 2006). Lysolecithin also can increase the distribution of stearic acid into the micellar phase and away from the particulate phase (Lock et al., 2006). After the secretion of bile and pancreatic juices into the duodenum, there is a significant increase of free and esterified unsaturated fatty acids into the lipid material in the small intestine (Noble, 1981). This is important because this could help with micelle formation and absorption of saturated fatty acids (Lock et al., 2006). In the intestinal epithelial cells, fatty acids are re-esterified into TAG and then packed into chylomicrons for transport in the lymph to the blood (Lock et al., 2006).

Ruminants absorb a greater proportion of fatty acids as FFA than nonruminants (Noble, 1981). In nonruminants, there is a wide separation in the digestibility of fatty acids (Freeman, 1984). Digestibility of individual fatty acids decreases as the length of the fatty acid increases but increases as more double bonds are present (Lessire et al., 1992). The average for total tract average digestibility was around 74%, whereas the range was 58-86% (Lock et al., 2006).

Doreau and Ferlay (1994) reported similar values for digestibility, ranging from 55-92%, without a relationship to the fatty acid intake. Differences in digestibility of individual fatty acids contribute very little to the extensive variation in total fatty acid digestibilities; most of the variation reflects differences among individual experiments (Lock et al., 2006). Stearic acid is the main fatty acid in the digesta and is the major contributor to total absorbed fatty acids (Lock et al., 2006). Free palmitic and stearic acids are poorly absorbed in nonruminants (Noble, 1981). There are some mild differences in the digestibility of individual fatty acids; mean digestibilities by ruminants for the fatty acids 16:0, 18:0, 18:1, 18:2, and 18:3 were 75, 72, 80, 78, and 77%, respectively (Lock et al., 2006). Doreau and Ferlay (1994) similarly observed mean digestibilities by ruminants of 77, 85, 83, and 76% for 18 carbon fatty acids with zero, one, two, and three double bonds, respectively. The absorbed fatty acids are close to the composition of the fatty acids entering the duodenum (Lock et al., 2006).

Rumen outflow of total fatty acids is similar in amount to dietary intake of fatty acids, although sometimes the total amount of fatty acids entering the duodenum can surpass fatty acid intake (Lock et al., 2006). This occurs when lipid intake is low, such as feeding ruminants forage-based diets, and bacterial lipid synthesis occurs in the rumen (Lock et al., 2006). Though, Noble (1981) noted that use of microbial lipids is extremely variable because of the effects of different diets on microbial synthesis. There is no significant absorption or modification of long chain or medium chain fatty acids that take place in the omasum or abomasum. The lipid material accessible for absorption in the small intestine is like the contents that are leaving the rumen (Moore and Christie, 1984). About 80-90% of FFA are attached to feed particles, and the remaining lipid contents are microbial phospholipids and small amounts of TAG and glycolipids from residual feed material that can be hydrolyzed by intestinal pancreatic lipases (Doreau and

Ferlay, 1994). The highly efficient absorption of saturated fatty acids in the ruminant versus nonruminant animal can be explained in multiple ways. As fatty acid intake increases from 0 to 1600 g/d, fatty acid duodenal flow increases to 1600 g/d, which indicates similarities between these two measures (Lock et al., 2006). The ability of ruminants to absorb fatty acids is higher than non-ruminants (Noble, 1981). The way the lipids in digesta interact with the small intestinal environment differs between animal types (Noble, 1981). Ruminants have a slow and continuous release of small amounts of fatty acids into the duodenum with most of the lipids in the form of FFA, whereas nonruminants mainly have esterified fatty acids entering the small intestine (Lock et a., 2006). Another reason for the difference is the lesser neutralization of the acidic digesta when it passes through the duodenum of the ruminant, which is a result of low concentration and rate of secretion of bicarbonate from the ruminant pancreas (Moore and Christie, 1984). The digestion and metabolism of dietary fats and fatty acids is extensive in the rumen, and the dietary unsaturated fatty acids leaving the rumen are mostly FFA that have been biohydrogenated (Lock et al., 2006).

Health Benefits of CLA

Lipids have health benefits for both ruminants as well as people who consume milk or beef. It is now recognized that fatty acids, both dietary and those produced in the rumen, could have specific and strong effects on ruminant metabolism and human health (Lock et al., 2006). The use of dietary fat supplements has increased as nutritionists aim to increase the energy density of diets to meet requirements, especially for high producing dairy cow (Lock et al., 2006). With the increased interest in nutrition, lipid metabolism in ruminant digestion could be useful in diet formulations, decision making, and recommendations (Lock et al., 2006). Positive health benefits have been correlated with CLA, an intermediate of ruminal biohydrogenation of fatty acids, including anti-obesity effects, decreases in fat accumulation, anti-diabetic effects, anti-atherosclerosis effects, enhanced bone mineralization, and modulation of immune system, such as anti-asthmatic effects (Pariza, 1997). The production of biohydrogenation intermediates is interesting due to the effects of individual fatty acids on ruminant metabolism and human health (Lock et al., 2006). In milk fat, there are *trans*-11 18:1 and *cis*-9, *trans*-11 CLA, which have anti-carcinogenic and anti-atherogenic properties in animal models of human health. *Trans*-10, *cis*-12 CLA is a regulator of milk fat synthesis in dairy cows (Lock et al., 2006).

Algae derived omega-3 fatty acids have positive effects on visual and neural development and on the prevention of diseases such as heart conditions, hypertension, cancer, diabetes, cystic fibrosis, asthma, arthritis, depression, and schizophrenia (Ohse et al., 2015). When n-3 PUFA was introduced into the diet of mammals, there was a reversal of ventricular fibrillation and the cells were electrically stabilized (Sinclair et al., 2002). There has been a reduction of cardiac deaths in mammals when n-3 PUFA was added to the diet (Sinclair et al., 2002). They can reduce inflammation and help lower the risk of chronic diseases, like heart disease (Gammone et

al., 2019). These fatty acids can regulate blood pressure, hematic clotting, and glucose tolerance, and aid nervous system development and function (Gammone et al., 2019). The algal PUFA can regulate the immune and inflammatory responses in the body (Gammone et al., 2019). Anti-inflammation comes from the inhibition of the 5-lipoxygenase pathway in neutrophils and monocytes and decreases levels of interleukins, subsequently inhibiting inflammation (Gammone et al., 2019). In skeletal muscle, DHA increases lipid oxidation (Gammone et al., 2019). The health benefits from adding omega-3 fatty acids to the diet is low cost and low risk (Gammone et al., 2019).

Fish consumption has been correlated with a decrease in sudden heart death in humans (Kris-Etherton et al., 2002). Possible mechanisms for omega-3 fatty acids to help with cardiac disease are through reducing blood TAG and cholesterol (Kris-Etherton et al., 2002). Dietary omega-3 fatty acids also have a small effect in reducing blood pressure (Kris-Etherton et al., 2002). Adding these fatty acids into the diet can decrease platelet aggregation for tolerable lengths of bleeding times (Kris-Etherton et al., 2002). Though the amount of omega-3 fatty acids needed to reduce coronary artery disease could be unattainable through the diet, dietary omega-3 fatty acid intake is a good prevention method (Kris-Etherton et al., 2002).

Linoleic acid is important for growth, reproduction, and skin function (Sinclair et al., 2002). It is a precursor for arachidonic acid, which is the substrate for enzymes that produce eicosanoids, including prostaglandins (Sinclair et al., 2002). The requirement for linoleic acid is lower when the diet contains lower ALA concentrations (Sinclair et al., 2002). ALA increases growth of fat-deficient rats and helps with skin lesions (Sinclair et al., 2002). DHA, a long chain n-3 PUFA, is known for playing a crucial role in the nervous system and has been connected to changes in brain function, including memory, appetite, and learning (Sinclair et al., 2002). For

skin, ALA can control water permeability (Sinclair et al., 2002). In mammals, the functions of ALA include being the precursor for long chain n-3 FA, a substrate for beta-oxidation and carbon recycling in the brain and tissues (Sinclair et al., 2002). The role of n-3 PUFA is important in the cardiovascular system (Sinclair et al., 2002).

There are positive effects when including dietary supplementation of fats for dairy cattle and feedlot cattle. Addition of fat to dairy cattle diets increases the energy content of the diet, allows optimization of the forage: concentrate ratio, partitions energy towards milk, increases the efficiency of energy utilization for milk products, reduces incidences of acidosis and frothy bloat, and reduces the overall dustiness of the feed (Palmquist and Chalupa, 1986). In feedlot cattle, there is an increase in the energy content of the diet, improvement of feed efficiency, reduced incidences of acidosis and frothy bloat, and reduced overall dustiness of the feed (Palmquist and Chalupa, 1986). With the positives, there are also negative effects that come with increased fat supplementation. These can include decreased feed intake, changes in the rumen with high FFA concentrations, including decreased fiber digestibility and acetate: propionate ratio, and changes in milk composition, such as decreased milk fat percent and milk protein percent (Palmquist and Chalupa, 1986).

Conclusion

There are positives and negatives to adding lipids into ruminant diets. It is important to know how ruminants digest lipids to allow optimal formulation of diets containing feed sources that have lipids. Also, it is important to know how lipids affect the body. Lipids can help with meeting energy requirements, improving feed efficiency, and decreasing bloat and acidosis by changing eating behavior. Biohydrogenation of lipids is important to reduce concentrations of fatty acids that are toxic for rumen microflora. There is metabolism of dietary unsaturated fatty acids in the rumen allowing lipid material to leave the rumen as FFA that are saturated. The ruminant animal can digest saturated fatty acids better than nonruminants. Humans can benefit from the lipids in meat and milk for health reasons.

References

Abedi E., and M. A. Sahari. 2014. Long-chain polyunsaturated fatty acid sources and evaluation of their nutritional and functional properties. Food Sci Nutr. 5:443-63. doi:10.1002/fsn3.121.

Bauman, D. E., J. W. Perfield II, M. J. de Veth, and A. L. Lock. 2003. New perspectives on lipid digestion and metabolism in ruminants. Proc. Cornell Nutr. Conf. p. 175-189.

Beauchemin, K. A., S. M. McGinn, and H. V. Petit. 2007. Methane abatement strategies for cattle: lipid supplementation of diets. Can. J. Anim. Sci. 87:431–440. doi:10.4141/CJAS07011.

Britannica, The Editors of Encyclopedia. 2022. Phospholipid. Encyclopedia Britannica. https://www.britannica.com/science/phospholipid. Accessed 17 October 2022.

Byers, F. M., and G. T. Schelling. 1988. Lipids in ruminant nutrition. In: The ruminant animal digestive physiology and nutrition. Prentice-Hall Inc., Englewood Cliffs, New Jersey. p. 298-312.

Chen H., C. Wang, S. Huasai, and A. Chen. 2021. Effects of dietary forage to concentrate ratio on nutrient digestibility, ruminal fermentation and rumen bacterial composition in Angus cows. Sci. Rep. 11:17023. doi: 10.1038/s41598-021-96580-5.

Devendra, C., and D. Lewis. 1974. Fat in the ruminant diet: a review. Indian J. Anim. Sci. 44:917-938.

Dewanckele, L., J. Jeyanathan, B. Vlaeminck, and V. Fievez. 2020. Identifying and exploring biohydrogenating rumen bacteria with emphasis on pathways including trans-10 intermediates. BMC Microbiol. 20:198-219. doi:10.1186/s12866-020-01876-7.

Doreau, M., and A. Ferlay. 1994. Digestion and utilisation of fatty acids by ruminants. Anim. Feed Sci Technol. 45(3-4):379–396. doi:https://doi.org/10.1016/0377-8401(94)90039-6.

Doreau, M., D. I. DeMeyer, and C. Van Nevel. 1997. Transformations and effects of unsaturated fatty acids in the rumen: consequences on milk fat secretion. In: Milk composition, production and biotechnology. p. 73–92. http://hdl.handle.net/1854/LU-406587.

Freeman, C. P. 1984. The digestion, absorption and transport of fats- non-ruminants. In: J. Wiseman, editor, Fats in Animal Nutrition 105–122. doi: https://doi.org/10.1016/B978-0-408-10864-5.50011-5.

Gammone, M. A., G. Riccioni, G. Parrinello, and N. D'orazio. 2019. Omega-3 polyunsaturated fatty acids: Benefits and endpoints in sport. Nutrients 11:1–16. doi:10.3390/nu11010046.

Gaynor, P. J., D. R. Waldo, A. V. Capuco, R. A. Erdman, L. W. Douglass, and B. B. Teter. 1995. Milk fat depression, the glucogenic theory, and trans-C18:1 fatty acids. J. Dairy Sci. 78:2008–2015. doi:10.3168/jds.S0022-0302(95)76826-6.

Gerson, T., A. John, and A. S. D. King. 1983. The effects of dietary starch and fiber on the in vitro rates of lipolysis and hydrogenation by sheep rumen digesta. J. Agric. Sci. 105:27–30. doi: https://doi.org/10.1017/S0021859600055659.

Giger-Reverdin, S., P. Morand-Fehr, and G. Tran. 2003. Literature survey of the influence of dietary fat composition on methane production in dairy cattle. Livest. Prod. Sci. 82:73–79. doi:10.1016/S0301-6226(03)00002-2.

Harfoot, C., and G. P. Hazlewood. 1997. Lipid metabolism in the rumen. In: P. N. Hobson C. S. Stewart, editor, The rumen microbial ecosystem. Springer, Dordrecht, Germany. p. 382–426. doi:10.1007/978-94-009-1453-7_9.

Henderson, C. 1973. The effects of fatty acids on pure cultures of rumen bacteria. J. Agric. Sci. Camb. 81(1):107-112. doi:10.1017/S0021859600058378.

Ikwuegbu, O. A., and J. D. Sutton. 1982. The effect of varying the amount of linseed oil supplementation on rumen metabolism in sheep. Br. J. Nutr. 48:365-375. doi: 10.1079/bjn19820120.

Jarvis, G., and E. R. B. Moore. 2010. 46. Lipid metabolism and the rumen microbial ecosystem. In: K. N. Timmis, editor, Handbook of hydrocarbon and lipid microbiology. Springer, Berlin, Heidelberg. p. 2246–2257. doi:10.1007/978-3-540-77587-4_163.

Jenkins, T. C. 1993. Lipid Metabolism in the Rumen. J. Dairy Sci. 76:3851–3863. doi:10.3168/jds.S0022-0302(93)77727-9.

Katz, I., and M. Keeny. 1966. Occurrence of ketostearic acids in the rumen. J. Dairy Sci. 49(8):967-970. doi:10.3168/jds.S0022-0302(66)87991-2.

Kemp, P., and D. J. Lander. 1984. Hydrogenation in vitro of α-linolenic acid to stearic acid by mixed cultures of pure strains of rumen bacteria. J. Gen. Microbiol. doi:130527-533. 527–533.

Kris-Etherton, P. M., W. S. Harris, and L. J. Appel. 2002. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. Circulation 106:2747–2757. doi:10.1161/01.CIR.0000038493.65177.94.

Kris-Etherton, P., D. S. Taylor, S. Yu-Poth, P. Huth, K. Moriarty, V. Fishell, R. L. Hargrove, G. Zhao, and T. D. Etherton. 2000. Polyunsaturated fatty acids in the food chain in the United States. Am. J. Clin. Nutr. 71(Suppl.1):179S-188S. doi:10.1093/ajcn/71.1.189s.

Krueger, N. A., R. C. Anderson, L. O. Tedeschi, T. R. Callaway, T. S. Edrington, and D. J. Nisbet. 2010. Evaluation of feeding glycerol on free-fatty acid production and fermentation kinetics of mixed ruminal microbes in vitro. Bioresour. Technol. 101:8469–8472. doi:10.1016/j.biortech.2010.06.010.

Lehnen T. E., M. R. da Silva, A. Camacho, A. Marcadenti, and A. M. Lehnen. 2015 A review on effects of conjugated linoleic fatty acid (CLA) upon body composition and energetic metabolism. J. Int. Soc. Sports Nutr. 12:36. doi: 10.1186/s12970-015-0097-4.

Lessire, M., M. Doreau, and A. Aumaitre. 1992. Digestion and metabolism of fats in domestic animals. In: Manuel des Corps Gras, Lavoisier, Paris. p. 683–694.

Lock, A. L., K. J. Harvatine, J. K. Drackley, and D. E. Bauman. 2006. Concepts in fat and fatty acid digestion in ruminants. Proc Intermountain. Nutr. Conf. pages 85–100. doi:https://www.researchgate.net/publication/266499830.

Lourenço, M., E. Ramos-Morales, and R. J. Wallace. 2010. The role of microbes in rumen lipolysis and biohydrogenation and their manipulation. Animal 4:1008-1023. doi:10.1017/S175173111000042X.

Maia, M. R., L. C. Chaudhary, C.S. Bestwick, A. J. Richardson, N. McKain, T. R. Larson, I. A. Graham, and R. J. Wallace. 2010. Toxicity of unsaturated fatty acids to the biohydrogenating ruminal bacterium, *Butyrivibrio fibrisolvens*. BMC Microbiol. 10:52. doi:10.1186/1471-2180-10-52.

McAllister, T. A., E. K. Okine, G. W. Mathison, and K. J. Cheng. 1996. Dietary, environmental and microbiological aspects of methane production in ruminants. Can. J. Anim. Sci. 76:231–243. doi:10.4141/cjas96-035.

Moore, J. H., and W. W. Christie. 1984. Digestion, absorption and transport of fats in ruminant animals. J. Wiseman, editor, Fats in Animal Nutrition. pages 123–149. https://doi.org/10.1016/B978-0-408-10864-5.50012-7.

Nagaraja, T. G., C. J. Newbold, C. J. van Nevel, D. I. Demeyer, D.I. 1997. Manipulation of ruminal fermentation. In: Hobson, P.N., Stewart, C.S., editors, The rumen microbial ecosystem. Springer, Dordrecht. doi:10.1007/978-94-009-1453-7_13.

National Research Council (US) Committee on Diet and Health. 1989. Fats and other lipids. In: Diet and health: implications for reducing chronic disease risk. National Academies Press, Washington DC. p. 159-258.

Noble, R. C. 1981. Digestion, transport and absorption of lipids. In: Biochemistry department, the Hannah research institute, editor. Prog. Lipid Res. Vol. 17, Ayr, Scotland. p. 57–93.

Ohse, S., R. Bianchini Derner, R. Ávila Ozório, R. Gordo Corrêa, E. Badiale Furlong, and P. C. Roberto Cunha. 2015. Lipid content and fatty acid profiles in ten species of microalgae. Idesia (Arica) 33:93–101. doi:10.4067/s0718-34292015000100010.

Palmquist, D. L. 1984. Use of fats in diets for lactating dairy cows. J. Wiseman, editor, Fats in Animal Nutrition 357–381. https://doi.org/10.1016/B978-0-408-10864-5.50025-5.

Palmquist, D., and W. Chalupa. 1986. New developments in feeding fat to dairy cows. Proc. Amer. Dairy Sci. Assoc.

Pariza, M. W. 1997. Conjugated linoleic acid: an anticarcinogenic nutrient. In: Ohigashi, H., Osawa, T., Terao, J., Watanabe, S., Yoshikawa, T, editors, Food Factors for Cancer Prevention. Springer, Tokyo. p. 63-69. doi:https://doi.org/10.1007/978-4-431-67017-9_71.

Roughan, P. G., and R. D. Batt. 1969. The glycerolipid composition of leaves. Phytochemistry. 8:363–369. https://doi.org/10.1016/S0031-9422(00)85432-1.

Ronning, M., and R.C. Laben. 1966. Response of lactating cows to free choice feeding of milled diets containing 10 to 100% concentrates. J. Dairy Sci. 49: 1080-1085. doi:https://doi.org/10.3168/jds.S0022-0302(66)88021-9.

Salsinha A.S., L. L. Pimentel, A. L. Fontes, A. M. Gomes, L. M. Rodríguez-Alcalá. 2018. Microbial production of conjugated linoleic acid and conjugated linolenic acid relies on a multienzymatic system. Microbiol Mol Biol Rev. 82(4):e00019-18. doi:10.1128/MMBR.00019-18.

Shingfield, K. J., and R. J. Wallace. 2014. Chapter 1: synthesis of conjugated linoleic acid in ruminants and humans. In: B. Sels and A. Philippaerts, editors, Conjugated Linoleic Acids and Conjugated Vegetable Oils, Royal Society of Chemistry, Cambridge, UK. p. 1-65 doi:10.1039/9781782620211-00001.

Sinclair, A. J., N. M. Attar-Bashi, and D. Li. 2003. What is the role of α-linolenic acid for mammals? Lipids 37:1113–1123. doi:10.1007/s11745-002-1008-x.

Sukenik, A., Y. Carmeli, and T. Berner. 1989. Regulation of fatty acid composition by irradiance level in the eustigmatophyte *Nannochloropsis* sp. J. Phycol. 25:686–692. doi:10.1111/j.0022-3646.1989.00686.x.

Voet, D., J. G. Voet, and C. W. Pratt. 2016. Fundamentals of biochemistry: life at the molecular level. 5th ed. Publisher, City, ST.

Van Nevel, C., and D. DeMeyer. 1996. Influence of pH on lipolysis and biohydrogenation of soybean oil by rumen contents in vitro. Reprod. Nutr. Dev. 36:53–63. doi:10.1051/rnd:19960105.

Wallace R. J., N. McKain, K. J. Shingfield, and E. Devillard. 2007. Isomers of conjugated linoleic acids are synthesized via different mechanisms in ruminal digesta and bacteria. J. Lipid Res. 48(10):2247-2254. doi: 10.1194/jlr.M700271-JLR200.

Wright, D. E. 1969. Fermentation of glycerol by rumen microorganisms. N. Z. J. Agric. Res. 12:281-286. doi: 10.1080/00288233.1969.10421180.