ABSTRACT

Title of Thesis:	EFFECT OF AIR ON RUMEN GAS PRODUCTION
	Rachel Youngah Rha, Master of Science, 2021
Thesis directed by:	Professor Richard A. Kohn Department of Animal and Avian Sciences

Ruminants may swallow air as they eat and ruminate throughout the day. However, it is unclear as to how the introduction of oxygen impacts fermentation pathways, bacteria, and yeast within this mostly anaerobic environment. Therefore, the focus of this thesis was to study air's impact on rumen fermentation and to determine if probiotics could offset air's impact on digestibility. An in vitro analysis of air and probiotics indicated the main effect of air decreased digestibility, the main effect of probiotics had variable effects, and probiotics had significant interactions with air. The interactions suggested yeast employing a potential alternative pathway with the introduction of oxygen. Utilizing published literature, a static and dynamic mathematical model was built to further analyze digestibility, gas composition, and uptake of oxygen within the rumen. Future studies will further develop this model with in vivo studies to further interpretation and understanding of rumen fermentation's complex system.

EFFECT OF AIR ON RUMEN GAS PRODUCTION

by

Rachel Youngah Rha

Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Master of Sciences
2021

Advisory Committee:
Professor Richard A. Kohn, Chair/Advisor
Professor Debabrata Biswas
Professor Nishanth E. Sunny

Rachel Youngah Rha
2021

DEDICATION

To my parents

And all of my friends

Thank you for your support throughout the years

ACKNOWLEDGEMENTS

I would like to thank the University of Maryland and the Department of Animal and Avian Sciences for allowing me to pursue my degree in Animal Science with a focus on Ruminant Nutrition. I would like to thank Dr. Rick Kohn, Dr. Debabrata Biswas, and Dr. Nishanth Sunny. Each of my committee members has helped me grow and further enhance my education and knowledge of ruminant nutrition. I truly appreciate the ability to pursue a project which challenged me and also enabled me to use my background in animal science to enhance my project.

I would sincerely like to thank Dr. Rick Kohn. Ever since I joined his lab as a sophomore, Dr. Kohn has been a wonderful advisor and most importantly a mentor. He has been supportive throughout my entire experience at Maryland and has always inspired me to pursue my interests in research. I truly appreciate all the guidance and time he has given me, and I hope that I am able to carry his lessons and passion forward as I pursue my own career.

I would like to thank my friends and officemates in room 4111: Jarvis, I thank you for the countless advice and support throughout the years and I am glad to have you as a fellow lab mate I can rely on; Chaitra, I am constantly amazed by your diligence and work ethic in everything you do; Amanda, I truly appreciate all of your kind words and support throughout the years and am grateful to have you as a friend.

Finally, I would like to thank my family for always being there for me and supporting my pursuit of my M.S. degree. I would like to thank my mother and father for instilling in me the importance of education at a young age and for supporting me in everything that I've done.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1:

LITERATURE REVIEW

Introduction

In numerous studies, probiotics within the agriculture industry have been surging in popularity due to their perceived health and production advantages from promoting good bacteria within the rumen. Specific yeast species such as *Saccharomyces boulardii* or *Saccharomyces cerevisiae*, as well as lactic acid bacteria species such as *Streptococci sp., Bifidobacteria sp., Megasphaera elsdenii, Bacillus subtilis*, have been reported to improve animal health and performance (Uyeno et al., 2015).

However, in order to comprehend how probiotics can promote health in cattle, it is important to understand the cattle rumen environment as well as rumen gas production. Cattle rely heavily on microbes in the rumen to ferment carbohydrates to make volatile fatty acids (VFA), specifically acetate, propionate, and butyrate. These VFA allow the cow to transform feed into usable protein and energy for everyday work within the rumen. With stoichiometric calculations, VFA are linked to gas production such as methane which is not necessarily dangerous on its own, but in copious amounts it begins to present issues related to global warming. Analyzing these VFA as well as gas production within the rumen can indicate the efficiency of fermentation within the gut.

The goal of these studies is to gain a better understanding of rumen fermentation and rumen digestibility. In particular, we aim to understand oxygen's role in rumen fermentation as well as the mechanisms behind probiotic effects within cattle. These studies can clarify our understanding of the rumen system in order to utilize probiotics more effectively and gain a better understanding of the rumen itself.

Literature Review

Ruminant Digestive System

Cattle are ruminants, which are ungulate mammals that chew on regurgitated cud, and specifically have four chambers within their gastrointestinal system: the rumen, reticulum, omasum, and abomasum prior to the small intestine (Huffman, 1948). The rumen, also known as "paunch", is the largest compartment. Together with the reticulum or "honeycomb", the rumen and reticulum in cattle has been reported to hold about 80-200L, depending on the size of the cattle itself (Evans & Hooser, 2010; Russell, 2009). Also called the "fermentation vat", the rumen allows the breakdown and digestion of forage ingested by cows (Huffman, 1948). In order to do this, the internal environment of the rumen is covered with tiny projections, papillae, allowing the increase in surface area of the rumen and increasing absorption of digested nutrients. The reticulum or "honeycomb" is next and able to collect smaller digesta particles, moving them directly to the omasum or "manyplies", which absorbs water and other substances consumed by the cow. Finally, the abomasum or "true stomach" is lined with glands, can release hydrochloric acid and enzymes, and break down feeds (Soest, 1982).

Rumen pH

The pH inside the rumen is an important factor to consider especially since the microbes required for fermentation need a suitable environment for growth. Microbes able to digest fiber within the rumen cannot grow in low pH, acidic environments. The optimal pH range should be around 5.7 (grain-based diets) and 7.3 (forage-based

diets) with the normal ruminal pH >6.0 (Russell, 1998). Symptoms such as acidosis can occur when a cow eats a large amount of rapidly digestible starch or sugar, overwhelming the rumen's buffering system and resulting in a rumen with a pH less than 5.5. The buffer system which consists of saliva (leading to the formation of bicarbonate in the rumen) in a cow can reduce the risk of acidosis. Rumination in cattle can trigger this saliva flow and allow the rumen to maintain a favorable pH for the microbes (Russell & Rychlik, 2001). When the rumen contracts, it mixes the feed consumed with microbes in order for volatile fatty acids to be absorbed. However, if cattle are fed fiber-deficient diets, "then mixing motions, eructation, rumination, and saliva flow decrease; fermentation acids accumulate; and ruminal pH declines" (Russell & Rychlick, 2001).

Rumen Fermentation

Rumen fermentation is important for the growth of microbes and the digestion of feedstuffs used for energy. Because of this, environmental conditions inside the rumen require a particular balance in pH, which if not stable, will lead to poor microbial growth and a decreased digestion which in turn leads to decreased milk production (Bayat et al., 2015). The rumen also requires a relatively constant temperature of 39°C in order for fermentation to occur and should be buffered well by salivary secretions (Russell & Hespell, 1981). During this process, glucose consumed by cattle is broken down to pyruvate, releasing hydrogen, and then to acetate releasing more hydrogen and CO_2. Some pyruvate is converted to propionate and butyrate consuming H_2 (Chalupa, 1977). The final products of fermentation include volatile fatty acids, NH_3, CO_2, and CH_4.

The rumen is an anaerobic environment, meaning there is little to no oxygen inside and many microbes that live inside the rumen are unable to grow and proliferate when air is present. However, even though the rumen is considered to be anaerobic, it has been shown that rumen gas contains less than 1% O_2 (McArthur & Multimore, 1961). In addition, rumen gas composition contains the average of 67% CO_2, 26% CH_4 (Kleiber et al., 1943). Furthermore, a study (Barry et al., 1977) with fistulated sheep show that some N_2 is present in rumen headspace and this indicates some amount of air must be swallowed. To offset the O_2, yeast may utilize oxygen in the rumen which encourages the growth of anaerobic bacteria (Newbold et al., 1996). Specifically, Newbold et al., 1996 suggested there are potentially two modes of action of yeast associated yeast respiratory activity protecting anaerobic rumen bacteria that may be damaged by O_2. The most relevant mode of action from Newbold et al., 1996 suggested the potential of yeast having the ability to increase the viable count of rumen bacteria. The second mode of action from Newbold et al., 1996 suggests yeast provides malic acid and other dicarboxylic acids that stimulate the growth of certain rumen bacteria.

Published values for O_2, uptake by *S. cerevisiae* (200-300pmol/min per g; Barford & Hall, 1979) suggest that they have respiratory rates several orders of magnitude greater than rumen fluid. Thus, even at the low inclusions used in ruminant diets, yeast might still be expected to exert an effect on the rate of O_2, uptake in rumen fluid

Volatile Fatty Acids

The total concentration of VFA should be discussed as well as the meaning of molar proportion. Most literature usually reports molar proportion and often total VFA in mmol/L. VFAs are a major energy source for ruminants and specific proportions can determine fat and protein content in milk.

Acetate

One of the end products of rumen fermentation in the rumen is acetate. This VFA is essential for milk-fat production, and if the molar proportion of acetate produced is too low, it can lead to milk-fat depression. One cause of milk-fat depression is diet fed to the cow that is high in grain and low in fiber (Bauman et al., 1971). This is important to note as farmers can be paid more for milk with a higher milk-fat content. Milk composition plays a large part in the economy of milk producers as some companies pay more for milk with a higher milk-fat content. The composition has averaged around 3.6 percent of fat, 3.2 percent protein, and 4.7 percent lactose (Young et al., 1986).

There are various theories surrounding milk-fat depression and its relationship to the acetate to propionate ratio. Specifically, an increased acetate to propionate ratio increases the milk-fat sample, therefore having a positive relationship (Rodger et al., 1982). Diets corresponding to changes in acetate to propionate ratios are specific types of carbohydrates in the diet, the forage-to-concentrate ratio, the processing of specific ingredients, additives, the physical form of the diet itself, and the frequency of feed offered (Sutton, 1980). Specifically, with decreasing the forage-to-concentrate ratio, the rumen fermentation decreases pH, which in turn increases propionic acid

production and reduces fiber digestion. Therefore, as the forage amount decreases, the milk-fat percentage will also fall proportionally to this value (Bauman et al., 1971).

Another theory related to milk-fat synthesis is centered around conjugated linoleic acid (CLA). In short, CLA are the intermediates in the biohydrogenation of linoleic acid and in ruminants, CLA come from the incomplete biohydrogenation of unsaturated fat by the means of rumen bacteria (Kelly et al., 1998). When the rumen pH decreases to a low level, biohydrogenation becomes inhibited. This then results in a buildup of trans fatty acid and CLA. Both of these causes a decrease in milk-fat synthesis in the mammary (Chouinard et al., 1999).

Propionate

Propionate is another VFA produced in the rumen at a concentration of 10-15 molar percent of total VFA. The sugar and starch utilizing bacteria reportedly produce a lower ratio of acetate to propionate, so high starch diets are thought to cause a decrease in acetate to propionate. The glucose needed for the mammary system to work efficiently and produce lactose is from the synthesis of propionate. In a study from the Journal of Dairy Science, it was found that a high-grain, low-fiber diet resulted in a decrease in a 50% milk-fat percent reduction and a decreased molar ratio of acetate to propionate (Bauman et al., 1971). This then showed how the change in the molar ratio of rumen volatile fatty acids for cows fed a high-grain vs. low-fiber diet "is the result of an increase in propionate production rather than a decrease in acetate production" (Bauman et al., 1971). It should be noticed if cattle are fed a high cereal grain diet or a diet high in fermentable carbohydrates, this can

lead to an increase in the starch digesting bacteria within the gut, therefore producing more propionate. It was reported there would be lower ruminal degradability when comparing products such as corn and barley, which would then result in a higher milk-fat percentage (Bauman et al., 1971).

Butyrate

Like propionate, butyrate is not as greatly produced as acetate in the rumen (5 -15 molar percent). Its main role is to serve as an energy source for epithelial cells in ruminants while also maintaining colonic health (Bugaut, 1987; Li et al., 2016). As butyrate stimulates this epithelial cell production, this leads to improved feed utilization by the animal, making them more efficient. In addition, butyrate can also prevent certain types of colitis, impact the mucosal barrier, feed passage, microbiome, immune system, and pathogens (Pierce et al., 2004; Kato et al., 2011; Scheppach et al., 1994). Essentially, it works to improve the health and performance of cattle (Canani et al., 2012).

Thermodynamics

Understanding the relationship between thermodynamics and fermentation is essential for the research completed in studies involving the rumen. Thermodynamics is a branch of physics that takes both heat and temperature and connects these two factors to energy and work expressed as three laws. The first law of thermodynamics is also known as the Law of Conservation of Energy and states that energy cannot be created or destroyed in an isolated system. Another way to describe this law is change

in the internal energy of a system is equal to the total heat and the work done on the system by surroundings (Pippard, 1964). The second law of thermodynamics emphasizes entropy of any isolated system will always increase. In other words, the second law explains how isolated systems spontaneously move towards thermal equilibrium or the maximum entropy of the system, therefore the entropy of the universe only increases and never decreases (Pippard, 1964). The third law of thermodynamics states that the entropy of a system approaches a constant value as the temperature approaches absolute zero. In simpler terms, the temperature of a system approaches absolute zero and then entropy will also become constant (Pippard, 1964).

In biological systems, the first and the second laws of thermodynamics are important. In the rumen, "thermodynamic control occurs when reactants are sufficiently limited relative to the products for the reactions not to be able to proceed" (Kohn & Boston, 2000).

In the rumen, fermentation results in the production of three main volatile fatty acids: butyrate, propionate, and acetate via the uptake of glucose (Russell, 1998). It is feasible to produce two acetate molecules per glucose molecule at a higher concentration than for production of propionate, butyrate, or three molecules of acetate. Therefore, a higher concentration of acetate is produced. If the system is thermodynamically limited (accounts for formation of product), the pathway should shift to propionate or butyrate when acetate concentration is high. When acetate is produced from glucose, 4 H_2 and 2 CO_2 molecules are also released per glucose molecule, and these gases can be converted downstream to methane, which is a particularly potent greenhouse gas. In addition, shifting fermentation from acetate to

propionate and butyrate can lead to an increase in energy of fermentation end products (Chalupa, 1977). This can be dependent on the feed and carbohydrates found in plants that cattle consume regularly. These carbohydrates can be broken down into small sugar molecules and then further broken down by microbes and fermentation to acetate, propionate, butyrate, and CO_2 (Russel & Hespell, 1981).

Microbes

Cows rely heavily on microbes within the rumen to convert their feed into metabolizable energy and protein. In fact, microbes within the rumen are the main source of protein in a cow's diet and are responsible for the degradation of carbohydrates to VFAs and gases such as carbon dioxide (CO_2) and methane (CH_4) (Dewhurst et al., 2000). Microbes within the rumen are abundant as they fill this compartment with approximately 10^{10} to 10^{11} bacterial and 10^6 protozoal cells per milliliter (Russell & Hespell, 1981). In addition to population, diversity within the microbiome is considered extensive as they constitute approximately 200 species of bacteria and 20 species of protozoa (Russell & Hespell, 1981).

The microbes responsible for feeding on the ingested forages are bacteria, protozoa, and fungi. These microbes are able to digest starch, sugar, and cellulose. The amounts and proportions of these microbes can vary depending on the specific diet of the individual cow. However, the efficiency of ruminants to break down and utilize various feeds is due to the highly diverse rumen microbial ecosystem which consists of bacteria (10^{10}-10^{11} cells/mL, representing more than 50 genera), ciliate protozoa (10^4-10^6/mL, from 25 genera), anaerobic fungi (10^3-10^5

zoospores/mL, representing five genera) and bacteriophages (10^8-10^9/mL) (Kamra, 2005).

Microbes break down rumen degradable protein and non-protein nitrogen into amino acids and ammonia to grow. Looking at microbial growth, these microbes are then digested by the omasum and the abomasum and absorbed by the small intestine. These can then synthesize protein that is absorbed by the rumen wall and in the small intestine.

Ruminants alone cannot produce fiber-degrading enzymes, but the microbes in their rumen such as, bacteria, fungi, and protozoa have the ability to do so (Russell & Rychlik, 2001). The rumen itself provides a suitable habitat for growth which allows microbes to supply protein, vitamins, and volatile fatty acids for the cattle. There are a few classifications of bacteria such as cellulolytic, amylolytic, and lactate utilizers or lactic acid bacteria. Cellulolytic or fiber-digesting bacteria are very sensitive to acid and pH levels within the rumen (Russell, 1988; Hungate, 1966). If the pH drops below 6.0, the fiber-digesting bacteria fail to produce an optimal amount of acetate, therefore decreasing the acetate to propionate ratio. Some of the most common cellulolytic bacteria in the cow's rumen are *Ruminococcus flavefacians, Ruminococcus albus*, *Bacteriodes succinogenes*, *Butyrivibrio fibrisolvens* and require cellulose, hemicellulose, and pectin for growth (Russell, 1988; Hungate, 1966). In addition, these fiber-digesting bacteria have a slow reproduction rate and a low tolerance to high fat diets, impacting how microbes can move nutrients into and out of the body (Russell, 1988; Hungate, 1966). Another classification of ruminal bacteria is amylolytic or starch and sugar-digesting bacteria. These compose a large portion of

the bacterial population and are heavily utilized since dairy cows can consume diets containing 30% starch and sugars (Russell, 1988; Hungate, 1966). Common amylolytic bacteria species in the rumen are *Bacteroides ruminicola*, *Bacteroides amylophilus*, *Selenomonas ruminatium*, *Streptococcus bovis*, *Succinomonas amylolytica* and require sugar, starch, peptides, amino acids, ammonia, and B-vitamins for growth (Russell, 1988; Hungate, 1966). These bacteria have fermentation products such as acetate, propionate, butyrate, lactate, hydrogen, and carbon dioxide and can tolerate a more acidic pH than cellulolytic bacteria. It is important to note, after starch and sugars are fed to cattle, a bacterium called *Streptococcus bovis* is present. These produce lactic acid and grow rapidly, endangering the animal with rumen acidosis (Russell, 1988; Hungate, 1966). Finally, there are lactate utilizers within the rumen such as *Lactobacilli sp.,* some *Streptococci sp., Bifidobacteria sp., and Megasphaera elsdenii* (Uyeno et al., 2014). These offset the lactic acid produced by *Streptococcus bovis* and use it to grow, increasing the pH of the system in the rumen (Uyeno et al., 2014). The chemical equation for bacteria producing methane is:

$$4H_2 + CO_2 \text{ ---------> } CH_4 + 2H_2O$$

Lactic Acid

Lactic acid is naturally produced by bacterial fermentation within the rumen and is an intermediate in the metabolism of carbohydrates (Chamberlain et al., 1983). Certain bacteria such as *Streptococcus bovis* promote an increase in lactic acid within the rumen by shifting fermentation away from acetic acid. Ruminal conditions often

make it thermodynamically infeasible to obtain energy from producing lactic acid (Kohn & Kim, 2008). However, when there is a high concentration of glucose, lactic acid can accumulate (Kohn & Kim, 2008). This high concentration of lactic acid occurs when acetate and propionate production are limited by lack of viable bacteria, low pH, or another inhibitor. The high amounts of lactic acid can promote acidosis, a nutritional disease caused by a sudden transition to a high starch or concentrate-based diet (Kleen et al., 2003). The fermentation end products will be propionate and butyrate when cattle are fed a high concentrate diet (Chamberlain et al., 1983). Symptoms of acute acidosis include reduced feed intake, reduced rumination, increased heart rate, increased breathing rate, diarrhea, lethargy, and even death (Kleen et al., 2003). In addition, low rumen pH "ruminal acidosis" leads to lactic acid production which leads to systemic "acidosis" from accumulation of lactate in blood. Since lactic acid is about 10 times stronger than VFA with a pKa of 3.9 versus 4.9, it is less protonated than VFA and accumulates in the rumen, contributing to a decreased pH (Giesecke & Stangassinger, 1980). The proportion of L+lactate and D-lactate, two isomers of lactic acid, are associated with lower pH and acidosis (Giesecke & Stangassinger, 1980; Omale et al., 2001). A study on acidosis associated with diarrheic calves found both lactate isomers contributed to this metabolic disease as the serum lactate concentrations were found to be higher in sick calves (Omale et al., 2001).

The importance of lactic acid relates to the potential of specific lactic acid bacteria (LAB) acting as probiotics within the rumen. LAB are gram positive and non-spore forming cocci. They ferment glucose consumed by the animal and turn it

into lactic acid, carbon dioxide, and ethanol (Matthews et al., 2019). LAB need an anaerobic environment and are considered "aerotolerant anaerobes" since they can grow if oxygen is present (Weinberg et al., 2003). In the cow's rumen, prominent LAB are *Lactobacillus, Bifidobacterium,* and *Enterococcus* (Uyeno et. al., 2015) and have been considered and studied as probiotics as they have shown to be beneficial to the host. LABs are able to utilize lactic acids to grow, increasing the pH of the system the rumen (Uyeno et. al., 2015). There have been a few notable papers on LAB studies looking at specific bacteria and how they may benefit the host. However, most papers are centered around LAB's impact on silage with variable results. For example, in a study completed by J.L. Ellis in the Journal of Animal Feed Science and Technology, they looked at *Lactobacillus plantarum* in vitro to see the effects it may have when used as either a probiotic or silage inoculant for various silages (Ellis et al., 2016). They saw *L. plantarum* increased organic matter (OM) digestibility in vitro when used as a probiotic. On the other hand, they also had various effects with LAB silage inoculants and concluded LAB depended on strain, dose, and substrate (Ellis et al., 2016). Another paper from the Journal of Dairy Science looked at the effect of LAB when combined with beet pulp to see how they impacted silage fermentation quality and in vitro ruminal dry matter (DM) digestion of certain vegetables (Cao et al., 2011). They concluded LAB-inoculated silage had high DM digestibility and low methane production while also noting LAB alone increased DM digestibility while decreasing ruminal methane production (Cao et al., 2011).

Modeling

Mathematical models allow scientists to express complex processes in the form of concise formulas (Lehman, 2008). Because of this, effective models should be simple and focused on a particular system or concept to address a problem at hand (Lehman, 2008). More specifically, these are systems commonly used in natural sciences and engineering. The process of developing a model includes mathematical language, concepts, and a set of linear equations, algebraic equations, or differential equations (Venkateshan et al., 2014). There are various types of models that exist, such as linear versus nonlinear, dynamic versus static, deterministic versus stochastic, and mechanistic versus empirical. All of these types will depend on what is exactly being studied (Venkateshan et al., 2014). More specifically, a mechanistic approach is needed to predict VFAs and gases from rumen fermentation and requires an understanding of the control mechanisms of metabolism (Kohn, 2007). The need for kinetics and thermodynamics is due to the fact that chemical reactions are controlled by both of these concepts, sometimes in a combination (Chang, 1981). With kinetics, enzyme kinetic theory is the assumption that substrate or enzyme concentration and activity control the rate of formation of products (Kohn, 2007). These biological products depend on the rate they are produced and can be quantified by the Michaelis-Menten equation (Chang, 1981; Kohn, 2007). However, the rumen system is known to not just follow enzyme kinetics, there is also thermodynamics that needs to be considered.

Isotope Tracing

A method of analyzing VFAs and VFA pathways can be through isotope tracing. Isotope tracing is a method prominently used in analyzing mammalian-cell metabolism (Fernández-García et al., 2020). More specifically, this method can be used to track an isotope through a reaction, metabolic pathway, or cell in order to "maximize the information extracted from *in vivo* measurements" (Fernández-García et al., 2020). In this case, evaluating rumen fluid and gaining a better understanding of pathways through isotope tracing can potentially improve probiotic studies within cattle.

Carbon is known to be radioactive and has the potential to decay during an experiment, however a stable molecule such as ^{13}C will not have this issue. The main advantage to stable isotopes is the fact that they do not give off radioactive particles that may cause cancer. ^{13}C, along with ^{15}N, have been reported to be used successfully in isotope-ratio mass spectrometry (IRMS) in the processes of disease control, authentication and certification of animal products (Bahar et al., 2008; Heaton et al., 2008), traceability (Silva et al., 2012), and evaluation of conventional and organic productions systems for beef (Bahar et al., 2008; Schmidt et al., 2005; Osorio et al., 2011; Ferreira et al., 2016).

Environmental Impact

Discussions of climate change and global warming revolve heavily around the topic of greenhouse gases (GHG). It should be noted, agriculture is both a source and a sink of GHG as livestock and crops are a source, contributing to GHG, and forests

are a sink, absorbing carbon dioxide from the atmosphere (Cole et al., 1993). There are many suspects to the earth's increasing temperature, but a known contributor are animals and the agriculture business as agricultural lands occupy 37% of the earth's land surface (Smith et al., 2008). The primary greenhouse gases emitted from agriculture are nitrous oxide (crops and manure management) and methane (enteric fermentation from livestock and manure management; Malik et al., 2015). Notably, global agricultural GHG emissions are approximately 14.5% (methane: 44% from livestock; 6.3% of total GHG emissions; Gerber et al., 2013). Nitrous oxide emissions dominate primarily from feed fertilization (Gerber et al., 2013). GHG emissions from cattle specifically represent around 65% of the livestock sector emissions which makes these animals the most significant contributor to the total sector emissions (Gerber et al., 2013).

Methane is produced during the fermentation of carbohydrates in the rumen. Cows excrete methane through eructation. Though low concentrations of methane are not hazardous on their own, accumulated methane gas contributes to global warming. Specifically, ruminant livestock can create about 250 to 500 liters of methane each day (Johnson & Johnson, 1995; Huhtanen et al., 2015). Although methane emissions are lower than CO_2 emissions, methane is still considered a threat as a major greenhouse gas since methane molecules have 25 times the global warming potential of a CO_2 molecule. It is estimated in the next 50 to 100 years, cattle may contribute to a little less than 2% of total global warming (Johnson & Johnson, 1995). Manipulation of: level of feed intake, type of carbohydrates in the diet, feed processing, addition of lipids or ionophores to the diet, and alterations in the ruminal

microflora can influence and possibly reduce methane emissions from cattle (Johnson & Johnson, 1995).

Methane can either be removed from the gastrointestinal tract of the cow through eructation or through the rumen wall itself. Methane cannot be utilized by the cow's body system therefore this production accounts for a loss of about 6% of total energy intake of cattle (Johnson & Johnson, 1995).

In addition to eructation, manure also poses as a problem due to the content of phosphorus and nitrogen in feces. Although a significant portion of it can be utilized as a fertilizer for farmland rather than pesticides, runoff with rainfall and watering crops can impact local waterways. This dangerous runoff contributes to HAB or "harmful algal blooms" (Anderson, 2009). The term HAB can be broad and cover many algal blooms of many types, however their common feature is that they can cause harm due to either the "production of toxins or to the manner in which the cells' physical structure or accumulated biomass affects co-occurring organisms and alters food-web dynamics" (Anderson, 2009).

A few decades ago, only a few countries were impacted by HABs, but now it is reported that most coastal countries are threatened by more than one harmful toxic species of algal blooms (Anderson, 1989; Hallegraeff, 1993). In addition, the concerns surrounding manure as fertilizers revolve around antibiotics remaining in manure-based fertilizers (Zhou et al., 2020). There is concern over the residues of certain antibiotics that could "depress seed germination, crop growth, and pose as a potential risk to soil ecosystem" (Liu et al., 2009; Hu et al., 2010; Malchi et al., 2014; Gros et al., 2019).

In order to prevent this risk, reducing the impact cows have on nutrient runoff can decrease the impact agriculture has on global warming and the ecosystem. Understanding the effect of probiotics on dairy cows can lead to numerous environmental and economic benefits.

Economic and Social Impact

Climate change poses a costly concern in terms of maintenance through loss of connectivity and repairs to infrastructure (Schweikert et al., 2014). In order to prevent this, pro-active adaptation measures are crucial in order to protect current and future infrastructure investments as well as the economic, social, and other functions they provide (Schweikert et al., 2014). These are only a few examples of how climate change could negatively influence 22 sectors of the economy by an increase in temperature, estimated to range in costing the U.S. hundreds of billions of US dollars each year by the end of the century (Martinich & Crimmins, 2019). Infrastructure damage can be due to rising sea-levels, floods, droughts, wildfires, and hurricanes, leading to serious repair of homes, roads, dams, and seawalls (Schweikert et al., 2014). Furthermore, economic impacts from climate change such as loss in productivity due to harm in trade, transportation, agriculture, fisheries, energy production, and even tourism, impact the economy negatively. In addition to the economy, social impacts can arise from climate change. There have been studies that have analyzed the serious implications of forced migrations and impacts on environmental, economic, and social vulnerabilities (Brown, 2007). These forced migrations are increasing discussions on climate refugees or “Climate Change

Displaced People”- defined as those whose habitat is threatened or at risk of being extinguished due to climate change (Hodgkinson et al., 2009). Although the extreme economic impacts of climate refugees are still being studied, it is important to consider the possibility of forced migration hindering economic development.

BIBLIOGRAPHY

Anderson. (1989). *Toxic algal blooms and red tides: A global perspective. | Semantic Scholar*. https://www.semanticscholar.org/paper/Toxic-algal-blooms-and-red-tides-%3A-a-global-Anderson/26331da50b8b3b67671cf867d79066367be47767

Anderson, D. M. (2009). Approaches to monitoring, control and management of harmful algal blooms (HABs). *Ocean & Coastal Management*, *52*(7), 342–347. https://doi.org/10.1016/j.ocecoaman.2009.04.006

Bahar, B., Schmidt, O., Moloney, A. P., Scrimgeour, C. M., Begley, I. S., & Monahan, F. J. (2008). Seasonal variation in the C, N and S stable isotope composition of retail organic and conventional Irish beef. *Food Chemistry*, *106*(3), 1299–1305. https://doi.org/10.1016/j.foodchem.2007.07.053

Barry, T. N., Thompson, A., & Armstrong, D. G. (1977). Rumen fermentation studies on two contrasting diets. 1. Some characteristics of the in vivo fermentation, with special reference to the composition of the gas phase, oxidation/reduction state and volatile fatty acid proportions. *The Journal of Agricultural Science*, *89*(1), 183–195. https://doi.org/10.1017/S0021859600027362

Bauman, D. E., Davis, C. L., & Bucholtz, H. F. (1971). Propionate Production in the Rumen of Cows Fed Either a Control or High-Grain, Low-Fiber Diet. *Journal of Dairy Science*, *54*(9), 1282–1287. https://doi.org/10.3168/jds.S0022-0302(71)86021-6

Bayat, A. R., Kairenius, P., Stefański, T., Leskinen, H., Comtet-Marre, S., Forano, E., Chaucheyras-Durand, F., & Shingfield, K. J. (2015). Effect of camelina oil or live yeasts (Saccharomyces cerevisiae) on ruminal methane production, rumen fermentation, and milk fatty acid composition in lactating cows fed grass silage diets. *Journal of Dairy Science*, *98*(5), 3166–3181. https://doi.org/10.3168/jds.2014-7976

Bedford, A., & Gong, J. (2018). Implications of butyrate and its derivatives for gut health and animal production. *Animal Nutrition*, *4*(2), 151–159. https://doi.org/10.1016/j.aninu.2017.08.010

Berni Canani, R., Di Costanzo, M., & Leone, L. (2012). The epigenetic effects of butyrate: Potential therapeutic implications for clinical practice. *Clinical Epigenetics*, *4*(1), 4. https://doi.org/10.1186/1868-7083-4-4

Brown, O. (n.d.). *Climate change and forced migration: Observations, projections and implications*. 35.

Brown—Climate change and forced migration Observations,.pdf. (n.d.). Retrieved April 16, 2021, from https://www.iisd.org/system/files/publications/climate_forced_migration.pdf?q=sites/default/files/publications/climate_forced_migration.pdf

Bugaut, M. (1987). Occurrence, absorption and metabolism of short chain fatty acids in the digestive tract of mammals. *Comparative Biochemistry and Physiology. B, Comparative Biochemistry*, *86*(3), 439–472. https://doi.org/10.1016/0305-0491(87)90433-0

Cao, Y., Cai, Y., Takahashi, T., Yoshida, N., Tohno, M., Uegaki, R., Nonaka, K., & Terada, F. (2011). Effect of lactic acid bacteria inoculant and beet pulp addition on fermentation characteristics and in vitro ruminal digestion of vegetable residue silage. *Journal of Dairy Science*, *94*(8), 3902–3912. https://doi.org/10.3168/jds.2010-3623

Chalupa, W. (1977). Manipulating Rumen Fermentation. *Journal of Animal Science*, *45*(3), 585–599. https://doi.org/10.2527/jas1977.453585x

Chamberlain, D. (1989). Optimizing compound feed use in dairy cows with high intakes of silage. *Recent Advances in Animal Nutrition-1989*. https://ci.nii.ac.jp/naid/10024581702/

Chouinard, P. Y., Corneau, L., Sæbø, A., & Bauman, D. E. (1999). Milk Yield and Composition During Abomasal Infusion of Conjugated Linoleic Acids in Dairy Cows1. *Journal of Dairy Science*, *82*(12), 2737–2745. https://doi.org/10.3168/jds.S0022-0302(99)75530-X

Cole, C. V., Flach, K., Lee, J., Sauerbeck, D., & Stewart, B. (1993). Agricultural sources and sinks of carbon. *Water, Air, and Soil Pollution*, *70*(1), 111–122. https://doi.org/10.1007/BF01104991

Dewhurst, R. J., Davies, D. R., & Merry, R. J. (2000). Microbial protein supply from the rumen. *Animal Feed Science and Technology*, *85*(1), 1–21. https://doi.org/10.1016/S0377-8401(00)00139-5

Ellis, J. L., Bannink, A., Hindrichsen, I. K., Kinley, R. D., Pellikaan, W. F., Milora, N., & Dijkstra, J. (2016). The effect of lactic acid bacteria included as a probiotic or silage inoculant on in vitro rumen digestibility, total gas and methane production. *Animal Feed Science and Technology*, *211*, 61–74. https://doi.org/10.1016/j.anifeedsci.2015.10.016

Evans, T. J., & Hooser, S. B. (2010). 10.16—Comparative Gastrointestinal Toxicity. In C. A. McQueen (Ed.), *Comprehensive Toxicology (Second Edition)* (pp. 195–206). Elsevier. https://doi.org/10.1016/B978-0-08-046884-6.00848-4

Fernández-García, J., Altea-Manzano, P., Pranzini, E., & Fendt, S.-M. (2020). Stable Isotopes for Tracing Mammalian-Cell Metabolism In Vivo. *Trends in Biochemical Sciences*, *45*(3), 185–201. https://doi.org/10.1016/j.tibs.2019.12.002

Fossato da Silva, D. A., Biscola, N. P., Souza, R. M. F., Caetano, D. A., Denadai, J. C., Sartori, M. M. P., da Silva, E. T., Ducatti, C., Martins, C. L., Jorge, A. M., dos Santos, L. D., Ferreira, R. S., & Barraviera, B. (2012). 45. Carbon-13 and Nitrogen-15 Turnover in Serum of Bubaline Donors of Biological Material for Medical Use. *Toxicon*, *60*(2), 117. https://doi.org/10.1016/j.toxicon.2012.04.046

Gerber, P. J., Steinfeld, H., Henderson, B., Mottet, A., Opio, C., Dijkman, J., Falcucci, A., & Tempio, G. (2013). Tackling climate change through livestock: A global assessment of emissions and mitigation opportunities. *Tackling Climate Change through Livestock: A Global Assessment of Emissions and Mitigation Opportunities.* https://www.cabdirect.org/cabdirect/abstract/20133417883

Giesecke, D., & Stangassinger, M. (1980). Lactic acid metabolism. In Y. Ruckebusch & P. Thivend (Eds.), *Digestive Physiology and Metabolism in Ruminants: Proceedings of the 5th International Symposium on Ruminant Physiology, held at Clermont—Ferrand, on 3rd–7th September, 1979* (pp. 523–539). Springer Netherlands. https://doi.org/10.1007/978-94-011-8067-2_25

Gros, M., Mas-Pla, J., Boy-Roura, M., Geli, I., Domingo, F., & Petrović, M. (2019). Veterinary pharmaceuticals and antibiotics in manure and slurry and their fate in amended agricultural soils: Findings from an experimental field site (Baix Empordà, NE Catalonia). *Science of The Total Environment*, *654*, 1337–1349. https://doi.org/10.1016/j.scitotenv.2018.11.061

Hallegraeff, G. (1993). A Review of Harmful Algal Blooms and Their Apparent Global Increase. *Phycologia*, *32*. https://doi.org/10.2216/i0031-8884-32-2-79.1

Heaton, K., Kelly, S. D., Hoogewerff, J., & Woolfe, M. (2008). Verifying the geographical origin of beef: The application of multi-element isotope and trace element analysis. *Food Chemistry*, *107*(1), 506–515. https://doi.org/10.1016/j.foodchem.2007.08.010

Hodgkinson, D., Burton, T., Young, L., & Anderson, H. (2009). Copenhagen, Climate Change "Refugees" and the Need for a Global Agreement. *Public Policy*. https://search.informit.org/doi/abs/10.3316/INFORMIT.117407333511939

Hu, X., Zhou, Q., & Luo, Y. (2010). Occurrence and source analysis of typical veterinary antibiotics in manure, soil, vegetables and groundwater from organic vegetable bases, northern China. *Environmental Pollution*, *158*(9), 2992–2998. https://doi.org/10.1016/j.envpol.2010.05.023

Huffman, C. F. (1948). The Role of the Rumen in the Nutrition of the Ruminant. *The Scientific Monthly*, *66*(4), 342–344.

Huhtanen, P., Cabezas-Garcia, E. H., Utsumi, S., & Zimmerman, S. (2015). Comparison of methods to determine methane emissions from dairy cows in farm conditions. *Journal of Dairy Science*, *98*(5), 3394–3409. https://doi.org/10.3168/jds.2014-9118

Hungate, R. (1966). *The Rumen and its Microbes*. Elsevier. https://doi.org/10.1016/C2013-0-12555-X

Johnson, K. A., & Johnson, D. E. (1995). Methane emissions from cattle. *Journal of Animal Science*, *73*(8), 2483–2492. https://doi.org/10.2527/1995.7382483x

Kamra, D. N. (2005). Rumen microbial ecosystem. *Current Science*, *89*(1), 124–135.

Kato, S.-I., Sato, K., Chida, H., Roh, S.-G., Ohwada, S., Sato, S., Guilloteau, P., & Katoh, K. (2011). Effects of Na-butyrate supplementation in milk formula on plasma concentrations of GH and insulin, and on rumen papilla development in calves. *The Journal of Endocrinology*, *211*(3), 241–248. https://doi.org/10.1530/JOE-11-0299

Kelly, M. L., Berry, J. R., Dwyer, D. A., Griinari, J. M., Chouinard, P. Y., Van Amburgh, M. E., & Bauman, D. E. (1998). Dietary Fatty Acid Sources Affect Conjugated Linoleic Acid Concentrations in Milk from Lactating Dairy Cows. *The Journal of Nutrition*, *128*(5), 881–885. https://doi.org/10.1093/jn/128.5.881

Kleen, J. L., Hooijer, G. A., Rehage, J., & Noordhuizen, J. P. T. M. (2003). Subacute Ruminal Acidosis (SARA): A Review. *Journal of Veterinary Medicine Series A*, *50*(8), 406–414. https://doi.org/10.1046/j.1439-0442.2003.00569.x

Kleiber, M., Cole, H. H., & Mead, S. W. (1943). Bloat in Cattle and Composition of Rumen Gases. *Journal of Dairy Science*, *26*(10), 929–933. https://doi.org/10.3168/jds.S0022-0302(43)92788-2

Kohn, R. A., & Kim, S. W. (2015). *Process for producing lower alkyl alcohols from cellulosic biomass using microorganisms* (United States Patent No. US9193979B2). https://patents.google.com/patent/US9193979B2/en

Lehman, E. M. (2008). Impoundments. In S. E. Jørgensen & B. D. Fath (Eds.), *Encyclopedia of Ecology* (pp. 1937–1942). Academic Press. https://doi.org/10.1016/B978-008045405-4.00058-6

Li, X., Højberg, O., Canibe, N., & Jensen, B. B. (2016). Phylogenetic diversity of cultivable butyrate-producing bacteria from pig gut content and feces. *Journal of Animal Science*, *94*(suppl_3), 377–381. https://doi.org/10.2527/jas.2015-9868

Liu, F., Ying, G.-G., Tao, R., Zhao, J.-L., Yang, J.-F., & Zhao, L.-F. (2009). Effects of six selected antibiotics on plant growth and soil microbial and enzymatic activities. *Environmental Pollution*, *157*(5), 1636–1642. https://doi.org/10.1016/j.envpol.2008.12.021

Malchi, T., Maor, Y., Tadmor, G., Shenker, M., & Chefetz, B. (2014). Irrigation of Root Vegetables with Treated Wastewater: Evaluating Uptake of Pharmaceuticals and the Associated Human Health Risks. *Environmental Science & Technology*, *48*(16), 9325–9333. https://doi.org/10.1021/es5017894

Malik, P. K., Bhatta, R., Takahashi, J., Kohn, R., & Prasad, C. S. (2015). *Livestock Production and Climate Change*. CABI.

Martinich, J., & Crimmins, A. (2019). Climate damages and adaptation potential across diverse sectors of the United States. *Nature Climate Change*, *9*(5), 397–404. https://doi.org/10.1038/s41558-019-0444-6

Matthews, C., Crispie, F., Lewis, E., Reid, M., O'Toole, P. W., & Cotter, P. D. (2018). The rumen microbiome: A crucial consideration when optimising milk and meat production and nitrogen utilisation efficiency. *Gut Microbes*, *10*(2), 115–132. https://doi.org/10.1080/19490976.2018.1505176

McArthur, J. M., & Miltimore, J. E. (2011). RUMEN GAS ANALYSIS BY GAS-SOLID CHROMATOGRAPHY. *Canadian Journal of Animal Science*. https://doi.org/10.4141/cjas61-026

Newbold, C. J., Wallace, R. J., & Mcintosh, F. M. (1996). Mode of action of the yeast Saccharomyces cerevisiae as a feed additive for ruminants. *British Journal of Nutrition*, *76*(2), 249–261. https://doi.org/10.1079/BJN19960029

Nocek, J. E., & Russell, J. B. (1988). Protein and Energy as an Integrated System. Relationship of Ruminal Protein and Carbohydrate Availability to Microbial Synthesis and Milk Production. *Journal of Dairy Science*, *71*(8), 2070–2107. https://doi.org/10.3168/jds.S0022-0302(88)79782-9

Omole, O. O., Nappert, G., Naylor, J. M., & Zello, G. A. (2001). Both L- and D-lactate contribute to metabolic acidosis in diarrheic calves. *The Journal of Nutrition*, *131*(8), 2128–2131. https://doi.org/10.1093/jn/131.8.2128

Osorio, M. T., Moloney, A. P., Schmidt, O., & Monahan, F. J. (2011). Beef Authentication and Retrospective Dietary Verification Using Stable Isotope Ratio Analysis of Bovine Muscle and Tail Hair. *Journal of Agricultural and Food Chemistry*, *59*(7), 3295–3305. https://doi.org/10.1021/jf1040959

Pippard, A. B. (1966). *Elements of Classical Thermodynamics for Advanced Students of Physics*. University Press.

Rogers, J. A., Davis, C. L., & Clark, J. H. (1982). Alteration of Rumen Fermentation, Milk Fat Synthesis, and Nutrient Utilization with Mineral Salts in Dairy Cows1. *Journal of Dairy Science*, *65*(4), 577–586. https://doi.org/10.3168/jds.S0022-0302(82)82235-2

Russell, J. B. (1998). The Importance of pH in the Regulation of Ruminal Acetate to Propionate Ratio and Methane Production In Vitro. *Journal of Dairy Science*, *81*(12), 3222–3230. https://doi.org/10.3168/jds.S0022-0302(98)75886-2

Russell, J. B. (2009). Rumen. In M. Schaechter (Ed.), *Encyclopedia of Microbiology (Third Edition)* (pp. 163–174). Academic Press. https://doi.org/10.1016/B978-012373944-5.00061-4

Russell, J. B., & Rychlik, J. L. (2001). Factors that alter rumen microbial ecology. *Science (New York, N.Y.)*, *292*(5519), 1119–1122. https://doi.org/10.1126/science.1058830

Russell, James B., & Hespell, R. B. (1981). Microbial Rumen Fermentation. *Journal of Dairy Science*, *64*(6), 1153–1169. https://doi.org/10.3168/jds.S0022-0302(81)82694-X

Russell, James B., & Rychlik, J. L. (2001). Factors That Alter Rumen Microbial Ecology. *Science*, *292*(5519), 1119–1122. https://doi.org/10.1126/science.1058830

Scheppach, W. (1994). Effects of short chain fatty acids on gut morphology and function. *Gut*, *35*(1 Suppl), S35–S38.

Schmidt, O., Quilter, J. M., Bahar, B., Moloney, A. P., Scrimgeour, C. M., Begley, I. S., & Monahan, F. J. (2005). Inferring the origin and dietary history of beef from C, N and S stable isotope ratio analysis. *Food Chemistry*, *91*(3), 545–549. https://doi.org/10.1016/j.foodchem.2004.08.036

Schweikert, A., Chinowsky, P., Espinet, X., & Tarbert, M. (2014). Climate Change and Infrastructure Impacts: Comparing the Impact on Roads in ten Countries through 2100. *Procedia Engineering*, *78*, 306–316. https://doi.org/10.1016/j.proeng.2014.07.072

Smith, P., Martino, D., Cai, Z., Gwary, D., Janzen, H., Kumar, P., McCarl, B., Ogle, S., O'Mara, F., Rice, C., Scholes, B., Sirotenko, O., Howden, M., McAllister, T., Pan, G., Romanenkov, V., Schneider, U., Towprayoon, S., Wattenbach, M., & Smith, J. (2008). Greenhouse gas mitigation in agriculture. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *363*(1492), 789–813. https://doi.org/10.1098/rstb.2007.2184

Soest, P. J. V. (1994). *Nutritional Ecology of the Ruminant*. Cornell University Press.

Stewart, C. S. (1992). Lactic Acid Bacteria in the Rumen. In B. J. B. Wood (Ed.), *The Lactic Acid Bacteria Volume 1: The Lactic Acid Bacteria in Health and Disease* (pp. 49–68). Springer US. https://doi.org/10.1007/978-1-4615-3522-5_3

Sutton, J. D. (1980). Digestion and end-product formation in the rumen from production rations. In Y. Ruckebusch & P. Thivend (Eds.), *Digestive Physiology and Metabolism in Ruminants: Proceedings of the 5th International Symposium on Ruminant Physiology, held at Clermont—Ferrand, on 3rd–7th September, 1979* (pp. 271–290). Springer Netherlands. https://doi.org/10.1007/978-94-011-8067-2_13

Sutton, J. D. (1985). Digestion and Absorption of Energy Substrates in the Lactating Cow. *Journal of Dairy Science*, *68*(12), 3376–3393. https://doi.org/10.3168/jds.S0022-0302(85)81251-0

Ungerfeld, E., & Kohn, R. (2006). The Role of Thermodynamics in the Control of Ruminal Fermentation. In *Ruminant Physiology: Digestion, Metabolism and Impact of Nutrition on Gene Expression, Immunology and Stress* (pp. 55–85).

Uyeno, Y., Shigemori, S., & Shimosato, T. (2015). Effect of Probiotics/Prebiotics on Cattle Health and Productivity. *Microbes and Environments*, *30*(2), 126–132. https://doi.org/10.1264/jsme2.ME14176

Venkateshan, S. P., & Swaminathan, P. (2014). Chapter 1—Preliminaries. In S. P. Venkateshan & P. Swaminathan (Eds.), *Computational Methods in Engineering* (pp. 1–15). Academic Press. https://doi.org/10.1016/B978-0-12-416702-5.50001-6

Young, C. W., Hillers, J. K., & Freeman, A. E. (1986). Production, Consumption, and Pricing of Milk and Its Components1, 2. *Journal of Dairy Science*, *69*(1), 272–281. https://doi.org/10.3168/jds.S0022-0302(86)80398-8

Zhou, X., Wang, J., Lu, C., Liao, Q., Gudda, F. O., & Ling, W. (2020). Antibiotics in animal manure and manure-based fertilizers: Occurrence and ecological risk assessment. *Chemosphere*, *255*, 127006. https://doi.org/10.1016/j.chemosphere.2020.127006

CHAPTER 2:

EFFECT OF AIR AND PROBIOTICS ON IN VITRO FERMENTATION

ABSTRACT: Many fiber-digesting microorganisms are strict anaerobes so fiber digestion could be decreased when ruminants swallow air during feed consumption. The objective of this study was to evaluate the effect of adding air on rumen fermentation, and to determine whether adding aerobic probiotics can ameliorate the effects of air. Twelve treatments were analyzed in a 4x3 factorial design with 4 levels of air treatments and 3 probiotic treatments. Air treatments included: no air added to chemically reduced medium, and 0, 25 mL or 50 mL air added to 40 mL unreduced medium with 10 mL rumen inoculum at the start of fermentation in 125 mL flasks. Probiotic treatments were: no additive (control), Dairyman's Edge (DE), and live yeast. Timothy hay (0.5 g) and corn grain (0.5 g) were incubated at 39°C for 24 hours with 5 replicates. Results were analyzed by the model: $Y = \mu + A + P + AxP + S + E$, where Y is the response variable, and A is a fixed effect of air or reducing agent, P is the effect of yeast or probiotic additive, S is sequence and E is error. Significant differences were accepted at $P < 0.05$, and tendencies at $P < 0.10$. Two runs were completed in this study. In both, increased air decreased NDF% digestibility ($P < 0.05$). Air in both studies also decreased butyrate ($P < 0.05$) from reduced media, to 0 mL of air, 25 mL of air and 50 mL of air. In Run 1 alone, air treatment tended to vary by reduction and air treatment, with lower gas volume for reduced media compared with unreduced, and with increasing gas volume as air addition increased. With the probiotic treatment, Run 1 tended ($P < 0.10$) to increase pH from 6.3 for no probiotic or with for yeast treatments, and 6.62 for DE while decreasing in Total VFA. In Run 2, there was a significant ($P < 0.05$) effect of 8 h gas with air as it decreased from reduced to 25 mL and increased from 25 mL to 50 mL. There was also a tendency (*P*

< 0.10) in Run 2 for that follows the same pattern as the 8 h gas data. Furthermore, Run 2 had a tendency ($P < 0.10$) for 4 h gas fluctuation between the gas treatments. Acetate production had a significant ($P < 0.05$) decrease with probiotics in Run 1, however had a significant increase in Run 2, with total VFA having a tendency ($P < 0.10$) to follow the same pattern. Propionate tended to increase in Run 2 as well, while having no effect in Run 1. There was no effect on acetate to propionate ratio in either study for both air and probiotics however there was an interaction between the two. Furthermore, Run 1 had an interaction between probiotic and air for 4 h gas and total gas. Run 2 had an interaction between probiotic and air for NDF, acetate, acetate:propionate, and total VFA. Use of probiotics did not ameliorate the decrease in fiber digestion due to presence of air.

Key Words: dairy, probiotics, air, rumen, fermentation

INTRODUCTION

Probiotics are defined by the World Health Organization as live microorganisms and yeasts, which when dispensed in appropriate amounts, can benefit the host they inhabit due to the similar qualities they share with the bacteria currently in the body (Mack, 2005). In addition to human studies on probiotics, many studies have investigated the effects of probiotics on other mammals, such as cattle (Moya et al., 2009). The potential benefits of certain probiotics in a cow's digestive system have been discussed at length (Uyeno et al., 2015). These strains have the potential to increase the production of volatile fatty acids (VFAs) which increase bovine milk production (Uyeno et al., 2015). Through the background research completed for this experiment, the probiotics: Dairyman's Edge (DE; Papillon Agricultural Company, Easton, MD) and live yeast (*Saccharomyces cerevisiae*) can possibly increase the VFAs within the rumen by adjusting certain pathways, promote fermentation, and therefore may increase the amount of milk produced from each cow. Furthermore, these probiotics can possibly offset effects of air. As cattle consume their food, they swallow air (Barry et al., 1977) which could potentially inhibit the growth of organisms within their rumen (Newbold et al., 1996). However, live yeast may decrease the amount of oxygen within the rumen and increase digestibility by utilizing the oxygen swallowed for growth. The objective of this study was to examine the impact of air in the rumen and how certain probiotics could decrease the negative effects of oxygen. Understanding air's impact on digestibility as well as yeast's ability to utilize oxygen can help farmers select certain probiotics to increase milk production in their cattle or decrease bloat.

MATERIALS AND METHODS

Probiotics Studied

Dairyman's Edge (DE; Papillon Agricultural Company, Easton, MD) is a probiotic that maximizes feed efficiency and production regardless of the lactation stage in a cow, specifically "increasing dry matter intake, supporting healthy rumen, and assisting dairy animals in capturing feed nutrients" (Papillon, 2020). Specifically, Dairyman's Edge contains live yeast such as active dry *Saccharomyces cerevisiae*; yeast cultures of *S. cerevisiae* grown on corn products, cane molasses, and malted barley; live bacterial cultures such as dried fermentation products of: *E. faecium*, *L. acidophilus*, *L. plantarum*, *L. brevis*, dried extracts of: *B. lentus*, *B. amyloliquefaciens*; exogenous enzymes; salt of glutamic acid, dried grain, molasses products, calcium carbonate, and mineral oil (Papillon, 2020). The typical analysis includes 18.7% minimum of protein, 4.3% crude fat, 5.5% acid detergent fiber, 18.5% neutral detergent fiber, 9.3% calcium, and 28.4% ash.

Live yeast used for this experiment is a Biomate YC-20 yeast concentrate, manufactured by Chr. Hansen and specific towards beef and dairy cattle. Primary ingredient is dried saccharomyces cerevisiae fermentation product and was stored in a cooler (36˚F).

Experimental design and treatments

The study analyzed twelve treatments in a 4x3 factorial design with 4 levels of air treatments and 3 probiotic treatments, and 3 randomized blocks of samples over time and space. Air treatments included: no air added to chemically reduced medium, and 0, 25 mL or 50 mL air added to 40 mL unreduced medium with 10 mL rumen

inoculum at the start of fermentation in 125 mL flasks. Probiotic treatments were: no additive (control), Dairyman's Edge (DE), and live yeast. Timothy hay (0.5 g) and corn grain (0.5 g) were distributed to Approximately two liters of rumen fluid (solid and liquid fraction) was collected from permanently non-lactating rumen-cannulated cow consuming a timothy hay diet. The contents were then blended and strained with a cheesecloth and run under CO_2 to remove air.

Stoppers fitted with two glass tubes with luer-lock fittings to attach tubing or balloons were attached to all flasks, and the gas was removed with the glass syringe. Carbon dioxide gas was perfused through each flask before and during filling with medium, and then treatments. Forty milliliters of prepared media were distributed to each Erlenmeyer flasks under CO_2 as well as 10 mL of rumen fluid. One milliliter of reducing agent (Cysteine and Na_2S) was added to specific flasks as well as 1 mL of Dairyman's Edge stock solution (140 mg/40 mL of media) as well as Yeast stock solution (140 mg/40 mL of media) was added to specific flasks. Gas treatments were added with the glass syringe and placed in the incubator. The pH was tested and VFA samples were taken from the time zeroes only and immediately placed into the freezer.

Sampling and Measurements

Gas Sampling

Syringes with balloons were attached in order to measure gas for the experiment and samples were placed in an incubator at 39 °C for a total of 24 hours. Gas was collected 4 and 8 h after the samples were placed in the incubator/water bath. The gas was measured with a glass syringe and expelled. After 24 hours, gas was

collected, and the pH was measured in the random order. Samples for VFA were taken and placed in the freezer.

NDF Sampling

Neutral Detergent Fiber (NDF) analysis was completed according to the method of Mertens. A few days before, NDF solution was made and beakers were labeled with the random identification number and laid out. Each sample, including the time zeroes and blanks were thawed and poured in a beaker with 100 mL of NDF solution. The analysis was completed by refluxing each beaker for one hour, adding amylase solution to prevent gelatinous material interfering with filtration, and pouring into a crucible. In the crucible, samples were aspirated and rinsed with acetone. Then, after drying, the crucibles were placed in a preheated 100˚C oven overnight. The following day, the crucibles were hot weighed, and the weights were recorded. The percentage of NDF was calculated as 100 times dry NDF residue divided by original feed. The percentage of NDF digested was the NDF percentage remaining after 24 h digestion divided by the NDF percentage of the NDF of feed that was not fermented.

Ash Sampling

Ash analysis was completed after NDF analysis. Each crucible was placed in the muffle furnace at 500˚C for a minimum of six hours. The furnace was then turned off and left to cool overnight in the furnace. The next morning, crucibles were moved into the preheated oven at 100˚C and warmed up for one hour. The crucibles were then hot-weighed and the ash percentage was calculated as 100 times the weight of

ash residue subtracted from the original sample weight divided by original sample weight.

Statistical Analysis

The data were analyzed using the model:

$$Y = \mu + A + P + AxP + S + E$$

where Y is the response variable, and A is a fixed effect of air or reducing agent, P is the effect of yeast or probiotic additive, S is the randomized sequence and E is error. Significant differences were accepted at $P < 0.05$, and tendencies at $P < 0.1$. A Student T test was run on JMP to determine significant effects between air treatments and probiotics.

RESULTS

Air Treatment for Run 1 Decreased Digestibility

Air decreased NDF digestibility ($P < 0.05$ from 51.11% for reduced medium to 45.83%, 45.00%, and 41.28 % with 0, 25, and 50 mL air respectively. For VFA, butyrate only had a main effect ($P < 0.05$) from air treatment, decreasing significantly from 12.3 for reduced medium to 12.1 for 0 mL of air unreduced, 10.9 for 25 mL of air unreduced and 11.0 for 50 mL of air. Finally, there was tendency ($P < 0.10$) for a decreased air effect on 8 h gas volume starting with 41 for 0 mL of air reduced, 44 for 0 mL of air unreduced, and 42 for 25 mL of air unreduced, to 32 for 50 mL of air unreduced. Student T test determined change between reduced 0 mL of air, 0 mL of air unreduced, and 25 mL of air unreduced was significant from 50 mL of air unreduced.

Probiotic Treatment for Run 1 Decreased VFA

Acetate decreased ($P < 0.05$) for both yeast and DE. We see acetate had a value of 97 with no probiotic, then decreased to 75 with yeast, and then 72 with DE. Total VFA also tended ($P < 0.10$) to decrease for both yeast and DE (starting with no probiotic having a value of 145, yeast with 125, and DE with 120. No other VFA was affected by probiotic, however pH increased ($P < 0.10$) starting with 6.3 for no probiotic, and 6.3 with yeast, and 6.6 with DE.

Air Treatment for Run 2 Increased Digestibility

Air decreased ($P < 0.05$) NDF digestibility from 45.9% for reduced medium to 44.75%, 40.56% and 40.17% with 0, 25, and 50 mL air respectively. For VFA, butyrate only had a main effect from air treatment, decreasing significantly ($P < 0.05$) from 17.2 for reduced medium to 14.6 for 0 mL of air unreduced, 13.8 for 25 mL of air unreduced, and then increasing at 50 mL of air with 14.6.

Air treatment also had a tendency to decrease both the 4 h gas and the total gas, while having a significant effect on the 8 h gas. Air tended ($P < 0.10$) to decrease the 4 h gas from 56 for reduced medium, 59 for 0 mL of air unreduced, and 46 for 50 mL of air, to 43 for 25 mL air. Air tended ($P < 0.10$) to decrease total gas from 240 for reduced medium, 225 for 0 mL of air unreduced, and 212 for 50 mL of air unreduced to 191 for 25 mL air unreduced. Finally, air had a significant ($P < 0.05$) decrease for air effect on 8 h gas starting with 28 for 0 mL of air reduced, 26 for 0 mL of air unreduced, and 25 for 50 mL of air unreduced to 19 for 25 mL of air unreduced.

Probiotic Treatment for Run 2 Decreased VFA

There were a few probiotic effects starting with Total VFA. Total VFA had a significant increase ($P < 0.05$) starting with 111 for no probiotic and 119 with yeast, to jump to 137 with DE.

Acetate production had a significant increase ($P < 0.05$) for the DE treatment jumping from a value of 61 for no probiotic and 60 for yeast, to 78 for DE. Propionate had a tendency ($P < 0.10$) to increase for both yeast and DE, moving from 33 for no probiotic, to 39 for yeast, and 39 for DE. There were no other significant probiotic effects for the 2nd run.

DISCUSSION

NDF Decreased with added Air

As seen in Table 2.1 and 2.3, there was a significant decrease in NDF for Run 1 and Run 2. In Run 1, the values for decreased NDF digestibility ($P < 0.05$) from 51.11% for reduced medium to 45.83%, 45.00%, and 41.28% with 0, 25, and 50 mL air respectively. In Run 2, the decrease ($P < 0.05$) in NDF digestibility due to air went from 45.89% for reduced medium to 44.75%, 40.06% and 40.17% with 0, 25, and 50 mL air respectively. The reason for this may be due to oxygen inhibiting the anaerobic, rumen bacteria. When certain anaerobic bacteria are exposed to oxygen, they can die which therefore slows fermentation within the rumen (Hentges, 1996). However, it should be noted strictly anaerobic species, e.g., methanogens, can survive in the rumen under oxygen conditions that were previously believed to be detrimental to the bacteria. In theory, the ruminal microbial population should have the ability to quickly use oxygen and remove it from the rumen which contains highly oxygen sensitive organisms (Ellis et al., 1989). However, the significant decrease between 0

mL of air unreduced and 25 mL of air could mean the limit of oxygen tolerance was surpassed and began to impact the bacteria within the flasks. This could also explain the plateau from 25 mL of air and 50 mL of air. 4 h gas and total gas had a significant interaction for Run 1. Both of these gases decreased between treatment 2 and 3 for the yeast probiotic. This could be due to yeast utilizing the O_2 within the rumen and decreasing the amount of gas however, this decrease in gas seems to be too high for just the disappearance of O_2. More gas was expected to be produced between probiotics and added air treatments due to aerobic respiration pushing the end products away from VFA and towards CO_2. The data here is suggesting there is an interaction with air and something significant is occurring, however could be further explored.

There was also a significant interaction between air and probiotic treatment on Run 2 with NDF% digested as seen in Figure 2.3. Specifically, both probiotics have a significant decrease moving from treatment 1 (0 mL of air reduced), to treatment 2 (0 mL of air unreduced), to treatment 3 (25 mL of air unreduced), with a slight increase at treatment 4 (50 mL of air unreduced). It was surprising to see the probiotic did not offset the air treatment within the rumen. This could possibly be due to the certain bacteria within the probiotic that were not able to utilize O_2 or the concentration of probiotic was too low.

VFA and Gas

The addition of air decreased expected butyrate production ($P < 0.05$) in Run 1 (Table 2.1) and also had a significant decrease ($P < 0.05$) for Run 2 (Table 2.3). Air did not have a significant impact or tendency for other VFAs.

However, there were some significant impacts and tendencies with probiotic effects in both runs. In Run 1, there was a significant decrease in acetate ($P < 0.05$) and a significant decrease in total VFA ($P < 0.10$). Run 2 however, had a few significant effects with probiotic treatment including a significant increase with total VFA ($P < 0.05$) starting with 111 for no probiotic and 119 with yeast, to then jump to 137 with DE (Table 2.4). In addition, Run 2 had acetate production with a significant increase ($P < 0.05$) for the DE treatment whereas propionate just had a tendency for both yeast and DE to increase ($P < 0.10$). Acetate to propionate ratio did not have a significant effect. The probiotics utilized contained species such as *Saccharomyces cerevisiae* and may have impacted the fermentation within the rumen and increased the level of other volatile fatty acids produced in the rumen wall as explained previously (Uyeno et al., 2015). The manipulation of the pathway can therefore increase the rumen fermentation efficiency and benefit the cattle which could explain the increase in acetate and propionate in Run 2. However, this is not in total agreement with previous studies (Erasmus et al., 1992), where yeast cultures (*S. cerevisiae*) would decrease acetate concentration while increasing propionate in order to lower the acetate to propionate ratio. An increase in total VFA from Run 2 and a decrease in total VFA from Run 1 are different from previous studies (Qadis et al., 2014) where they reported no significant change in total VFA related to their probiotic study.

Furthermore, a few interactions between probiotics and air have been seen through VFA analysis in Run 2. Acetate had a significant interaction ($P < 0.05$) with a slight increase in live yeast and then decrease at treatment 4, then DE had a sharp

decrease from treatment 1 to 3 then a slight increase at treatment 4 (Figure 2.4). Acetate to propionate ratio had a slight interaction between air and probiotic ($P < 0.10$) with a slight increase in live yeast and then decrease at treatment 4, then DE had a sharp decrease from treatment 1 to 3 then a slight increase at treatment 4 (Figure 2.5). Total VFA also had a notable interaction ($P < 0.05$) with a slight increase in live yeast and then decrease at treatment 4, then DE had a sharp decrease from treatment 1 to 3 then a slight increase at treatment 4 (Figure 2.6).

It is interesting to see the similar trend Total VFA, acetate, and the acetate to propionate ratio follow. The increase in yeast could mean the probiotics as well as the bacteria within the rumen were utilizing the air added into the system. These results support previous research of a relationship between oxygen uptake in the rumen and the ability of yeast to stimulate bacterial growth was discovered (Ellis et al., 1989; Amin & Mao, 2021). The slight decrease at 50 mL of air unreduced could indicate a max level of air was reached and no fermentation and VFA production occurred at this treatment. Furthermore, when analyzing the interaction between DE and VFAs, the decrease in VFA could be explained by aerobic metabolism within the system, ending with CO_2 instead of VFA. However, the various responses of acetate and total VFA need to be further explored as probiotic and yeast in cattle are not completely understood. Other probiotic and yeast studies also suggest that although probiotics have the capacity to impact and change the gut microbiology, the definite mode of action for probiotics and yeast have yet to be discovered (Amin & Mao, 2021). Yeast may affect metabolism and prevent the production of gas by utilizing oxygen and possibly using a unique pathway to decrease production.

Another theory related to the acetate and total VFA having a significant decrease in Run 1 is related to a few factors. The disappearance of fatty acids can be due to either the absorption of fatty acids in the rumen as the amount of lipids from the diet increases, catabolism of fatty acids in ketone bodies in the cells of the ruminal epithelium, and finally oxidation of fatty acids by bacteria adherent to the rumen wall that utilize oxygen from epithelial cells (Doreau & Ferlay, 1994; Fiorentini et al., 2015).

pH

Probiotic treatment tended ($P < 0.10$) to increase pH from 6.27 for no probiotic to 6.25, to 6.29 for yeast, and 6.62 for DE. An explanation of this could be DE altering the fermentation process in the rumen and raise and stabilize ruminal pH. This is done through stimulation of specific populations of protozoa that consume starch and compete effectively with amylolytic lactate-producing bacteria (Uyeno et al., 2015). These results agree with previous studies (Desnoyers et al., 2009) where yeast, *S. cerevisiae*, increased pH with *in vitro* rumen experiments.

Gas

In Run 1, there was tendency ($P < 0.10$) for an increased and decreased air effect on 8 h gas starting with 41.00 for 0 mL of air reduced, 43.89 for 0 mL of air unreduced, 41.72 for 25 mL of air unreduced, and 32.33 50 mL of air unreduced. Whereas, in Run 2, air treatment had a tendency to increase and decrease both the 4 h gas and the total gas, while having a significant effect on the 8 h gas. Air tended ($P < 0.10$) to increase and decrease the 4 h gas from 56.44 for reduced medium to 59.00,

42.89 and 45.67 with 0, 25, and 50 mL air respectively. Air tended ($P < 0.10$) to increase and decrease total gas from 243.33 for reduced medium to 224.56, 190.89 and 211.78 with 0, 25, and 50 mL air respectively. Finally, air had a significant ($P < 0.05$) increase and decrease for air effect on 8 h gas starting with 27.89 for 0 mL of air reduced, 25.78 for 0 mL of air unreduced, 18.78 for 25 mL of air unreduced, and 24.89 50 mL of air unreduced. There are very few studies on gas production in vitro. A rumen gas model could further explain and clarify the reasons for fluctuations with gas production. It is interesting to note how certain gas time points had significant effects from the air treatment yet there were no significant effects from air on acetate or propionate since gas production in the rumen is stoichiometrically related to VFA. As a future direction, it would be interesting to analyze the gas composition of the gas produced throughout this *in vitro* experiment.

Sequence Effect

Analyzing the data regarding the probiotic treatment and the air treatment, has shown sequence ID having a significant effect with both of the runs. We can see both the first and second runs have a sequence effect only with volatile fatty acids and pH. There are a few possibilities as to how this could have occurred. With the prospect of interconversion of VFAs, this could potentially explain the reason as to why a sequence effect appeared in both of the runs.

Future Research

Understanding the significance of the effect of probiotics in dairy cows, is important as it allows understanding of the role of these microorganisms in animal

nutrition. It has been shown that these specific strains can improve milk production in dairy cows. These findings could lead to further investigations of other potential benefits of probiotic supplementation with different strains and in different feeds.

Looking in the direction of methods, previous experiments have shown in vitro methods have been successful in measuring digestibility, however, these may not accurately estimate volatile fatty acids. We can look to new methods to measure VFA production to better understand the mechanisms of rumen fermentation. It has been noted there have been very few in vivo experiments completed due to the complications of handling and working directly with the animal. As a future study, analyzing the difference between in vitro and in vivo methods and results could lead to new questions and directions.

CONCLUSION

These results confirm probiotics and air have an impact on rumen fermentation as we have seen significant effects with gas, certain VFAs, and NDF from both runs. We identified air treatment can significantly decrease NDF% digested for both runs while also decreasing butyrate concentration. We also saw air decrease butyrate production in both runs as well as have a significant effect on 4 h gas and 8 h gas for Run 2 and just 8 h gas on Run 1. In Run 2, probiotics increased Total VFA and acetate, while having a tendency to increase propionate whereas in Run 2, had a decrease in acetate, a tendency to decrease total VFA, while tending to increase pH. The reason for these differences is uncertain. Further research is needed, such as a rumen gas model, to explain how certain VFAs and certain gas time points increase

or decrease in concentration due to probiotics and air. One theory for differences in gas time points can be due to the fact that some yeast species may utilize oxygen within a certain time frame. In addition, an isotope study can be completed to look at the preferred pathway of glucose utilization in the rumen by analyzing VFA. This can be done by gaining a better understanding of citric acid cycle, testing which level of glucose in rumen fluid will be converted to lactate, succinate, propionate, or acetate. From there, different levels of lactate with rumen fluid and succinate with rumen fluid will be analyzed to see when saturation occurs.

ACKNOWLEDGMENTS

The author would like to thank Dr. Shirron Ratiff and Jarvis G. Scott for their assistance in this experiment.

TABLES AND FIGURES

Table 2.1. Least square means of air treatments (R, 0 mL, 25 mL, 50 mL) with SEM and P value for Run 1

	Reduced	0 mL	25 mL	50 mL	SE[d]	*P* <
NDF% digested	51.11[a]	45.83[ab]	45.00[b]	41.28[b]	0.019	< 0.05
Total VFA (mmol)	130	128	128	129	4.6	NS[e]
Acetate (mmol)	81	74	72	76	3.5	NS
Prop (mmol)	35	41	44	41	5.0	NS
But (mmol)	12.3[a]	12.1[ab]	10.8[bc]	11.0[c]	0.39	< 0.05
Acetate/Prop	2.4	2.0	1.8	2.1	0.26	NS
4 h gas (mL)	68	68	63	59	4.3	NS
8 h gas (mL)	41[a]	44[a]	42[a]	32[b]	3.0	< 0.10
24 h gas (mL)	102	102	93	87	7.3	NS
Total gas (mL)	210	214	198	179	11.0	NS
pH	6.39	6.25	6.24	6.26	0.064	NS

[a,b,c]Within a row, means without a common superscript letter differ
[d]Standard error
[e]Not significant

Table 2.2. Least square means of probiotic treatments (R, 0 mL, 25 mL, 50 mL) with SEM and P value for Run 1

	Control	Yeast	DE	SE[d]	*P* <
NDF% digested	53.83	49.17	50.33	0.033	NS[e]
Total VFA (mmol)	145[a]	125[ab]	120[b]	7.7	< 0.10
Acetate (mmol)	97[a]	75[b]	72[b]	6.0	< 0.05
Prop (mmol)	34	36	35	8.5	NS
But (mmol)	12.1	12.3	12.4	0.66	NS
Acetate/Prop	3.0	2.1	2.2	0.43	NS
4 h gas (mL)	65	74	64	7.3	NS
8 h gas (mL)	38	44	41	5.1	NS
24 h gas (mL)	81	110	114	12.0	NS
Total gas (mL)	184	228	220	18.7	NS
pH	6.3[b]	6.3[b]	6.6[a]	0.11	< 0.10

[a,b,c]Within a row, means without a common superscript letter differ
[d]Standard error
[e]Not significant

Table 2.3. Least square means of air treatments (R, 0 mL, 25 mL, 50 mL) with SEM and P value for Run 2

	Reduced	0 mL	25 mL	50 mL	SE[d]	*P* <
NDF% digested	45.89[a]	44.75[a]	40.56[b]	40.17[b]	0.010	< 0.05
Total VFA (mmol)	122	121	116	120	4.2	NS[e]
Acetate (mmol)	66	66	63	66	2.9	NS
Prop (mmol)	37	37	37	38	1.3	NS
But (mmol)	17.2[a]	14.6[b]	13.8[b]	14.6[b]	0.63	< 0.05
Acetate/Prop	1.83	1.18	1.70	1.75	0.077	NS
4 h gas (mL)	56[ab]	59[a]	43[b]	46[ab]	5.1	< 0.10
8 h gas (mL)	28[a]	26[a]	19[b]	25[a]	1.6	< 0.05
24 h gas (mL)	160	140	130	140	11	NS
Total gas (mL)	240[a]	225[ab]	191[b]	212[ab]	14	< 0.10
pH	6.30	6.31	6.33	6.34	0.033	NS

[a,b,c]Within a row, means without a common superscript letter differ
[d]Standard error
[e]Not significant

Table 2.4. Least square means of control, yeast, DE treatments with P value for Run 2

	Control	Yeast	DE	SE[d]	*P* <
NDF % digested	45.5	43.6	48.6	0.017	NS
Total VFA (mmol)	111[b]	119[ab]	137[a]	7.3	< 0.05
Acetate (mmol)	61[b]	60[b]	78[a]	5.1	< 0.05
Prop (mmol)	33[b]	39[ab]	39[a]	2.2	< 0.10
But (mmol)	16	18	18	1.1	NS
Acetate/Prop	1.9	1.6	2.0	0.13	NS
4 h gas (mL)	57	55	57	8.8	NS
8 h gas (mL)	27	28	29	2.7	NS
24 h gas (mL)	162	156	160	18.5	NS
Total gas (mL)	246	239	246	24.1	NS
pH	6.35	6.28	6.26	0.057	NS

[a,b,c]Within a row, means without a common superscript letter differ
[d]Standard error
[e]Not significant

INTERACTIONS

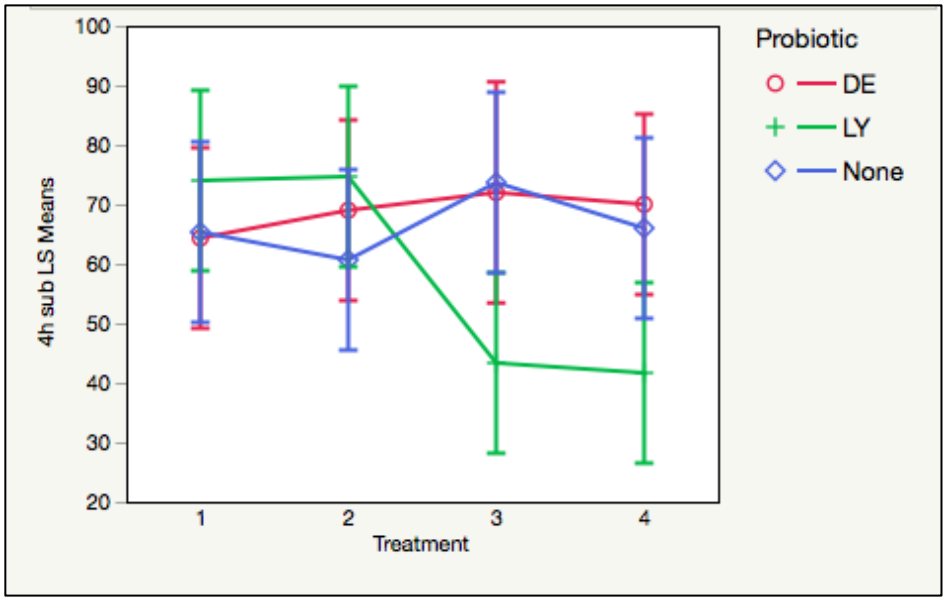

Figure 2.1. Interaction between Treatment and Probiotic for 4 h gas in Run 1 (*P* < 0.05). Treatment 1: 0 mL of air with chemically reduced medium, treatment 2: 0 mL of air with unreduced medium, treatment 3: 25 mL of air with unreduced medium, treatment 4: 50 mL of air with unreduced medium

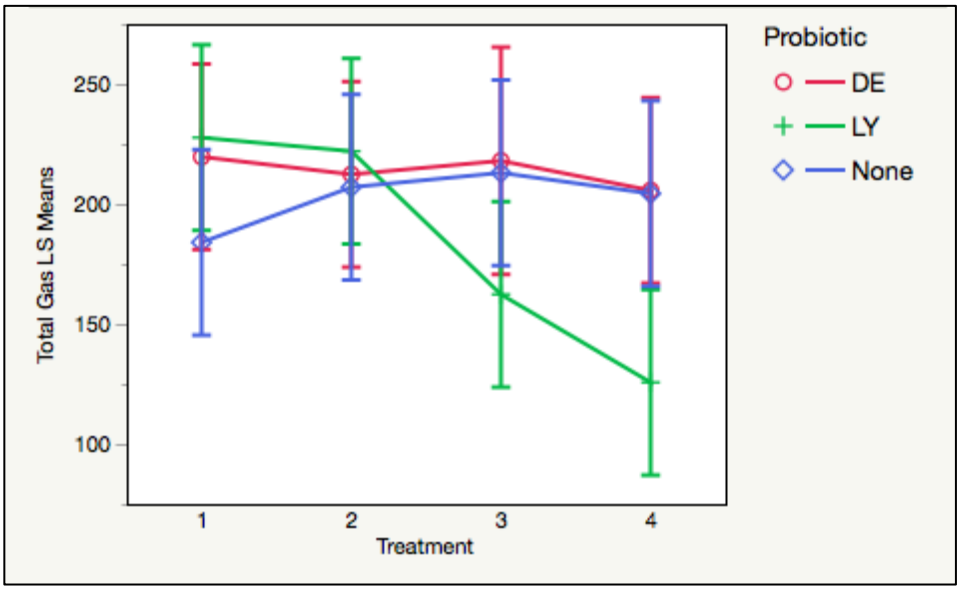

Figure 2.2. Interaction between Treatment and Probiotic for Total gas in Run 1 (*P* < 0.05). Treatment 1: 0 mL of air with chemically reduced medium, treatment 2: 0 mL of air with unreduced medium, treatment 3: 25 mL of air with unreduced medium, treatment 4: 50 mL of air with unreduced medium

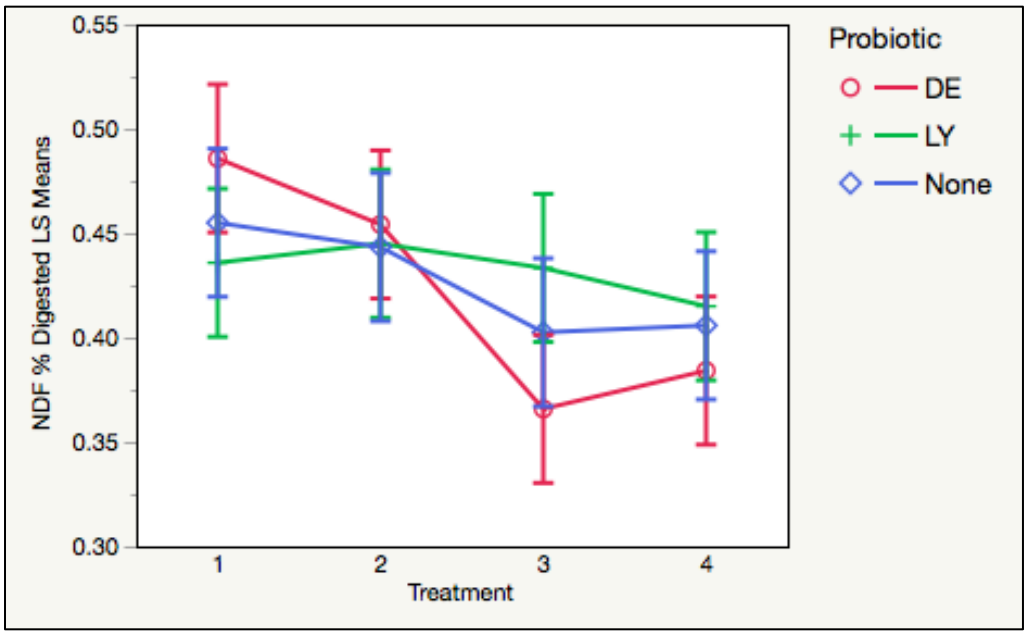

Figure 2.3. Interaction between Treatment and Probiotic for NDF% digested in Run 2 (*P* < 0.05). Treatment 1: 0 mL of air with chemically reduced medium, treatment 2: 0 mL of air with unreduced medium, treatment 3: 25 mL of air with unreduced medium, treatment 4: 50 mL of air with unreduced medium

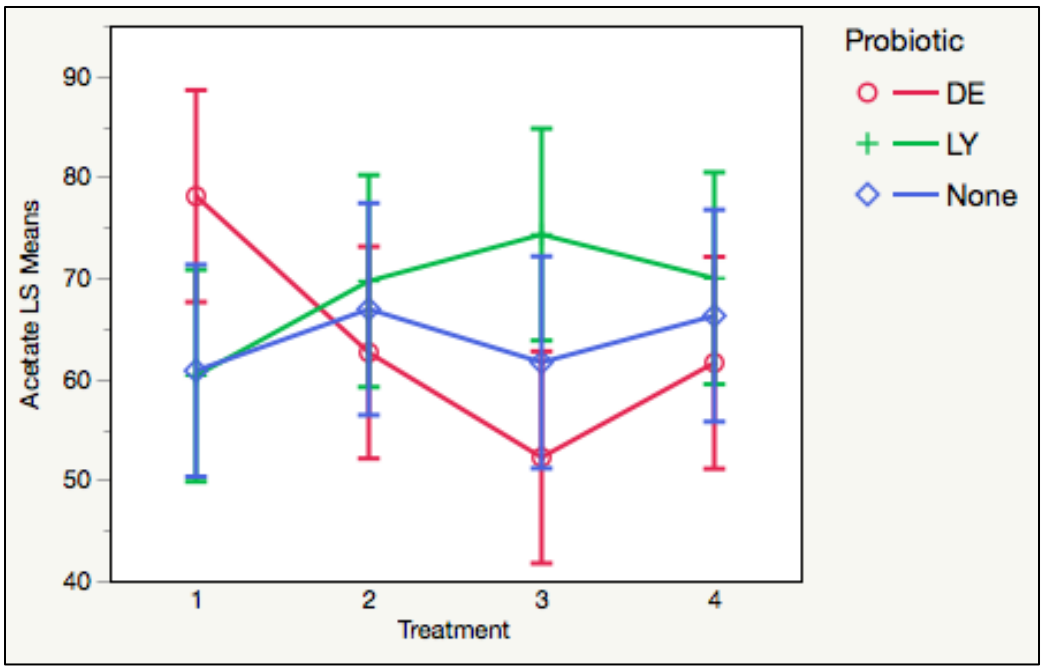

Figure 2.4. Interaction between Treatment and Probiotic for Acetate in Run 2 (*P* < 0.05). Treatment 1: 0 mL of air with chemically reduced medium, treatment 2: 0 mL of air with unreduced medium, treatment 3: 25 mL of air with unreduced medium, treatment 4: 50 mL of air with unreduced medium

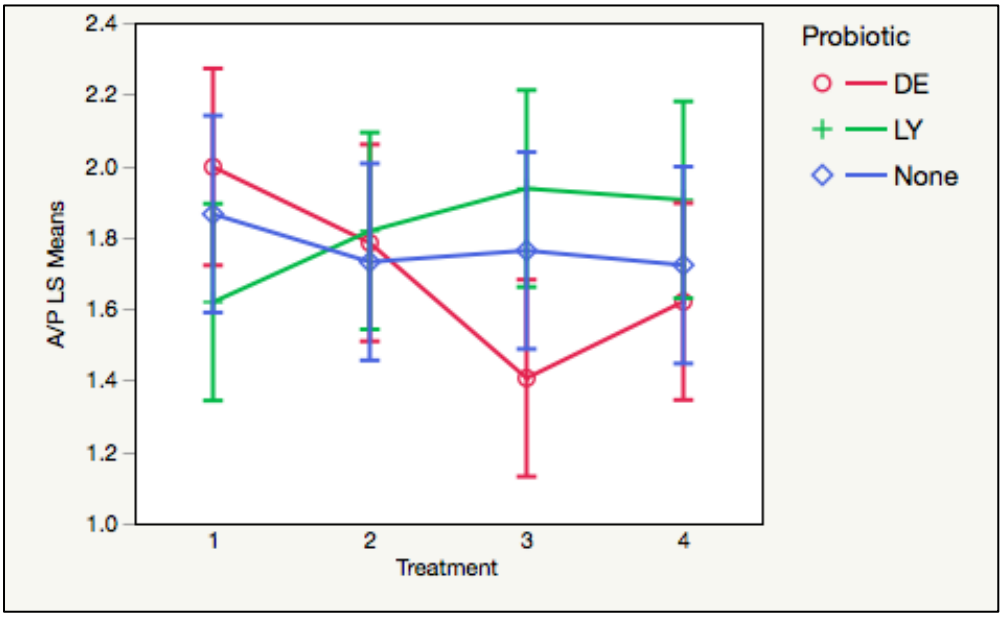

Figure 2.5. Interaction between Treatment and Probiotic for Acetate to Propionate Ratio in Run 2 (*P* < 0.10). Treatment 1: 0 mL of air with chemically reduced medium, treatment 2: 0 mL of air with unreduced medium, treatment 3: 25 mL of air with unreduced medium, treatment 4: 50 mL of air with unreduced medium

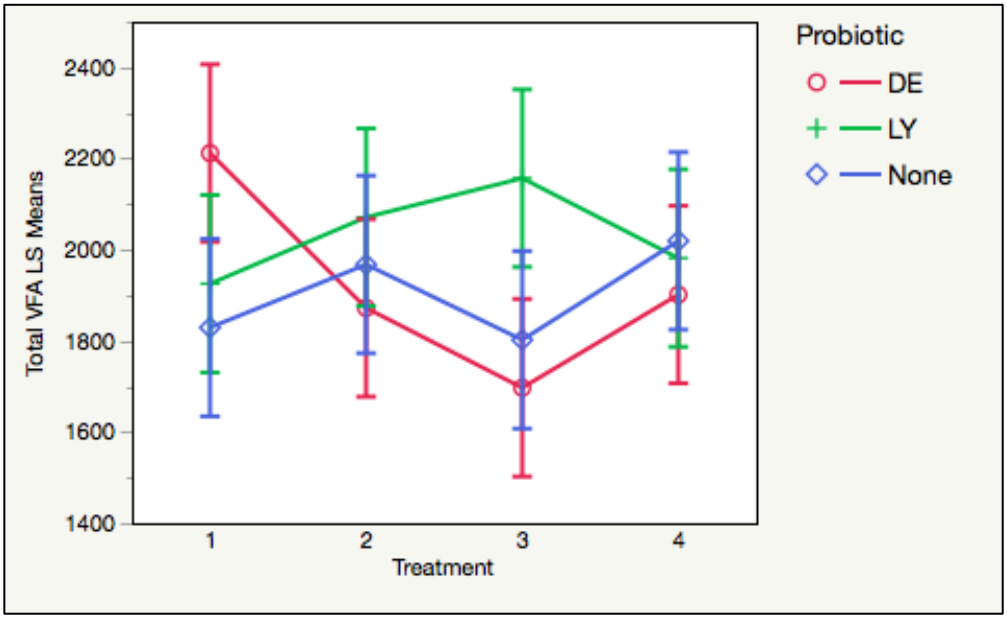

Figure 2.6. Interaction between Treatment and Probiotic for Total VFA in Run 2 (*P* < 0.05). Treatment 1: 0 mL of air with chemically reduced medium, treatment 2: 0 mL of air with unreduced medium, treatment 3: 25 mL of air with unreduced medium, treatment 4: 50 mL of air with unreduced medium

BIBLIOGRAPHY

Amin, A. B., & Mao, S. (2021). Influence of yeast on rumen fermentation, growth performance and quality of products in ruminants: A review. *Animal Nutrition*, *7*(1), 31–41. https://doi.org/10.1016/j.aninu.2020.10.005

Desnoyers, M., Giger-Reverdin, S., Bertin, G., Duvaux-Ponter, C., & Sauvant, D. (2009). Meta-analysis of the influence of Saccharomyces cerevisiae supplementation on ruminal parameters and milk production of ruminants. *Journal of Dairy Science*, *92*(4), 1620–1632. https://doi.org/10.3168/jds.2008-1414

Doreau, M., & Ferlay, A. (1994). Digestion and utilisation of fatty acids by ruminants. *Animal Feed Science and Technology*, *45*(3), 379–396. https://doi.org/10.1016/0377-8401(94)90039-6

Ellis, J. E., Williams, A. G., & Lloyd, D. (1989). Oxygen consumption by ruminal microorganisms: Protozoal and bacterial contributions. *Applied and Environmental Microbiology*, *55*(10), 2583–2587.

Erasmus, L. J., Botha, P. M., & Kistner, A. (1992). Effect of Yeast Culture Supplement on Production, Rumen Fermentation, and Duodenal Nitrogen Flow in Dairy Cows1. *Journal of Dairy Science*, *75*(11), 3056–3065. https://doi.org/10.3168/jds.S0022-0302(92)78069-2

Fiorentini, G., Carvalho, I. P. C., Messana, J. D., Canesin, R. C., Castagnino, P. S., Lage, J. F., Arcuri, P. B., & Berchielli, T. T. (2015). Effect of Lipid Sources with Different Fatty Acid Profiles on Intake, Nutrient Digestion and Ruminal Fermentation of Feedlot Nellore Steers. *Asian-Australasian Journal of Animal Sciences*, *28*(11), 1583–1591. https://doi.org/10.5713/ajas.15.0130

Hentges, D. J. (1996). Anaerobes: General Characteristics. In S. Baron (Ed.), *Medical Microbiology* (4th ed.). University of Texas Medical Branch at Galveston. http://www.ncbi.nlm.nih.gov/books/NBK7638/

Mack, D. R. (2005). Probiotics. *Canadian Family Physician*, *51*(11), 1455–1457.

Moya, D., Calsamiglia, S., Ferret, A., Blanch, M., Fandiño, J. I., Castillejos, L., & Yoon, I. (2009). Effects of dietary changes and yeast culture (Saccharomyces cerevisiae) on rumen microbial fermentation of Holstein heifers1. *Journal of Animal Science*, *87*(9), 2874–2881. https://doi.org/10.2527/jas.2008-1446

Papillon Product Specification Sheet. (2020, April 10). *Dairyman's Edge Core*. https://www.papillon-ag.com/wp-content/uploads/2020/06/01-DECORE-TB-Product-Specification-Sheet-09-11-19.pdf

Qadis, A. Q., Goya, S., Ikuta, K., Yatsu, M., Kimura, A., Nakanishi, S., & Sato, S. (2014). Effects of a bacteria-based probiotic on ruminal pH, volatile fatty acids and bacterial flora of Holstein calves. *The Journal of Veterinary Medical Science*, *76*(6), 877–885. https://doi.org/10.1292/jvms.14-0028

Uyeno, Y., Shigemori, S., & Shimosato, T. (2015). Effect of Probiotics/Prebiotics on Cattle Health and Productivity. *Microbes and Environments*, *30*(2), 126–132. https://doi.org/10.1264/jsme2.ME14176

Weigand, E., Young, J. W., & McGilliard, A. D. (1975). Volatile Fatty Acid Metabolism by Rumen Mucosa from Cattle Fed Hay or Grain1. *Journal of Dairy Science*, *58*(9), 1294–1300. https://doi.org/10.3168/jds.S0022-0302(75)84709-6

CHAPTER 3:

TITLE OF CHAPTER 3

ABSTRACT: Rumen headspace gas may affect the fermentation process. We developed a steady state model to investigate effects of rumen metabolism and swallowing of air on rumen headspace gases. Fitting the models using data from published experiments provided parameters to estimate the volume of air swallowed during feeding and how much O_2 swallowed was chemically reduced in the rumen. Headspace gases reported from previous publications were used to fit rates of swallowing and fermentation gas production. This model considers the inflow from swallowing air and gases produced from feed digestion and metabolism, and the disappearance from eructation of certain gases with the assumption of 35 L of rumen volume and 10 L of headspace in sheep. It is noted rumen headspace gas from swallowing air, net metabolism of CO_2, and metabolism of CH_4 contribute to the amount of O_2, N_2, CO_2, and CH_4 within the rumen as well as resulting in the release of the four gases through eructation. Metabolism of oxygen is also considered as oxygen swallowed from eating throughout the day may be utilized by the aerobic species within the rumen. We developed a 4-compartment model in which the compartments were rumen headspace CO_2, CH_4, N_2, and O_2 to test changes of gases before and during feeding the hay diet. Both steady state models had a rate of CO_2 and CH_4 production of 171 L per day, with 70% CO_2 and 30% CH_4. Rate of air (80% N_2, 20% O_2) swallowing was set to 10% per day from inflow of gas before feeding and set to 60% per day from inflow of gas during feeding. Utilization of O_2 was set to 10% per day. Eructation of gases was set to reset the rumen gas volume to 10 L after each timestep. Before feeding, the model approached steady state where volumes of gases were: 151.2 L, 64.8 L, 30.5 L, 6.3 L for CO_2, CH_4, N_2 and O_2 respectively.

During feeding, the model approached steady state where volumes of gases were: 71.2 L, 30.5 L, 122.0 L, 25.3 L for CO_2, CH_4, N_2 and O_2 respectively. Metabolism of O_2 of the steady state model not during feeding was around 1.3 L/day and during feeding the metabolism of O_2 was about 5.1 L/day. These gas concentrations were similar to limited measurements in the literature. A subsequent dynamic model was created to show changes in gas concentrations throughout the day. The composition of gases not during feeding were: 69%, 20%, 10%, 1% for CO_2, CH_4, N_2 and O_2 respectively. The composition of gases during feeding were: 20%, 10%, 60%, 10% CO_2, CH_4, N_2 and O_2 respectively.

Key Words: mathematical modeling, fermentation, rumen, oxygen, gas, air

INTRODUCTION

Mathematical models are systems commonly used in natural sciences and engineering. The process of developing a model includes mathematical language, concepts, and a set of linear equations, algebraic equations, or differential equations (Venkateshan et al., 2014). In rumen fermentation studies, previous papers have described the rumen system through mechanistic models in order to explain ruminal fermentation (France et al., 1982; Baldwin et al., 1987). More specifically, certain models incorporated thermodynamics, such as the model of Kohn and Dunlap (2000), and others began to evaluate gas production and determine Michaelis-Menten equations (Kohn and Boston, 2000; Dhanoa et al., 2000). The purpose of the present model is to gain a better understanding of rumen fermentation gases to determine how gas profiles come about and how they affect ruminal metabolism. The objective of this study is to model the effects of certain feeds and even probiotics within the rumen by measuring gas composition and production. The expected results of this model will be to analyze and understand rumen fermentation beginning with type of feed fed to a cow and the ending with final products such as VFAs and gases. The objective of this chapter is to model Barry's paper, "Rumen fermentation studies on contrasting diets". They determined large differences in gas volumes between a concentrate diet versus a hay-based diet (Barry et al., 1977). Specifically, the concentrate diet had additional peaks of O_2 and N_2 compared to the hay diet and whenever there was a peak for these two gases, there was a decrease in CO_2 and CH_4 (Barry et al., 1977). Time of feeding was also a factor analyzed. During feeding it

was noted O_2 and N_2 in rumen gas increased while CO_2 and CH_4 decreased whereas after feeding CO_2 and CH_4 increased rapidly (Barry et al., 1977).

METHODS

Paper of Interest

This model is a meta-analysis using data from the literature. There is very little data on rumen fermentation gases, therefore results from T.N. Barry's paper titled, *Rumen Fermentation Studies on Two Contrasting Diets* (Barry et al., 1977) comprise most of the data used for developing a steady state and a dynamic model. In Barry's study, sheep were given one of two different diets: high concentrate or hay (Barry et al., 1977). Oxygen gas (O_2), and nitrogen (N_2) as well as gas end products such as, methane (CH_4), carbon dioxide (CO_2), were measured over 33 h time period (Barry et al., 1977). Gas results describing the percentage composition of the four gases throughout the day from Barry et al., are listed in Table 3.1. Notably, the gas data shows sharp increases in N_2 and O_2 and sharp decreases in CO_2 and CH_4 during feeding in both diets, but especially the hay diet (Table 3.1). Before feeding the gas composition was around 15% of air and 85% of CO_2 and CH_4. After feeding the gas composition was around 60% of air and 40% of CO_2 and CH_4. The concentrate diet has similar peaks and valleys throughout feeding, however the data shows increased fluctuation of gases throughout the day even after feeding. To analyze this data more closely, Table 3.2 was calculated based on the Barry et al., data. The rates of each of the collection time points is described in Table 3.2 and illustrate clear increased rates of N_2 and O_2 and decreased rates of CO_2 and CH_4 during feeding times (Table 3.2).

These rates also observed a larger ratio of N_2 to O_2 and higher rates of O_2 disappearance.

Description of the Model

There are two different models created in this study, a steady state model and a dynamic model. A steady state model, or static model, indicates rational rates of rumen digestion as well as passage to help predict digestibility over various parameters and compartments (Mertens, 1987). These steady state outcomes and models can be useful as they are used to predict the digestibility associated with gas production and composition over the course of 24 h and various feed intakes. A dynamic model takes time into consideration as it focuses on the mechanisms of how components or parameters change over a period of time.

Overall, there have been a large number of mathematical model attempts at identifying and modelling specific processes to determine the outflow of rumen digesta (Greg et al., 2005). Despite these efforts, mathematical models that analyze rumen gas production are scarce. Since gas production is a measure of digestibility within ruminants, it would be interesting to model this data in order to gain a better understanding of the mechanisms within the rumen. However, there is a lack of in-depth gas collection data from ruminants potentially due to the difficulty of gaining clean gas samples from the rumen.

Model Inputs/Assumptions

Rumen Volume

Sheep rumen values have been reported to be between 20 L to 37 L in volume, depending on the size of the sheep (Sheep Production Handbook, 2002). As this model considers the inflow from swallowing air and gases produced from feed digestion and metabolism, and the disappearance from eructation of certain gases, the rumen volume is assumed to be 35 L of total rumen volume and 10 L of headspace for gas.

Gases of Interest

It is noted rumen headspace gas from swallowing air, net metabolism of CO_2, and metabolism of CH_4 contribute to the amount of O_2, N_2, CO_2, and CH_4 within the rumen as well as resulting in the release of the four gases through eructation. Metabolism of oxygen is also considered as O_2 swallowed from eating throughout the day may be utilized by the aerobic species within the rumen. For this model, the compartments were rumen headspace CO_2, CH_4, N_2, and O_2. The model included the following percent compositions from literature: [CO_2]= 0.7 or 70%, [CH_4]= 0.3 or 30% where a study noted molar percentage composition of rumen gas from cows on alfalfa pasture has been reported as 67% CO_2 and 26% CH_4 (Wolin, 1977), [swallowing air]= 0.10 or 10% with air containing (80% N_2, 20% O_2) from inflow of gas (Marty, 2008). Utilization of O_2 was assumed to be about 10% per day. Eructation of gases was set to reset the rumen gas volume to 10 L after each timestep. Each main gas required an inflow and an outflow as described by the differential equations below:

Table 3.0. Differential Equations of Gases (O_2, N_2, CO_2, CH_4)

Gases	Differential Equations
O_2	dO_2/dt= (swallowing* O_2% in air) - Metabolism O_2 - Eructation of O_2
N_2	dN_2/dt= (swallowing* N_2% in air) - Eructation of N_2
CO_2	dCO_2/dt= (net metabolism of CO_2 %) - Eructation of CO_2
CH_4	dCH_4/dt= Metabolism CH_4 - Eructation CH_4

Glucose/Feed Calculations

The diets included in this model were from Barry et al., 1977. Two diets: hay diet consisting of 100% hay and concentrate diet consisting of 20% hay and 80% cooked flaked maize were fed at the maintenance level of energy intake as two equal portions per day (Barry et al., 1977). For the hay diet, 900 g of air-dried hay or 795 g of dry matter (D.M.) were fed per day and for the concentrate diet, 150 g of hay (132g D.M) and 600 g of flaked maize (528g D.M.) were fed per day (Barry et al., 1977). In order to convert D.M. of the hay and cooked flaked maize to glucose, it was assumed the flaked maize was multiplied by 0.9 (about 90%) of the total would be converted into glucose and the hay was multiplied by 0.5 (about 50%) to be converted to glucose as well. Hay on average has been reported to contain about 35%-80% of glucose content (Wedig et al., 1986; Jenset et al., 2014). Cooked flaked maize of concentrates in sheep diets are primarily converted to glucose. Equations depicted in Table 3.2 and Table 3.3, equation 1.

To convert grams into moles, the values of grams of glucose were divided by glucose's molecular weight (180 g/mol) minus the molecular weight of one water molecule (18 g/mol) to equal 162 mol/g. The reason for this is to find the true molar

mass of glucose from glycogen. The storage form of glycogen has about three or four parts of water per glycogen molecule and is stored in the liver, muscles and fat cells (Kreitzman et al., 1992). This hydrated form would be inaccurate to use for this calculation, so when the molar mass of glucose this case is 162 g/mol.
This conversion equates the hay diet to consist of 3.34 mol of glucose and the concentrate diet to consist of 2.45 mol of glucose. Equations depicted in Table 3.2 and Table 3.3, equation 2.

Volatile Fatty Acids (VFA) Calculations

After calculating moles of glucose, moles of total volatile fatty acids (VFA) needed to be calculated. The model assumes about 1.875 mol of total VFA will be converted from 1 mol of glucose. The ratios of acetate, propionate, and butyrate used in this model have been taken from literature. The molar proportions of these VFAs found in rumen fluid are acetate around 65%, propionate around 20%, and butyrate around 15% (Wolin, 1960). These proportions represent proportions in which these products are produced from fermented substrates such as feed (Wolin, 1960) and described in Table 3.2 and Table 3.3, equations 3-6.

CO_2 Calculations

To convert VFAs to CO_2, the conversion for VFA to CO_2 in moles was needed. Previous studies have calculated and outlined the pathways of the breakdown of glucose during ruminal fermentation from glucose to CO_2 (Ungerfeld & Kohn, 2006). This conversion assumes two acetate molecules and one butyrate molecule

contribute to production of CO_2 in the rumen described in Table 3.2 and Table 3.3, equation 7.

This then converted CO_2 to 9.08 mol/day, which then was converted into liters of CO_2 per day from VFA. This required the ideal gas law equation shown below:

$$PV=nRT$$

Where P is pressure at 1 atmosphere, V is volume to be solved for, n is the given value of moles at 9.08 mols, R is the ideal gas constant 0.0821, and T is the temperature of the rumen which is 39°C or 312.32 Kelvin. This is depicted mathematically in Table 3.3 and Table 3.4, equation 8.

The steady state model ran two separate times: before and during feeding (Barry et al., 1977). Before feeding, swallowing was set to 15% per day from inflow of gas from Period 1 (Table 3.1). During feeding, swallowing was set to 60% per day from inflow of gas from Period 2 (Table 3.1). The rate of swallowing was calculated by the difference of the sum of N_2 and O_2 minus the sum of CO_2 and CH_4. Utilization of O_2 was assumed to be about 10% per day of oxygen.

The dynamic model followed the same parameters as the steady state model at 15% swallowing of air, however required an input of time. In this case, time was added through the pulse function in Stella Professional, represented by a flow function labeled “Feed Intake”. The pulse function follows the format: amount where the amount of that function returns during a pulse, first pulse which is the initial time of the first pulse, and interval which is the length between pulses.

PULSE(<initial amount>, [<first pulse>,<interval>])

The feeding times were at 9:15 and 16:15 as described in the equation below:

PULSE(12, 9, 24)+PULSE(12, 16, 24)

RESULTS

Steady State Model

The steady state model runs had a total gas input of 244.1 L/day and a total gas output of 244.1 L/day. The steady state model run before feeding had an eructation of 242.8 L/day. Before feeding, the model approached steady state where volumes of gases were: 151.2 L, 64.8 L, 30.5 L, 6.3 L for CO_2, CH_4, N_2 and O_2 respectively. The percent composition of gases was: 62%, 27%, 12%, 2% for CO_2, CH_4, N_2 and O_2 respectively. Metabolism of O_2 was set to 1.3 L/day.

The steady state model run during feeding had an eructation of 236 L/day. During feeding, the model approached steady state where volumes of gases were: 71.2 L, 30.5 L, 122.0 L, 25.3 L for CO_2, CH_4, N_2 and O_2 respectively. The percent composition of gases was: 29%, 12%, 49%, 10% for CO_2, CH_4, N_2 and O_2 respectively. Metabolism of O_2 was set to 5.1 L/day.

Dynamic Model

With the introduction of a pulse function, the dynamic model values (Figure 3.2) emulated changes in gas composition similar to Table 3.1. The peaks of percent oxygen and nitrogen gas within the rumen are shown in Figure 3.3 and 3.4 as these gases increased during feeding time, similarly to the Barry et al. Figure 3.3 and 3.4 shows percent CO_2 and percent CH_4 changes throughout the day with feeding with noticeable decreases during feeding times. The composition of gases not during feeding were: 69%, 20%, 10%, 1% for CO_2, CH_4, N_2 and O_2 respectively. The

composition of gases during feeding were: 20%, 10%, 60%, 10% CO_2, CH_4, N_2 and O_2 respectively.

DISCUSSION

The purpose of this model was to highlight this information in order to investigate effects of rumen metabolism and swallowing of air on rumen headspace gases. This could provide insight on digestibility within ruminants to hopefully evaluate the effectiveness of various feeds and probiotics in the future. In order to do this, various gasses (CO_2, CH_4, O_2, and N_2) were measured and rates from the Barry paper were plugged into Stella Professional to determine if known values in rumen fermentation match the gas patterns from the model and (Barry et al., 1977).

The results of the steady state and dynamic model detected O_2 presence within the rumen before, during, and after feeding. This suggests swallowing of air does not occur only at mealtimes but could occur throughout the day as cattle and sheep continue to chew and ruminate consistently. Although some studies argue (Russel, 2009) that rumen is strictly anaerobic, rumen gas during feeding can contain around 1.3% and 10.2% O_2 gas as represented by Barry et al., and both mathematical models. Previous literature supports this finding, arguing rumen gas contains between 5 to 10 mL of O_2 that can be detected in the liquid phase (MacArthur and Multimore, 1962). In addition, Czerkawski et al., calculated O_2 transfer from diffusion of blood, saliva, and food may total to 38 L of O_2 entering the rumen daily (Czerkawski et al., 1969). This mathematical model supports Czerkawki et al., as the volume of O_2 entering the rumen daily through only swallowing of air approached a steady state value of 25.3 L. O_2 transfer from saliva as well as diffusion of blood of the host animal may account

for the additional 12.7 L. Moreover, the model shows that if air is only swallowed during eating, N_2 and O_2 concentrations would quickly decrease to close to zero. The fact that Barry et al. found the O_2 concentration to stay above a threshold throughout the day suggests that there is some air entering the rumen all day, or possibly there was a low-level contamination of air in the sampling.

The steady state model before and during feeding calculated O_2 metabolism to be 1.3 L/day and 5.1 L/day respectively. In Newbold et al., the rates of O_2 uptake by rumen fluid were measured at between 60 to 100 nmol/min per mL or 11.5 to 16.1 L/day (Newbold et al., 1996). This discrepancy alludes to the fact that metabolism of O_2 may actually be higher than the assumed 10% from the model. Furthermore, the ratio of N_2 to O_2 from Barry et al., was calculated as 5 during feeding and around 7 after feeding whereas the mathematical model calculated the ratio of N_2 to O_2 to be about 5. Because we see an increased ratio between N_2 to O_2, Barry et al., is suggesting O_2 is disappearing faster and being utilized by the rumen. The ratio between N_2 to O_2 is critical in our understanding of fermentation and O_2 metabolism. If in fact the air in the ratio of N_2 to O_2 is similar to atmospheric air which is around 4, then the O_2 may not be reduced and may not impact fermentation. Yeast or microbes within the rumen may not utilize the oxygen and the air in the headspace perhaps does not get mixed within the rumen. There are many potential biological explanations or artifacts that could be explored in a further model.

One of the limitations of this model was that the literature did not report individual measurements of gas, just percent composition, therefore certain values had to be assumed. To test the overall performance of the model, it was important to

evaluate the data based on other studies. In addition, the amount of literature regarding gas data for both sheep and cattle are very limited. This required many models to be built and adjusted, requiring a large amount of trial and error through the building process. Adding converters and adjusting rates in order to replicate rumen gas production while also exploring new concepts such as, swallowing of air and metabolism of O_2 required the construction of 3 base models in order to create 2 developed models (steady state and dynamic).

When considering future studies with mathematical models, it is understood that they can continue to be built and improved upon. Specific future studies of this model could evaluate the impact of other ruminal gases such as, hydrogen or fermentation factors such as, specific microbes, feeds, or probiotics. More specifically, a meta-analysis of specific yeast species and gas production within the rumen could highlight and clarify the mechanisms behind probiotics.

CONCLUSION

There is very little data available on rumen headspace gases. A mathematical model using limited data that exists in literature suggests air is swallowed with meals and possibly swallowed in smaller amounts continuously throughout the day. Only a small amount of the O_2 in swallowed air appears to be metabolized in the rumen which suggests limited mixing of rumen headspace gas with rumen contents. Future studies centered on yeast and O_2 within both sheep and cattle, are necessary in order to analyze O_2 metabolism and uptake by the rumen. Although previous publications suggest the ability of yeast to utilize oxygen and both the model and Barry et al.,

observed a larger ratio of N_2 to O_2 due to rapid O_2 disappearance, the mechanisms behind oxygen utilization have yet to be understood (Amin & Mao, 2021; Newbold et al., 1996).

TABLES AND FIGURES

Table 3.1. Barry values of percent composition of gas, cattle fed at 9:15 and 16:15

Time Point	8	9:15-10:00	12	16	16:15-17:00	20:00	24:00	4:00	8:00	9:15-10:00	12:00
Period	1	2	3	4	5	6	7	8	9	10	11
CO_2%	47.1	24.5	47.5	49.8	31.7	55.8	53.7	49.4	46.8	33.3	48
CH_4%	36.2	12	33	34.2	14.6	33.8	35	36.4	34.7	18.3	34.3
O_2%	2.1	10.2	2.4	2.2	9.2	1.3	1.6	1.7	1.6	7.9	2.1
N_2%	14.9	51.4	17.1	13.4	43.3	8.8	9.4	12	16.5	39.7	15.8

Table 3.2. Model Equations from initial diet to CO_2 in liters in the Hay diet

Equations	Hay Diet
1) Diet to glucose (g)	(795g D.M. of hay)*0.5= 397.5g of glucose
2) Glucose (g) to glucose (mol)	397.5g of glucose ÷ 162 g/mol= 2.45mol of glucose
3) Glucose to Total VFA (mol)	2.45 mol of glucose*1.875 = 4.58 mol of Total VFA
4) Total VFA to acetate (mol)	4.58 mol of Total VFA*0.65 = 2.977
5) Total VFA to butyrate (mol)	4.58 mol of Total VFA*0.15 = 0.687
6) Total VFA to propionate (mol)	4.58 mol of Total VFA*0.2 = 0.916
7) Acetate and Butyrate to CO_2 (mol)	(2.977*2)+ 0.687 = 6.641
8) CO_2 (mol) to CO_2 (L)	*(1atm)(Volume)=(6.67mols)(0.0821)(312.32 Kelvin)*
8) CO_2 (mol) to CO_2 (L)	*(1atm)(Volume)=(6.67mols)(0.0821)(312.32 Kelvin)*

Table 3.3. Model Equations from initial diet to CO_2 in liters in the Concentrate diet

Equations	Concentrate Diet
1) Diet to glucose (g)	(132g D.M. of hay*0.5)+(528g D.M of cooked flaked maize)*0.9= 541.2g of glucose
2) Glucose (g) to glucose (mol)	541.2g of glucose ÷ 162 g/mol= 3.34mol of glucose
3) Glucose to Total VFA (mol)	3.34 mol of glucose*1.875 = 6.26 mol of Total VFA
4) Total VFA to acetate (mol)	6.26 mol of Total VFA*0.65 = 4.069
5) Total VFA to butyrate (mol)	6.26 mol of Total VFA*0.15 = 0.939
6) Total VFA to propionate (mol)	6.26 mol of Total VFA*0.2 = 1.252
7) Acetate and Butyrate to CO_2 (mol)	(4.069*2)+ 0.939 = 9.077
8) CO_2 (mol) to CO_2 (L)	*(1 atm)(Volume)=(6.67 mols)(0.0821)(312.32 Kelvin)*

Table 3.4. Model Equations of swallowing of air, metabolism of O_2, total gas into rumen, total eructation, and eructation of each gas

Converters	Equations
1) Swallowing of air before/after feeding	Gas into rumen*0.15
2) Swallowing of air during feeding	Gas into rumen*0.60
3) Metabolism of O_2	O_2 *0.10 per hour
4) Total Gas into Rumen	Swallowing (N_2+O_2)+Metabolism (CO_2+CH_4)
5) Total Eructation	Total Gas into Rumen – 10 L
6) Eructation of each gas	(Gas(x)/Total Gas into Rumen)*Total Eructation

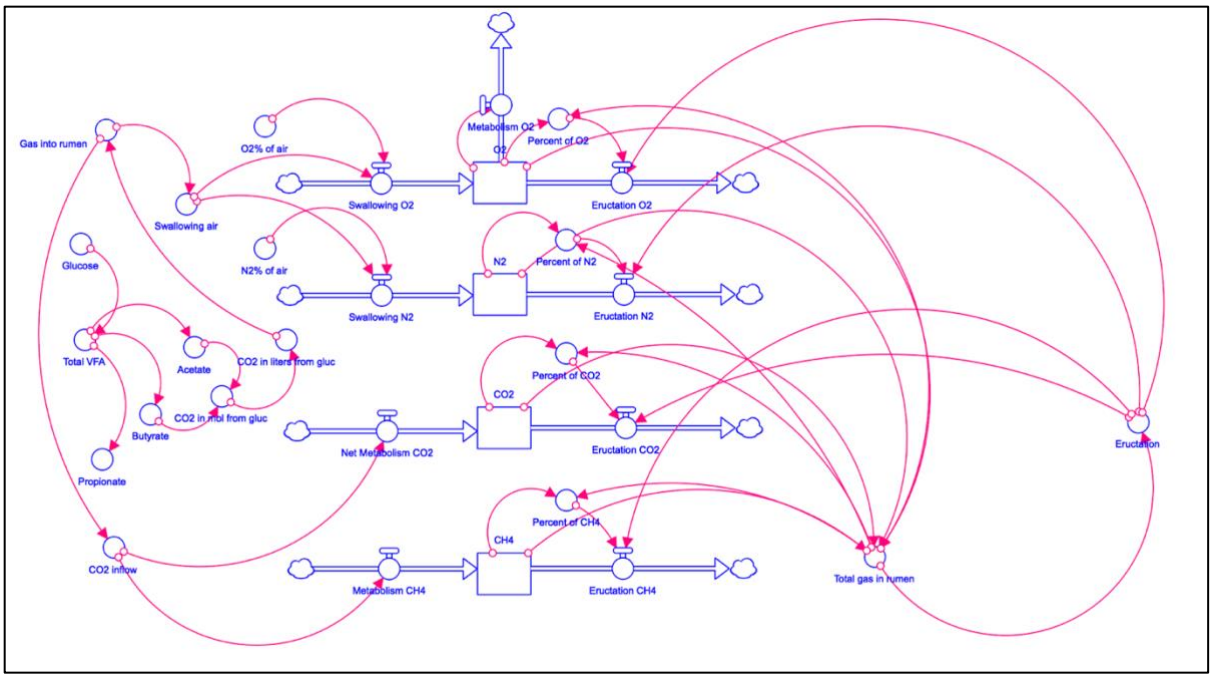

Figure 3.1. Steady State 2 model from Stella Professional

Table 3.5. Steady State 2 Table of gas values per day during feeding on hay diet

	Before Feeding (Swallowing 15%)				During Feeding (Swallowing 60%)			
Gases	CO_2	CH_4	N_2	O_2	CO_2	CH_4	N_2	O_2
Gas per day (L)	151.2	64.8	30.5	6.3	71.2	30.5	122.0	25.3
% Composition	62	27	12	2	29	12	49	10
Total Gas (in)	244.1 L				244.1 L			
Eructation (out)	242.8 L				239 L			
Metabolism of O_2	1.3 L/day				5.1 L/day			

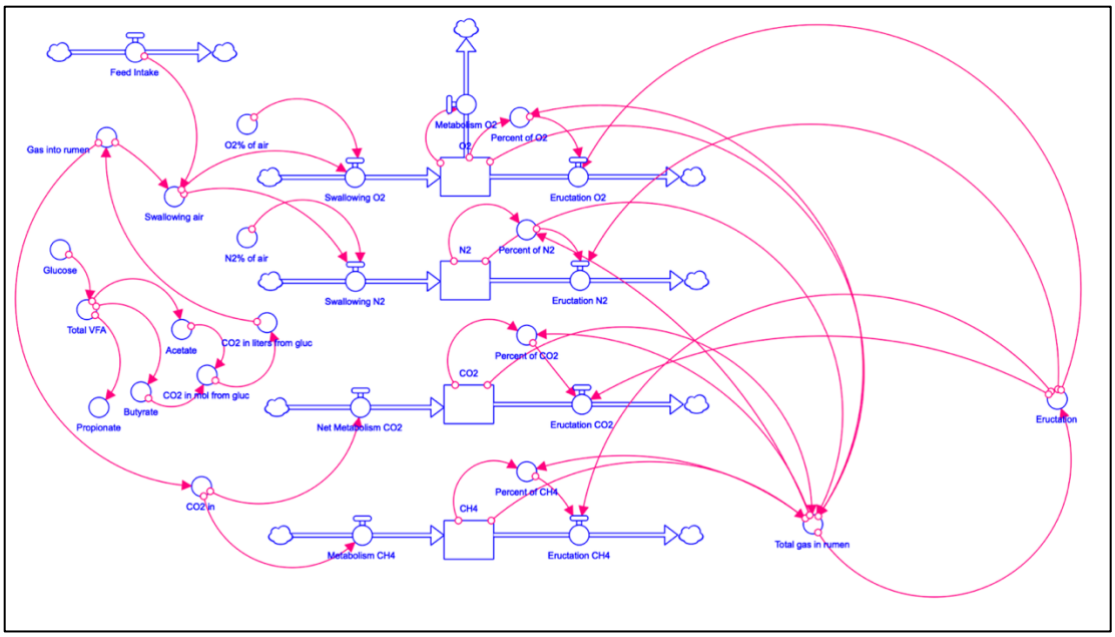

Figure 3.2. Dynamic Model from Stella Professional

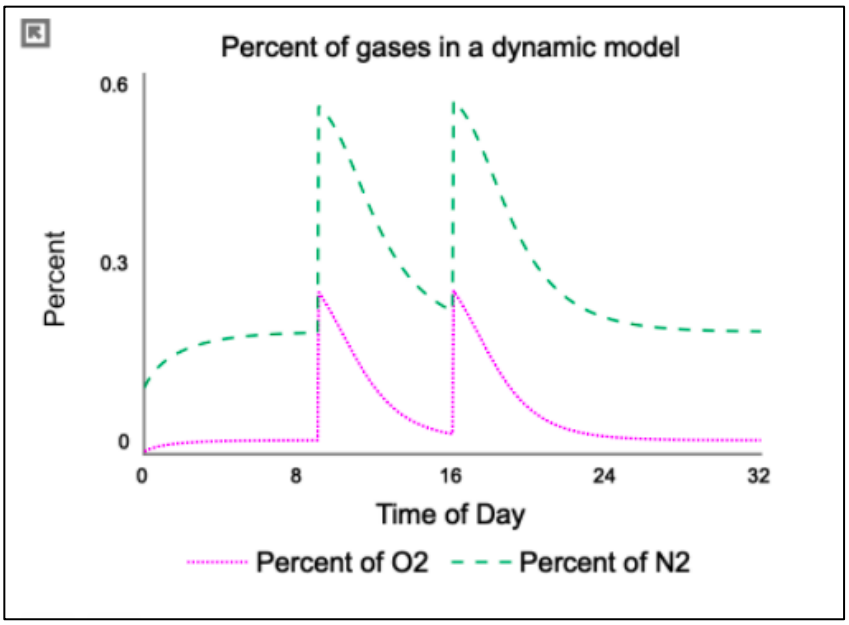

Figure 3.3. Percentage composition of gas for O_2 and N_2 for the dynamic model

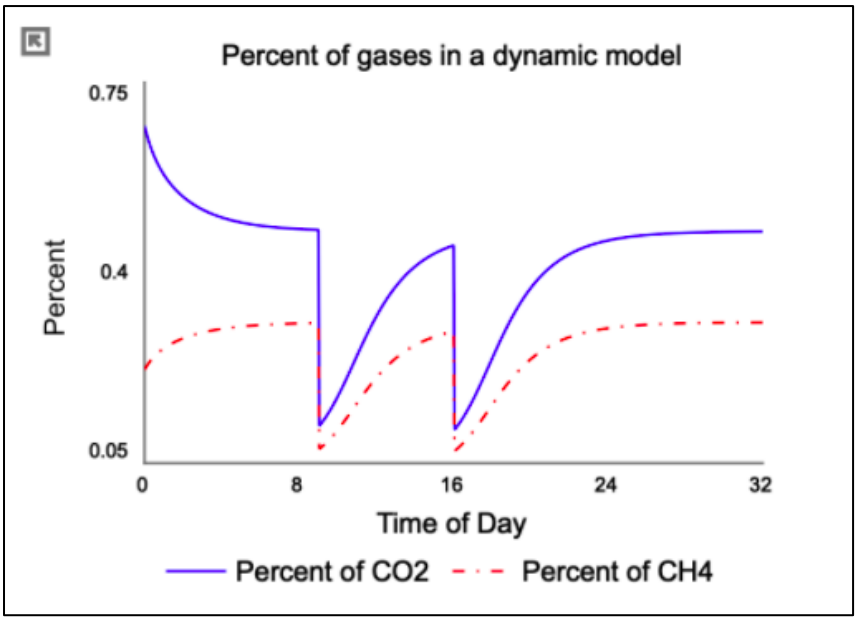

Figure 3.4. Percentage composition of gas for CO_2 and CH_4 for the dynamic model

BIBLIOGRAPHY

Amin, A. B., & Mao, S. (2021). Influence of yeast on rumen fermentation, growth performance and quality of products in ruminants: A review. *Animal Nutrition*, *7*(1), 31–41. https://doi.org/10.1016/j.aninu.2020.10.005

Baldwin, R. L., France, J., & Gill, M. (1987). Metabolism of the lactating cow: I. Animal elements of a mechanistic model. *Journal of Dairy Research*, *54*(1), 77–105. https://doi.org/10.1017/S002202990002522X

Barry, T. N., Thompson, A., & Armstrong, D. G. (1977). Rumen fermentation studies on two contrasting diets. 1. Some characteristics of the in vivo fermentation, with special reference to the composition of the gas phase, oxidation/reduction state and volatile fatty acid proportions. *The Journal of Agricultural Science*, *89*(1), 183–195. https://doi.org/10.1017/S0021859600027362

Dhanoa, M. S., Lopez, S., Dijkstra, J., Davies, D. R., Sanderson, R., Williams, B. A., Sileshi, Z., & France, J. (2000). Estimating the extent of degradation of ruminant feeds from a description of their gas production profiles observed in vitro: Comparison of models. *British Journal of Nutrition*, *83*(2), 131–142. https://doi.org/10.1017/S0007114500000179

France, J., Thornley, J. H. M., & Beever, D. E. (1982). A mathematical model of the rumen. *The Journal of Agricultural Science*, *99*(2), 343–353. https://doi.org/10.1017/S0021859600030124

Garza-Ulloa, J. (2018a). Chapter 5 - Methods to develop mathematical models: Traditional statistical analysis. In J. Garza-Ulloa (Ed.), *Applied Biomechatronics using Mathematical Models* (pp. 239–371). Academic Press. https://doi.org/10.1016/B978-0-12-812594-6.00005-6

Garza-Ulloa, J. (2018b). Chapter 5 - Methods to develop mathematical models: Traditional statistical analysis. In J. Garza-Ulloa (Ed.), *Applied Biomechatronics using Mathematical Models* (pp. 239–371). Academic Press. https://doi.org/10.1016/B978-0-12-812594-6.00005-6

Gregorini, P., Beukes, P., Waghorn, G., Pacheco, D., & Hanigan, M. (2015). Development of an improved representation of rumen digesta outflow in a mechanistic and dynamic model of a dairy cow, Molly. *Ecological Modelling*, *313*, 293–306. https://doi.org/10.1016/j.ecolmodel.2015.06.042

Jensen, R., Austbø, D., Knudsen, K., & Tauson, A.-H. (2014). The effect of dietary carbohydrate composition on apparent total tract digestibility, feed mean retention time, nitrogen and water balance in horses. *Animal : An International Journal of Animal Bioscience*, *8*, 1–9. https://doi.org/10.1017/S175173111400175X

Kreitzman, S. N., Coxon, A. Y., & Szaz, K. F. (1992). Glycogen storage: Illusions of easy weight loss, excessive weight regain, and distortions in estimates of body composition. *The American Journal of Clinical Nutrition*, *56*(1 Suppl), 292S-293S. https://doi.org/10.1093/ajcn/56.1.292S

Marty, B. (2011). Earth's Atmosphere, Origin and Evolution of. In M. Gargaud, R. Amils, J. C. Quintanilla, H. J. (Jim) Cleaves, W. M. Irvine, D. L. Pinti, & M. Viso (Eds.), *Encyclopedia of Astrobiology* (pp. 464–467). Springer. https://doi.org/10.1007/978-3-642-11274-4_470

Mertens, D. R. (1987). Predicting Intake and Digestibility Using Mathematical Models of Ruminal Function. *Journal of Animal Science*, *64*(5), 1548–1558. https://doi.org/10.2527/jas1987.6451548x

Murphy, M. R., Baldwin, R. L., & Koong, L. J. (1982). Estimation of Stoichiometric Parameters for Rumen Fermentation of Roughage and Concentrate Diets. *Journal of Animal Science*, *55*(2), 411–421. https://doi.org/10.2527/jas1982.552411x

Newbold, C. J., Wallace, R. J., & McIntosh, F. M. (1996). Mode of action of the yeast Saccharomyces cerevisiae as a feed additive for ruminants. *The British Journal of Nutrition*, *76*(2), 249–261. https://doi.org/10.1079/bjn19960029

Physical Chemistry and Its Biological Applications—1st Edition. (n.d.). Retrieved April 16, 2021, from https://www.elsevier.com/books/physical-chemistry-and-its-biological-applications/brey/978-0-12-133150-4

Russell, J. B. (2009). Rumen. In M. Schaechter (Ed.), *Encyclopedia of Microbiology (Third Edition)* (pp. 163–174). Academic Press. https://doi.org/10.1016/B978-012373944-5.00061-4

Sheep Production Handbook (7). (2003). https://www.biblio.com/book/sheep-production-handbook-7/d/1388090130

Ungerfeld, E., & Kohn, R. (2006). The Role of Thermodynamics in the Control of Ruminal Fermentation. In *Ruminant Physiology: Digestion, Metabolism and Impact of Nutrition on Gene Expression, Immunology and Stress* (pp. 55–85).

Venkateshan, S. P., & Swaminathan, P. (2014). Chapter 1—Preliminaries. In S. P. Venkateshan & P. Swaminathan (Eds.), *Computational Methods in Engineering* (pp. 1–15). Academic Press. https://doi.org/10.1016/B978-0-12-416702-5.50001-6

Wedig, C. L., Jaster, E. H., & Moore, K. J. (1986). Composition and Digestibility of Alfalfa and Orchardgrass Hemicellulose Monosaccharides by Holstein Steers. *Journal of Dairy Science*, *69*(5), 1309–1316. https://doi.org/10.3168/jds.S0022-0302(86)80537-9

Wolin, M. J. (1960). A Theoretical Rumen Fermentation Balance. *Journal of Dairy Science*, *43*(10), 1452–1459. https://doi.org/10.3168/jds.S0022-0302(60)90348-9