ABSTRACT

EUN, JONG-SU. Characterizing microbial dynamics in continuous cultures and lactation performance of cows fed gamagrass. (Under the direction of Vivek Fellner and Jerry W. Spears)

Three experiments were performed to investigate the dynamics of fermentation in continuous cultures and lactation performance of cows fed gamagrass. We first studied the effects of dilution rate and forage to concentrate ratio on fermentation by rumen microbes cultured in fermentors. Methane production, when calculated from stoichiometric equations, did not change with dilution rate or forage to concentrate ratio. When measured from gas taken from fermentor headspace, methane production increased with dilution rate and level of forage in the diet. Compared to actual concentrations, stoichiometric equations consistently underestimated methane output at higher dilution rates and with high forage diets. Higher dilution rates resulted in an increase in microbial yield and microbial efficiency. Increasing the level of concentrate in the diet only increased microbial efficiency. Overall results show that dilution rate and forage to concentrate ratio can alter the partitioning of substrate by rumen microbes. Further that gas production, in particular methane, may not be accurately estimated using stoichiometrics of end product appearance.

Twenty lactating Holstein cows were fed gamagrass hay or silage without or with supplemental corn to determine their effects on milk production. Milk yield did not differ among cows fed gamagrass hay or gamagrass silage. Feeding supplemental corn increased milk yield but only at the medium and high levels of corn inclusion. Gamagrass silage increased the conversion of feed nitrogen to milk nitrogen compared to gamagrass hay. Contrary to expectation, gamagrass silage lowered milk urea nitrogen compared to hay. Supplemental corn further reduced milk urea nitrogen.

In the last experiment, we investigated the characteristics of microbial fermentation of gamagrass. Increasing corn supplementation in gamagrass silage linearly decreased culture pH whereas ammonia nitrogen was similar across treatments. Corn supplementation to gamagrass silage was an effective strategy to increase microbial capture of rumen degradable protein from gamagrass via enhanced availability of ruminal fermentable energy. However, gamagrass silage without corn supplementation resulted in higher efficiency of microbial growth but at the expense of microbial yield.

CHARACTERIZING MICROBIAL DYNAMICS IN CONTINUOUS CULTURES AND LACTATION PERFORMANCE OF COWS FED GAMAGRASS

by

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DEDICATION

This dissertation is dedicated to my heavenly Father from whom all strength comes, who is always there when I need Him.

The Sovereign LORD is my strength;

HE makes my feet like the feet of a deer,

HE enables me to go on the heights.

- HABAKKUK 3:19 -

BIOGRAPHY

Jong-Su Eun was born in Kimje, Chunbok, South Korea on May 5, 1965 to Mr. Kapcheul Eun and Mrs. Oksoon Choi. Shortly after his birth, his family moved to Iksan, Chunbok where he finished his education until high school. He then went to the Kon-Kuk University in Seoul where he studied Dairy Science. Jong-Su received his Bachelor of Science degree in February 1993. He served in the Korean Air Force from August 1986 to July 1989. From November 1992 to May 1994 Jong-Su conducted an internship at Animal Resources Research Center, Kon-Kuk University under the guidance of Dr. Changwon Kim and Dr. Wansup Kwak. In August 1994, he entered Virginia Polytechnic Institute and State University's Dairy Science program in pursuit of a Master of Science degree under the guidance of Dr. Joseph H. Herbein. Jong-Su completed his degree in May 1996. Shortly after graduation, he returned to his home country and worked for a private feed manufacturing company as a ruminant nutritionist. During that time, he traveled to the Netherlands, Germany, and Belgium to work with the nutritionists in the Hendrix-UTD of Nutreco. He married Eunsook Chung on November 2, 1996, and they had their first child, Jaemin Eun on December 9, 1997. Jong-Su began pursuing a Doctor of Philosophy degree in North Carolina State University's Animal Science program in the summer of 1999 under the guidance of Dr. Vivek Fellner. While conducting his research he became a master at measuring methane production, and learned a great amount about rumen microbiology.

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CHAPTER 1

INTRODUCTION

The rumen enables ruminants to digest high fiber plant materials less suitable for most nonruminants. The rumen has evolved to benefit from the fiber digesting activities of the microbes. The end-products of fermentation, volatile fatty acids (VFA), are absorbed across the rumen wall and used for energy and protein synthesis. The principle benefit of the rumen foregut fermentation, in comparison with hindgut fermentation, is that the microbial cells formed during fermentation pass to the abomasum and intestine, where they are digested and their amino acids are absorbed. Unlike hindgut fermentation, microbial amino acids become available to the host. The outflow of microbial biomass and VFA from the rumen will impact the nutritional status of the animal as well as efficiency of nutrient utilization. The rumen is therefore a highly efficient organ in the context of the evolution of an herbivore subsisting on forage with its attribution to maintaining rumen functions.

Rumen fermentation also brings some disadvantages. Methane is produced as a natural consequence of the anaerobic fermentation; it is a potent greenhouse gas, so in this sense, ruminants damage the environment. The ruminant is also less efficient than other species in the utilization of dietary protein. Nitrogen losses from ruminants are exceptionally high, particularly in grazing animals. This is an environmental problem with the impact of nitrogen-rich animal wastes as well as an economic one with energetic inefficiency to the whole animal. Furthermore, there are digestive disorders unique to ruminants which occur as a direct consequence of rumen microbial fermentation being impaired.

Chemical and antibiotic feed additives have been used to alleviate some of these problems. The presence of such materials in the food chain is becoming ever less acceptable

to regulatory authorities and the consumer. Thus, solutions to the problems of ruminant livestock production must be natural and sustainable. In order to minimize nutrient waste and maximize its use by dairy cows, we need to better understand microbial dynamics in the rumen.

Among various factors influencing microbial metabolism in the rumen, liquid turnover rate and forage to concentrate ratio have been found to alter the balance of microbial species and consequently impact their fermentation pathways. Ruminal output or flow to the omasum divided by ruminal volume gives the fractional passage rate. For liquid, this is often called "dilution rate". Slow growing microorganisms, especially certain protozoa, would wash out of the rumen if the dilution rate exceeded their growth rate (Carro et al., 1995). In addition, Hoover et al. (1984) suggested that high dilution rates result in energetic uncoupling with diminished protein digestion and microbial growth. In contrast, if the dilution rate is reduced, accumulated metabolites may adversely affect microorganisms (Fuchigami et al., 1989). Variation in the forage to concentrate ratio is one of the most commonly modified characteristics of practical ruminant diets. With medium to high forage diets, rates of fermentation are normal and avoid the accumulation of fermentation end-products to inhibitory levels. Conversely, high concentrate diets results in a rapid rate of acid production which causes VFA to accumulate and possibly inhibit certain fermentation reactions. However, at very high rates of forage inclusion, depression in microbial yield is observed due possibly to higher microbial recycling in the rumen and slower microbial growth, resulting in a greater proportion of energy diverted for maintenance (Sniffen and Robinson, 1987).

Digestibility estimates of feedstuffs are often derived from in vitro gas production (Menke et al., 1979) and fermentation rates of the rumen microbes are derived from

continuous gas production measurements (Blümmel et al., 1997). Measuring gas production is relatively simple, but interactions between fermentation end-products, buffering system, and amount of gas produced are very complex (Beuvink and Spoelstra, 1992). Schofield and Pell (1995) suggest that it is important to monitor the molar proportions of VFA which results in different amount of gas production. Recently, Doane et al. (1997) pointed out that changes in microbial metabolism or yield may alter the relationship between substrate digestion and gas production and could affect the estimation of digestion rate from gas measurements. However, there is no clear explanation for the discrepancy between actual gas production and estimates based on stoichiometric equations.

Good quality forages like corn silage have been sought to support high milk yields and consequently improve profitability for dairy farmers. In the Southeastern USA, several forages have the potential to be economically more feasible than traditional sources such as corn silage. Among these is eastern gamagrass, which is a subtropical bunch-type, warm-season perennial tall grass, possessing agronomic characteristics that make it suitable for hay or silage production. Horner et al. (1985) compared the nutritive value of eastern gamagrass and alfalfa hay for dairy cows and reported that although neutral detergent fiber (NDF) and acid detergent fiber (ADF) concentrations were greater in eastern gamagrass, the digestibility of these fiber components was also higher (62 vs. 45% in NDF and 57 vs. 40% in ADF). Burns et al. (1992) reported that eastern gamagrass had a greater proportion of leaf and lower proportion of stem than either flaccidgrass or bermudagrass, and the digestibility of the leaf and stem components were higher in eastern gamagrass than the other two grasses. There is currently much interest in the importance of the synchrony of releasing available energy and nitrogen (N) in the rumen. It is assumed that a lack of synchrony leads to inefficient

microbial capture of the N and hence to reduced efficiency of microbial protein synthesis (Chamberlain and Choung, 1995). Grass silages, in general, are characterized by markedly asynchronous rates of release of energy and N in the rumen resulting in low rates of microbial protein synthesis (Beever and Cottrill, 1994). Ruminal conditions in this situation could be improved through addition of ruminal degradable starch (corn or sorghum grain). It has also been shown that at relatively modest levels (about 15% of DM) of fermentable carbohydrate, ruminal bacteria are buffered against fluctuation in the release of energy and N (Kim et al., 1999). In addition, degradability characteristics of N play an important role in microbial protein synthesis; for example, the highly degradable nature of N in alfalfa can lead to poor utilization of potentially available N in lactating dairy cows (Broderick, 1985). Degradation rates of N were reported to be slower for gamagrass than for legumes because of its large proportion of N associated with neutral detergent insoluble N (> 51% on a whole-plant basis; Coblentz et al. 1998). Therefore, synchronization of release of energy and N should consider physiological characteristics of N storage in forage and appropriate energy source with optimum level of supplementation.

Overall research contained in this dissertation will show that dilution rate and forage to concentrate ratio can alter the partitioning of substrate by rumen microbes and that gas production, in particular methane, may not be accurately estimated using stoichiometrics of end product appearance. While corn supplementation to gamagrass silage will be proved to be an effective strategy to increase microbial capture of rumen degradable protein from gamagrass by enhancing the availability of ruminal fermentable energy, gamagrass silage without corn supplementation will result in higher efficiency of microbial growth at the expense of microbial yield.

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CHAPTER 2

REVIEW OF LITERATURE AND DISSERTATION OBJECTIVES

CONTINUOUS CULTURE METHODOLOGY

Because it is an open and continuous ecosystem, the rumen is an ideal environment for maintaining a stable microbial population. Even if we are given access to it by means of suitable surgical techniques, the quantitative study of reactions occurring therein is difficult due to simultaneous actions of fermentation, passage, absorption, and secretion. In addition, increased public awareness elicited by the animal rights movement make the use of invasive surgical procedures for nutritional research more difficult to justify (Mansfield et al., 1995). Therefore, over many years attempts have been made to isolate small proportions of rumen contents and to allow them to continue to ferment under more controlled laboratory conditions (Czerkawski, 1986). Continuous culture methodology has been widely used for a means to evaluate the effects of nutrients on the metabolism of microbes maintained under controlled conditions of turnover rate and nutrient intake. A continuous culture is an "open" system in which a well-mixed culture is continuously provided with fresh nutrients. A continuous culture consists of a thoroughly mixed suspension of biomass into which medium is added at a constant rate and the culture is removed at the same rate so that the culture volume in the fermentor vessel stays constant. For any microbial species to survive in a continuous culture, its specific growth rate must exceed the dilution rate (Theodorou and France, 1993).

Various continuous culture fermentation systems have been designed to simulate the ruminal environment, enabling the study of factors affecting microbial ecology and digestion

of nutrients (Hoover et al., 1976a; Czerkawski and Breckenridge, 1977; Teather and Sauer, 1988; Fuchigami et al., 1989). Advantages of these systems compared with in vivo measurements include decreased cost, time, and variation among experimental units. Furthermore, there are no complications from endogenous sources, and digesta flow rate markers are not required because passage rates are regulated and measured directly. However, similar to in vivo measurements, reliable techniques are required for isolation of microbial cells and for differentiation of effluent digesta into microbial and dietary N fractions. Additionally, we need to simplify ruminal ecosystem in vitro in attempts to understand microbial metabolism and microbial interactions.

Types of Continuous Culture Systems

Two types of continuous culture fermentor systems are currently in use for measuring nutrient digestion by ruminal microbes. The rumen simulation technique (Rusitec), developed by Czerkawski and Breckenridge (1977), has a single outflow, and residence time in the rumen is simulated by placing feedstuffs into nylon bags and suspending these bags inside the reaction vessel for 48 h. Prevot et al. (1994) evaluated the Rusitec system and found that in its present form this system cannot reproduce the in vivo state of conventionally reared animals. Ciliated protozoal populations and probably many bacterial species are eliminated in the fermentor; therefore, certain variables need to be studied and perhaps modified. Carro et al. (1995) studied the effects of bag pore size (40, 100, and 200 μm) and dilution rate (2.3 and 3.5 %/h) on fermentation patterns and the ciliated protozoal population in Rusitec fermentors. The slow dilution rate decreased pH from 6.36 to 6.17, but digestion of dry matter (DM) and NDF was not affected. Pore size of the bags incubated in Rusitec

fermentors influenced not only DM and NDF digestion of the diet, but also the microbial population in the system and, therefore, the fermentation (Carro et al., 1995).

In the other type of continuous fermentor, described by Hoover et al. (1976a), both liquids and solids exit via an overflow and the differential rates of solids and liquids depend on setting and control of the mixing rate. Liquid rates are precisely regulated, and solid turnover is measured. Mansfield et al. (1995) used ruminally and duodenally cannulated lactating dairy cows and dual-flow continuous culture fermentors to compare fermentation of total-mixed diets and microbial ecology of in vivo and in vitro systems. Organic matter (OM) truly digested (corrected for microbial OM) did not differ between the in vivo and dual-flow continuous culture methods. Partitioning OM into carbohydrate fractions revealed that digestion of total nonstructural carbohydrate (NSC) was greater (74.6 vs. 60.6%) and NDF was less (47.4 vs. 58.3%) in continuous culture than in vivo. Most of this effect was a result of extreme values obtained from continuous culture fermentation of diets containing 40% nonfibrous carbohydrate (NFC). The continuous culture system seemed to have difficulty simulating NSC and NDF digestion for the 40% NFC diets, despite the fact that OM digestion was similar and pH was controlled at 6.4. The moisture, pressure, and heat applied during pelleting of diets used in continuous culture may have partially gelatinized the corn starch in the 40% NFC diets, rendering the starch more available to microbial fermentation. Amylolytic bacteria concentration did not differ in the rumen and fermentors, but there was a culture × NFC interaction. Amylolytic bacteria concentrations were greater in vitro than in vivo when 40% NFC diets were fed, which is consistent with the NSC digestion data, indicating that in vitro, 40% NFC diets exhibited the greatest digestion. Cellulolytic bacterial concentrations decreased in vitro. These data paralleled the decreased NDF digestion that

occurred in the fermentors. Digestion of crude protein (CP) was less in the rumen of cows than in the continuous culture fermentors (38.8 vs. 42.9%). Correcting the in vivo value using a factor of 3.6 g of endogenous N per kg of duodenal DM flow (Brandt et al., 1980) produced a mean ruminal CP digestion of 47.6%. Regardless of the in vivo estimate used, the difference in CP digestion in vivo and in vitro was less than 5 percentage units. Other differences observed between fermentors and the rumen can be attributed to lack of absorptive capacity and defaunation in vitro. However, overall data of VFA concentration and composition, NH_3-N concentration and microbial synthesis, microbial ecology, bacterial composition, and amino acid metabolism in vivo and in vitro reported by Mansfield et al. (1995) were similar for 80% of the individual measurements that were evaluated, supporting the dual-flow continuous culture system as an excellent model for studying ruminal microbial fermentation.

Completely mixed fermentors equipped with overflows quickly lose part or all of the protozoal component of the rumen microbial population. Fermentors of this type differ from the rumen in that the entire components have a uniform turnover time, generally as fast or faster than the protozoal generation time, which eventually leads to defaunated condition. Teather and Sauer (1988) introduced a naturally compartmented rumen simulation system for the continuous culture of rumen bacteria and protozoa. This unit provided reasonable estimates of ruminal fermentation with achievement in maintaining protozoal populations over extended periods and permitted the measurement of gas production from ruminal culture. In this system, they incorporated a glass “T” inside the fermentor vessel in order for culture contents to be allowed to stratify and for independent fractional dilution rates to be maintained, which is similar to the differential turnover that occurs within the ruminal

environment. Obviously, these are particularly important in studies related to the digestion of fibrous feeds or those concerning the role of ciliate protozoa (Fuchigami et al., 1989). The protozoa in the fermentors declined, however, below inoculum values (2.6×10^5 cells/ml) to about 2×10^4 cells/ml (10% of the inoculum value) with twice daily feeding. This was eliminated by continuous feeding rather than feeding twice daily.

Limits of Continuous Cultures for Simulating Microbial Fermentation In Vivo

Although the continuous culture methodology has provided valuable information with respect to basic phenomena in rumen microbial ecology, the main drawbacks are the lack of some important qualities, including the absence of end-product absorption from the cultures. These flaws cause differences in substrate degradation, end-product formation, and microbial protein synthesis compared with the in vivo situation. Hannah et al. (1986) reported that ammonia-N (NH_3-N) concentrations were numerically greater in vitro compared with in vivo. Concentration of NH_3-N is a product of utilization and release by the microbial population. The absence of absorption in continuous culture and possible differences in N recycling combine to make measurement of NH_3-N alone a poor indicator of proteolytic activity and protein degradation within the in vitro and in vivo fermentation environments (Mansfield et al., 1995).

One would expect greater concentrations of total VFA in continuous culture fermentors because VFA are not absorbed. In one study, fermentors accumulated 68 to 84 m*M* more total VFA than mean concentrations in vivo (Hannah et al., 1986) due possibly to the large input of DM (75 g/d) relative to the fermentor volume (1,030 ml), but in other reports, VFA concentrations were more reasonable (Hoover et al., 1976b; Bas et al., 1989). Efficiency of bacterial synthesis may be greater in vivo than in vitro due to the fact that excessive VFA

accumulation in a continuous culture may have increased maintenance requirements of the population by diverting energy away from growth towards maintenance of intracellular pH and cell integrity (Mansfield et al., 1995). In addition, the authors pointed out that efficiency of N capture in the fermentors was greater than 100% suggesting that urea N in the artificial saliva which is added to simulate urea recycling may be a more important source of N in vitro compared with in vivo.

Given all the pitfalls, the greatest difference in microbial ecology between the rumen environment and continuous culture is the drastic decline in protozoa from the fermentation vessels (Hoover et al., 1976a; Crawford et al., 1980; Hannah et al., 1986; Mansfield et al., 1995). This effect can be partially reversed by elevating solids retention time in fermentation vessels (Crawford et al., 1980). Holotrichs are especially vulnerable because they have a relatively long generation time (Williams, 1986) and cannot sequester themselves in the homogeneous conditions of the fermentors. Many attempts have been made to retain protozoa in continuous culture and these usually involve low substrate input (Merry et al., 1987; Teather and Sauer, 1988), low turnover (Hoover et al., 1976a), or complex artificial matrices composed of nylon string, sugar beet pulp, or hay (Hillman et al., 1991). However, minimizing agitation and allowing stratification may be the most promising technique for retaining protozoa (Teather and Sauer, 1988; Fuchigami et al., 1989).

The cultures in the laboratory allow examination of the factors influencing microbial growth and activity, but these cultures can only give an idea of the potential that specific microorganisms have under those specific conditions employed within a culture; the results have to be extrapolated to the in vivo situation. Even though continuous culture systems are not suitable for routine analysis of microbial digestion for individual feed ingredients due to

elaborate and expensive facilities and required inoculum with ruminal digesta (Stern et al., 1997), the technique is an excellent model for studying ruminal microbial fermentation.

GAS PRODUCTION AND RUMINAL FERMENTATION

In vitro gas production techniques have become popular for characterizing the rate and extent of digestion of ruminant feeds. Innovations in equipment design, including automated pressure recording systems and mathematical descriptions of the gas production profiles themselves, make the techniques both simple and precise and therefore a valuable laboratory procedure. The technique of measuring gas is of value in ruminant nutrition because the kinetics of gas production and substrate degradation are very closely correlated. However, although it is relatively easy to measure gas volumes and to determine the kinetics of gas production, the underlying processes that give rise to the gas in the first place are complex and are not well understood. Therefore, there is concern about what is being measured in gas production studies and how this relates to the digestion process in the ruminant animal.

Production of Gas by Ruminal Fermentation

When an energy-yielding substrate in a culture medium, which is predominantly bicarbonate-buffered, is inoculated with rumen fluid, the substrate will be converted initially by 'primary' microorganisms. These fermentation end-products are acetate, propionate, and butyrate, representing the bulk of the VFA produced, and the gases, carbon dioxide (CO_2), methane (CH_4), and hydrogen (H_2). The need to convert substrate to product is fundamental to energy metabolism, resulting in the production of ATP for the maintenance and growth of microbial cells. Some of the primary fermentation end-products, however, contain sufficient energy to make them attractive as substrates in a subsequent or 'secondary' fermentation.

Thus the process of methanogenesis permits the methanogenic bacteria to derive energy for maintenance and growth by the conversion of CO_2 and H_2 to CH_4 (Wolin et al., 1997). This is a key process in the rumen which results in a net loss of energy to the animal but is essential for effective redox balance. Subject to rate of passage, methanogenesis ensures that substrates are digested to their maximum extent in the rumen (Hungate, 1966).

The three reactions giving rise to the main end-products of carbohydrate fermentation in the rumen were summarized by Hungate (1966) as follows:

$C_6H_{12}O_6 + 2H_2O \rightarrow 2C_2H_4O_2$ (acetic acid) $+ 2CO_2 + 4H_2$

$C_6H_{12}O_6 + 2H_2 \rightarrow 2C_3H_6O_2$ (propionic acid) $+ 2H_2O$

$C_6H_{12}O_6 \rightarrow C_4H_8O_2$ (butyric acid) $+ 2CO_2 + 2H_2$.

The carbon dioxide produced as an end-product of the above reactions can then be combined with hydrogen to form CH_4:

$CO_2 + 8H^+ \rightarrow CH_4 + 2H_2O$.

Production of methane does not influence the total amount of gas produced from fermentation because a large proportion of the hydrogen is incorporated into methane and 1 mol of methane simply replaces 1 mol of carbon dioxide. The amount of gas produced is therefore dependent upon the amount of hexose fermented and the amount and molar proportions of the VFA produced. Any shift in the pattern of fermentation to increase the proportion of acetic and butyric acids and decrease that of propionic acid would result in the production of an increased volume of gas. Conversely, changing the fermentation stoichiometry to produce more propionic acid at the expense of acetic and butyric acids would result in less gas from fermentation. Changes in fermentation pattern related to diet are not uncommon in ruminant nutrition and are, in fact, viewed as a way of manipulating animal

production responses. High concentrate diets tend to enhance propionate production at the expense of acetic and butyric acids while high fiber diets favor the production of acetic and butyric acids at the expense of propionic acid (Russell and Wallace, 1997). Thus, the VFA molar proportions need to be taken into account when making comparisons between gas production profiles from widely different substrates. Moreover, the assumption that rumen microorganisms produce only VFA and CO_2 gas as their major fermentation end-product may not be valid under all circumstances. Some rumen microorganisms such as *Selenomonas ruminantium* and *Streptococcus bovis* can switch between the formation of VFA plus carbon dioxide and lactate minus carbon dioxide, depending on their growth rate (Russell and Wallace, 1997). This mechanism permits rapidly growing microorganisms to maximize the amount of ATP produced per unit of time instead of per unit of substrate. In a gas production study with energy-rich substrates, rumen populations containing those microorganisms produce lactate in addition to VFA and gas, where lactic acid is produced during the initial fermentation period and is subsequently replaced by VFA formation as the rapidly fermentable components are utilized and availability of substrates begin to limit microbial growth (Beuvink and Spoelstra, 1992).

Stoichiometry of Gas Production

Gas, VFA, and microbial mass are all end-products of ruminal digestion. The quantity of each end-product measured at the end of fermentation is directly related to the mass of material digested (Menke and Steingass, 1988; Pitt et al., 1996). Gas production is linearly related to both the net VFA produced (O'Hara and Ohki, 1973; Opatpatanakit et al., 1994) and microbial synthesis (El-Shazly and Hungate, 1965; Krishnamoorthy et al., 1991; Theodorou et al., 1995). However, the absolute volume of gas produced per unit substrate

digested reflects microbial metabolism. Because the in vitro methods use bicarbonate-based buffering solutions, CO_2 is released into the gas phase as VFA enters the medium (O'Hara et al., 1974; Menke and Steingass, 1988; Beuvink and Spoelstra, 1992). While all acids entering the system produce CO_2 indirectly from bicarbonate in medium, CO_2 produced directly from metabolism is primarily related to the production of acetate and butyrate (Wolin, 1960; Hungate, 1966; Beuvink and Spoelstra, 1992). As a result, the production of two moles of acetate or one mole of butyrate (from a mole of hexose) leads to the production of 4 or 3 moles of CO_2 respectively, while two moles of propionate is only responsible for the release of two moles of CO_2 indirectly by reaction with bicarbonate (Table 1). In practice, the in vitro medium is not a pure bicarbonate buffer, but contains phosphate as well. The phosphate buffer therefore neutralizes a portion of the acid equivalents entering the medium and reduces the CO_2 release from the 1:1 relationship of bicarbonate to approximately 0.87 moles CO_2 per mole acid for the buffer used by Beuvink and Spoelstra (1992). The specific reduction is determined by the concentration of phosphate in the medium.

A few studies have used the stoichiometry related to VFA production to calculate a theoretical or expected gas production based on the observed VFA profile. In the study of Beuvink and Spoelstra (1992), the expected gas volumes were very close for rice starch but greater than the observed gas production for the fermentation of glucose and purified cellulose (10 and 20%, respectively). Opatpatanakit et al. (1994) observed a close relationship ($r^2 = 0.94$) between the expected and measured gas volumes for a variety of starches, but again the expected volumes were slightly greater than observed. Blümmel and Ørskov (1993) also reported a high correlation between expected and observed volumes with forages, but noted that this tended to be more accurate at 24 h than at 48 h. Groot et al.

(1998) also reported that the measured gas volume for cell contents of Italian ryegrass was considerably lower than predicted from VFA produced due to a lower absolute gas production rate, which was most evident during the first 24 h of the incubation. The approximately 25% higher gas production predicted by stoichiometric calculations, than measured in their study, is similar to overestimations of gas production reported for cellulose (by 20%) and glucose (by 10%) by Beuvink and Spoelstra (1992). Typically, these overestimations occur for substrates producing high proportions of propionic acid. The use of pure substrates by Beuvink and Spoelstra (1992) seems to rule out the possible mechanism of reduction of gas production by bonding of CO_2 to ammonia. A second process which could reduce the gas produced per mole VFA would be the conversion of CO_2 and H_2 to acetate by acetogenic bacteria instead of CH_4 (Miller, 1995), which would result in the net consumption of one mol CO_2. This process mainly occurs when low roughage diets containing high proportions of sugar and protein are fed (Leedle and Greening, 1988). A third possibility could relate to the large CP fraction of cell contents. It proved that the lower gas production upon the fermentation of protein could be explained by the production of ammonia, inhibiting gas release from the buffer (Cone, 1998). In addition, Debersaques et al. (1998) reported that the form of nitrogen supply to microorganisms can affect the total gas production of carbohydrate, gas production being inhibited by increasing amounts of N originating from ammonia. This suggests that care must be taken with stoichiometric balances for feedstuffs which contain large amounts of protein.

Relationship between Gas Production and Microbial Growth

As stated previously for VFA production, microbial growth simultaneously involves catabolic pathways producing gas (both directly and indirectly) and anabolic mechanisms

sequestering carbon. It is clear that the relationship between microbial mass and gas volume is complex. It may vary with the type of substrate, nature of the inoculum, growth conditions, and time of observation (Blümmel et al., 1997a). This relationship was first explored in the gas system by Krishnamoorthy et al. (1991). Using a syringe technique and defined substrates (starch, cellulose, and glucose-starch-cellulose mixture), they found a curvilinear relationship between the rate of microbial protein synthesis and the rate of gas production. High rate of gas production corresponded to high rates of protein synthesis, but the curvature of this relationship varied with the type of substrate; cellulose produced a steep upward curve, starch a less steep curve. With the mixed carbohydrate substrate, they also found a linear relationship between microbial protein synthesis and cumulative gas production over an 8 h time span. The important conclusion from this study was that one should not rely solely on cumulative gas production as an index of the microbial growth potential of feeds.

Microbial yield is the efficiency of bacterial growth from digestion (g bacteria/g substrate digested) and therefore, as yield increases, the proportion of the substrate appearing as gas and VFA is reduced. This leads to the negative relationship between microbial growth rate and gas or VFA production in a 2 h fermentation observed by Naga and Harmeyer (1975). Blümmel et al. (1997b) have done some work relating gas production and microbial growth using Hohenheim system. They demonstrated that some substrates with potentially high gas volumes had comparatively low microbial biomass yields. Gas volume measurements were complemented by a determination of the truly degraded substrate in vitro to avoid selection of substrates with proportionally high VFA production and low microbial biomass yield, which is relevant for forages with a low N content.

Many workers (Beuvink and Spoelstra, 1992; Blümmel and Ørskov, 1993; Opatpatanakit et al., 1994) have already shown a close association of VFA production and gas volumes in vitro. It is also well established that the relationship between VFA production and microbial biomass is not a constant (Leng, 1993), the explanation for which resides in the variation of microbial biomass production per unit ATP generated. This can impose an inverse relationship between gas production and microbial biomass yield. The evaluation of roughages by in vitro gas tests might result in selection against the maximum microbial biomass yield by favoring substrates with proportionally high VFA yield. This intrinsic disadvantage with in vitro gas production can be overcome by combining gas measurements with the determination of the undegraded residue.

Although Blümmel et al. (1997b) proposed an inverse relationship between gas production and in vitro microbial biomass yield, it seems unlikely that such a relationship would hold true for microbial protein yield under all rumen conditions. It may be that the combination of estimates of hexose available and ATP production, together with a suitable model of rumen function, would provide substantially improved estimates of microbial protein production, compared with current relationships based solely on OM apparently degraded in the rumen (Givens et al., 2000). To make sound nutritional decisions based on gas data, we need to be aware that gas yield and microbial yield are substrate-dependent variables. If the gas volume from digestion of a given feed is low, we should measure the gas yield (based on OM disappearance) and the microbial yield (based on microbial mass produced) before concluding that the feed value is low (Schofield, 2000).

Ecology of Methanogens and Factors Influencing Methanogenesis

None of the carbohydrate-fermenting bacteria and protozoa produce CH_4, but many of them produce formate, H_2, and CO_2 as fermentation products. Methanogenic species of bacteria can then transform the H_2 and CO_2 into CH_4. Formate is converted into H_2 and CO_2 by methanogens that use formate as the primary substrate for the production of CH_4. The methanogenic bacteria are now classified as part of the domain archaea, ancient prokaryotes (Wolin et al., 1997). Methods of reducing methanogenesis in ruminants have been investigated as part of the overall attempt to improve the efficiency of rumen metabolism. However, methanogenic bacteria play an important role in the complex ecology of the rumen by maintaining a low partial pressure of H_2, indicating that methanogenesis, rather than being a waste process to the ruminants, promotes a more efficient fermentation by keeping the H_2 concentration low in the rumen.

Methanogenesis involves the uptake of H_2 and the stepwise reduction of CO_2. Formate can also serve as a substrate for ruminal methanogenesis. However, Hungate et al. (1970) indicated that most of the ruminal formate is converted to H_2 and CO_2 (formate-hydrogen lyase) prior to methanogenesis. This is based on the observation that the *K*m for formate was much higher than the concentration in ruminal fluid and that the rate of methane production was more closely correlated with the concentration of dissolved hydrogen than with formate. Conversion of acetate into CH_4 and CO_2, and conversion of propionate and butyrate and other VFA into acetate, H_2, and CO_2, with subsequent conversion into CH_4, seldom occurs in the rumen. The production of CH_4 from VFA is a very slow process. The turnover of the rumen is too rapid to permit conversion of any of the VFA into CH_4 (Wolin et al., 1997).

The reduction of CO_2 is initiated by the methanofuran, and this C1 carrier catalyses the formation of formylmethanofuran. Tethydrohydromethanopterin then accepts the formyl group from formylmethanofuran, and the carbon is then reduced to a methenyl derivative. The reduction of methenyl to methylene is mediated by factor F_{420}, a flavin analog. It functions as a two electron acceptor in H_2 oxidation and plays a major role in the transfer of electrons during CH_4 synthesis. The last step of CO_2 conversion to methane is mediated by coenzyme M (2-mercaptoethanesulphonic acid) and it serves as the terminal acceptor of the methyl groups. The reduction of methyl-coenzyme M is the most exergonic step of CO_2 conversion to methane, and this step can create a proton motive force that then drives ATP synthesis (Miller, 1995; Deppenmeier et al., 1996).

Methanogenic bacteria are very diverse with respect to their morphology, nutritional requirements, cell wall composition, and optimal growth temperature. On the basis of these characteristics and their 16S rRNA homologies methanogenic chemoautotrophs have been divided into three major phylogenic groups: the Methanococcales (e. g. *Methanococcus*), the Methanobacterials (e. g. *Methanobacterium*), and the Methanomicrobials (e. g. *Methanosarcina*). All of these are distributed within the euryarchaeotal branch of archaea (Palmer and Reeve, 1993). Methanogenic archaea are present at the 10^7 and 10^8/ml concentrations and constitute one of the major functional groups in the rumen (Ushida et al., 1995). The principal species of methanogens isolated from the rumen include *Methanobacterium formicicum*, *Methanobrevibacter ruminantium, Methanosarcina barkeri, Methanosarcina mazei,* and *Methanomicrobium mobile* (Stewart et al, 1997). The distribution of these species in the rumen of the general population of ruminants is uncertain and there may be yet an unidentified major species of rumen methanogens (Wolin et al.,

1997). Smith and Hungate (1958) isolated from the bovine rumen a methanogen that formed CH_4 from H_2 and CO_2 and was present in high concentrations; it was named *Methanobacterium ruminantium*. A similar methanogen, strain M1, was isolated by Bryant (1965) and became the type strain of the species *Methanobrevibacter ruminantium* (Balch et al., 1979). It has been generally assumed that *Methanobrevibacter ruminantium* is the dominant H_2- and CO_2-using rumen methanogen. It requires coenzyme M and was, until recently, the only methanogen known to have this requirement. However, both coenzyme M-requiring and -nonrequiring *Methanobrevibacter* species have been detected at high concentrations in bovine rumen contents (Lovley et al., 1984; Miller et al., 1986). *Methanomicrobium mobil* and the species of *Methanobrevibacter* grow rapidly with H_2 and CO_2 and do not form CH_4 from methyl groups (Wolin et al., 1997). *Methanosarcina barkeri*, which has been isolated from cow and sheep rumen contents (Patterson and Hespell, 1979), grows slowly with H_2 and CO_2 and forms CH_4 from methyl group. It is sustained in the rumen by its ability to produce CH_4 from methanol or methylamines. *Methanosarcina barkeri* does not compete for H_2 with the other methanogens. *Methanobrevibacter* species are probably the most important bovine rumen methanogens, but more investigations are needed in order to be certain about the importance, and the nature, of the species of *Methanobrevibacter* found in the bovine rumen (Wolin et al., 1997).

The stoichiometric relationship presented by Hungate (1966) indicates that CH_4 production is related to the amount and ratios of VFA produced in the rumen. Factors that contribute to higher ratios of acetate and butyrate result in increased CH_4 production; a high ratio of propionic acid results in decreased CH_4 production. Fermentation biochemistry suggests that carbohydrates may differ in the amount of CH_4 produced in the rumen

(Wilkerson et al., 1995). Structural carbohydrates (SC) such as hemicellulose and cellulose typically result in higher proportions of acetate during fermentation than do NSC and should result in greater CH_4 production. Factors that increase the proportion of propionate during fermentation, such as high dietary NSC concentrations, decrease CH_4 production. Based on regression analysis of literature data, however, Johnson and Johnson (1995) noted that as a greater amount of any carbohydrate fraction is fermented per day, whether it is fiber or starch, relative CH_4 production is decreased. This observation was confirmed by direct measurements of CH_4 production by steers fed beet pulp, a highly digestible fiber source (Kujawa, 1994).

In habitats such as lake sediments, in which unidirectional mass flow occurs but retention times are long, most methane is produced from acetic acid by slow-glowing (generation time ~130 h) "aceticlastic" methanogens, while some methane is produced from other faster-growing (generation time 4 to 12 h) methanogenic species reduce CO_2 with H_2 (Weimer, 1998).

In the rumen, however, methanogenesis occurs mostly by the faster-growing methanogenic species that reduce CO_2 with H_2 because ruminal retention times are too short to permit establishment of the slow-growing aceticlastic species. Consequently, growth of methanogens requires an environment with an extremely low dilution rate, while many methanogens, such as short chain fatty acid oxidizers, barely survive in the rumen with a relatively high dilution rate (Ushida et al., 1997). Isaacson et al. (1975) observed a large decrease in CH_4 formation with increase in the proportion of propionate and decrease in the proportion of butyrate when dilution rate increased from 2 to 12 %/h in continuous culture. In a trial using rumen simulation technique, Carro et al. (1995) reported no effect on CH_4

production in response to dilution rate at 2.3 and 3.5 %/h. A small change in dilution rate may result in no change on CH_4 production. Grinding and pelleting of forages can markedly decrease CH_4 production (Blaxter, 1989). Increased rate of passage of the ground or pelleted forage likely contributes to the reduced CH_4 production.

Cereal grain fermentation often causes a decrease in ruminal pH, and this effect is most dramatic when feed intake is high (Slyter, 1976). Van Kessel and Russell (1996) indicated that ruminal methanogens are sensitive to even modest decreases in pH and thus, the rate of CH_4 production was pH-dependent. Hino et al. (1993) reported that CH_4 production was decreased by increasing concentrate without pH control. However, when pH was kept at 6.7 using a pH controller which allowed the inflow of 2% Na_2CO_3 into the fermentor when pH was dropped to the set-point, CH_4 production was doubled with the high concentrate diet. These results suggest that the growth of hydrogen producers and methanogens is suppressed by low pH. Russell (1998) observed that low pH caused a marked decrease in the acetate to propionate ratio, which was mirrored by a reduction in CH_4 production. This result is consistent with the idea that propionate production and methanogenesis are competing and alternative mechanisms of reducing equivalent disposal. Van Kessel and Russell (1996) indicated that ruminal methanogens lose their ability to take up H_2 at low pH, and they detected H_2 when the final pH of in vitro incubations was less than 5.5. This suggests that low pH drives a decrease in methanogenesis and not just an increase in propionate.

Ciliated protozoa in the rumen are potential hydrogen producers. Elimination of protozoa from the rumen, i.e. defaunation, often reduces ruminal methanogenesis (Vermorel and Jouany, 1989). Methanogenic bacteria have been observed both on the cell surface and in cytosol of rumen ciliates (Finlay et al., 1994). The ciliates increase acetate to propionate ratio

and this metabolic shift has been suggested as the cause of the differing rates of CH_4 formation in faunated and ciliate-free animals (Whitelaw et al., 1984). The contribution of ciliate protozoa and their adherent methanogens to ruminal methanogenesis has been estimated to be a few nmol (less than 5 nmol) per ciliate per day (Ushida and Jouany, 1996). This methanogenic population appeared to not fully consume H_2 produced by the protozoa because H_2 still accumulated in the culture vessels, even when CH_4 was detected. Tokura et al. (1997) reported that the number of methanogens associated with ciliates was modulated by feeding. These authors suggest that the large amount of change in methanogen numbers occurring in the 3 h after feeding results from a rapid change in the number of methanogens extracellularly associated with the ciliates.

Energetics of Methanogenesis

The mechanism by which ATP synthesis is coupled with methanogenesis from H_2 and CO_2 is controversial. Formation of ATP has been proposed either by electron transport phosphorylation (ETP) or by substrate level phosphorylation (SLP). Since H_2 is the electron donor for CO_2 reduction it seems most likely that ETP is the mechanism for ATP synthesis; furthermore, the free energy changes associated with methanogenesis from H_2 and CO_2 from acetate are too low (–30 to –40 kJ/mol) to be coupled with stoichiometric ATP formation (Schönheit, 1993). This also suggested ATP synthesis to occur via ETP, since ETP allows fractional numbers of ATP molecules to be formed (Thauer and Morris, 1984). The mechanism of ETP according to the Mitchell (1966) hypothesis implies that methane formation from CO_2 and H_2 generates an electrochemical potential of protons across the cytoplasmic membrane, which constitutes the driving force for the synthesis of ATP via a membrane-bound H^+-translocating ATP synthase.

The energetics of methane production was not well understood until recently. From the standpoint of free energy change, the initial steps of CO_2 formation are low or even positive, and only the last step, methanol reduction, has a free energy change (-26.9 kcal) that would be sufficient to drive ATP formation (Russell and Wallace, 1997). Growth of methanogens on CO_2 and H_2 as sole energy and carbon source provided evidence that the reduction of CO_2 to CH_4 is an exergonic process that is coupled with the synthesis of ATP (Schönheit, 1993). The free energy change of CO_2 reduction to CH_4 under natural habitats in which methanogens grow at a H_2 partial pressure of 10^{-4}-10^{-5} atm (1-10 Pa) is about –30 to –40 kJ/mol CH_4 (Schönheit, 1993). The energy requirement for the synthesis of ATP from ADP and Pi under cellular conditions is about 60-80 kJ/mol assuming a thermodynamic efficiency of energy conversion of 60-70% (Thauer, 1988). Thus, in in vivo less than 1 mol ATP/mol CH_4 can be formed. In accordance with the thermodynamics, the experimentally determined molar growth yields (g of cell dry mass formed per mol CH_4 produced) have been reported to be 1.6-6.0 g/mol CH_4 for various methanogens growing in batch and continuous cultures (Roberton and Wolfe, 1970; Zehnder and Wuhrmann, 1977; Weimer and Zeikus, 1978; Schönheit et al., 1980; Schauer and Ferry, 1980; Fardeau and Belaich, 1986; Fardeau et al., 1987). The stoichiometry of coupling of methanogenesis with ADP phosphorylation appears not to be constant. The growth yield was found to increase, for example in *Methanobacterium thermoautotrophicum*, from 1.6 g/mol CH_4 up to 3-6 g/mol CH_4 (Schönheit et al., 1980) when the hydrogen partial pressure in the culture medium was lowered. This indicates that CH_4 formation and ATP synthesis are coupled more efficiently when the H_2 concentration is low, as is the case in natural habitats. With decreasing H_2 concentrations the free energy change associated with CO_2 reduction to CH_4 decreases from

–131 kJ/mol CH_4 at 10^5 Pa H_2 to only approximately –30 kJ/mol CH_4 at the H_2 concentrations prevailing in methanogenic ecosystems where the H_2 partial pressure is only between 1 and 10 Pa (Thauer, 1998).

Production of CH_4 promotes a more efficient fermentation and higher yields of ATP synthesis by keeping the H_2 concentration low in the rumen rather than being a waste process to the ruminant. Hence, CH_4 production *per se* does not only provide energy for microbial growth, but it also improves the overall fermentation process and contributes to microbial growth.

CARBOHYRATE AND PROTEIN METABOLISM AND RUMINAL FERMENTATION

An efficient and economically sound approach to ensuring adequate flow of nutrients from the rumen to support high milk production should first take maximum advantage of rumen fermentation. The diet should then be supplemented with additional bypass energy and protein as required for high milk production (Stokes et al., 1991). Maximum rumen fermentation is characterized by high ruminal digestion of DM in conjunction with an optimum level of efficiency of microbial protein synthesis. These functions appear to depend largely on providing dietary energy and protein in appropriate ratios and amounts. Therefore, the importance of energy and protein interactions within the rumen has been established over the last 20 years with quantitative description of the event as influenced by diet composition, along with consideration of how such processes may be manipulated in order to improve the overall efficiency of ruminant livestock production (Beever, 1993).

Relationship between Carbohydrate and Nitrogen in the Rumen

Microbial metabolism in the rumen is primarily regulated by the amount and rate of degradation of carbohydrate and protein, which largely depends on the chemical and physical characteristics of a diet. The nutrient components of diet used for microbial maintenance and growth in the rumen have been subdivided into more specific aspects of ADF, NDF, structural carbohydrate (SC) and NSC, rumen degradable protein (RDP), and rumen undegradable protein (RUP). These different nutrient components have different physical characteristics and utilization as well as providing evaluative tools for the nutritive values contained in feedstuffs (Van Soest, 1994).

The overall process illustrating the coordination of N and carbohydrate is shown in Figure 1. Carbohydrate produces the carbon skeleton and energy as a form of ATP for ruminal microbial growth. Carbohydrates in feeds consist of starch, cellulose, hemicellulose, and pectin with cellulose and hemicellulose accounting for the major components of dietary fiber fraction which can be degraded by rumen microbes. Starch is the primary energy component in grains, and is considered the primary driver of microbial protein synthesis in the rumen. Hexoses and pentoses produced through microbial breakdown are subsequently used by the ruminal microbial population to supply the precursors of macromolecules and energy needed for growth and maintenance (Van Soest, 1994). It is generally accepted that the energy available for ruminal microbial yield is largely dependent on the rate of carbohydrate digestion in the rumen (Hoover and Stokes, 1991). Even though protein sources and NDF are major nutrients supplying NH_3-N and energy, respectively, NSC in ruminal fermentation plays a significant role with respect to optimization of microbial protein yield in the rumen.

In a study designed to test the roles of NSC and dietary protein in rumen fermentation and the performance of dairy cows with four diets formulated for high and low ruminal availabilities of NSC and protein, Aldrich et al. (1993) found that the highest duodenal passage of bacterial N (262 g/d) was observed when cows consumed a combined diet of high rumen-available NSC and protein. Furthermore, Hoover (1986) suggested that microbial protein yield in the rumen was optimized when diets contained 56% of the total carbohydrate as NSC. A proportion of NSC from 8 to 32% of concentrate had no influence on the amount of microbial N flow to the duodenum. In contrast, it is interesting to note that a higher value in 4% fat corrected milk (FCM) production was shown in cows fed a diet containing low rumen-available NSC; this possibly resulted from decreases in DM intake and OM passage to the duodenum when cows consumed high rumen-available NSC diets. Possible explanations for the reduction of feed intake observed in high NSC diets are: lower ruminal pH caused by rapid breakdown of readily fermentable NSC and the palatability of the NSC source in place of a less fermentable carbohydrate source (McCarthy et al., 1989).

After analyzing both in vitro and in vivo data obtained from various studies to determine the effects of NSC and RDP on microbial yield, Hoover and Stokes (1991) concluded that NSC affected total carbohydrate digestion while RDP content had a major influence on both efficiency of microbial protein yield and carbohydrate digestion. From a study of the effects of NSC:RDP ratio employing a rumen simulated continuous culture technique, Stokes et al. (1991) demonstrated that bacterial protein yields increased as RDP levels increased within each level of NSC; increasing NSC content of the diet had a major effect on the bacterial protein production, illustrating the importance of NSC and RDP in ruminal microbial protein yield. After analyzing various data, Hoover and Stokes (1991) concluded that an optimum

ratio of dietary NSC to RDP for maximization of microbial protein yield is 2. In addition, Huber and Herrera-Saldana (1994) suggested that a desirable ratio of dietary rumen degradable starch (RDS) to RDP, a practically valuable tool for evaluating energy and nitrogen availability in the rumen fed high energy diets, would be from 1.5 to 2.25.

Ruminal Synchronization of Energy and Nitrogen Availability

Rooke et al. (1987) demonstrated the principle of increased microbial protein synthesis in response to improved energy and nitrogen synchronization when glucose was infused into the rumen of cows consuming grass silage-based diets with or without casein. Since then there have been many other attempts to test the 'synchrony' hypothesis. The basic assumption on the effect of synchronization of energy and N availability is that a lack of synchrony between the rates at which energy and N become available to the microbes will lead to a reduced efficiency of microbial capture of N. Also, ATP production from fermentation of dietary carbohydrate will be inefficiently used for microbial growth (Chamberlain and Choung, 1995). Therefore, synchronizing the availability of energy and N in the rumen is seen as offering considerable potential to enhance the output of microbial protein from the rumen for certain dietary circumstances, many of which are common under practical feeding regimens.

Herrera-Saldana et al. (1990) reported that synchronization of rapid fermentation with fast degradable starch and protein stimulated greater microbial protein passage than asynchronized or slowly degradable synchronized diets. Further, starch digestibility in the rumen affected utilization of nutrients more than protein digestibility. Sinclair et al. (1993) supported the beneficial effects of synchrony in ruminal releases of energy and N in sheep fed synchronous or asynchronous diets formulated based upon in situ digestibility of

individual ingredients. The authors concluded that the ruminal synchronization of substrate release can enhance the amount and efficiency of microbial protein yield.

Wheat is more rapidly digested than sorghum in the rumen. In addition, sorghum has the lowest whole tract digestibility among cereal grains due to a hard peripheral endosperm layer which is resistant to digestion (Rooney and Pflugfelder, 1986). From a study designed to determine whether different proportions of two grain sources in the same diet would affect the ruminal fermentation, Miron et al. (1996) observed that there were no differences in the amount of nonammonia N (NAN) flow to the duodenum. In this study cows were fed a total mixed rations which differed in the ratio of sorghum to wheat (70:30% vs. 30:70%). McAllister and Cheng (1996) reported that the degradation in the rumen of a protein matrix surrounding the starch granule in grains is a prerequisite to starch digestion. Therefore, similar rates of the starch and protein digestion by ruminal bacteria may occur for sorghum and wheat grains. This phenomenon can explain the synchronization between the wheat starch and wheat protein plus urea N, readily digested in the rumen of a 70:30 diet fed to cows to produce microbial mass. In addition, similar synchronization may be obtained in the rumen of a 30:70 diet fed to cows having slowly digested sorghum and corn silage starch and protein, and readily digested wheat and urea.

In addition, a substantial number of studies have reported conflicting results, showing a lack of benefit from the synchronization of energy and N availability in the rumen. Henning et al. (1993) demonstrated that the degree of energy and N synchronization had no influence on either the amount of microbial flow to the small intestine or the efficiency of microbial yield in the rumen when various dietary patterns, i.e. continuous infusion or pulse-doses of synchronous and asynchronous diets, were intraruminally supplied to sheep. This indicated

that efforts should be made to maintain a continuous ruminal energy supply pattern while supplying the appropriate quantity of rumen degradable N instead of simply pursuing synchronization of energy and N release.

Effects which are attributed to synchrony may be the result of individual nutrients, particularly protein and energy fractions. It is useful to consider the 'synchrony' hypothesis in this context and its interpretation from the standpoint of two quite different forage-based feeding systems, one being the grazing of fresh forage and the other, the feeding of conserved forages such as silage and hay. Chamberlain and Choung (1995) presented the case for 'asynchrony' with conserved forage-based feeds in which there are little readily-available energy at any time and when there is an abundance of protein degradation products (peptides, amino acids, and ammonia; McDonald et al., 1991). Kim et al. (1999a) observed an increase in microbial N flow in dairy cows when synchronous conditions in the rumen were created by infusion of sugars at different times with the basal diet of silage plus concentrates. In a similar experiment where silage was fed alone (Kim et al., 1999b), however, there was no effect of the infusion treatment. The authors suggested that the degree of synchrony will only influence microbial protein synthesis in diets already containing high levels of readily fermentable carbohydrates, although this is only likely if the capacity of the microbes to store starch is exceeded.

Pasture generally has high levels of soluble protein which are rapidly and extensively degraded in the rumen. Consequently, extensive grazing of fresh grass can be associated with excessive ammonia-N (NH_3-N) production in the rumen, negatively affecting animal performance and the environment. Ruminal NH_3-N concentration can be reduced and non-ammonia N flow to the duodenum increased when cows consuming pasture are

supplemented with non-structural carbohydrate (Van Vuuren et al., 1990). Factors such as level, type, and balance of different carbohydrate sources may assume greater importance, particularly when variations in soluble sugar concentration are taken into account. Chamberlain et al. (1993) have demonstrated that the soluble sugars, sucrose, lactose, and fructose are superior to starch (cereals are usually chosen for practical supplementation rather than sucrose) as an energy source for fixation of microbial N in the rumen. This finding may have significance for microbial synthesis in grazing animals. Although starch levels are very low in grasses the storage polysaccharide fructan (a polymer of fructose), can form up to 70% of the water soluble carbohydrate content of some cool-season grasses (McGrath, 1988).

Leaving aside the issue of synchronization, information on the rate and pattern of fermentation of carbohydrate sources is urgently needed if we are to uncover the mechanisms underlying the effects of diet on metabolism, and hence on milk composition, and their use to advance feeding of the dairy cow.

Starch Digestion, Ruminal Fermentation, and Effects on Lactation Performance

In the ruminant, the majority of starch in the diet is fermented to VFA in the rumen. Rumen fermentation varies with type of grain as well as conservation or processing method, and this variation can greatly affect animal performance. Optimal levels of starch in the diet, and rumen fermentability of that starch depend on animal and dietary factors.

Rumen fermentation of starch is determined by the rate at which starch is fermented and the retention time of the starch in the rumen, both of which vary by species and physiological status of animal, grain type, grain genotype, growing conditions, and physical and chemical processing method (Table 2; Herrera-Saldana et al., 1990; Nocek and Tamminga 1991; Huntington, 1997). Herrera-Saldana et al. (1990) compared rates of starch digestion of five

cereal grains with in vitro and in situ methods. When processed similarly, oats had the fastest rate of starch digestion, followed by wheat, barley, corn, and finally sorghum.

Although in vivo rumen digestion is a function of both rate of fermentation and rate of passage, rumen starch digestion has been reported to follow the same trend. In a comparison of sorghum-, corn-, and barley-based diets containing approximately 82% grain (Spicer et al., 1986), the rumen digestion of sorghum (75.2%) was significantly less than corn (83.7%) or barley (87.7%). In lactating dairy cow rations, in vivo rumen starch digestion was much greater on barley diets than on ground corn diets (77.4 vs. 48.6%; McCarthy et al., 1989), and on barley than on sorghum diets (80 vs. 49%; Herrera-Saldana and Huber, 1989).

Within a grain type, dry and wet processing methods have been used to alter digestibility of grains fed to ruminants. Most grain processing methods increase both rate of starch fermentation and rumen starch digestibility. Unprocessed grains with low rumen digestibilities such as sorghum seem to respond to processing to the greatest extent (Theurer, 1986).

Other factors that may influence rumen starch digestion include rumen protozoa (Mendoza et al., 1993), level of DM and starch intake (Russell et al., 1981; Brink and Steele, 1985), and grain hybrid (Wester et al., 1992; Ladely et al., 1995). Protozoa reduce the rate of starch digestion in the rumen through ingestion of starch digesting bacteria, and through ingestion of starch granules and sugars. Defaunation of sheep has been shown to increase rumen starch digestibility (Mendoza et al., 1993). In addition, level of starch intake affects starch digestion. Brink and Steele (1985) reported that ruminal starch digestion (g/d and % of total starch intake) increased as amount of corn in the diet fed to steers increased.

Although we can manipulate rumen starch digestion fairly easily, the effect on milk yield varies. Increased milk yield with increased rumen available starch was observed for early-lactation cows when fed barley in place of sorghum (Herrera-Saldana and Huber, 1989), or steam flaked sorghum instead of dry rolled sorghum (Moore et al., 1992; Poore et al., 1993), or when fed ground corn instead of cracked corn (Knowlton et al., 1996). Similar increases in milk yield were observed in mid-lactation cows fed corn ground more finely (Moe and Tyrrell, 1976), or steam flaked sorghum in place of dry rolled sorghum (Chen et al., 1994; Chen et al., 1995), or steam flaked corn instead of dry rolled corn (Plascencia and Zinn, 1996). However, diets with increased ruminally degraded starch did not affect milk yield or FCM in other studies (Oliveira et al., 1993; Oliveira et al., 1995). Milk or FCM yield was decreased when higher ruminally degraded starch was fed to early-lactation cows fed barley instead of corn (McCarthy et al., 1989), or dry rolled barley versus dry ground corn (Casper et al., 1990), or high moisture shelled corn in place of dry ear corn (Aldrich et al., 1993). This occurred for mid-lactation cows fed untreated barley instead of the more slowly digested ammoniated barley (Robinson and Kennelly, 1989).

Increasing the energy content of the diet by increasing grain feeding generally increases milk protein concentration (Poore et al., 1993; Chen et al., 1994). This increase is generally attributed to increased microbial protein synthesis with increasing levels of ruminally fermented carbohydrates. Recent research suggests that increased feeding of fermentable starch may also increase milk protein concentration through changes in whole body glucose and insulin response (McGuire et al., 1995). Increased digestion of starch in the rumen generally increases availability of propionate and other glucose precursors. Increased

synthesis of glucose elicits an insulin response, and elevated insulin and glucose can increase milk protein concentration.

Increasing grain content of the diet usually decreases milk fat concentration, a change most commonly explained by changes in rumen fermentation. High grain diets, and diets higher in ruminally degraded starch typically increase rumen propionate concentrations and decrease the acetate to propionate ratio (McCarthy et al., 1989; Moore et al., 1992; Aldrich et al., 1993; Oliveira et al., 1993; Knowlton et al., 1996; Plascencia and Zinn, 1996). Propionate is a precursor for the synthesis of glucose by the liver, while acetate is a precursor for the synthesis of fat by the mammary gland and body tissue. The decrease in milk fat with high grain diets has, therefore, been classically explained by a general shift from lipogenesis to gluconeogenesis. Recent research, however, suggests that the depression in milk fat may instead be caused by an accumulation of *trans*-fatty acids in the rumen caused by low rumen pH on high grain diets (Gaynor et al., 1995; Kalscheur et al., 1997a; Kalscheur et al., 1997b).

PRODUCTION AND UTILIZATION OF GRASSES IN RUMINANTS

Environmental sustainability and nutrient management challenge dairy farmers with increased public concern. Many dairy farmers are now turning to grass forage crops and pasture systems as solutions to some of the nutrient management challenges they now encounter (Cherney and Cherney, 1998). Since a shift to grass-based dairying is seen as a mechanism to increase the sustainability of our dairy farms, perennial grass has attracted much attention, but its application in practical dairy operations is in its infancy as far as knowledge is related to intensive management of the lactating cow. Notable improvements in grasses from breeding program and the management of grass for dairy cattle consumption

makes grass an increasingly desirable forage option. Properly managed pastures or hay fields can produce high quality grass for lactating dairy cows, as well as improve the degree of nutrient management needed on most dairy farms.

Plant Anatomical Factors Influencing Forage Intake and Digestibility

Forage contains five different types of tissues: vascular bundles containing phloem and xylem cells, parenchyma bundle sheath surrounding the vascular tissue, sclerenchyma patches connecting the vascular bundles to the epidermis, mesophyll cells between the vascular bundles and epidermal layers, and, on the exterior, a single layer of epidermal cells covered by a protective cuticle (Minson, 1990). These tissues are digested to varying extents in the rumen. In addition, the proportion of these tissues varies among species, plant parts, and stage of growth and is affected by management factors. Figure 2 illustrates a simple model which links plant anatomy to chemical composition and is the basis for differences in the potential digestibility of the various fractions. The general ranking of digestibilities of the various cell types present in forages follows the order: phloem = mesophyll = undifferentiated parenchyma > epidermis > parenchyma bundle sheath > sclerenchyma > lignified vascular tissue (Wilson, 1990).

Plant maturity

The predominant feature of increasing physiological maturity of most forages is a tremendous reduction in the leaf to stem ratio (Albrecht et al., 1987). Among grasses, both leaf and stem increase in cell wall and lignin contents with advancing maturity (Jung and Vogel, 1992). Consequently, the digestibilities of both leaves and stems of grasses decline with increasing forage maturity, although the rate of decrease is greater for stems than leaves (Mowat et al., 1965). Albrecht et al. (1987) found that alfalfa stems increased in cell wall and

lignin concentrations with increasing maturity, but alfalfa leaves maintained relatively constant composition across maturity levels. Thus, alfalfa stem digestibility decreases considerably with increasing maturity, whereas digestibility of alfalfa leaves is little affected (Albrecht et al., 1987).

Grasses versus legumes

Legumes usually contain lower cell wall concentrations but greater lignin concentrations than grasses at similar maturities. Also, hemicelluloses comprise a smaller percentage of total cell wall in legumes than in grasses (Van Soest, 1994). Temperate, but not tropical, legumes generally are consumed in greater quantities than grasses by ruminants (Minson, 1990). The greater intake potential of temperate legumes is related to their lower cell wall contents and their lower resistance to breakdown during eating and rumination. Thornton and Minson (1973) found that the voluntary intake of legumes was 28% higher than that of grasses of equal digestibility, an increase which was associated with a 17% shorter ruminal retention time of digesta for legumes.

Digestibility of legume cell walls is usually lower than that of grass cell walls, an effect that is related to the higher lignin concentration in cell walls of legumes (Van Soest, 1994). Smith et al. (1972) found a similar lignin:cellulose ratio in indigestible residues of grasses (0.94) and legumes (1.09), suggesting that lignin protects similar quantities of cell wall polysaccharides from digestion in both grasses and legumes. Wilson and Kennedy (1996) suggested that the greater digestibility of legumes compared with grasses may reflect leaf length. Grass particles are inherently long and buoyant, with a low functional specific gravity, and easily entangled in the rumen, while chewed legume vascular particles are short and chunky with high functional specific gravity and therefore likely to escape the rumen

quickly. Thus, potential intake is dependent not only on the fiber content, but also on the anatomy of the plant and the way in which it breaks down during digestion.

Temperate versus tropical forages

Tropical forages generally contain higher cell wall and lignin concentrations than temperate forages (Van Soest, 1994). Minson (1990) found that voluntary intake by sheep of temperate forages was usually greater than that of tropical forages, a difference that applied to both grasses and legumes. However, Reid et al. (1988) did not found differences in voluntary intake of C_3 (temperate) and C_4 (tropical) grasses by cattle. Dry matter digestibility of temperate forages is usually greater than that of tropical forages (Reid et al., 1988; Minson, 1990). Minson and McLeod (1970) estimated that temperate grasses were an average of 13 percentage units higher in DM digestibility than tropical grasses. The difference was estimated to be only 4 percentage units greater for temperate vs. tropical legumes (Minson and Wilson, 1980). Lagasse et al. (1990) found greater voluntary intakes and total tract NDF digestibilities when cattle consumed vegetative orchardgrass compared to vegetative bermudagrass. However, both extent and site of NDF digestion were similar when cattle consumed mature orchardgrass or mature bermudagrass at 1.5% of body weight (Jones et al., 1988).

The greater digestibility usually observed for temperate grasses has been attributed both to differences in anatomical structure of C_3 and C_4 grasses and to the higher temperature at which tropical grasses are normally grown. Temperate grass leaves usually contain smaller proportions of slowly digested parenchyma bundle sheath and epidermis cells and larger proportions of rapidly digested mesophyll cells than do tropical grass leaves (Akin, 1989). The significance of these anatomical variations has been confirmed by the greater

digestibility of C_3 vs. C_4 species of the switchgrass grown under the same environmental conditions (Wilson et al., 1983). Higher environmental temperature during growth increased cell wall and lignin contents and decreased digestibility of a variety of forages without altering anatomical composition of leaves and stems (Wilson et al., 1991). Wilson and Kennedy (1996) suggested that the lower digestibility of tropical grasses compared with temperate grasses and legumes reflects an interlocking and therefore more rigid cell structure.

Ensiling As a Method of Preservation of Forage

The conservation of forage for winter and dry-period feed is essential for efficient operation of animal production system. The objective of preservation is to conserve digestible nutrients as efficiently as possible. Traditionally this has been accomplished by making either hay or silage. Silage fermentation results in large changes in both the amounts and types of nutrients in silage as compared to fresh forages (Table 3). The ensiling process results in fermentation of soluble carbohydrates and proteins leaving behind the less digestible fraction of forage DM (Table 3). At the same time, ensiling results in organic acid production, principally lactic acid, but also including substantial amounts of acetic, propionic, butyric, and other acids. In addition, soluble forage proteins are degraded which results in production of amines, ammonia, and other N containing compounds. These changes in composition are required for forage preservation to occur. It is the production of lactic acid and the resulting drop in pH which preserves silage in storage and simultaneously improves stability in the feed bunk.

Fermentation of NSC in silage has a direct effect on the pattern of VFA production in the rumen. Silages that have undergone extensive homolactic fermentation contain almost no

soluble sugars. In the rumen, VFA are directly absorbed and lactic acid is metabolized primarily to propionate (Charmley, 2001). This shifts the balance of fermentation end-products away from lipogenic to glucogenic precursors (Jaakkola and Huhtanen, 1992; Martin et al., 1994). Whereas in the past this was regarded as a problem, today it may be possible to exploit this as a tool to modify bovine milk composition.

In addition to poor efficiency of microbial protein synthesis, the proportion of dietary proteins escaping degradation in the rumen is also generally low for forages and for silages in particular (Tamminga et al., 1991). Charmley and Veira (1991) showed that the problem of high N solubility was not attributed to microbial fermentation, but to proteolysis by plant enzymes. Plant enzyme systems were inhibited by short-duration heat treatment, which reduced protein solubility, increased CP flow to the duodenum and increased CP gain in lambs (Chamley and Veira, 1990). Similar responses have been achieved with rapid acidification (Charmley et al., 1995) or wilting (Muck, 1987) in order to inhibit proteolytic activity.

Wilting (field drying) is a most popular method to restrict fermentation during the ensiling process. Since reducing the moisture content of the crop retards the activity of all microorganisms, wilting reduces microbial activity in silage, thus restricting the extent of fermentation. However, it also tends to favor lactic acid bacteria over other types, thus reducing the risk of less useful microbial pathways dominating the ensilage process (McDonald et al., 1991). Wilting resulted in an increase in silage intake (Zimmer and Wilkins, 1984), but did not improve animal performance (Charmley and Thomas, 1987). This was attributed to reduced digestibility of wilted, compared with unwilted silage, and reduced efficiency of energy utilization (Unsworth and Gordon, 1985). Rook and Gill (1990)

concluded that the response to wilting was curvilinear with no benefits seen when DM content was above about 25%. More recently, however, Wright et al. (2000) reported linear response to increasing DM concentration in the wilted silage with a wider set of data from 79 comparisons. These authors concluded that the wilting rate, and the extent of moisture loss in wilted silage were variables most highly correlated with improved intake and performance from wilted silage relative to unwilted silage. This suggests that benefits to the animal are greatest under good wilting conditions.

Animal studies have linked the reduction in protein solubility by wilting to increased amino acid flow to the intestine (Charmley and Veira, 1990), and milk yield in lactating cows (Broderick et al., 1993). Thus, the benefits of wilting on animal performance may be related primarily to an improvement in the utilization of N by the ruminant.

Forage Quality and Microbial Protein Synthesis in the Rumen

In general, the most important source of protein reaching the lower gut of ruminants is the microbial protein produced in the rumen. Microbial protein is of high quality with a well balanced amino acid profile (Merchen and Titgemeyer, 1992). Protein in many forages is extensively degraded and this increases the dependency of the host on microbial protein as a source of amino acid for production. The average value for the efficiency of microbial protein synthesis (EMPS) from literatures summarized in Table 4 is 33.4 g microbial N (MN)/kg OM apparently digested in the rumen (OMD_A). A large range of values (17.0 to 74.0) is noted although most values are in the range of 24 to 37 g MN/kg OMD_A. Some distinction should be made between net microbial protein synthesis (e. g., the quantity of microbial protein entering the lower gut) and true microbial protein synthesis. The difference between the two quantities is the appreciable intraruminal recycling of microbial N that

occurs due to cell death and lysis and protozoal predation (Leng and Nolan, 1984). The yield and efficiency of synthesis of microbial protein has been recorded as high (30-45 g MN/kg OMD_A) when high-quality grass is grazed (Beever et al., 1986; Dove and Milne, 1994; Carruthers et al., 1997; Jones-Endsley et al., 1997; Elizalde et al., 1998) or fed as fresh grass harvested (Beever et al., 1978; O'Mara et al., 1997). Much lower microbial efficiencies (< 20) have been noted with lower-quality autumn-grass (Dove and Milne, 1994; Carruthers et al., 1997). Dove and Milne (1994) noted that the reduced microbial efficiency in autumn was closely related to much lower levels of rumen propionate and thus higher acetate:propionate ratios in the rumen. Beever et al. (1978) indicated that such a VFA pattern in the rumen would be the likely consequence of very low water soluble carbohydrate levels in the forages. Feeding forages at advanced stages of maturity results in decreases in the quality of microbial protein entering the postruminal gut of sheep (Kawas et al., 1990). Net microbial protein production is a function of EMPS and of the quantity of OM digested in the rumen. Reductions in net synthesis associated with more mature forages result largely from reductions in ruminal OM digestion, although EMPS is sometimes at least modestly reduced as well. Kawas et al. (1990) observed that net microbial protein synthesis decreased by 48% when full-bloom alfalfa hay was fed compared to pre-bloom hay. In that experiment, the quantity of OMD_A decreased by 30% while EMPS decreased from 34.0 to 25.0 g MN/kg OMD_A for pre- vs. full-bloom hays, respectively.

Preservation of forages as silages involves fermentation of carbohydrates in the silo and so it is not surprising that in silage-fed animal yields of microbial protein are lower than for fresh forages (Charmley, 2001). Poor N utilization from silages is a product of two consequences of ensiling: solubilization of protein and fermentation of soluble sugars to VFA

and lactic acid. Energy yield and utilization from VFA and lactic acid are considerably less than from non-fermented carbohydrate (Thomas and Thomas, 1985). A high proportion of VFA is absorbed directly across the rumen wall and not utilized by rumen microbes. Thus, the efficiency of utilization of fermented OM for microbial protein synthesis is approximately 60 to 70% of that for energy from non-fermented feeds (Agricultural Research Council, 1984). Jaakkola and Huhtanen (1993) found high EMPS with a silage compared to hay made from the same silage (Table 4). This unusual result may reflect the restricted fermentation (high residual sugar and true protein) because of a high level of formic acid used as an additive. On the other hand, higher EMPS in orchardgrass hay compared to silage (Holden et al., 1994; Table 4) may have resulted from the early stage of harvest when making silage. Because silage fermentation depletes soluble carbohydrate concentration, many researchers have added sugars back to silage rations in an attempt to improve protein utilization and their results have been equivocal (Chamberlain et al., 1985; Charmley and Veira, 1991; Khalili and Huhtanen, 1991). Chamberlain et al. (1985) concluded that sucrose supplementation improved efficiency of microbial protein synthesis in limit-fed sheep. However, ruminal fermentation patterns under these conditions are characterized by extremes in rumen ammonia concentration and are not conducive to efficient ammonia capture by rumen microbes. Such conditions are not found in animals fed ad libitum (Charmley et al., 1995). Khalili and Huhtanen (1991) concluded that the beneficial effects of sucrose on the amounts of amino acids reaching the intestine was due to increased ruminal turnover rate rather than improved microbial efficiency. In support of this, Keady and Murphy (1998) found that sucrose supplementation alone did not increase intake or milk yield of dairy cows, but when sucrose plus fishmeal was fed there was a response. Elizalde et al. (1998) reported

that different energy sources (cracked corn vs. corn gluten feed) fed with tall fescue did not affect EMPS (Table 4). Differences in energy sources (starch vs. fiber) of supplements fed to cattle that consumed forage diets had minimal effects on EMPS when supplements were fed at a level below 60% of the total DM intake (Thomas, 1988). In the study of Elizalde et al. (1998), cracked corn and corn gluten feed represented 27% of total OM intake.

There are no consistent effects of cutting date or stage of maturity of forages when ensiled on microbial protein synthesis, so that effects in individual experiments are probably related to other factors, including interactions with level and type of concentrate, and not maturity *per se*. McAllan et al. (1994) attributed higher microbial protein synthesis with a grass silage prepared in early June (compared with one prepared in late June), to higher levels of amino acids and peptides. On the other hand, Rinne et al. (1997) found no effect of stage of maturity on microbial protein synthesis, while Hart and Leibholz (1990) only found an effect with silages made from kikuyugrass. Conversely, Thomas et al. (1980) found higher efficiency of microbial protein synthesis with more mature silage, when comparing autumn-cut silages.

Utilization of Eastern Gamagrass for Ruminants

Forage crops that economically compete with grain crops include alfalfa, corn for silage, forage sorghum, sorghum-sudan hybrids, and sudan grass (Vogel et al., 1985). Among these crops, corn silage is the most widely grown and highest yielding in terms of DM per acre. However, corn production can result in considerable soil erosion on marginal and sloping cropland (Martin and Cassel, 1992). Minimum soil erosion is best achieved when perennial crops are used (Gantzer et al., 1991). To create more sustainable silage production systems and help reduce soil erosion, high yielding, high quality perennial grass silage crops are

needed as alternatives to corn for silage production on marginal and sloping cropland (Brejda et al., 1994).

Eastern gamagrass [*Tripsacum dactyloides* (L.) L.] is a warm season perennial bunch grass. Waller and Lewis (1979) determined that gamagrass possesses the same C_4 (warm season) photosynthetic pathway as does corn. This wonder grass has been called the **"Queen of the Grasses"** because of its high yields and rapid growth. It has recently gained renewed interest, not only because of its nutritive value as a forage crop, but also because of its ability to penetrate acid, compact, and marginal soil and to survive both flooding and drought. Eastern gamagrass forage quality is excellent if harvested at the proper stage of maturity. Horner et al. (1985) compared the nutritional value of eastern gamagrass and alfalfa hay for dairy cows and reported that although NDF and ADF concentrations were greater in eastern gamagrass, the digestibility of these fiber components was also greater with eastern gamagrass (NDF = 62 vs. 45%; ADF = 57 vs. 40%). Lactating dairy cows offered the same forages consumed more alfalfa than gamagrass (9.85 vs. 8.99 kg/d). Burns et al. (1992) compared diet characteristics, digesta kinetics, and DM intake of steers grazing eastern gamagrass, flaccidgrass, and bermudagrass and reported that eastern gamagrass had a greater proportion of leaf and lower proportion of stem than either flaccidgrass or bermudagrass. The digestibility of the leaf and stem components were higher in eastern gamagrass than the two other grasses.

The pattern of growth for gamagrass contrasts sharply with that of most other warm season perennials due to plentiful growth during spring and early summer (Coblentz et al., 1998). Thus, the opportunity for multiple harvest exists, and growth is sustained throughout the summer without the dormant period in the southeastern USA that is common to most cool

season grasses. Furthermore, at growth states that produce reasonable yields (boot and early anthesis stages), N concentrations can be high [2.2 to 3.2% N (Brejda et al., 1996; Burns et al., 1992; Horner et al., 1985)] relative to the N concentrations of other perennial warm season grasses at similar growth stages and comparable with N concentrations commonly reported for red clover hay and midbloom alfalfa hay (NRC, 1989). This high N potential poses some intriguing possibilities for the use of gamagrass in dairy cattle rations. Despite the high N concentrations found naturally in alfalfa, the highly degradable nature of this N can lead to poor utilization of potentially available N in lactating dairy cows (Broderick, 1985). Mullahey et al. (1992) suggested that anatomical differences between C_3 and C_4 grasses may be responsible for the greater concentrations of RUP in switchgrass (C_4) compared with smooth bromegrass (C_3). Similar differences might occur between gamagrass and alfalfa or red clover. Coblentz et al. (1998) reported that most of the N in leaves of gamagrass was neutral detergent insoluble N (NDIN), implying association with the cell wall; this fraction ranged from 51.3 to 64.5% of the total leaf N and increased as the gamagrass matured. On a whole-plant basis, NDIN concentration was high in gamagrass at all stages of growth (> 51%), reflecting the leafy nature (> 69%) of the plant at all growth stages. Degradation rates of N were faster for leaf tissues of alfalfa and red clover than for leaf tissues of gamagrass due to larger proportions of immediately degradable N (fraction A) in the leaf tissue of legumes than in corresponding leaf tissue of gamagrass. Assuming 6% passage rate, these authors found that effective degradabilities would be 82 and 54% for alfalfa and gamagrass, respectively, implying that 113% more actual forage N (1.30 vs. 0.61% of DM) would be available in the small intestine with gamagrass than with alfalfa.

However, calculated effective ruminal degradability may be limited by slow degradation rate of gamagrass, which may result in reduced DM intake in lactating dairy cows.

Ensiling as a method of preservation has been recommended for warm season grasses in areas where cool temperatures, high humidity, and frequent precipitation make field curing of hay difficult (Moser, 1980). However, Ball et al. (1991) cautioned that warm season grasses are more difficult to ensile due to low concentrations of fermentable carbohydrates in their tissues. Brejda et al. (1994) reported that eastern gamagrass silage had 2.6 to 4.2 percentage units greater CP, 11.5 to 15.3 percentage units greater ADF, 8.0 to 15.5 percentage units greater NDF, 1.8 to 6.1 percentage units greater lignin concentrations, 10.9 to 21.3 percentage units lower in vitro DM digestibility (IVDMD) than corn silage. Eastern gamagrass harvested at inflorescence emergence or vegetative stages had 2.0 to 3.5 percentage units greater CP, 3.2 to 7.5 percentage units lower ADF, and 2.3 to 17.0 percentage units lower NDF concentrations than eastern gamagrass harvested at the seed development stage. Consequently, if harvested at the vegetative or inflorescence emergence stage and proper moisture concentration, eastern gamagrass can make good quality silage, but lower in IVDMD than corn. Producers interested in using eastern gamagrass in place of corn for silage production on marginal and erosive cropland will have to weigh the potential benefits of reduced annual input costs and reduced soil erosion against possible reduction of forage quality and consequent animal performance. However, the literature is void of comparative evaluation of nutritional value of eastern gamagrass conserved as hay or silage.

DISSERTATION OBJECTIVES

The results presented in this dissertation are primarily directed towards two goals. The first goal is to provide validation of methane production as a method to estimate microbial production in response to changes in fermentation environment. This led to a study of the effects of dilution rates and dietary forage to concentrate ratios on fermentation kinetics in continuous cultures. Direct measurements of methane concentration in fermentor headspace are compared with estimates of methane production based on stoichiometric equations in the study. The second goal is to develop feeding strategy of grass silage as an alternative forage source for corn silage with the concept of synchronizing energy and nitrogen availability in the rumen. Research was undertaken to assess the feeding value of gamagrass as hay or silage for lactating dairy cows and to determine the effect of supplemental corn in gamagrass silage-based diets. Gamagrass silage was chosen based on the fact that it has potential to be economically more feasible than traditional sources such as corn silage and on the expectation that it would provide atypical nutritional values to lactating dairy cows due to its high association of N to neutral detergent insoluble fraction. After the initial evaluation of the nutritional value of gamagrass in lactation performance trial, further research was undertaken to determine the effects of gamagrass offered as hay or silage without or with supplemental corn on ruminal fermentation. Discussion of the microbial fermentation and its contribution to lactation performance of dairy cows is presented in the final section with a summary of each study conducted in this dissertation.

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Table 1. Direct and indirect gas production from 1 mole of glucose fermented to different end-products in ruminal fermentation. (Beuvink and Spoelstra, 1992)

Fermentation end-product	Moles, end-product produced	Direct CO_2[1], mole	Indirect CO_2[2], mole	Total CO_2, mole
Acetate	2	2	2	4
Butyrate	1	2	1	3
Propionate	2	-	2	2
Lactate	2	-	2	2

[1]CO_2 production directly from microbial metabolism.
[2]Assumed a pure bicarbonate buffer (a 1:1 release of CO_2 per acid equivalent).

Table 2. Starch content and rumen digestibility of cereal grains commonly fed to dairy cows. (Herrera-Saldana et al.,1990; Huntington, 1997)

Grain	% starch, ± SD	Processing method	Rumen digestibility, % intake
Wheat	70.3 ± 2.9	Dry rolled	88.3
		Steam rolled	88.1
Barley	64.3 ± 3.3	Dry rolled	80.7
		Steam rolled	84.6
Oats	58.1 ± 4.3	Dry rolled	92.7
		Steam rolled	94.0
Corn	76.0 ± 1.8	Dry rolled	76.2
		Steam flaked	84.8
		Steam rolled	72.1
		High moisture	89.9
		Ground	49.5
Sorghum	71.3 ± 2.7	Dry rolled	59.8
		Steam flaked	78.4
		High moisture ground	73.2
		Ground	70.0

Table 3. Examples of changes in forage composition occurring during ensiling.

Item	Fresh grass-legume[1]	Grass-legume silage[1]	Fresh alfalfa[1]	Alfalfa haylage[2]	Whole corn plant[2]	Corn silage[2]
	% DM					
DM, %	20.9	25.8	35.4	35.0	29.4	30.0
pH	-	4.62	5.56	4.52	5.20	3.91
CP, %	17.9	19.6	20.2	20.9	8.2	8.0
Soluble protein, % of total CP	59.2	45.3	50.9	28.1	68.0	62.0
ADF, %	34.0	40.4	34.2	36.4	24.5	24.5
NDF, %	45.3	52.8	44.0	43.1	45.5	45.3
Lactic acid, %	-	6.6	-	7.4	-	4.6
Total organic acids, %	-	11.2	-	11.7	-	6.7
Ammonia N, % of total N	2.9	8.0	3.0	11.9	5.5	8.0
Sugars, %	5.1	0.6	6.4	1.4	10.8	1.6

[1]Adapted from Waldo (1976).
[2]Adapted from McDonald et al. (1991); Shaver et al. (1885); Wilkinson et al. (1976)

Table 4. In vivo measurements of efficiency of microbial protein synthesis (EMPS) in animals fed forage-based diets.

Diet	Animal	EMPS, g MN/kg OMD_A[1]	Reference
Alfalfa hay	Sheep		Kawas et al., 1990
Pre-bloom		34.0	
Early bloom		27.0	
Mid-bloom		24.0	
Full bloom		25.0	
Perennial ryegrass	Lactating dairy cows		O'Mara et al., 1997
Fresh grass only		37.7	
Fresh grass + 3 kg/d molassed beet pulp pellets		42.0	
Perennial ryegrass silage	Lactating dairy cows		Teller et al., 1992
Direct-cut (17.0% DM)		28.6	
Wilted (38.0% DM)		32.0	
Timothy	Bulls		Jaakkola and Huhtanen, 1993
Hay, chopped		20.3	
Silage, unwilted + formic acid		24.8	
Orchardgrass	Dry cows		Holden et al., 1994
Pasture		17.0	
Hay, chopped (full bloom)		22.7	
Silage (early bloom)		28.4	
Tall fescue	Steers		Elizalde et al., 1998
Pasture only		40.4	
+ 3.11 kg/d of corn gluten feed		44.2	
+ 3.11 kg/d of cracked corn		34.2	
+ 1.55 kg/d of cornstarch-corn gluten meal		43.0	
Alfalfa + orchardgrass, pasture	Lactating dairy cows		Jones-Endsley et al., 1997
+ 6.4 kg/d supplement		74.0	
+ 9.6 kg/d supplement		71.0	

[1]Efficiency of ruminal microbial protein synthesis expressed as g microbial N per OM apparently digested in the rumen.

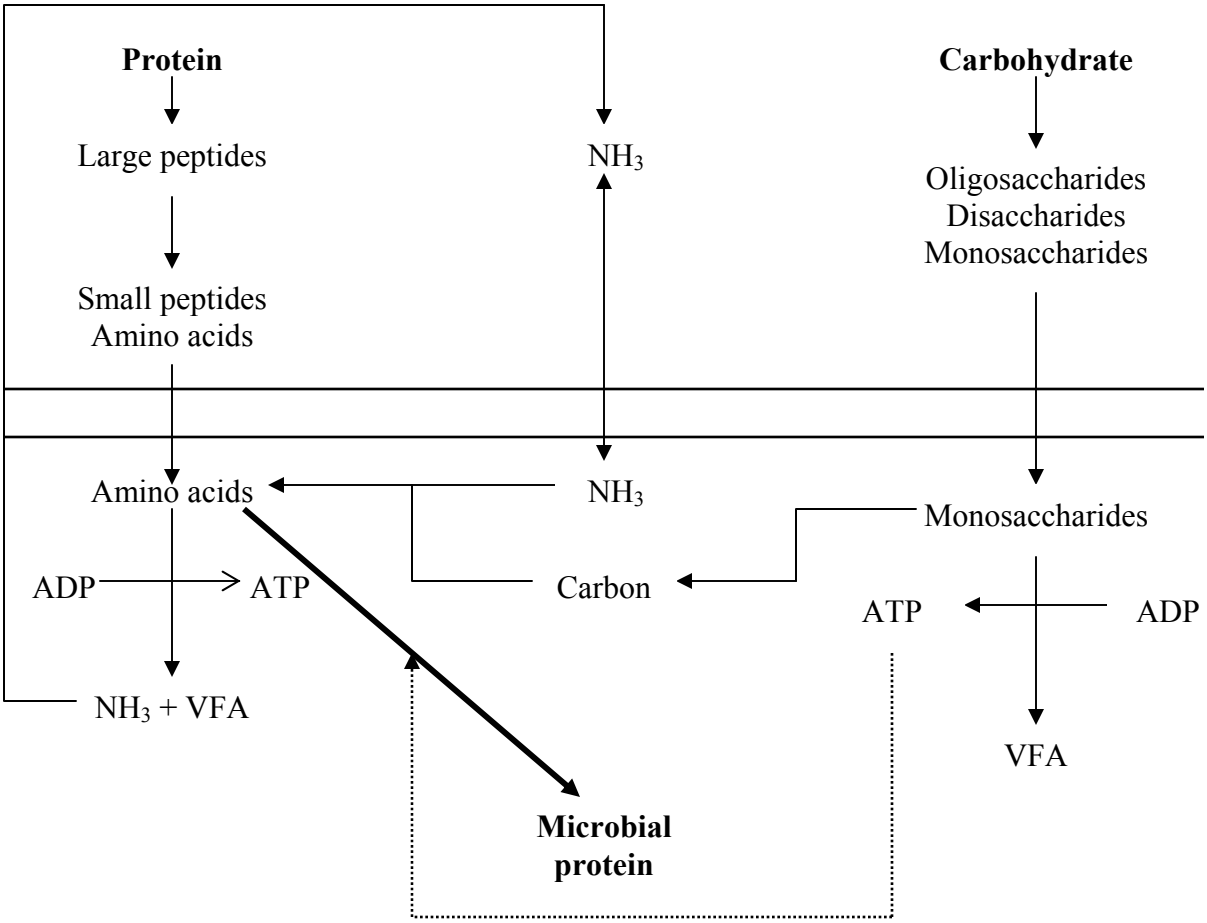

Figure 1. A theoretical scheme showing carbohydrate and protein utilization by ruminal bacteria. (Cotta and Russell, 1996)

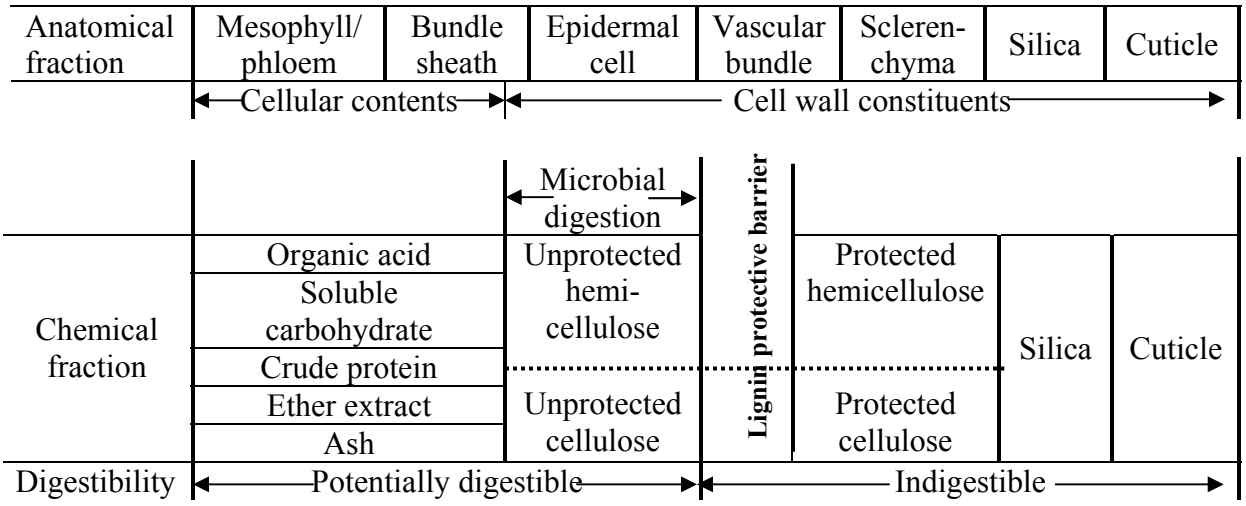

Figure 2. Conceptual model of the relation between plant anatomy and chemical fractions indicating areas of potential digestibility. (Minson, 1990)

CHAPTER 3

RUNNING HEAD: METHANE PRODUCTION BY RUMEN MICROBES

EFFECT OF DILUTION RATE AND FORAGE TO CONCENTRATE RATIO ON FERMENTATION AND METHANE PRODUCTION BY MICROBES INCUBATED IN DUAL-FLOW CONTINUOUS CULTURES

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ABSTRACT

In vitro gas production is commonly used as a measure of carbon metabolism to estimate digestibility of feed. This study was designed to monitor the effects of dilution rate and forage to concentrate ratio on fermentation by rumen microbes cultured in fermentors. Dual-flow continuous cultures were used to test three dilution rates (3.2, 6.3, or 12.5 %/h) and three forage to concentrate ratios (70:30, 50:50, or 30:70). Filtered ruminal contents were incubated in the fermentors and allowed 6 d of adaptation to dilution rate and diets followed by 7 d of sample collection. Liquid dilution rate was manipulated by changing the flow rate at which artificial saliva was infused into the culture vessels. Forage consisted of pelleted alfalfa and the concentrate mix included ground corn, soybean meal, and a mineral and vitamin premix. Approximately 13 g of dry feed per d was offered to each fermentor in two equal portions. Samples of fermentor contents and headspace gas were analyzed for volatile fatty acids, ammonia-N, and methane. The experiment was replicated in a split-plot design. Production of total volatile fatty acids was not affected by dilution rate and level of concentrate in the diet and averaged 58.0 mmol/d. Molar proportion of acetate was not affected by dilution rate but increased with increasing forage to concentrate ratio. Molar proportion of propionate tended to decrease with an increase in dilution rate and increased with the level of concentrate. The acetate to propionate ratio increased with increasing dilution rate or level of forage in the diet. Ruminal culture pH increased with increasing dilution rate but did not change in response to forage to concentrate ratio. Methane production when calculated from stoichiometric equations did not change with dilution rate or forage to concentrate ratio. Methane production based on actual concentrations in the fermentor increased with dilution rate and level of forage in the diet. Compared to actual

concentrations in fermentor headspace, stoichiometric equations consistently underestimated methane output at higher dilution rates and with high forage diets. Fermentability of diets averaged 60.0% and increased with dilution rate. Higher dilution rates resulted in an increase in microbial yield and microbial efficiency. Increasing the level of concentrate in the diet only increased microbial efficiency. Our results show that dilution rate and forage to concentrate ratio can alter the partition of substrate by rumen microbes and that gas production, in particular methane, may not be accurately estimated using stoichiometrics of end product appearance.

(**Key words:** continuous culture, methane production, stoichiometric equations, microbial energetics)

Abbreviation key: DR = dilution rate, **F:C** = forage to concentrate ratio, **H** = high forage diet (70% forage:30% concentrate), **M** = medium forage diet (50% forage:50% concentrate), **L** = low forage diet (30% forage:70% concentrate), **A:P** = acetate to propionate ratio, **CO_2** = carbon dioxide, **CH_4** = methane, **NH_3-N** = ammonia-N.

INTRODUCTION

Carbon dioxide (CO_2) and methane (CH_4) are the two major gases produced as end products of anaerobic fermentation of feeds in the rumen. In addition to these gases, VFA, ATP, and microbial biomass are also produced. Stoichiometric equations relating substrate degradation to VFA and gas production have been developed and are commonly used to estimate digestibility of ruminant feeds (Wolin, 1960; Russell and Baldwin, 1979; Menke et al., 1979; Van Soest, 1994). Total gas production is increasingly used as a measure of the fermentative quality of feeds (Beuvink and Spoelstra, 1992; Pell and Schofield, 1993). Since the composition of the end products influences the amount of gas produced a close relationship between the two has been reported (Naga and Harmeyer, 1975; Menke and Steingass, 1988; Taya et al., 1980). However, rate of fermentation can vary with carbohydrate fraction; rapidly fermenting carbohydrates may not always result in higher amounts of total gas production (Beuvink and Spoelstra, 1992). Also, total gas yield can vary considerably due to the incorporation of carbons into microbial mass as well as the different metabolic pathways by which carbohydrate fractions can be degraded by rumen microbes (Van Soest, 1994; Beuvink and Spoelstra, 1992; Krishnamoorthy et al., 1991). The relationship between microbial biomass and gas yield can vary also with growth conditions (Krishnamoorthy et al., 1991; Blümmel et al., 1997). With short duration incubation studies, fermentation rate as measured by gas production has been used as an index of net growth rate of rumen microorganisms (El-Din and El-Shazly, 1969). Extending the period of incubation reduced net growth of microorganisms even though gas production continued to increase (Raab, 1980). Increased lysis of microbial cells as a consequence of substrate exhaustion and uncoupled fermentation may contribute to reduced net growth at longer incubation times

(Van Nevel and Demeyer, 1977). Changes in microbial yield or metabolism may alter the relationship between substrate digestion and gas production and could affect the estimation of digestion rate from gas measurements (Doane et al., 1997). However, there is no clear explanation for the discrepancy between actual gas production and estimates based on stoichiometric equations.

We know that the production of microbial biomass per unit of ATP is not a constant (Van Soest, 1994;Russell and Wallace, 1997), and therefore similar amounts of VFA can result in different microbial yields. Previous studies do not make any distinction between CO_2 and CH_4 and assume that the latter arises directly from the former. There is evidence to suggest that more than one substrate may be used for the production of CH_4 (Miller, 1995). The generation of microbial biomass can be a sink for reducing equivalents and if ignored it can result in the over prediction of CH_4 output (Hungate, 1967; Wolin, 1960). Methanogens have been shown to have variable affinities for hydrogen and are capable of altering their growth rates depending upon the availability of hydrogen and CO_2 (Morgan et al., 1997).

The objective of the present study was to evaluate the effects of dilution rates and dietary forage to concentrate ratios on fermentation kinetics in continuous cultures. Direct measurements of CH_4 concentration in fermentor headspace were compared with estimates of CH_4 production based on stoichiometric equations.

MATERIALS AND METHODS

Incubation Conditions

A mature lactating Holstein cow fitted with a rumen cannula was fed a diet consisting of 63% roughage and 37% concentrate. Grab samples of ruminal contents were taken from

various sites within the reticulo-rumen, filtered through double-layered cheesecloth, and transported to the laboratory in sealed, pre-heated containers. In the laboratory, contents from the containers were filtered again through double-layered cheesecloth into a large wide mouth beaker and mixed thoroughly before pouring into the fermentors. The preparation time of the ruminal contents in the laboratory did not exceed 15 min. Approximately 700 ml of the strained ruminal fluid were transferred into fermentors with a continuous overflow system (Teather and Sauer, 1988). Several hours prior to the addition of the ruminal fluid, the system was purged with CO_2 gas. In order to displace O_2 and maintain anaerobic condition in the vessels, the rate of CO_2 flow through the fermentors was fixed at 20 ml/min throughout the experiments. A circulating water bath was used to maintain the temperature of the fermentors at 39°C. Continuous stirring of fermentor contents was achieved with the aid of a central paddle set at a speed of 10 rpm. Artificial saliva was prepared as described by Slyter et al. (1966) and delivered continuously at 0.73 ml/min. Liquid turnover rate was increased or decreased by adjusting the saliva flow rate as described below.

Dietary Treatments and Dilution Rates

Experimental diets consisted of three forage to concentrate ratios: high forage (H) 70:30, medium forage (M) 50:50, and low forage (L) 30:70. The forage comprised 100% pelleted alfalfa (16.5% CP) and the concentrate (13.1 % CP) consisted of 81.4% ground corn, 10.4% soybean meal (48% CP), 2.1% bentonite, 1.5% sodium bicarbonate, 1.3% phosphate, 1.1% limestone, 1.0% soybean oil, 1.0% salt, and 0.2% vitamin-mineral premix. The chemical composition of dietary treatments is as follows and was estimated based on NRC (1989) values (H: 31.8% NDF, 15.5% CP, 67.8% TDN, 3.0 DE Mcal/kg; M: 25.2% NDF, 14.8% CP, 72.8% TDN, 3.2 DE Mcal/kg; L: 18.6% NDF, 14.1% CP, 77.7% TDN, 3.4 DE Mcal/kg).

A total of 15 g of the diet was added daily in two equal amounts to each of three fermentors. All fermentors were offered the high forage diet (H) for the first 2 d (stabilization period). At the end of d 2, one fermentor was maintained on the H diet and the other two received the M diet for an additional 2 d. On d 5, one of the two fermentors receiving the M diet was switched to the L diet and was allowed to stabilize for an additional 2 d. By the end of d 6, all three fermentors had been stabilized for at least 2 d on the respective dietary treatments. Data were collected from d 7 through d 13 (treatment period).

The effect of the three diets was tested at three different dilution rates of 0.032, 0.063, and 0.125 per h, approximating 0.8, 1.5, and 3.0 volume turnovers per d. These dilution rates were chosen to cover the physiological range of fluid turnover rates that are typically observed in vivo. Throughout all experiments, fermentors were allowed to stabilize for 2 d at a saliva flow rate of 0.73 ml/min. Following stabilization, saliva flow rate was either maintained at 0.73 ml/min resulting in a fractional dilution rate of 6.3 %/h or it was adjusted to obtain fractional dilution rate of 3.2 %/h or 12.5 %/h (0.37 ml/min or 1.46 ml/min, respectively).

Sampling and Analyses

Five ml of thoroughly mixed fermentor contents were collected twice daily for 7 d and analyzed for VFA by gas chromatography (model CP-3380; Varian, Walnut Creek, CA) and for ammonia-N (NH_3-N) using a colorimetric assay (Beecher and Whitten, 1970). Ten µl of headspace gas samples from the fermentor were drawn into a gas tight syringe (Hamilton Co., Reno, NV) and analyzed for CH_4 using gas chromatography (model CP-3800; Varian, Walnut Creek, CA). The pH of the ruminal cultures was monitored continuously and recorded when samples for CH_4 were taken.

Assuming that carbohydrates (hexoses) are the major source of VFA in the rumen, partitioning of substrate use was expressed as the amount of substrate fermented to VFA, gas (CH_4 + CO_2 direct and indirect), or microbial biomass. The direct source of CO_2 is the fermentation of glucose by various pathways yielding VFA, ATP, and CO_2. Because the in vitro methods use bicarbonate-based buffering solutions, CO_2 is released into the gas phase as VFA enter the medium (Beuvink and Spoelstra, 1992). This source of CO_2 is considered as indirect CO_2 production. The production of microbial biomass was estimated from the total ATP production (2, 3, and 3 mol/mol acetic, propionic, and butyric acids, respectively; Groot et al.,1998) and the yield of microbial biomass per mol ATP (Y_{ATP}, assumed to be 7.5, 11.6, and 16.7 mg/mmol ATP for 3.2, 6.3, and 12.5 %/h, respectively regardless of forage to concentrate ratio; Hespell and Bryant, 1979). Also assumed was that 80% of bacterial components were synthesized from glucose skeletons (Groot et al., 1998). Consequently, microbial biomass from glucose consumption was calculated as:

Microbial biomass (g/d) = (0.8 Y_{ATP} (2 Acetate, mmol/d + 3 Propionate, mmol/d + 3 Butyrate, mmol/d + CH_4, mmol/d))/1,000.

Energy contents of acetate, propionate, butyrate, valerate, isobutyrate, and isovalerate were used to estimate digestible energy (DE). Similarly energy content of CH_4 was used to estimate contribution of energy in CH_4 to total DE. In addition to direct measurement of CH_4, the production of fermentation gases, CO_2 and CH_4, and the associated production of H_2O, was calculated using the equation outlined by Wolin (1960) and Blümmel et al. (1997).

Experimental Design and Statistical Analyses

Within a run an experimental period lasted 13 d which included 6 d for adaptation followed by 7 d for data collection. Daily values were averaged across the 7 d within each

run. A single run comprised of three fermentors that were inoculated simultaneously with ruminal contents obtained from the same cow. Each fermentor was randomly assigned to one of three diets with different forage to concentrate ratios. Each run was replicated (n = 2) at each of the three dilution rates.

Data were analyzed using the general linear model procedure of SAS (1999). Split-plot design was used with dilution rate as whole plot and forage to concentrate ratio as subplot. The model used is described by:

$$Y_{ijk} = \mu + DR_i + Run_j (DR_i) + FC_k + DR_i \times FC_k + e_{ijk}$$

where

Y_{ijk} = individual response variable measured,

μ = overall mean,

DR_i = fixed effect of dilution rate (i = 1 to 3),

$Run_j (DR_i)$ = whole plot error,

FC_k = fixed effect of forage to concentrate ratio (k = 1 to 3),

$DR_i \times FC_k$ = fixed effect of interaction between dilution rate and forage to concentrate ratio, and

e_{ijk} = subplot error.

Dilution rates were compared with the whole plot error term. Forage to concentrate ratios and interaction between dilution rate and forage to concentrate ratio were tested using the subplot error term. Standard errors appropriate for comparisons among different means were calculated as follows (Steel et al., 1997):

for comparing dilution rate: $SE(\overline{y}_{1\cdot\cdot} - \overline{y}_{2\cdot\cdot}) = \sqrt{\frac{2\,MSE_{whole\ plot}}{6}}$,

for comparing forage to concentrate ratio: SE $(\overline{y}_{\cdot\cdot 1} - \overline{y}_{\cdot\cdot 2}) = \sqrt{\frac{2\,MSE_{subplot}}{6}}$.

Comparison of dilution rate and forage to concentrate ratio means was done by contrast test with Fisher's protected LSD test when the effect of dilution rate or forage to concentrate ratio ($P \leq 0.10$) was detected by the model. The level of significance accepted was $P \leq 0.05$ and 0.10 for trend.

RESULTS

Increasing the DR reduced ($P < 0.01$) the concentration of total VFA (Table 1). Molar proportion of acetate ranged from 49 to 56 and was not affected by DR. Propionate remained unchanged ($P > 0.10$) when DR increased from 3.2 %/h to 6.3 %/h, but tended to decrease ($P < 0.07$) at 12.5 %/h. The acetate to propionate (A:P) ratio increased ($P < 0.01$) with increasing DR from 3.2 or 6.3 %/h to 12.5 %/h. Concentration of valerate decreased ($P < 0.01$) with increasing DR. Higher concentrate in the diet decreased ($P < 0.01$) the molar proportion of acetate with the molar proportions of propionate being lowest for the high F:C ratio and increased ($P < 0.03$) for the medium and low F:C ratio. Molar proportions of valerate were similar for the high and medium F:C diets but increased for the low F:C diet. The A:P ratio was highest for the high F:C ratio and decreased ($P < 0.01$) for the medium and low F:C ratio.

There were DR × F:C interactions for molar percentages of butyrate and isovalerate. At 3.2 %/h butyrate proportion was highest in the low forage diet and similar between the medium and high forage diets. When DR was increased to 6.3 %/h there was no difference in butyrate concentration between F:C ratios. At DR of 12.5 %/h molar proportions of butyrate

were highest for the low F:C diet and lowest for the medium F:C diet with the high F:C being intermediate. Molar proportions of isovalerate were higher in the high forage diet when compared to either the medium or low forage diets at a DR of 3.2 %/h. At DR of 6.3 %/h isovalerate was numerically higher for both the medium and low forage diets. Increasing the DR to 12.5 %/h increased isovalerate in ruminal cultures receiving medium forage diets and was higher compared to both the high and low forage diets.

Daily production of total VFA (mmol/d) was not affected by DR or F:C ratio (Table 2). The rate of production of acetate and propionate did not change as a result of changing the DR. Increasing DR decreased ($P < 0.01$) the production of valerate which was consistent with lower concentrations in ruminal cultures. There were interactions for butyrate, isobutyrate, and isovalerate. Daily output of butyrate followed the same trend as the molar proportions of butyrate in the cultures. The low F:C diets resulted in the highest production of butyrate at DR of 3.2 and 12.5 %/h. The medium and high F:C diets were similar at 3.2 %/h and 6.3 %/h but increasing DR to 12.5 %/h increased butyrate production in cultures receiving the high F:C compared with the medium F:C diet. Daily output of isobutyrate was very low across all F:C ratios at DR of 3.2 %/h. Increasing DR to 6.3 %/h increased isobutyrate production for all F:C treatments; the increase was greater for cultures receiving the medium F:C diet. At DR of 12.5 %/h, rate of isobutyrate production increased even further for the low F:C diet and decreased for both the medium and high forage diets. The effect of DR and F:C ratio on the rate of isovalerate production was similar to changes observed in molar proportions.

Ruminal culture pH tended ($P < 0.08$) to increase with increasing DR (Table 3). Concentration of NH_3-N was highest at 6.3 %/h and lowest at 3.2 %/h with 12.5 %/h being

intermediate. There were no effects of F:C on pH and concentration of NH_3-N. There was a tendency for DR × F:C interaction for the daily output (g/d) of NH_3-N ($P < 0.07$). Increasing DR resulted in an increase in the rate of production of NH_3-N. At 3.2 %/h and 6.3 %/h F:C ratio did not seem to affect ammonia production but increasing DR to 12.5 %/h increased ammonia output for the low forage diets compared with the medium and high forage diets.

There was no effect of either DR or F:C ratio on methane production (mmol/d) calculated from stoichiometric equations. Based on actual measurements however, there was an interaction for methane output. At each DR, methane output was highest for the high forage diet (Table 3 and Figure 1). At DR of 3.2 %/h methane output was similar for the medium and low forage diets but at 6.3 %/h cultures receiving the medium F:C diet had higher amounts of daily methane production compared with those receiving the low F:C diet. Increasing the DR to 12.5 %/h did not increase methane output for the medium F:C diet, but there was a substantial increase for the low F:C diets which was numerically higher compared to the medium F:C diets. When expressed as mmol of methane produced per g of DM fed the effects of DR and F:C were similar to the total daily production rates described above.

The main effect of increasing DR or F:C ratio on methane production calculated from stoichiometric equations and methane production measured directly from the headspace in the fermentors was a linear increase (Figures 2 and 3). This linear response was less evident for methane values based on stoichiometric calculations. At the low DR (3.2 %/h) methane output based on actual measurements was almost half of that based on stoichiometric estimation. At the higher DR (6.3 and 12.5 %/h), however, estimated methane values were

lower. Stoichiometrically estimated values tended to underestimate or overestimate methane output for the high and the low forge diets, respectively.

Based on the percentage of substrate used for VFA production from DM fed, apparent digestibility ranged from 27.9% to 31.5% and was not affected by DR or F:C ratio (Table 4). However, the amounts of substrate used for gas (CH_4 + direct and indirect CO_2) and microbial biomass were affected by DR, resulting in an increase in the total amount of substrate fermented when DR increased from 3.2 to 6.3 %/h ($P < 0.03$). Overall diet fermentability was very similar across DR and F:C ratios averaging 60.0%. It was reduced only when dilution rate was 3.2 %/h.

Increasing DR increased microbial yield and microbial growth efficiency ($P < 0.01$). Increasing the proportion of concentrate in the diet did not affect microbial yield ($P > 0.10$) but improved ($P < 0.02$) microbial growth efficiency (Table 4). At DR of 6.3 %/h microbial growth efficiency in cultures receiving low F:C diet was numerically higher than cultures receiving medium F:C diet, but increasing DR to 12.5 %/h resulted in numerically higher efficiency of microbial growth in fermentors receiving medium F:C diet compared to those receiving low F:C diet.

The amount of energy produced daily in the form of VFA and as a percentage of DE fed was not affected by DR (Table 5). A higher proportion of concentrate tended to increase ($P < 0.08$) the amount of energy (kcal/d) captured in VFA due primarily to the increased DE fed. The VFA energy as a % of DE fed was not affected by F:C ratio. There was an interaction for the amount of energy released in CH_4 ($P < 0.01$). At DR of 3.2 %/h and 6.3 %/h, cultures receiving the low forage diet had the lowest rate of methane production; however, increasing DR to 12.5 %/h resulted in the medium F:C diet having the lowest methane output.

DISCUSSION

Altering liquid turnover rates had no effect on daily production of total VFA or in the proportion and production of acetate by ruminal cultures. These results are similar to those reported by Isaacson et al. (1975) and Hoover et al. (1984). Molar proportion of propionate tended to decrease and that of butyrate increased with an increase in dilution rate, but production of both fatty acids remained unchanged. Ruminal pH and ruminal methane output increased with increasing dilution rate. The effect of dilution rate on VFA production and fermentation profile in other studies has been variable. Total organic acid production decreased (Carro et al., 1995) or increased (Fuchigami et al. 1989) when fractional dilution rates increased. Molar percentages of propionate in ruminal fluid were reported to be higher (Isaacson et al., 1975; Hoover et al., 1984) or lower (Thomson et al., 1978; Crawford et al., 1980) as a result of increasing dilution rates.

In the present study, dilution rate had a significant effect on the isoacids. Rapid liquid turnover lowered the proportion of ruminal valerate but increased that of the branched chain isoacids, isobutyrate and isovalerate. Part of the valerate produced in the rumen comes from the fermentation of carbohydrates and part comes from the fermentation of amino acids. The branched chain isoacids arise almost exclusively from the oxidative deamination of amino acids. The inability of the cellulolytic bacteria to transport preformed branched chain amino acids across their cell wall makes the branched chain isoacids essential for normal growth of fiber digesting bacteria (Bryant, 1973). The decrease in valerate may be due to reduced retention time of the fermentable carbohydrates at higher liquid passage rates. An increase in branched chain isoacids suggests enhanced deaminative activity at higher dilution rates since a reduced uptake should have resulted in a lower acetate production.

Increasing liquid turnover increased NH_3-N output. Ammonia-N concentration, similar to the isoacids, is a function of the rate of release and rate of uptake by microbial populations. Ruminal pH can also influence NH_3-N production (Erfle et al., 1982) and increasing dilution rates increased NH_3-N with a concomitant increase in culture pH similar to results reported earlier (Hoover et al., 1984). Ammonia-N and branched chain isoacids increased at higher dilution rates suggesting either enhanced rate of production or reduced utilization. Both are used predominantly by cellulolytic organisms and since acetate production was not affected, higher liquid dilution rates seem to have enhanced rates of production rather than reduced rates of utilization.

Similar values for DM digestibility have been reported earlier with no effect of dilution rate (Hoover et al., 1984; Carro et al., 1995). In the study by Hoover et al. (1984) increasing dilution rates did not seem to reduce NDF digestibility or the digestibility of the more rapidly fermentable carbohydrate fraction. In fact, in some cases, increasing dilution rate seemed to increase cellulose and NDF digestibility (Hoover et al., 1984).

Increasing dilution rates did not affect the amount of substrate used for VFA production but increased the amount used for gas and microbial biomass production. Actual methane production increased significantly at each successive increase in dilution rate, but the proportion of substrate used for total gas output (CH_4 + CO_2) increased only when dilution rates were increased from 3.2 %/h to 12.5 %/h. The growth rate of methanogens is relatively slow which results in reduced numbers during rapid rates of transit from the rumen (Wolin et al., 1997). The highest dilution rate in our study (12.5 %/h) did not seem to have a negative impact on methane production. Microbial yields and efficiencies increased with an increase in turnover rate. With faster dilution rates microbial growth is faster and a greater proportion

of substrate energy is used for bacterial synthesis (Hespell and Bryant, 1979). Increasing dilution rate did not affect total organic acid production but actual gas output (in particular, methane output) increased suggesting increased microbial activity. Higher dilution rates are associated with higher efficiencies and increased microbial biomass due primarily to reduced maintenance requirements and enhanced utilization of substrate energy for growth related processes.

Altering the forage:concentrate ratio affects the nature of fermentation in the rumen. Higher proportion of concentrate typically increases ruminal propionate and lowers methane production as was observed in this study. Total VFA production and culture pH did not change with F:C ratio. A higher energy content of VFA production due to reduced methane production in the high concentrate diets resulted in increased microbial efficiencies.

According to Wolin (1960) the amount of total gas produced ($CH_4 + CO_2$) can be determined from the amount and molar proportion of acetate, propionate and butyrate. Hence, based on the stoichiometric equation variations in the molar proportion of acetate, propionate and butyrate will have a direct influence on gas volumes. Blümmel et al. (1997) reported that the total substrate required for the production of equal amounts of gas from widely different VFA patterns was very similar. If, however, one accounts for the efficiency of ATP use by microbial populations which is not a constant but can vary widely the amount of substrate required for microbial biomass can vary substantially even at similar amounts of VFA proportion (Blümmel et al., 1997). Hence, bacterial cell yields may have a greater influence on gas estimation.

Total gas yield can vary considerably due to the fact that some of the substrate fermented is used to produce microbial mass. The Cornell Net Carbohydrate and Protein System

(CNCPS) suggests a maximum incorporation of 40% of the fermented feed carbohydrate into microbial biomass (Russell et al., 1992). With substrates consisting predominantly of structural carbohydrates, microbial biomass yield was negatively correlated to gas production over a 24-h period of incubation (Blümmel et al., 1997). In another study (Krishnamoorthy et al., 1991), a curvilinear relationship was reported over a 2-h period between microbial protein synthesis and gas production. The curvature varied with the type of substrate with cellulose producing a steep upward curve and starch producing a less steep curve. The relationship between microbial mass and gas volume is complex and can vary with the type of substrate as well as with the time of sampling.

In all previous experiments no distinction was made between CO_2 and CH_4 and it is assumed that the latter arises directly from the former. As per the stoichiometric equations outlined previously (Wolin, 1960; Blümmel et al., 1997) we included both the direct CO_2 production from the fermentative route as well as the indirect contribution from the reaction of the VFA with the bicarbonate supplied in the saliva in estimating total gas production. Total gas production was not affected by the F:C ratio and dilution rates in the present study. Methane production, when estimated using stoichiometric equations, was also not altered by either the dilution rates or F:C ratio. However, actual measurements of methane production decreased significantly with an increase in the level of concentrate and increased with an increase in dilution rate (Figure 1). The decrease in methane in cultures receiving a higher proportion of concentrate is consistent with the shift in reducing equivalents toward propionate formation. However, the increased methane formation at higher dilution rates was unexpected.

Gas production and VFA formation are closely related processes, but gas production can vary without any change in total VFA production (Beuvink and Spoelstra, 1992). The amount of gas released indirectly is assumed to be a constant; therefore, the variation is attributed primarily to gas produced directly as an end product of ruminal fermentation. Direct gas production varies with the pattern of VFA and given the complex nature of mixed feedstuffs and fermentative pathways of microbial metabolism it can vary considerably. Most species of rumen microbes are capable of fermenting various substrates resulting in similar end products (Hungate, 1966) and some have been shown to switch end products depending upon their growth rate (Russell and Wallace, 1997).

Hydrogen and CO_2 are the major precursors of CH_4 formation in the rumen (Hungate, 1967) and most methanogens can utilize these substrates to generate ATP (Thauer et al., 1977). The distribution of methanogenic species in the rumen is not known and whilst some species can occur in high concentration others may be present in low concentration (Wolin et al., 1997). Methanogens have a slower growth rate compared with other rumen bacteria, but there are some species, *Methanobrevibacter* in particular, that will grow more rapidly with H_2 than other methanogens (Wolin et al., 1997). The success of microorganisms to survive depends upon their ability to maximize growth rate or growth yield (Russell and Wallace, 1997; Neijssel and de Mattos, 1994). Culture of *M. thermoautotrophicum* did not grow when H_2 supply was low, but they continued to produce methane. At higher availability of H_2, growth of *M. thermoautotrophicum* occurred but methanogenesis remained constant as long as growth was H_2 limiting (Morgan et al., 1997). When H_2 supply was not growth limiting, CH_4 production increased rapidly (Morgan et al., 1997). Changing dilution rates has a profound impact on the growth rate of bacteria and alters the metabolic pathways of

fermentation. Given the complex interactions between microbial growth and the fermentation environment by altering dilution rates could interfere with growth by changing the concentration and or shifting the metabolic pathways by which specific microbes utilize substrates.

CONCLUSIONS

Methane production and microbial fermentation pattern in continuous cultures were measured to determine if they were altered by dilution rate and forage to concentrate ratio. Increasing levels of concentrate increased propionate and decreased methane production. Increasing dilution rate decreased molar proportion of propionate and increased methane output. Methane production estimated from stoichiometric equations remained unchanged, irrespective of the dilution rate or level of concentrate in the diet. Actual measurements of methane output were higher compared with stoichiometric estimations at higher dilution rates and with high forage diets. Our results indicate that the composition of the diet and rate of passage of culture contents can interfere with normal metabolic events altering the complex interaction between microbial growth and substrate utilization.

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Table 1. Effect of dilution rate (DR) and forage to concentrate ratio (F:C) on the concentration and molar proportion of VFA in continuous cultures (n = 2)[1].

	DR, %/h									SE	Significance of effect		
	3.2			6.3			12.5						
VFA	H	M	L	H	M	L	H	M	L		DR	F:C	DR × F:C[2]
Total, m*M*	97.7	100.4	104.0	56.9	63.2	59.5	27.3	25.7	29.6	2.69	0.01	NS[3]	NS
Individual, m*M*													
Acetate	51.9	52.7	51.7	31.3	30.4	26.5	16.5	14.3	15.0	1.63	0.01	NS	NS
Propionate	28.1	30.1	30.9	14.9	19.9	21.2	5.1	6.3	6.6	1.65	0.02	0.08	NS
Butyrate	11.7	11.5	14.5	6.9	8.0	7.0	4.2	3.0	6.1	0.52	0.02	0.01	0.03
Valerate	3.8	5.0	5.7	1.7	2.0	2.2	0.5	0.4	0.7	0.21	0.01	0.01	0.04
Isobutyrate	0.3	0.3	0.3	0.5	0.6	0.5	0.2	0.1	0.3	0.05	0.09	NS	0.09
Isovalerate	2.0	0.9	0.9	1.8	2.5	2.3	0.9	1.7	1.1	0.16	0.01	NS	0.01
Individual, mol/100 mol													
Acetate (A)	53.0	52.7	50.0	54.9	48.6	44.6	60.6	54.4	49.9	2.07	NS	0.01	NS
Propionate (P)	28.9	29.8	29.4	26.1	31.2	35.5	18.7	25.2	22.4	1.64	0.07	0.03	NS
Butyrate	12.0	11.5	14.1	12.1	12.5	11.8	15.5	11.9	20.8	0.38	0.02	0.01	0.01
Valerate	3.9	5.0	5.5	2.9	3.1	3.7	1.8	1.7	2.4	0.26	0.01	0.01	NS
Isobutyrate	0.3	0.3	0.3	0.9	0.9	0.8	0.7	0.4	1.0	0.13	0.01	NS	NS
Isovalerate	2.0	0.9	0.9	3.2	3.9	3.8	3.0	6.5	3.7	0.31	0.01	0.01	0.01
A:P	1.9	1.8	1.8	2.2	1.6	1.3	3.3	2.3	2.3	0.20	0.01	0.01	NS

[1]H = High forage (70% forage:30% concentrate); M = Medium forage (50% forage:50% concentrate); L = Low forage (30% forage:70% concentrate).
[2]DR × F:C = Interaction between dilution rate and forage to concentrate ratio.
[3]NS = Not significant ($P > 0.01$).

Table 2. Effect of dilution rate (DR) and forage to concentrate ratio (F:C) on VFA production in continuous cultures (n = 2)[1].

	DR, %/h									SE	Significance of effect		
	3.2			6.3			12.5						
VFA[2]	H	M	L	H	M	L	H	M	L		DR	F:C	DR × F:C[3]
Total, mmol/d	51.8	53.2	55.1	59.8	66.3	62.5	57.3	54.0	62.3	3.19	NS[4]	NS	NS
Individual, mmol/d													
Acetate	27.5	27.9	27.4	32.9	31.9	27.8	34.7	30.0	31.5	2.11	NS	NS	NS
Propionate	14.9	16.0	16.4	15.6	20.9	22.2	10.7	13.3	13.8	1.21	NS	0.02	NS
Butyrate	6.2	6.1	7.7	7.2	8.4	7.4	8.8	6.3	12.8	0.69	NS	0.01	0.02
Valerate	2.0	2.7	3.0	1.7	2.1	2.3	1.0	0.9	1.5	0.21	0.01	0.02	NS
Isobutyrate	0.2	0.2	0.2	0.5	0.6	0.5	0.4	0.3	0.6	0.05	NS	NS	0.03
Isovalerate	1.1	0.5	0.5	1.9	2.6	2.4	1.7	3.4	2.3	0.13	0.01	0.01	0.01

[1]H = High forage (70% forage:30% concentrate); M = Medium forage (50% forage:50% concentrate); L = Low forage (30% forage:70% concentrate).
[2]Production parameters are based on 1 L of ruminal cultures.
[3]DR × F:C = Interaction between dilution rate and forage to concentrate ratio.
[4]NS = Not significant ($P > 0.01$).

Table 3. Effect of dilution rate (DR) and forage to concentrate ratio (F:C) on ruminal pH, ammonia-N (NH_3-N), and methane (CH_4) production in continuous cultures (n = 2)[1].

	DR, %/h										Significance of effect		
	3.2			6.3			12.5			SE	DR	F:C	DR × F:C[3]
Item[2]	H	M	L	H	M	L	H	M	L				
pH	5.3	5.0	5.0	5.7	5.5	5.6	6.7	6.3	6.3	0.20	0.08	NS[4]	NS
NH_3-N, mg/100 ml	15.6	14.7	12.1	27.3	31.1	25.5	18.2	19.5	21.9	1.54	0.01	NS	NS
NH_3-N[5], g/d	0.09	0.08	0.06	0.29	0.33	0.27	0.38	0.41	0.46	0.016	0.01	NS	0.07
Gas estimated[6], mmol/d	88.5	89.4	104.2	102.8	109.7	99.9	106.5	92.1	115.2	7.21	NS	NS	NS
CH_4 estimated[7], mmol/d	13.1	13.1	13.4	16.1	14.9	12.1	19.1	14.8	18.7	1.39	NS	NS	NS
CH_4 measured													
mmol/d	9.6	6.1	4.2	25.5	19.6	11.2	29.1	20.1	22.0	1.15	0.01	0.01	0.02
mmol/g DM fed	0.8	0.5	0.3	2.0	1.5	0.9	2.3	1.6	1.7	0.10	0.01	0.01	0.04

[1]H = High forage (70% forage:30% concentrate); M = Medium forage (50% forage:50% concentrate); L = Low forage (30% forage:70% concentrate).
[2]Production parameters are based on 1 L of ruminal cultures.
[3]DR × F:C = Interaction between dilution rate and forage to concentrate ratio.
[4]NS = Not significant ($P > 0.01$).
[5]NH_3-N (g/d) = (NH_3-N concentration, mg/100 ml × fermentor volume (700 ml) × turnover rate of fermentor)/1,000. Turnover rate of fermentor is 0.8, 1.5, and 3.0 for 3.2 %/h, 6.3 %/h, and 12.5%/h, respectively.
[6]Fermentative CO_2 + fermentative CH_4 + buffering CO_2. All gas productions were estimated.
[7](Acetate, mmol/d) + (2 × Butyrate, mmol/d) – (CO_2, mmol/d).

Table 4. Effect of dilution rate (DR) and forage to concentrate ratio (F:C) on the partitioning of substrate, fermentability, and microbial growth in continuous cultures (n = 2)[1].

	DR, %/h									SE	Significance of effect		
	3.2			6.3			12.5						
Item	H	M	L	H	M	L	H	M	L		DR	F:C	DR × F:C[2]
DM fed, g/d	12.8	12.9	13.0	12.8	12.9	13.0	12.8	12.9	13.0				
Substrate used, g/d													
For VFA[4]	3.3	3.4	4.2	3.8	4.2	4.0	3.7	3.3	4.0	0.29	NS[3]	NS	NS
For gas[5]	1.9	1.8	2.0	2.2	2.3	2.0	2.5	2.0	2.7	0.16	0.02	NS	NS
For microbial biomass[6]	1.0	1.0	1.2	1.7	1.9	1.8	2.5	2.2	2.7	0.13	0.01	NS	NS
Total[7]	6.1	6.2	7.5	7.7	8.4	7.7	8.6	7.6	9.4	0.56	0.03	NS	NS
Fermentability, %[8]	48.0	48.4	57.3	60.2	65.0	59.6	66.9	58.6	72.3	4.30	0.03	NS	NS
Microbial synthesis													
g/d[9]	0.79	0.81	0.98	1.39	1.54	1.45	1.96	1.78	2.16	0.104	0.01	NS	NS
g DM/kg DM fermented[10]	132.7	133.5	136.4	189.2	193.0	196.6	243.4	250.8	243.9	1.41	0.01	0.02	0.05

[1]H = High forage (70% forage:30% concentrate); M = Medium forage (50% forage:50% concentrate); L = Low forage (30% forage:70% concentrate).

[2]DR × F:C = Interaction between dilution rate and forage to concentrate ratio.

[3]NS = Not significant ($P > 0.01$).

[4](Acetate, mol/d × 60.05) + (Propionate, mol/d × 74.08) + (Butyrate, mol/d × 88.10).

[5]Substrate used for (CO_2, mol/d × 44) + (CH_4, mol/d × 16) + ($2H_2O$, mol/d × 36).

[6]Substrate used for microbial biomass = ATP (mmol) × Y_{ATP} (7.5 mg, 11.6 mg, and 16.7 mg for 3.2 %/h, 6.3 %/h, and 12.5%/h, respectively).

[7]Substrate used for VFA, CO_2 + CH_4 + $2H_2O$, and microbial biomass.

[8]Total substrate fermented expressed as a percentage of DM fed.

[9]Microbial biomass (g/d) = (((0.8 × Y_{ATP} ((2 × Acetate, mmol/d) + (3 × Propionate, mmol/d) + (3 × Butyrate, mmol/d) + (CH_4, mmol/d))))/1000). Y_{ATP} is 7.5 mg, 11.6 mg, and 16.7 mg for 3.2 %/h, 6.3 %/h, and 12.5%/h, respectively.

[10]Microbial growth efficiency (g DM/kg DM fermented) = ((Microbial biomass, g/DM fermented, g) × 1,000).

Table 5. Effect of dilution rate (DR) and forage to concentrate ratio (F:C) on the partitioning of digestible energy (DE) into VFA and methane (CH_4) obtained by actual measurement in continuous cultures (n = 2)[1].

	DR, %/h									SE	Significance of effect		
	3.2			6.3			12.5						
Item	H	M	L	H	M	L	H	M	L		DR	F:C	DR × F:C[2]
DE fed, kcal/d	38.1	41.2	44.4	38.1	41.2	44.4	38.1	41.2	44.4				
VFA													
kcal/d[4]	16.6	17.1	18.3	19.2	22.2	21.3	17.9	17.5	21.2	1.04	NS[3]	0.08	NS
% of DE	43.6	41.6	41.1	50.3	53.9	47.8	46.9	42.5	47.8	2.47	NS	NS	NS
CH_4													
kcal/d[5]	2.0	1.3	0.9	5.4	4.1	2.4	6.2	4.2	4.7	0.23	0.01	0.01	0.01
% of DE	5.3	3.2	2.0	14.1	10.0	5.3	16.1	10.3	10.5	0.56	0.01	0.01	0.01

[1]H = High forage (70% forage:30% concentrate); M = Medium forage (50% forage:50% concentrate); L = Low forage (30% forage:70% concentrate).

[2]DR × F:C = Interaction between dilution rate and forage to concentrate ratio.

[3]NS = Not significant ($P > 0.01$).

[4](Acetate, mol/d × 209.4 kcal/mol) + (Propionate, mol/d × 367.2 kcal/mol) + (Butyrate, mol/d × 524.3 kcal/mol) + (Valerate, mol/d × 681.6 kcal/mol) + (Isobutyrate, mol/d × 524.3 kcal/mol) + (Isovalerate, mol/d × 681.6 kcal/mol).

[5]CH_4 (kcal/d) = (CH_4, mol/d × 210.8 kcal/mol).

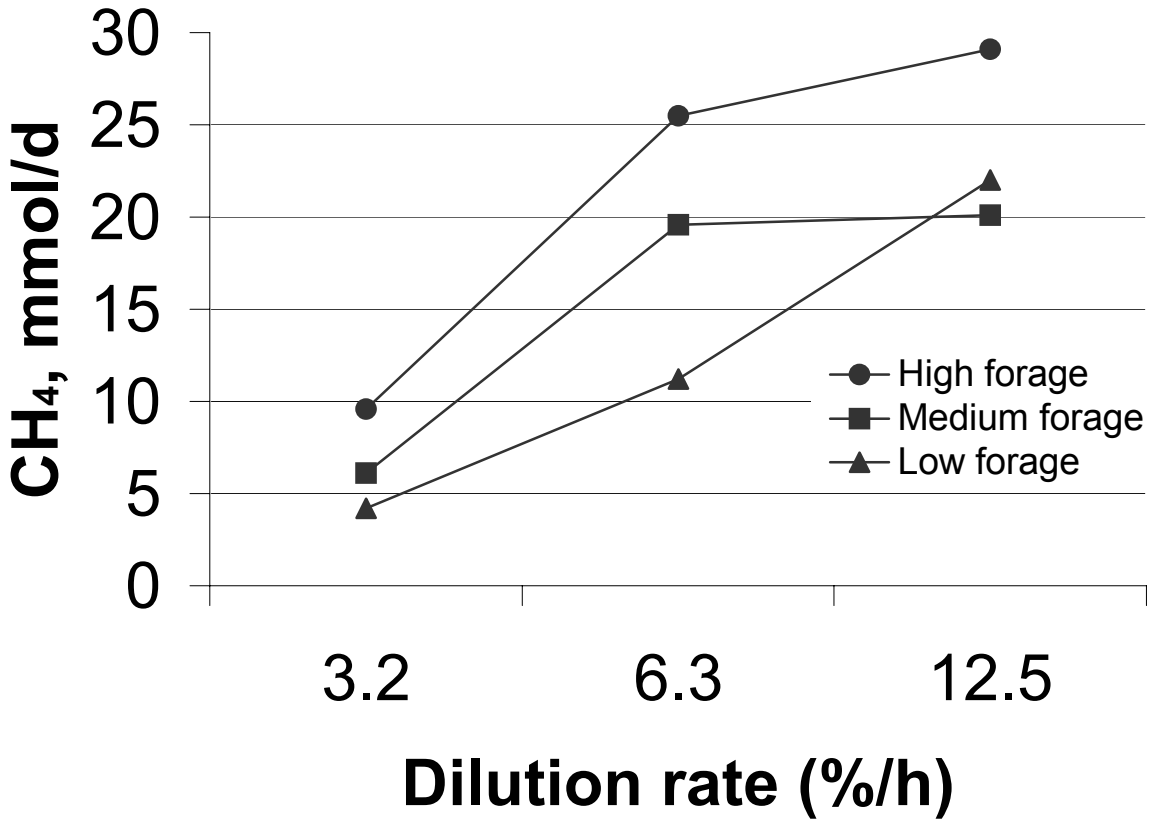

Figure 1. Interaction between dilution rate and forage to concentrate ratio on methane (CH_4) production calculated from measured concentrations in headspace gas in continuous cultures (High forage = 70% forage and 30% concentrate diet; Medium forage = 50% forage and 50% concentrate diet; Low forage = 30% forage and 70% concentrate diet).

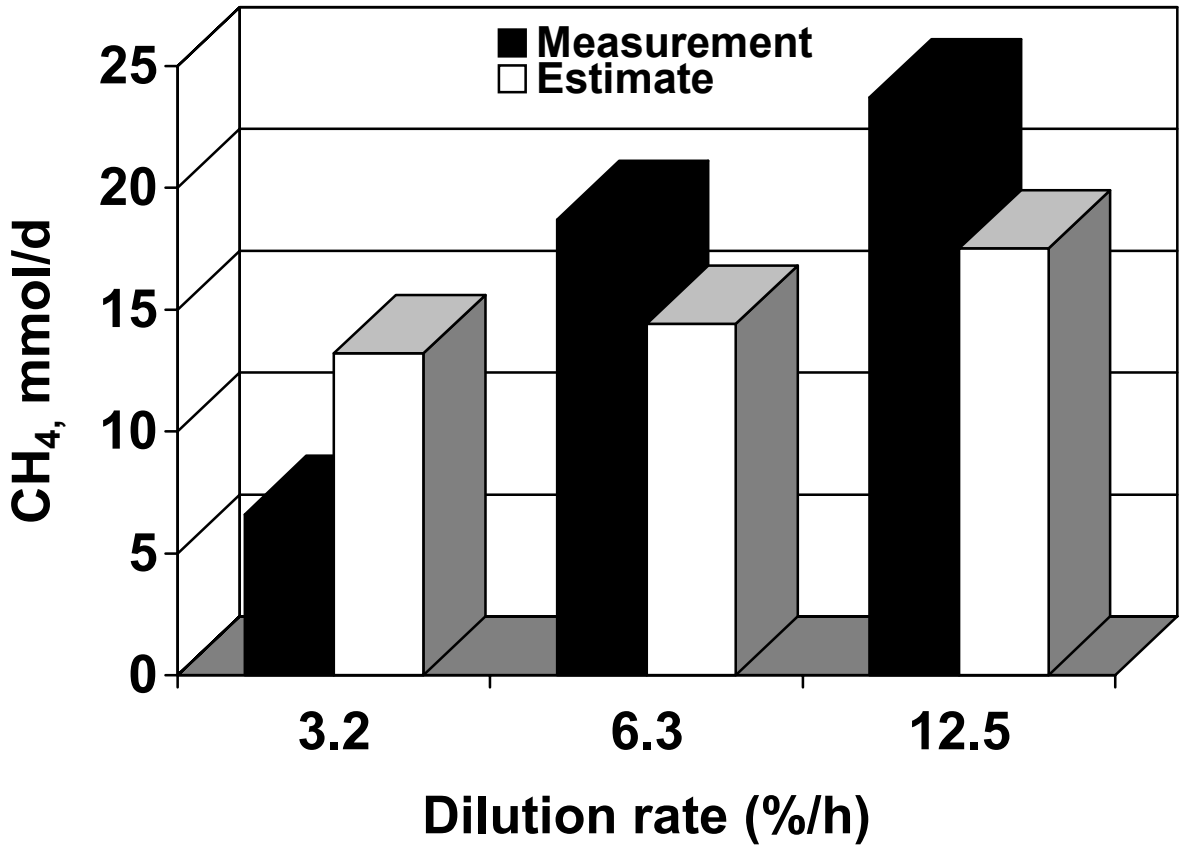

Figure 2. Difference in total methane (CH_4) production calculated either from measured concentrations in headspace gas or estimated from stoichiometric equations in response to dilution rate in continuous cultures.

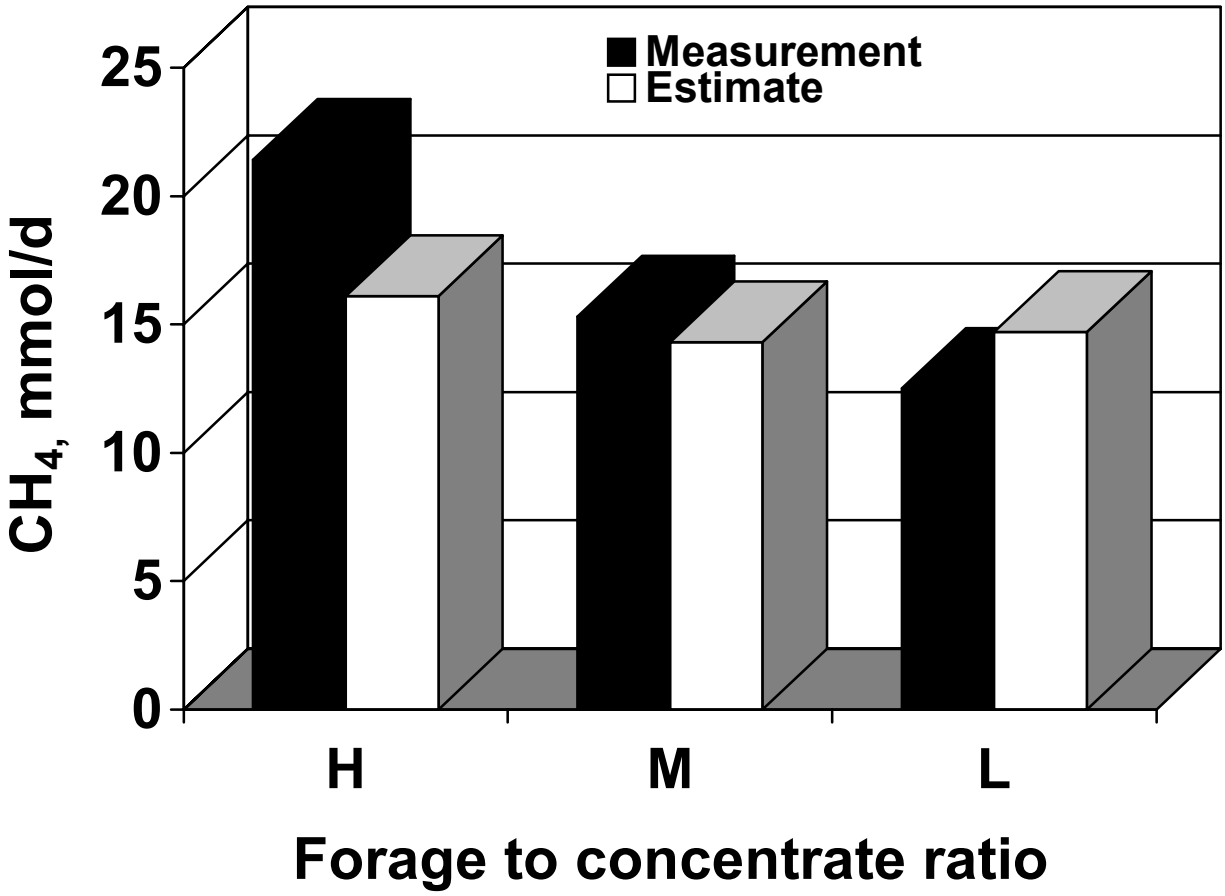

Figure 3. Difference in total methane (CH_4) production calculated either from measured concentrations in headspace gas or estimated from stoichiometric equations in response to forage to concentrate ratios in continuous cultures (H = 70% forage and 30% concentrate diet; M = 50% forage and 50% concentrate diet; L = 30% forage and 70% concentrate diet).

CHAPTER 4

Running head: **Gamagrass for lactating dairy cows**

Utilization of eastern gamagrass fed as hay or silage to lactating dairy cows[1]

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ABSTRACT

Nutritional value of eastern gamagrass [*Tripsacum dactyloides* (L.) L.] was determined as a single source of forage for dairy rations. Twenty lactating Holstein cows were assigned by days in milk and parity into one of 5 dietary treatments (4 cows per treatment) in a completely randomized design. The treatments consisted of 1) gamagrass hay (GH) and no corn (GHNC), 2) gamagrass silage (GS) and no corn (GSNC), 3) GS and low corn (GSLC), 4) GS and medium corn (GSMC), and 5) GS and high corn (GSHC). A protein supplement mix was offered to all cows to keep N intake similar across treatments (0.49 ± 0.03 kg/d). Diets containing GS were offered for 6 weeks and GHNC was offered for 3 weeks. Feeding gamagrass as hay or silage did not change milk yield (28.6 ± 1.3 kg/d). Compared to GSNC, feeding supplemental corn tended to increase milk yield at the medium or high level of corn inclusion ($P < 0.08$). Milk fat, protein, and lactose concentrations were similar across all treatments. There was a tendency for milk protein concentration to be higher with GSHC ($P < 0.07$). Yields of milk protein, lactose, and SNF tended to be higher with GSNC compared with GHNC ($P < 0.08$) and corn supplementation supported higher yields when compared to GSNC. Gamagrass fed as silage resulted in a higher feed conversion efficiency compared to gamagrass fed as hay ($P < 0.01$). Including corn with the silage resulted in a lower feed efficiency with GSHC being the lowest. Conversion of feed N to milk N was greater ($P < 0.01$) with gamagrass fed as silage (0.30) compared to hay (0.23). Supplementation of GS with corn at low or medium level failed to improve N efficiency. Milk urea N (MUN) concentration was significantly higher ($P < 0.01$) for cows fed GHNC (30.2 mg/100 ml) compared to all other treatments. Feeding GS significantly lowered MUN concentration, and corn supplementation at the medium (14.7 mg/100 ml) and high (13.3 mg/100 ml) levels

further reduced MUN concentration ($P < 0.05$). Milk lipid profiles were similar between GHNC and GSNC. Supplementing corn at the high level increased $C_{18:0}$ and $C_{18:2}$ concentrations ($P < 0.05$). Gamagrass silage supported similar milk yields compared with gamagrass hay. Increased energy from supplemental corn at the high level increased milk yield and tended to increase conversion of feed N into milk protein. Gamagrass fed as silage without or with corn improved the N status of the cows as indicated by lower MUN concentrations.

Key Words: Gamagrass Hay, Gamagrass Silage, Corn Supplementation, Dairy Cows

Introduction

In the Southeast region, several forages have been shown to be economically more feasible than traditional forages such as corn silage or alfalfa hay (Brejda et al., 1994). Among those, eastern gamagrass [*Tripsacum dactyloides* (L.) L.], a subtropical bunch-type and warm-season perennial native grass, is noted for its high production potential and quality (Coblentz et al., 1998). Horner et al. (1985) compared the nutritive value of eastern gamagrass and alfalfa hay for dairy cows and reported that although NDF and ADF concentrations were greater in eastern gamagrass, the digestibility of these fiber components was also higher (62 vs. 45% and 57 vs. 40% in NDF and ADF, respectively). However, the literature is void of comparative evaluation of the nutritional value of eastern gamagrass conserved as hay or silage fed to lactating dairy cows.

There is currently much interest in the importance of synchrony of releasing available energy and N in the rumen, it being assumed that a lack of synchrony leads to inefficient

microbial capture of the N and hence to a reduced efficiency of microbial protein synthesis (Chamberlain and Choung, 1995). The need for synchrony has been considered especially important with diets based on grass silage due to loss of fermentable carbohydrate during the ensiling process (Beever and Cottrill, 1994). However, synchronization of energy supply should consider physiological characteristics of N storage in forages, particularly gamagrass, because of the large proportion of its N associated with neutral detergent insoluble N (**NDIN**). The concentration of NDIN has ranged from 51.1 to 63.5% of total N across a wide range of plant maturities (Coblentz et al. 1998). The objectives of this study were to assess the feeding value of gamagrass as hay or silage when fed to lactating dairy cows and to determine the effect of supplemental corn in gamagrass silage-based diets.

Materials and Methods

Forage Harvest and Composition

A second year (excluding year of establishment) stand of 'Iuka' gamagrass, seeded in 15-cm rows into a prepared (deep disked) seed bed, was the source of forage. The area was top-dressed with lime, P, and K according to soil test prior to seeding and annually thereafter. In the year of the experiment (2001) the initial harvest was removed as hay and the field was then top-dressed with about 89 kg of N/ha applied as ammonium nitrate. The regrowth forage was cut on August 22 in the late vegetative stage (about 1.0 m tall with a few heads showing) using a conventional mower-conditioner. A field chopper immediately followed the mower-conditioner and the chopped (6 to 12.5 cm in length) forage was blown directly into a wagon. The contents of the wagon were dumped into a truck for transport to the ensiling site. The green forage (mean DM = 32.7 %) was dumped into a bagging unit which delivered the

forage into a horizontal plastic (8 mil) silo bag (sausage silo) that was partially filled with corn silage. The leading edge of the corn silage was squared off and eastern gamagrass added. When the last of the gamagrass was added, its leading edge was squared off and the remainder of the sausage silo filled with corn forage for ensiling. This provided a good seal for the experimental eastern gamagrass.

Eastern gamagrass for the hay treatment (cut the same time from the same area) was spread lightly over the stubble with a hay tedder to speed drying. The forage was tedded twice daily and was baled 3 d later at 87.3% DM using a conventional baler. The hay was stored in a metal barn until the time of the trial. Just prior to starting the trial the baled hay was passed through a hydraulic bale processor (Van Dale 5600, J. Starr Industries, Fort Atkinson, WI) with stationary knives spaced 10 cm apart. The processed hay was cut into predominantly 7 to 13 cm lengths in preparation for feeding, thereby avoiding leaf loss and excessive fine particles that occur when grinding. The processed hay was transported to the dairy center where it was placed under shelter in a holding bin ready for feeding.

Animals and Diets

The experiment was carried out at the Dairy Education Unit at North Carolina State University. Twenty lactating Holstein cows (mean ± SD) of 570 ± 71 kg of BW, parity 2.2 ± 1.0, 51 ± 17 days in milk (DIM), and 36 ± 6 kg of milk per day were used during early to midlactation. Prior to the start of the trial cows were grouped by parity and DIM into 1 of 5 groups. They were allowed at least 2 wk to adjust to Calan® feeding gates (American Calan, Inc., Northwood, NH) prior to being randomly assigned to one of five dietary treatments which were gradually introduced over 10 d. Before the trial was initiated with the experimental diets, milk yield of cows was recorded for 7 d and was used as a covariate in

statistical analysis. The five dietary treatments were: 1) **GHNC** = gamagrass hay (**GH**) + no corn, 2) **GSNC** = gamagrass silage (**GS**) + no corn, 3) **GSLC** = GS + low level of corn, 4) **GSMC** = GS + medium level of corn, and 5) **GSHC** = GS + high level of corn (Table 2). All diets contained a protein supplement composed of soybean meal (48% CP) and vitamin and mineral premix. We formulated the diets based on estimated intake of DM from cows fed corn silage (NRC, 1989). In addition, we considered DM intake of gamagrass from lactating cows as reported by Horner et al. (1985). These allowed us to vary dietary CP level to maintain similar N intakes by cows per day across the diets. Diets were prepared by weighing each ingredient and blending in a drum-type mixer (Data Ranger®; American Calan, Inc., Northwood, NH). The amount of diet offered was adjusted daily to achieve 5 to 10% feed refusal. The total experimental periods with dietary treatments lasted for 31 d and 52 d for cows fed GHNC and GS with or without ground corn, respectively; 10 d were for dietary adaptation followed by 21 d and 42 d of data collection. Quantity of gamagrass hay was enough to feed cows with GHNC for 3 wk of data collection. Body weights were measured in the beginning and at the end of the experiment.

Sample Collection and Analytical Procedures

Except for cows fed GHNC, all other cows were individually fed the TMR once daily for ad libitum intake with amounts fed and feed refusal recorded daily. Gamagrass hay was delivered to the cows twice daily. Protein supplement was provided on the top of GH in two equal portions and usually was consumed within 1 h. Samples of the total mixed ration (**TMR**), and feed refusal were obtained daily during the trial and frozen (-20°C) immediately. Weekly samples were composited by cow, dried at 65°C for 48 h, ground through a Wiley mill (1-mm screen), and stored for subsequent analyses. Protein supplement,

ground corn, GH, and GS were sampled weekly during the trial and processed as described above. Samples were analyzed for DM, ash, and Kjeldahl N using AOAC (1984, 1999) procedures. Neutral detergent fiber and ADF were sequentially determined using an Ankom 200 fiber extractor (Ankom Technologies, Fairport, NY) according to the method of Van Soest et al. (1991). Fat concentration in feed was measured by lipid extraction according to the method of Fellner et al. (1995). Non-protein N (**NPN**) concentration of feed samples was determined using a trichloroacetic acid precipitation (Licitra et al., 1996). Nonfibrous carbohydrate (**NFC**) was calculated as 100 – (CP + fat + NDF + ash) to provide one estimate of the NFC concentration (Mertens, 1988).

Cows were milked twice daily, and milk weights were recorded at each milking. Milk samples from individual cows were taken once per wk (a.m.and p.m.), preserved with 2-bromo-2-nitropropane-1,3 diol. The samples were shipped to the United DHIA Laboratory (Blacksburg, VA) where they were analyzed for fat, protein, lactose, solids-not-fat (**SNF**), and milk urea N (**MUN**) by infrared spectrometry (Multispec Mark I®; Foss Food Technology, Eden Plains, MN). A 5-ml raw milk sample proportional to a.m. and p.m. milk weight from each cow was stored at -70°C for later analysis of the milk fatty acid (**FA**) profile. Total lipids of 2 ml milk sample taken during the last week with each experimental diet were extracted by adding 10 ml of chloroform:methanol:ammonium hydroxide (12:12:1, vol/vol). The procedure of Kramer et al. (1997) was used for methylation of the milk lipids and analysis of fatty acid methyl esters using gas chromatography (model CP-3800; Varian, Walnut Creek, CA).

Calculations and Statistical Analysis

Yields of milk constituents were computed as the weighted means from p.m. and a.m. milk yields on each test day. Efficiency of conversion of feed DM into milk was computed by dividing milk yield by DMI. The efficiency of utilization of feed N to milk N was similarly calculated for each cow by dividing milk N output (total milk protein/6.38) by N intake. Fat-corrected milk (**FCM**; 3.5%) and energy content of milk (Tyrrell and Reid, 1965) were calculated as follows:

3.5% FCM (kg/d) = (16.22 × Milk fat, kg/d) + (0.43 × Milk yield, kg/d) and

NE_L (energy content of milk, Mcal/d) = ((Milk fat, % × 41.84) + (SNF, % × 22.29) – (25.58/1,000)) × (Milk yield, kg/d × 2.2).

Data for intakes, changes in BW, and lactation performance were analyzed as repeated measures by the GLM procedure of SAS (1999) with the following model:

$$Y_{ij} = \mu + T_i + \varepsilon_{ij},$$

where; Y_{ij} = dependent variable, μ = overall mean; T_i = effect of the ith treatment, and ε_{ij} = residual error associated with the ijth observation. Significant differences among means were determined using the PDIFF option of SAS (1999) when the *F*-test was significant. Body weight measured at the beginning of the trial was used as a covariate in the analysis of adjusted final BW. Since an interaction between initial BW and treatment was not significant, the interaction term was deleted in the model. Milk yield recorded for 7 consecutive days before introducing experimental diet was used as a covariate in the analysis of milk production. As an interaction between initial milk yield and treatment was not significant, the interaction term was removed from the model.

Significance was set at $P < 0.05$, and a tendency toward significance was set at $P < 0.10$.

Results

Composition of the Diet

Gamagrass hay and GS had similar CP, ADF, NDF, and fat concentrations (Table 1). As expected for C4 (warm season) grasses, the NDF concentration was high. Concentration of non-protein N (NPN) of GS (48.2% of total N) was higher than that of GH (32.7% of total N), which was attributed to the ensiling process. Intake of GH was expected to be lower compared to GS without or with supplemental corn. Consequently, we added more protein supplement to GH in order to achieve similar N intake compared to other diets. Corn supplementation linearly reduced CP concentration in the GS diets (Table 2). A lower inclusion of GS also lowered concentrations of NDF and ADF. Supplemental corn increased dietary fat and NFC concentration.

Feed Intake and Body Weight

Intake of DM was similar between cows fed GHNC and GSNC (Table 3) both of which contained similar levels of NDF (61.1% vs. 63.8%). Intake of DM was linearly increased ($P < 0.01$) with increasing corn supplementation to GS. Silage intake decreased ($P < 0.03$) with increasing corn supplementation due to a lower inclusion rate of GS. Due to the higher CP concentration and similar DMI, N intake was greater ($P < 0.01$) for cows fed GHNC compared to cows fed GSNC. Supplemental corn increased N intake due to the higher intake of DM with increasing corn supplementation.

Initial and final BW tended to be higher ($P < 0.09$) in cows fed GSNC compared to cows fed GHNC. However, final BW adjusted by covariate analysis to account for differences in initial BW were similar among cows fed GHNC or GSNC. The ratio between DMI and BW was numerically higher in cows fed GHNC compared to cows fed GSNC, but it was not

statistically significant ($P > 0.10$). In addition, there were no significant differences in initial and final BW of cows fed GS without or with supplemental corn. Cows fed GS with corn supplementation had a higher DMI to BW ratio compared to those fed GS without corn. Cows fed GSHC had a higher ($P < 0.05$) DMI to BW ratio than cows fed GSLC or GSMC.

Milk Production and Composition

There were no differences in milk yields between cows fed GSNC and GHNC (Table 4). Fat-corrected milk tended ($P < 0.09$) to be higher from cows fed GSNC compared with those fed GHNC due to numerical increases in milk fat yield. Concentrations of the milk components did not differ between GHNC and GSNC except for SNF which tended ($P < 0.09$) to be higher from cows fed GHNC. Yields of milk protein, lactose, and SNF tended to be higher ($P < 0.08$) from cows fed GSNC compared with those fed GHNC.

Cows fed GS with supplemental corn tended ($P < 0.08$) to produce more milk than cows fed GSNC (Table 4). However, milk yield was significantly increased only at the high level of corn inclusion. With the exception of milk fat, corn supplementation increased concentrations of all other milk components. The addition of corn to GS also increased yields of all milk components and energy content of milk; both were higher with GSHC and higher but similar between GSLC and GSMC.

Milk from cows fed GHNC or GSNC had similar concentrations of $C_{12:0}$ (Table 5). Cows receiving supplemental corn maintained similar concentrations of $C_{12:0}$, $C_{14:0}$, and $C_{16:0}$ in milk regardless of level of corn supplementation. Stearic acid concentration averaged 13.5 g/100 g of total FA across all diets and tended to increase ($P < 0.08$) with supplementing corn at the medium or high level. Cows fed GHNC had a higher concentration ($P < 0.06$) of *trans*-$C_{18:1}$ compared to cows fed GSNC. Linoleic acid concentration was lower ($P < 0.05$) in milk

from cows fed GSLC or GSMC. Concentration of $C_{22:0}$ was higher ($P < 0.01$) in milk from cows fed GSHC compared to those fed other diets. Cows fed supplemental corn tended ($P < 0.09$) to be higher in saturated fat (**S**) and lower in unsaturated fat (**U**) percentages. This resulted in lower S:U ratios ($P < 0.05$) in milk from cows fed GHNC or GSNC compared to cows fed other diets. Percentage of monounsaturated FA tended ($P < 0.09$) to be higher in milk from cows fed GH or GS alone. However, polyunsaturated FA tended ($P < 0.09$) to be higher in milk from cows fed only GHNC.

Efficiencies of Utilization of Feed and Nitrogen

Greater feed efficiency, expressed as milk/DMI ($P < 0.01$), occurred for cows fed GSNC compared to those fed GHNC and was due to higher milk yields with similar DM intake (Table 6). Supplementing GS with corn decreased feed efficiency ($P < 0.05$). Feed efficiency, when expressed as FCM/DMI, tended ($P < 0.08$) to increase for cows fed GSNC compared to cows fed GHNC, and it tended ($P < 0.06$) to increase for cows fed GSNC and GSLC compared to cows fed GSHC. Feed N was more efficiently converted ($P < 0.01$) to milk N in cows fed GSNC compared to those fed GHNC. Corn supplementation to GS improved N efficiency ($P < 0.04$) only at the high level. Milk urea N concentration was significantly higher ($P < 0.01$) from cows fed GHNC than from cows fed GSNC. The concentration of MUN decreased linearly ($P < 0.05$) with increasing level of supplemental corn to GS.

Discussion

Similar CP between GH and GS reflects little N loss during the handling and ensiling process. Hay generally has a higher NDF concentration than silage and likely due to some

hydrolysis of hemicellulose and carbon losses during silage fermentation (Nelson and Satter, 1992). However, the NDF concentration in GS did not decrease, implicating conservation of the cell-wall associated carbohydrate during the ensiling process. The nitrogenous components of well-preserved silages are mainly in a non-protein soluble form composed of short-chain peptides, ammonia, non-volatile amines, nitrate, and amino acids, which are about 20-30% and 60-70% of total N in fresh forages and in silages, respectively (Van Soest, 1994). Solubilization of protein has been considered as a main cause for poor N utilization from silages. In a survey of 35 grass silages, Tamminga et al. (1991) found that between 40 and 70% (average 61%) of CP was instantly solubilized in the rumen and only 4 to 24% was undegradable (average 9%). The concentration of NPN in GS was not high as observed in other grass silages. Most of the N in the leaf of gamagrass is associated with the NDIN fraction. Coblentz et al (1998) reported that this fraction ranges from 51.3 to 64.5% of the total leaf N, which may resist protein degradation during ensiling. The relatively lower NPN concentration in GS should improve CP utilization by lactating cows. In addition, the increased concentration of NFC with corn supplementation to GS would provide more readily fermentable carbohydrate for optimal ruminal fermentation.

The amount of silage intake was negatively related to DMI, which indicates that decreasing the NDF in a diet with ground corn increases DMI. Our results are in agreement with Petit and Veira (1991) who showed that intake of silage DM was decreased by concentrate supplementation, and that total DMI was lower for unsupplemented than for supplemented cows. The NDF concentration of the GS diets decreased from 63.8 to 31.0% with increasing corn supplementation. The higher NDF concentration, and consequently a lower percentage of soluble carbohydrates may contribute to a lower consumption of

gamagrass (Horner et al., 1985). Intake of DM increased with decreasing NDF when NDF concentration of diets exceeded 25% (Allen, 2000). In addition, Coblentz et al. (1998) pointed out that feeding gamagrass to lactating dairy cows may be associated with lower effective ruminal degradabilities due to a slower rate of fiber degradation. Only at the high level of corn supplementation did GS not limit DMI. Measurement of activity of carboxymethylcellulase (thought to initiate cellulolysis) of particle-associated bacteria (**PAB**) has been suggested as a method of estimating relative colonization of forages by cellulolytic bacteria (Bowman and Firkins, 1993). The authors reported that carboxymethylcellulase (**CMCase**) activity in gamagrass peaked relatively late in the incubation period (36 h) and stayed high for the remainder of the incubation period compared to activity of the same enzyme in orchardgrass and red clover. In addition, gamagrass had a low proportion of cellulolytic PAB in the residue in bags during the first 30 h of incubation and a relatively high proportion of cellulolytic PAB after 30 h. This supports the low initial rate of NDF and ADF disappearance for gamagrass, followed by a sustained disappearance of NDF and ADF at later incubation times.

Milk protein concentration and its yield were lower for cows fed GSLC or GSMC compared with cows fed GSHC. Grass silages supply little rumen undegradable protein (**RUP**). Thus, there should be a need for energy to increase the capture of N for microbial protein synthesis (**MPS**) in cows fed GS with low or medium level of supplemental corn. In a lactating trial with dairy cows fed perennial ryegrass silage, Dewhurst et al. (1996) reported a reduction in the efficiency of conversion of metabolizable protein into milk protein at a low energy supply.

Lower milk protein concentration than expected in the current study might also be explained by energy intake. The amount of NPN that can be incorporated into microbial protein is dependent on energy content of the ration (Huber and Kung, 1981). Microbial utilization of NPN for protein synthesis can be depressed when the supply of available energy is insufficient.

Milk lipid profile was very similar between cows fed GHNC and GSNC. *Trans*-$C_{18:1}$ FA was higher for GHNC but milk fat was not affected. The addition of concentrate typically inhibits ruminal biohydrogenation and decreases the formation of stearic acid. However, inclusion of corn to gamagrass silage resulted in an increase in stearic acid, suggesting a normal rate of ruminal biohydrogenation. It seems that gamagrass silage ameliorated the effects of corn even at the highest inclusion rate.

Petit and Tremblay (1995) reported lower feed efficiencies, when expressed as FCM/DMI, for unsupplemented than for supplemented cows fed grass silage-based diets. In the present study, lower DMI by cows fed GSNC could have resulted in increased digesta retention time, thereby increasing DM digestibility. The efficiency of utilization of N, assessed as milk N/N intake, ranged from 0.23 to 0.30 across treatments. Dewhurst et al. (1996) reported similar N efficiencies (0.24 to 0.32) with perennial ryegrass. However, the efficiency of dietary N utilization was higher for grass hay than grass silage-based diets using herbage from mixed timothy and meadow fescue (Shingfield et al., 2002). Difference in the efficiencies may be related to the different type of grasses (cool-season vs. warm-season grass). In addition, Shingfield et al. (2002) reported that increases in concentrate feeding had no effect on N efficiency. In their study, the amount of concentrate fed was between 7 and 10 kg. In the present study, intake of concentrate (ground corn + protein premix) averaged 4.7

kg, 8.1 kg, and 15.9 kg for cows fed GSLC, GSMC, and GSHC, respectively. Improved N efficiency in cows fed GSNC suggests that relatively lower levels of NPN in GS compared to other grass silages contribute to improved N utilization. Overall lactation performance data indicate that the optimum balance in terms of energy and protein for milk protein production is achieved with gamagrass silage-based diet containing between 28 and 55% of DM as supplemental corn.

Contrary to expectation, MUN concentration was higher with GHNC compared to GSNC. The higher N intake by cows fed GHNC may partly be responsible for the increased MUN concentration. The rate of degradation of silage NPN and soluble-protein N in the rumen is characteristically high and the fermentation of silage is followed by a pronounced peak in rumen ammonia concentration. The slow rate of degradation of silage N reported in our companion study (Eun et al., 2002) suggests that the ensiling process may affect the nature of fiber characteristics due possibly to the action of plant enzymes and acid hydrolysis. These can both contribute to the improvement of N utilization. Broderick (1995) reported that MUN more clearly reflected dietary CP intake rather than ruminal ammonia concentration. Excess N intake (total N intake – milk N secretion) was higher on GHNC than on GSNC (0.35 vs. 0.30 kg). Efficiencies of feed and N in cows fed GHNC may be negatively affected due to an additional energy cost of metabolizing excess N to urea.

Implications

The current study compared the nutritional value of gamagrass conserved as hay or silage when fed to lactating cows and the effects of varying corn supplementation levels to gamagrass silage. Ensiled gamagrass maintained its nutritional value as cows fed silage had

better feed efficiency than cows fed hay. Due to the higher concentration of extensively fermentable fiber in gamagrass and the close association of N with the neutral detergent insoluble fraction, MUN concentration was low resulting in high efficiencies of N utilization. Supplemental corn increased total DMI and improved utilization of dietary N compared to gamagrass alone. With gamagrass as the only source of forage, corn may be included at 50% of the diet DM to increase milk yield without any detrimental effects on ruminal fermentation or depression in milk fat. This perennial, native warm-season grass has characteristic that makes it a potential alternative forage for dairy enterprise and can be grown throughout much of the southern US.

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Table 1. Chemical composition of the dietary ingredients[1]

Item	Gamagrass hay	Gamagrass silage	Corn, ground	Protein supplement[2]
	---------- % of DM ----------			
DM, %	87.3	31.8	84.8	88.9
CP	13.5	13.7	8.2	38.1
NPN, % of total N	32.7	48.2	-	-
ADF	34.7	36.2	2.8	4.0
NDF	74.6	74.9	9.5	11.0
Fat	1.9	1.8	4.3	1.7
Ash	6.2	9.1	1.7	28.3

[1]n = 3 for gamagrass hay and n = 6 for other ingredients.

[2]Protein supplement included 78.6% soybean meal (48% CP), 8.5% deflourinated rock phosphate, 2.5% calcitic limestone, 2.7% salt, 1.6% magnesium oxide, 5.4% sodium bicarbonate, 0.6% McNess 1401®.

Table 2. Ingredient and chemical composition of the diets

Composition	Diet[1]				
	GHNC	GSNC	GSLC	GSMC	GSHC
Ingredient	---------- % of DM ----------				
Gamagrass hay	76.7	-	-	-	-
Gamagrass silage	-	83.3	71.1	56.0	29.2
Corn, ground	-	-	12.6	27.8	54.6
Protein supplement[2]	23.3	16.7	16.3	16.2	16.2
Chemical analyses	---------- % of DM ----------				
DM, %	85.2	36.7	37.3	41.5	54.6
CP	19.1	17.8	17.0	16.2	14.7
NPN, % of total N	27.8	35.0	31.6	32.9	28.7
ADF	29.1	32.2	28.1	22.6	13.3
NDF	61.1	63.8	58.7	48.3	31.0
Fat	1.9	1.8	2.1	2.4	3.2
Ash	10.5	11.4	10.7	9.3	7.7
NFC[3]	7.4	5.2	11.5	23.8	43.4

[1]GHNC = gamagrass hay + no corn; GSNC = gamagrass silage + no corn; GSLC = gamagrass silage + low level of corn; GSMC = gamagrass silage + medium level of corn; GSHC = gamagrass silage + high level of corn.

[2]Protein supplement included 78.6% soybean meal (48% CP), 8.5% deflourinated rock phosphate, 2.5% calcitic limestone, 2.7% salt, 1.6% magnesium oxide, 5.4% sodium bicarbonate, 0.6% McNess 1401®.

[3]NFC (Nonfibrous carbohydrate) = 100 – (CP + NDF + fat + ash).

Table 3. Intakes of DM and N and body weights in cows fed gamagrass hay (GH) or silage (GS) without or with supplemental corn [1]

Item	GH vs. GS[2]		SE	GS without or with corn[3]				SE
	GHNC	GSNC		GSNC	GSLC	GSMC	GSHC	
Intake, kg/d								
DM	14.3	14.6	0.3	16.4^{a}	18.4^{b}	18.5^{b}	22.5^{c}	0.3
Silage	-	-		13.6^{h}	13.1^{g}	10.4^{f}	6.6^{e}	0.2
Corn, ground	-	-		-	2.1^{a}	5.1^{b}	12.3^{c}	0.4
N	0.46^{b}	0.43^{a}	0.01	0.46^{i}	0.51^{k}	0.49^{j}	0.53^{k}	0.01
Cow wt., kg								
Initial	505.9^{i}	618.5^{j}	38.9	618.5	579.3	568.9	575.3	32.0
Final	513.7^{i}	623.7^{j}	37.6	607.9	607.2	568.6	588.2	32.4
Adjusted final[4]	567.3	570.1	8.3	576.2^{i}	613.2^{k}	584.6^{ij}	597.9^{ijk}	10.6
DMI:BW, %	2.7	2.4	0.2	2.4^{e}	2.9^{f}	3.1^{f}	3.8^{g}	0.2

[1]GHNC = GH + no corn; GSNC = GS + no corn; GSLC = GS + low level of corn; GSMC = GS + medium level of corn; GSHC = GS + high level of corn.

[2]Data for the comparison of cows fed GH vs. GS were obtained for 3 wk (n = 21) with treatment diet.

[3]Data for the comparison of cows fed GS without or with corn were obtained for 6 wk (n = 42) with treatment diet.

[4] Adjusted final BW were calculated by covariate analysis to account for differences in initial BW.

[a,b,c]Means within the same row without a common superscript differ ($P < 0.01$).

[e,f,g,h]Means within the same row without a common superscript differ ($P < 0.05$).

[i,j,k]Means within the same row without a common superscript differ ($P < 0.10$).

Table 4. Yields and concentrations of milk and milk components in cows fed gamagrass hay (GH) or silage (GS) without or with supplemental corn [1]

Item	GH vs. GS[2]		SE	GS without or with corn[3]				SE
	GHNC	GSNC		GSNC	GSLC	GSMC	GSHC	
Milk yield, kg/d	27.6	29.5	1.2	28.9^{i}	31.3^{ij}	33.1^{j}	37.2^{k}	1.6
3.5% FCM, kg/d	27.3^{i}	32.6^{j}	2.2	29.2^{e}	32.6^{f}	31.6^{ef}	36.0^{g}	1.2
Fat, %	3.81	3.83	0.17	3.44	3.72	3.35	3.43	0.13
Fat, kg/d	1.00	1.19	0.09	1.02^{i}	1.17^{jk}	1.09^{ij}	1.25^{k}	0.05
Protein, %	2.69	2.58	0.06	2.46^{i}	2.60^{j}	2.60^{j}	2.71^{k}	0.04
Protein, kg/d	0.69^{i}	0.80^{j}	0.04	0.73^{e}	0.82^{f}	0.84^{f}	1.00^{g}	0.03
Lactose, %	4.78	4.65	0.07	4.58^{e}	4.68^{ef}	4.76^{f}	4.71^{f}	0.05
Lactose, kg/d	1.23^{i}	1.44^{j}	0.08	1.35^{a}	1.48^{ab}	1.54^{b}	1.73^{c}	0.06
SNF, %	8.26^{j}	8.02^{i}	0.09	7.86^{a}	8.09^{b}	8.16^{b}	8.21^{b}	0.07
SNF, kg/d	2.12^{i}	2.48^{j}	0.13	2.31^{i}	2.55^{j}	2.65^{j}	3.02^{k}	0.09
NE_L,[4] Mcal/d	18.1	21.4	1.4	19.1^{i}	21.5^{j}	21.1^{j}	24.2^{k}	0.8

[1]GHNC = GH + no corn; GSNC = GS + no corn; GSLC = GS + low level of corn; GSMC = GS + medium level of corn; GSHC = GS + high level of corn.

[2]Data for the comparison of cows fed GH vs. GS were obtained for 3 wk (n = 21) with treatment diet.

[3]Data for the comparison of cows fed GS without or with corn were obtained for 6 wk (n = 42) with treatment diet.

[4]NE_L = energy content of milk.

[a,b,c]Means within the same row without a common superscript differ ($P < 0.01$).

[e,f,g]Means within the same row without a common superscript differ ($P < 0.05$).

[i,j,k]Means within the same row without a common superscript differ ($P < 0.10$).

Table 5. Fatty acid (FA) composition of milk from cows fed gamagrass hay (GH) or silage (GS) without or with supplemental corn[1]

FA	Diet[2]					SE
	GHNC	GSNC	GSLC	GSMC	GSHC	
	g/100 g of total FA					
$C_{10:0}$	1.59	1.60	2.09	2.04	0.76	0.39
$C_{12:0}$	2.37^{i}	2.57^{ij}	3.37^{jk}	3.98^{k}	3.17^{ijk}	0.39
$C_{14:0}$	9.89^{i}	10.35^{i}	13.30^{j}	13.94^{j}	11.56^{ij}	1.10
$C_{16:0}$	33.93	35.35	38.65	36.81	35.66	1.82
$C_{18:0}$	12.40^{i}	12.44^{ij}	11.92^{i}	14.29^{j}	16.25^{k}	0.72
trans-$C_{18:1}$	3.58^{j}	1.50^{i}	2.24^{ij}	1.69^{i}	2.09^{ij}	0.68
cis-$C_{18:1}$	30.25^{j}	32.12^{j}	24.25^{i}	23.41^{i}	24.49^{i}	2.22
$C_{18:2}$	3.76^{f}	3.04^{ef}	2.35^{e}	2.42^{e}	3.74^{f}	0.42
$C_{18\text{-conjugated diene}}$	0.55	0.24	0.49	0.49	0.41	0.25
$C_{18:3}$	1.19^{j}	0.43^{ij}	0.81^{ij}	0.53^{ij}	0.30^{i}	0.32
$C_{22:0}$	0.12^{a}	0.09^{a}	0.03^{a}	0.05^{a}	1.08^{b}	0.18
Saturated (S)	60.4^{i}	62.4^{ij}	69.4^{k}	71.1^{k}	68.7^{jk}	2.72
Unsaturated (U)	39.3^{k}	37.3^{jk}	30.5^{i}	28.5^{i}	31.0^{ij}	2.64
S:U	1.60^{e}	1.69^{ef}	2.32^{fg}	2.59^{g}	2.22^{efg}	0.25
MUFA[3]	33.83^{j}	33.62^{j}	26.49^{i}	25.09^{i}	26.59^{i}	2.54
PUFA[4]	5.50^{j}	3.72^{i}	4.06^{i}	3.44^{i}	4.44^{i}	0.40

[1]Samples taken in week 3 or week 6 were analyzed for milk fatty acid profile in cows fed GHNC or the other diets, respectively.

[2]GHNC = GH + no corn; GSNC = GS + no corn; GSLC = GS + low level of corn; GSMC = GS + medium level of corn; GSHC = GS + high level of corn.

[3]MUFA = monounsaturated fatty acid.

[4]PUFA = polyunsaturated fatty acid.

[a,b]Means within the same row without a common superscript differ ($P < 0.01$).

[e,f,g]Means within the same row without a common superscript differ ($P < 0.05$).

[i,j,k]Means within the same row without a common superscript differ ($P < 0.10$).

Table 6. Efficiencies of feed and N utilization for milk production and concentration of milk urea N (MUN) from cows fed gamagrass hay (GH) or silage (GS) without or with supplemental corn[1]

Item	GH vs. GS[2]		SE	GS without or with corn[3]				SE
	GHNC	GSNC		GSNC	GSLC	GSMC	GSHC	
Milk/DMI	1.88[a]	2.16[b]	0.06	1.90[g]	1.78[f]	1.81[f]	1.65[e]	0.03
FCM/DMI	1.92[i]	2.23[j]	0.15	1.85[k]	1.83[jk]	1.73[ijk]	1.61[i]	0.08
Milk N/N intake	0.23[a]	0.30[b]	0.02	0.25[e]	0.26[e]	0.28[ef]	0.29[f]	0.01
MUN, mg/100 ml	30.2[b]	18.4[a]	1.4	20.2[g]	19.5[g]	14.7[f]	13.3[e]	0.5

[1]GHNC = GH + no corn; GSNC = GS + no corn; GSLC = GS + low level of corn; GSMC = GS + medium level of corn; GSHC = GS + high level of corn.

[2]Data for the comparison of cows fed GH vs. GS were obtained for 3 wk (n = 21) with treatment diet.

[3]Data for the comparison of cows fed GS without or with corn were obtained for 6 wk (n = 42) with treatment diet.

[a,b]Means within the same row without a common superscript differ ($P < 0.01$).

[e,f,g]Means within the same row without a common superscript differ ($P < 0.05$).

[i,j,k]Means within the same row without a common superscript differ ($P < 0.10$).

CHAPTER 5

Running head: **Microbial fermentation of gamagrass**

Fermentation of eastern gamagrass by mixed cultures of ruminal microorganisms without or with supplemental corn[1]

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ABSTRACT

Five dual-flow fermentors (700 ml) were used to determine the effects of eastern gamagrass (*Tripsacum dactyloides* [L.] L.) diets on microbial metabolism by mixed rumen cultures. Fermentors were incubated with filtered ruminal contents and allowed to adapt for 4 d to diets followed by 3 d of sample collection. Five dietary treatments were tested: gamagrass hay (GH) + no corn (GHNC), gamagrass silage (GS) + no corn (GSNC), GS + low corn (GSLC), GS + medium corn (GSMC), and GS + high corn (GSHC). The experimental design was a randomized complete block with three replicates (runs). A concentrate mix consisting of soybean meal and mineral and vitamin premix was added to all diets to keep CP levels similar across treatments. Total VFA concentrations were not affected by diets (48.5 ± 2.8 m*M*). Corn supplementation at the medium and high level resulted in lowest proportion of ruminal acetate ($P < 0.01$). In contrast, molar proportion of propionate was reduced in GSLC ($P < 0.01$) and remained similar across all other diets. Corn supplementation linearly increased molar proportion of butyrate ($P < 0.03$). The acetate + butyrate to propionate ratio was lowered in cultures offered GSLC ($P < 0.01$) and remained similar across all other diets. Feeding GSNC resulted in a higher ruminal pH ($P < 0.01$) compared to GHNC (6.3 vs. 6.1). Increasing the level of corn supplementation in GS linearly decreased culture pH ($P < 0.04$). Concentration of ammonia-N was similar across treatments (27.9 ± 1.5 mg/100 ml) and tended to decrease with increasing levels of corn. All diets resulted in similar methane production with the exception of GSMC which lowered methane output ($P < 0.04$). Total fermentability tended to be higher ($P < 0.09$) in cultures receiving GHNC compared to GSNC (48.3 vs. 43.9%). Supplemental corn increased total fermentability of GS. Fiber (NDF) digestibility was similar between GH and GS and was not

affected by supplemental corn. Efficiency of microbial growth increased ($P < 0.02$) at the high level of corn supplementation but was similar to GSNC (33.3 vs. 29.3 g N/kg OM fermented). Microbial N flow increased in cultures offered GSHC ($P < 0.01$). Corn supplementation at the medium and high level decreased $C_{18:0}$ ($P < 0.05$) and increased *trans*-$C_{18:1}$ ($P < 0.02$). Total $C_{18:1}$ increased at the high level of corn inclusion. Including corn at the high level with GS did not have a detrimental effect on ruminal fermentation. Efficiency of microbial growth was high with gamagrass silage but supplemental corn at the high level increased microbial yield.

Key Words: Gamagrass, Fermentors, Continuous Culture, Microbial Growth

Introduction

Ensiling causes changes in the chemical composition of forage that typically lowers microbial protein synthesis (**MPS**) in silage-fed animals. The main factors affecting microbial assimilation of degraded N in the rumen with a silage-based diet are the amount of non-protein N in silage, the dietary source of carbohydrate, and the source and level of dietary N (Dewhurst et al., 2000). Siddons et al. (1985) reported that the efficiency of MPS was 21 and 26 g microbial N/kg apparently digestible OM in the rumen on a silage diet and a hay diet, respectively. This reduction was explained in terms of an imbalance of fermentable energy and degradable N in the rumen of cows receiving a grass silage diet. Silages typically contain large amounts of soluble protein but slower carbohydrate fermentation which tends to increase the mismatch between the rate at which energy and N are supplied to the microbes, causing a decreased capture of N as microbial protein. Supplementing silage-based diets with

moderate levels of starch often results in an increase in MPS. However, starch can have negative effects through decreased rumen pH and fiber degradation, increased energy spilling reaction, and a higher requirement for preformed AA (Russell and Wallace, 1997). Consequently, synchronizing energy and N release has not always benefited the ruminal environment supporting the argument that perhaps a continuous supply of energy is more important.

The NDF concentration in gamagrass is high but it is extensively degraded in the rumen (Coblentz et al., 1998). Much of the N in gamagrass is associated with the neutral detergent insoluble fraction (Coblentz et al., 1998). The potential to improve microbial capture of gamagrass N with supplemental energy has not been investigated. Our objective was to determine the effects of eastern gamagrass offered as hay or silage without or with supplemental corn on microbial fermentation. Kinetics of ruminal fermentation is discussed to better understand synchronization of N and energy to optimize MPS.

Materials and Methods

Apparatus, Diet, and Experimental Design

An in vitro study was designed to monitor the effects on ruminal fermentation when eastern gamagrass was fed as hay (**GH**) or silage (**GS**) without or with supplemental corn. Rumen inoculum was obtained from a nonlactating, ruminally cannulated Holstein cow fed alfalfa (*Medicago sativa* L.) hay. Rumen contents (approximately 3.5 L) were obtained using a hand vacuum pump and immediately placed in a preheated insulated containers. In the laboratory, ruminal contents were filtered through double layered cheesecloth, slowly mixed, and added into the fermentors within 15 min. Mixed ruminal contents with dual flow were

used as described earlier (Teather and Sauer, 1988). Anaerobic conditions in the culture vessels were maintained by infusion of CO_2 gas at a rate of 20 ml/min throughout the experiment. Culture pH was monitored during the entire duration of the experiment. A circulating water bath was used to maintain the temperature of the fermentors at 39°C. Continuous stirring of fermentor contents was achieved with the aid of a central paddle set at a speed of 10 rpm. The liquid dilution rate of the cultures was maintained at 6.3%/h by regulating the addition of artificial saliva prepared as described by Slyter et al. (1966). Alfalfa hay pellets (15 g as-fed/d) were added to the fermentors twice daily for 2 d during the adaptation period followed by 2 d of gradual transition on to the gamagrass treatment diets. On d 5, 100% experimental diets were added to the fermentors for 3 d. In order to monitor the changes of ammonia-N (**NH_3-N**) concentration from gamagrass hay and silage, 4 fermentors were inoculated (2 fermentors per diet) as described above. Two days were allowed for adaptation with alfalfa hay pellets followed by 2 d of treatment for sample collection.

A second year stand of 'Iuka' eastern gamagrass was used for this experiment. Details on harvesting condition, fertilization, and storage of the forage were described earlier (Eun et al., 2002b). Five dietary treatments (Table 1) were compared in a randomized complete block design with 3 blocks as repeated runs. Treatments consisted of 1) GH + no corn (**GHNC**); 2) GS + no corn (**GSNC**); 3) GS + low corn (**GSLC**); 4) GS + medium corn (**GSMC**); and GS + high corn (**GSHC**). Gamagrass hay collected during an in vivo study was ground through a Wiley mill (2-mm screen). Gamagrass silage, also collected and frozen (-20°C) during an in vivo trial, was thawed, and then processed in a food chopper (approximate length = 1 cm; model FC 19, G. S. Blakeslee & Co., Cicero, IL). All diets contained a protein supplement

composed of soybean meal (48% CP) and a vitamin and mineral premix. Dietary CP levels were formulated to be similar across all diets based on CP estimates of gamagrass hay and silage. Daily feed was added, on a DM basis, to average 13.9 g across all diets. Adding different amounts of cracked corn attained different levels of supplemental energy to GS. Diets were prepared by weighing and blending each ingredient manually.

Sample Collection and Analytical Procedures

Feed samples were analyzed for chemical constituents according to procedures described earlier (Eun et al., 2002b). Five ml of thoroughly mixed culture contents were collected twice daily during treatment period and analyzed for VFA by GLC (model CP-3380; Varian, Walnut Creek, CA) and for NH_3-N using a colorimetric assay (Beecher and Whitten, 1970). In addition, 5 ml of mixed culture contents were collected hourly for 7 hr after a.m. feeding for 2 d to measure NH_3-N concentrations from GHNC and GSNC in an independent run. Ten μl of headspace gas samples from the fermentor were drawn into a gas tight syringe (Hamilton Co., Reno, NV) and analyzed for methane (**CH_4**) using GLC. The pH of the ruminal cultures was monitored continuously and recorded when samples for CH_4 were taken.

Separate 5 ml samples of the mixed culture contents were taken on the last day of each period at 4 h after the morning feeding and frozen (-70°C) for lipid analysis of the culture contents. The frozen samples were thawed, methylated (Kramer et al., 1997), and then analyzed for fatty acid (**FA**) composition by GLC. Lipids in feed were extracted according to the method of Fellner et al. (1995) and analyzed for FA composition as described above.

On the last day, the liquid flow effluent containers were drained and 50 ml of the particulate matter were obtained and frozen for subsequent analysis. Frozen samples were

thawed, centrifuged at 1,300 × g for 10 min, and analyzed for DM and NDF according to procedures described earlier (Eun et al., 2002b). On the last day of each period, 50 ml of mixed culture contents were placed in conical tubes and frozen (-20°C). After thawing, the samples were mixed with 25 ml 0.9% NaCl and 1.5 ml 30% formaldehyde and divided into 2 aliquots. Both aliquots were used to isolate microbial cells by differential centrifugation, first at 1,074 × g for 5 min to precipitate particles, then at 47,800 × g for 20 min to sediment the bacteria, at 670 × g for 5 min to discard pellet, and at 47,800 × g for 20 min to re-sediment the bacteria. The resulting microbial pellet was dried for 4 h at 50°C and analyzed for microbial N concentrations.

Calculations and Statistical Analysis

Substrate quantity used for VFA, gas (CH_4 + CO_2), and microbial biomass determination was calculated according to stoichiometrical equations (Wolin, 1960; Blümmel et al., 1997) as outlined in a previous paper (Eun et al., 2002a). The yield of microbial biomass was set at 11.6 mg/mmol ATP (Hespell and Bryant, 1979). Total substrate used for VFA, CH_4 + CO_2, and microbial biomass was divided by DM fed to calculate total fermentability. Microbial N flow and efficiency of microbial protein synthesis were calculated as follows:

Microbial N flow, g/d = (Microbial DM flow, g/d) × (Microbial N %/100),

Microbial efficiency, g N/kg OM fermented = [(Microbial N flow, g/d) ÷ (OM fermented, g/d)] × 1,000.

Data were analyzed as repeated measures according to a randomized complete block design by the mixed model procedure of SAS (1999). The following model was used: $Y_{ij} = \mu + T_i + e_{ij}$, where Y_{ij} = the dependent variable, μ = the overall mean, T_i = the treatment effect,

and e_{ij} = residual error. Each independent run was considered random in the mixed model. Ammonia -N concentration over time in cultures receiving GHNC or GSNC was analyzed as repeated measures by the GLM procedure of SAS (1999) with the following model: $Y_{ij} = \mu + TR_i + TI_j + TR_i \times TI_j + e_{ij}$, where Y_{ij} = the dependent variable, μ = the overall mean, TR_i = the treatment effect, TI_j = the time effect, $TR_i \times TI_j$ = the interaction effect between treatment and time, and e_{ij} = residual error. Significant differences among means were determined using the PDIFF option of SAS (1999), where the *F*-test was significant. Significant treatment difference was declared at $P < 0.05$, and a tendency towards significance was set at $P < 0.10$.

Results

A protein supplement was included to formulate diets (Table 1) with similar CP concentration. The GHNC and GSNC treatments, however, tended to have higher CP concentration compared to GS with supplemental corn. This occurred because actual CP concentration in GS was higher than the value used to formulate the diets (13.7% vs. 11.0%). Overall, dietary CP concentrations were similar to those in diets fed to lactating cows in a companion paper (Eun et al., 2002b). Concentrations of non-protein N (**NPN**), NDF, and ADF decreased with increasing supplemental corn. Corn supplementation increased the fat concentration of the diet as well as the calculated nonfibrous carbohydrate (**NFC**) concentration. Linolenic acid comprised almost 50% of total FA in GHNC and GSNC. Both $C_{18:0}$ and *trans*-$C_{18:1}$ FA were present in the forage in amounts less than 3% of total FA. Including corn with GS decreased $C_{18:3}$ and increased $C_{18:2}$ FA concentrations.

Total VFA concentrations averaged 48.5 m*M* and were not affected by treatment (Table 2). Millimolar concentrations of acetate, propionate, and butyrate were similar between GHNC and GSNC ($P > 0.10$). Supplemental corn at the high level increased propionate ($P < 0.05$) and butyrate ($P < 0.04$).

Molar proportions of acetate (**A**) were higher for GHNC ($P < 0.01$) compared with GSNC (Table 2). Corn supplementation at the medium or high level resulted in the lowest molar proportion of ruminal acetate. Molar proportions of propionate (**P**) were not affected by diets with the exception of GSLC which lowered the propionate proportion ($P < 0.01$). The proportion of butyrate (**B**) was higher in GSNC compared with GHNC ($P < 0.01$). Corn supplementation increased the molar proportion of butyrate ($P < 0.03$) and decreased the molar proportion of valerate ($P < 0.05$). There were no major differences in the molar ratio of isoacids. The acetate to propionate (**A:P**) ratio tended to increase at the low corn inclusion but decrease at the medium and high levels of corn ($P < 0.08$). The lipogenic to glucogenic VFA ratio (**(A + B):P**) increased with the low level of corn supplementation to gamagrass silage ($P < 0.01$) but remained unchanged across all other treatments.

Feeding GSNC resulted in a higher ruminal pH ($P < 0.01$) compared to GHNC (Table 3). Increasing the level of corn supplementation in GS linearly decreased culture pH ($P < 0.04$). Concentrations and yields of NH_3-N were not affected ($P > 0.10$) by dietary treatments (average of 27.9 mg/100 ml and 0.29 g/d) but tended to decrease numerically with increasing levels of supplemental corn. Methane output remained unchanged across diets with the exception of GSMC which showed a lower methane output ($P < 0.04$).

Concentrations of NH_3-N at hourly intervals in cultures receiving either GHNC or GSNC showed no effects ($P > 0.10$) of treatment or time (Figure 1). Initial concentration of NH_3-N,

however, was numerically higher in cultures receiving GSLC compared to GHNC until 2 h after feeding.

Substrate used for VFA, gas ($CH_4 + CO_2$), and microbial biomass was similar between GHNC and GSNC (Table 4). The high level of corn with GS increased the amount of substrate used for VFA, gas, and microbial biomass. Total fermentability tended to be higher in cultures receiving GHNC compared to GSNC ($P < 0.09$), and it increased with increasing level of corn supplementation. Neutral detergent fiber digestibility was similar for all diets. Efficiency of MPS was not affected by gamagrass fed as hay or silage (Table 4). Including corn at the low and medium level resulted in lower microbial protein efficiencies when compared to GSNC. Cultures receiving the high level of corn had significantly higher efficiencies when compared with either GSLC or GSMC but were similar to efficiencies in cultures receiving GSNC. Microbial N flow increased only in cultures receiving GSHC ($P < 0.01$).

The fatty acid composition of ruminal cultures receiving GHNC and GSNC did not differ (Table 5). More than 60% of the total FA consisted of $C_{18:0}$ in cultures offered GHNC and GSNC. The concentration of the *trans* isomer of $C_{18:1}$ averaged less than 5.2% in cultures fed GHNC and GSNC. Supplementing gamagrass silage with corn reduced the $C_{18:0}$ concentration ($P < 0.05$) in ruminal cultures which was significant at the medium and high level. A decrease in $C_{18:0}$ was accompanied by an increase in the *trans*-$C_{18:1}$ concentration ($P < 0.02$) which comprised nearly 20% of the total FA in ruminal cultures receiving GSMC and GSHC. However, concentration of *cis*-$C_{18:1}$ remained unchanged across all treatments. Linoleic acid was less than 2% in all cultures except the case receiving GSHC. Inclusion of

corn to gamagrass silage increased the unsaturated FA and lowered the saturated:unsaturated FA (**S:U**) ratios in ruminal cultures.

Discussion

Molar percentages of VFA, in particular the relative concentrations of acetate and propionate, changed with the ratio of forage to concentrate. Lowering the forage to concentrate ratio by including medium and high levels of corn supplementation resulted in a lower acetate to propionate ratios. The higher molar proportions of butyrate in cultures receiving GSNC compared to GHNC may be related to a higher concentration of protozoa. Huhtanen (1992) and Jaakkola and Huhtanen (1993) reported that an increase in the number of protozoa is accompanied by an increase in ruminal butyrate. Ushida et al. (1986) also showed that faunated sheep had higher molar proportions of butyrate than did defaunated sheep. Jaakkola and Huhtanen (1993) reported a tendency for a higher number of protozoa with grass silage compared with grass hay.

The decrease in acetate proportions in cultures receiving medium or high supplemental corn seems to be compensated by an increase in the proportion of butyrate, resulting in a similar (A + B):P ratio, when compared to cultures receiving no supplemental corn. Sutton et al. (1988) concluded that the (A + B):P ratio is probably one of the best measures of rumen fermentation characteristics in terms of milk fat production as acetate and butyrate are independently and positively but propionate negatively correlated with milk fat concentration. Cultures fed gamagrass silage with a low level of supplemental corn had the highest (A + B):P ratio. Lactating cows fed a similar diet tended to have higher milk fat concentration compared with other treatments (Eun et al., 2002b).

When readily fermentable carbohydrates are added to forage diets, ruminal pH decreases, reducing cellulolytic microbes and limiting fiber digestion. Although supplemental corn lowered ruminal pH, the decrease was not as great as one would expect with the amount of corn included at the high level. This could be explained, in part, by the buffering effect of protozoa when feeding gamagrass silage. Structural carbohydrates, which are high in gamagrass silage and slow to degrade, may permit sequestration of protozoa. They are adept at accumulating polysaccharide by engulfing starch and soluble carbohydrates thereby reducing the rate of acid production. Protozoa maintain a gradual fermentation without high concentrations of lactate (Jaakkola and Huhtanen, 1993). This may prevent a rapid decrease in ruminal pH and maintain similar production of methane in cultures receiving gamagrass silage supplemented with medium or high levels of corn. However, the reason for the depressed methane production in cultures fed gamagrass silage with medium corn is not apparent.

De Visser et al. (1998b) observed a decrease in both the rate and extent of NDF digestibility in diets which were based primarily on grass silage in the presence of supplemental rumen degradable starch. In another study, De Visser et al. (1998a) reported a numerical decrease in ruminal pH from 6.2 to 5.9 with increasing flaked corn starch in the diet. The authors could not explain the reduction in the NDF digestibility with the small change of pH. They speculate that there may be competition between cellulolytic and amylolytic bacteria for growth factors including N (personal communication with Van Vuuren). In this study, NDF digestibility was similar across all diets, and culture pH ranged from 6.3 to 5.9. The higher concentrations of fermentable carbohydrate from corn added to gamagrass silage, even at the high level, did not reduce the activity of cellulolytic

microorganisms in the current study. Under normal circumstances, ammonia is the most abundant N compound available for microbial growth; indeed, most cellulolytic organisms have an absolute requirement for ammonia. Polan et al. (1976) reported that ammonia might be limited when rapidly fermentable carbohydrates are fed, thus reducing cellulose digestion (Stern et al., 1978). Since the concentration of NH_3-N and NDF digestibility were not decreased in the gamagrass silage cultures supplemented with high level of corn, ammonia may not have limited the growth of cellulolytic microorganisms. However, NDF digestibility in the current study was lower than that reported by Horner et al. (1985; 42.5 vs. 62.2%). In that study, NDF digestibility was measured on diets consisting of 100% chopped gamagrass hay fed to wethers. In this study, however, NDF digestibility was measured on diets consisting of gamagrass hay or silage with a concentrate mix composed of protein supplement and ground corn.

The efficiency of MPS ranged from 24.7 to 33.3 g microbial N/kg OM fermented and is within the range of values previously reported (Agricultural Research Council, 1984; Scollan et al., 1996; Siddons et al., 1985). Nitrogen utilization from the ensiling process is affected by the solubilization of protein and fermentation of soluble sugars to VFA and lactic acid (Charmley, 2001). Silages are typically associated with a lower energy yield because the utilization of VFA and lactic acid are considerably less when compared to the fresh non-fermented feed. This is due to direct absorption of a high proportion of VFA across the rumen wall (Thomas and Thomas, 1985). Thus, the efficiency of utilization of fermented OM for microbial protein synthesis is only approximately 60 to 70% of that for energy from non-fermented feeds (Agricultural Research Council, 1984). However, gamagrass silage tended to support numerically higher efficiency of MPS compared to gamagrass hay. This may, in

part, be related to the tendency for a slower rate of NH_3-N release from gamagrass silage compared to gamagrass hay (Figure 1). Silage diets contain a high proportion of NPN compounds, and synchronizing availability of energy and N may be a problem since the peak in ammonia production is attained rapidly. Jaakkola and Huhtanen (1993) also reported higher efficiency of MPS with grass silage compared to hay. This unusual result may reflect the restricted fermentation from use of a high level of formic acid which inhibits plant protease enzymes through a rapid reduction in pH and preserves high residual sugar and true protein available for MPS (Charmley, 2001). Since a large proportion of the N in gamagrass is associated with the neutral detergent insoluble fraction, which accounted for 51.1 to 63.5% of total N across a wide range of plant maturities (Coblentz et al. 1998), restricted fermentation of the neutral detergent insoluble N during the ensiling process could result in an unusually lower concentration of NPN (48.2%) compared with other grass silages (60-70% of total N; Van Soest, 1994). It is not clear, but a mechanism related to the availability of the substrate may exist for limiting the extent of proteolysis of gamagrass silage.

Synchronization of energy and N release has been regarded as being particularly relevant to grass silage-based diets due to rapid release of ammonia from the relatively large pool of NPN compounds. This requires a similarly rapid release of energy in the rumen to ensure the most efficient microbial fixation of ammonia (Chamberlain and Choung, 1995). However, supplementing gamagrass silage with corn failed to increase the efficiency of MPS in this study. Furthermore, low or medium levels of corn supplementation to gamagrass silage decreased the efficiency of MPS compared to gamagrass silage without supplemental corn. If silages tend to support increased population of protozoa, then some of the lack of response to supplemented corn may be related to this shift in culture fauna. An increase in protozoa can

increase the intraruminal recycling of ammonia thereby reducing microbial efficiency (Firkins et al., 1992). Chamberlain et al. (1985) reported that the number of total protozoa increased with starch supplementation. Jaakkola and Huhtanen (1993) also reported that the number of total protozoa increased linearly with the level of concentrate in the diet up to 75% DM. It appears that when gamagrass silage is supplemented with starch concentrates the potential impact of an increased energy supply in reducing ruminal ammonia concentration is offset by the effect of the concentrates on the rumen protozoa to a certain level. Furthermore, increasing the supply of starch changes the balance between fractions of N needed by microbial population in the rumen. A lack of AA for amylolytic microbes in the high corn diet may limit their growth and impact MPS. A lower pH may further limit conditions necessary for optimal MPS.

It is well accepted that the critical level of readily fermentable carbohydrate in the diet for MPS is difficult to define (Kim et al., 1999). Scollan et al. (1996) found that neither the efficiency of MPS nor the microbial N flow to the duodenum in grass silage-based diets fed to growing steers was significantly different among various sources of carbohydrate when supplemented at 10% of silage intake. Since microbial N flow was increased in cultures offered gamagrass silage with high levels of supplemental corn, feeding gamagrass silage with high level of supplemental concentrates can be implemented to increase microbial yield.

Grass is rich in $C_{18:3}$ FA, and preservation as silage should not decrease its concentration (Doreau and Poncet, 2000). Both gamagrass hay and silage contained a similar concentration of $C_{18:3}$, suggesting that there was no oxidation damage during the ensiling process. Because corn contains high amounts of polyunsaturated FA, which are precursors for *trans*-$C_{18:1}$, gamagrass silage with medium or high level of supplemental corn resulted in increased

production of *trans*-$C_{18:1}$ in the culture contents. This is a consequence of incomplete biohydrogenation (Harfoot and Hazlewood, 1997). It has become evident that an increase in the *trans*-$C_{18:1}$ concentration of milk fat across a wide range of diets is correlated with a reduction in milk fat yield. Recent work by Griinari et al. (1998) showed that the decrease in milk fat yield is associated with a specific increase in *trans*-10 $C_{18:1}$ rather than an increase in the total *trans*-$C_{18:1}$ concentration of milk fat. Piperova et al. (2000) have confirmed the increase in the milk fat concentration of *trans*-10 $C_{18:1}$ in milk-fat depressed lactating cows. Feeding similar diets to lactating cows (Eun et al., 2002b) did not depress milk fat yield. It is likely that the ruminal *trans*-$C_{18:1}$ had a lower *trans*-10 $C_{18:1}$ proportion and consisted of other *trans* isomers. Also gamagrass silage-based diets supplemented with corn, even at the high level, did not seem to decrease the (A + B):P ratio.

Implications

Gamagrass silage supported ruminal fermentation similar to gamagrass hay. Addition of corn to gamagrass silage lowered culture pH but increased total fermentability of diets. Contrary to expectation, feeding a high level of corn added to gamagrass silage did not impair microbial efficiencies compared to feeding gamagrass silage alone. No detrimental effect of supplemental corn (up to 55% of diet DM) on fermentation in cultures receiving gamagrass silage was noted and was associated with the buffering effect of protozoa. Increasing the supply of starch seemed to have shifted microbial populations, increased ruminal protozoa, and altered nutrient requirements that affect microbial protein synthesis. The efficiency of microbial protein synthesis was higher with gamagrass silage without supplemental corn but at the expense of microbial yield. However, corn supplementation at

the high level increased microbial yield and may be an effective strategy to increase passage of microbial protein.

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Table 1. Ingredient and chemical composition of diets

Item	Diet[1]				
	GHNC	GSNC	GSLC	GSMC	GSHC
Ingredient	---------- % of DM ----------				
Gamagrass hay	78.0	-	-	-	-
Gamagrass silage	-	81.5	70.6	56.7	29.7
Corn, ground	-	-	12.6	27.8	54.6
Protein supplement[2]	22.0	18.5	16.8	15.5	15.7
Composition	---------- DM ----------				
DM, %	89.1	38.9	40.6	44.3	58.3
CP, %	19.5	18.4	16.9	16.4	15.7
NPN, % of total N	28.2	34.3	31.5	31.6	28.7
ADF, %	28.1	30.6	26.9	21.4	12.2
NDF, %	60.2	61.9	55.2	45.7	30.2
Fat, %	1.9	1.8	2.1	2.5	3.3
Ash, %	10.1	11.9	10.9	9.0	7.6
NFC[3], %	8.3	6.0	14.9	26.4	43.2
Fatty acid profile	---------- g/100 g of total fatty acid ----------				
$C_{16:0}$	24.6	22.9	17.4	13.9	10.8
$C_{18:0}$	2.6	2.9	1.9	2.7	2.3
trans-$C_{18:1}$	2.7	2.9	0.8	1.1	1.2
cis-$C_{18:1}$	-	0.8	13.8	15.0	19.1
$C_{18:2}$	17.2	18.6	24.5	34.7	46.3
$C_{18:3}$	47.8	46.4	36.5	27.2	15.7
$C_{20:0}$	1.1	0.9	1.1	0.8	0.7

[1]GHNC = gamagrass hay + no corn; GSNC = gamagrass silage (GS) + no corn; GSLC = GS + low level of corn; GSMC = GS + medium level of corn; GSHC = GS + high level of corn.

[2]Protein supplement included 78.6% soybean meal (48% CP), 8.5% deflourinated rock phosphate, 2.5% calcitic limestone, 2.7% salt, 1.6% magnesium oxide, 5.4% sodium bicarbonate, 0.6% McNess 1401®.

[3]NFC (Nonfibrous carbohydrate) = 100 – (CP + NDF + Fat + ash).

Table 2. Concentration and molar ratio of VFA in continuous cultures receiving gamagrass hay (GH) or silage (GS) without or with supplemental corn[1]

Item	Diet[2]					SE
	GHNC	GSNC	GSLC	GSMC	GSHC	
Total, m*M*	47.0	44.9	49.0	49.1	52.4	2.11
Individual, m*M*						
Acetate	30.0	27.7	30.0	28.9	30.3	1.3
Propionate	8.73^{ij}	8.21^{i}	8.33^{i}	9.23^{jk}	9.77^{k}	0.42
Butyrate	5.38^{e}	5.91^{e}	7.46^{f}	7.72^{f}	9.05^{g}	0.44
Valerate	0.98	0.97	0.97	1.02	0.96	0.05
Isobutyrate	0.50	0.53	0.56	0.52	0.50	0.04
Isovalerate	1.38	1.68	1.67	1.76	1.88	0.15
Individual, mol/100 mol						
Acetate (A)	63.9^{c}	61.5^{b}	61.3^{b}	58.8^{a}	57.9^{a}	0.6
Propionate (P)	18.6^{b}	18.3^{b}	17.0^{a}	18.8^{b}	18.7^{b}	0.4
Butyrate (B)	11.5^{e}	13.1^{f}	15.2^{g}	15.7^{g}	17.1^{h}	0.6
Valerate	2.09^{g}	2.16^{g}	1.99^{f}	2.07^{fg}	1.82^{e}	0.06
Isobutyrate	1.04^{ab}	1.17^{b}	1.14^{b}	1.06^{ab}	0.94^{a}	0.06
Isovalerate	2.91	3.73	3.38	3.54	3.54	0.24
A:P	3.45^{j}	3.37^{j}	3.61^{k}	3.13^{i}	3.11^{i}	0.08
(A + B):P	4.08^{a}	4.09^{a}	4.51^{b}	3.97^{a}	4.08^{a}	0.11

[1]Each value is the mean of three runs.

[2]GHNC = GH + no corn; GSNC = GS + no corn; GSLC = GS + low level of corn; GSMC = GS + medium level of corn; GSHC = GS + high level of corn.

[a,b,c]Means within the same row without a common superscript differ ($P < 0.01$).

[e,f,g,h]Means within the same row without a common superscript differ ($P < 0.05$).

[i,j,k]Means within the same row without a common superscript differ ($P < 0.10$).

Table 3. Ruminal pH, concentration and production of ammonia-N (NH_3-N), and methane (CH_4) output in continuous cultures receiving gamagrass hay (GH) or silage (GS) without or with supplemental corn[1]

Item	Diet[2]					SE
	GHNC	GSNC	GSLC	GSMC	GSHC	
pH	6.1[f]	6.3[g]	6.1[f]	6.0[ef]	5.9[e]	0.08
NH_3-N, mg/100 ml	29.2	28.1	29.4	27.0	25.8	1.4
NH_3-N[3], g/d	0.31	0.29	0.31	0.28	0.27	0.02
CH_4 mmol/d	21.0[f]	19.0[f]	19.2[f]	16.2[e]	20.6[f]	1.0

[1]Each value is the mean of three runs.

[2]GHNC = GH + no corn; GSNC = GS + no corn; GSLC = GS + low level of corn; GSMC = GS + medium level of corn; GSHC = GS + high level of corn.

[3]NH_3-N (g/d) = (NH_3-N concentration, mg/100 ml × fermentor volume (700 ml) × 1.5 (turnover rate of fermentor))/1,000.

[e,f,g]Means within the same row without a common superscript differ ($P < 0.05$).

Table 4. Partitioning of substrate, fermentability, and microbial growth in continuous cultures receiving gamagrass hay (GH) or silage (GS) without or with supplemental corn[1]

Item	Diet[2]					SE
	GHNC	GSNC	GSLC	GSMC	GSHC	
DM fed, g/d	13.7	14.5	14.0	13.6	13.6	
Substrate used, g/d						
For VFA[3]	3.1^{e}	2.9^{e}	3.2^{ef}	3.3^{ef}	3.5^{f}	0.12
For CH_4 + CO_2[4]	2.2^{i}	2.2^{i}	2.3^{ij}	2.1^{i}	2.5^{j}	0.08
For microbial biomass	1.3^{i}	1.2^{i}	1.3^{i}	1.3^{i}	1.4^{j}	0.04
Total fermentability[5], %	48.3^{j}	43.9^{i}	49.0^{j}	49.2^{j}	54.8^{k}	1.6
NDF digestibility, %	42.9	42.7	42.6	39.9	44.2	2.06
Microbial efficiency[6]	29.3^{ef}	33.3^{f}	26.0^{e}	24.7^{e}	33.3^{f}	1.9
Microbial N flow, g/d	1.48^{a}	1.43^{a}	1.27^{a}	1.29^{a}	1.98^{b}	0.09

[1]Each value is the mean of three runs.

[2]GHNC = GH + no corn; GSNC = GS + no corn; GSLC = GS + low level of corn; GSMC = GS + medium level of corn; GSHC = GS + high level of corn.

[3](Acetate, mol/d × 60.05) + (Propionate, mol/d × 74.08) + (Butyrate, mol/d × 88.10).

[4]Substrate used for (CO_2, mol/d × 44) + (CH_4, mol/d × 16) + ($2H_2O$, mol/d × 36).

[5][(Substrate used for VFA, CO_2 + CH_4 + $2H_2O$, and microbial biomass) ÷ (DM fed)] × 100.

[6]Efficiency of microbial protein synthesis expressed as g of N/kg OM fermented.

[a,b]Means within the same row without a common superscript differ ($P < 0.01$).

[e,f]Means within the same row without a common superscript differ ($P < 0.05$).

[i,j,k]Means within the same row without a common superscript differ ($P < 0.10$).

Table 5. Fatty acid (FA) composition of culture contents in continuous cultures receiving gamagrass hay (GH) or silage (GS) without or with supplemental corn[1]

FA	Diet[2]					SE
	GHNC	GSNC	GSLC	GSMC	GSHC	
	g/100 g of total FA					
$C_{14:0}$	0.37	0.52	0.71	0.79	0.68	0.18
$C_{16:0}$	23.0	23.7	21.7	22.9	21.0	0.8
$C_{18:0}$	64.4^{f}	61.4^{f}	60.4^{f}	50.8^{e}	49.6^{e}	3.3
trans-$C_{18:1}$	4.47^{e}	5.17^{e}	9.03^{e}	17.95^{f}	18.68^{f}	2.31
cis-$C_{18:1}$	4.04	5.47	4.05	3.89	5.46	2.33
$C_{18:2}$	1.32^{i}	0.73^{i}	1.57^{i}	1.61^{i}	3.02^{j}	0.54
$C_{18:3}$	0.32	0.32	0.22	0.46	0.26	0.18
$C_{20:0}$	1.53^{j}	1.49^{j}	1.41^{j}	1.10^{i}	1.08^{i}	0.12
$C_{22:0}$	0.51^{ij}	0.73^{j}	0.78^{j}	0.54^{j}	0.20^{i}	0.17
Saturated (S)	89.9^{f}	87.8^{f}	85.1^{f}	76.1^{e}	72.5^{e}	3.2
Unsaturated (U)	10.2^{e}	12.2^{e}	15.0^{e}	23.9^{f}	27.5^{f}	3.2
S:U	11.52^{j}	9.27^{j}	6.10^{ij}	3.27^{i}	2.88^{i}	2.76
Monounsaturated	8.51^{e}	10.64^{e}	13.08^{e}	21.84^{f}	24.14^{f}	2.78
Polyunsaturated	1.64	1.55	1.87	2.07	3.33	0.67

[1]Each value is the mean of three runs.

[2]GHNC = GH + no corn; GSNC = GS + no corn; GSLC = GS + low level of corn; GSMC = GS + medium level of corn; GSHC = GS + high level of corn.

[e,f]Means within the same row without a common superscript differ ($P < 0.05$).

[i,j]Means within the same row without a common superscript differ ($P < 0.10$).

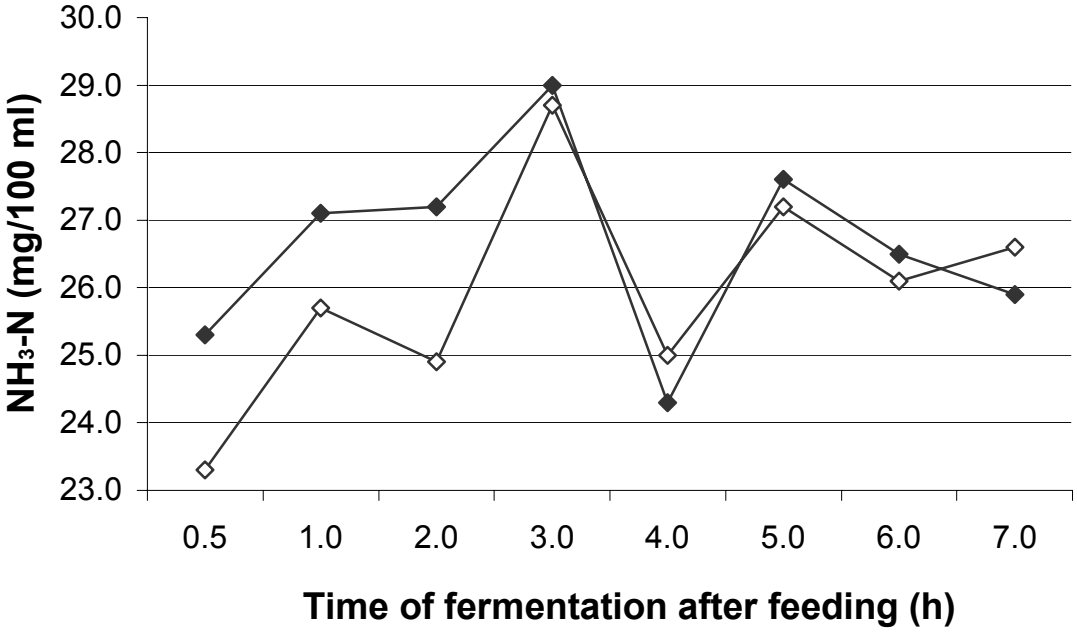

Figure 1. Pattern of ammonia-N (NH_3-N) concentration in continuous cultures receiving gamagrass hay (GH) or silage (GS). Data represent least square mean values (associated SE = 1.6). Legend: GH + no corn, GHNC (◇, n = 2); GS + no corn, GSNC (◆, n = 2). The effects of treatment, time, and interaction of time × treatment were not significant ($P > 0.10$).

CHAPTER 6

SUMMARY AND CONCLUSIONS

Renewed pressure from governmental and consumer agencies has necessitated minimizing nutrient waste and maximizing its use by the animal production industry. In dairy nutrition, we need a better understanding of microbial dynamics in the rumen to improve sustainability, productivity, and animal welfare. The outflow of microbial biomass and VFA from the rumen will impact the nutritional status of the animal as well as the efficiency of nutrient utilization. An inherent part of this fermentation process is the production of methane and ammonia, which represent energetic inefficiencies to the whole animal. The work presented here has addressed characteristics of ruminal fermentation and their contribution to lactation performance of dairy cows.

The effects of dilution rates and forage to concentrate ratios on fermentation kinetics of rumen microbes cultured in fermentors were studied in the first experiment. It was found that fractional dilution rate and forage to concentrate ratio greatly influence both substrate digestion and microbial metabolism, particularly microbial growth and methane production. Based on the estimates with stoichiometric equations, total gas production was not affected by forage to concentrate ratios or by dilution rates. Methane production, also estimated from stoichiometric equations, remained unchanged irrespective of the dilution rate or level of concentrate in the diet. However, actual methane production decreased significantly with an increase in the level of concentrate and increased with an increase in dilution rate. In addition, actual methane output compared with stoichiometric estimations was higher at higher dilution rates and with high forage diets. Since the amount of substrate required for microbial biomass can vary substantially due to variable efficiency of ATP use by ruminal

microorganisms, microbial cell yields may have a great influence on the difference in methane production between actual measurements and stoichiometric estimations. The decrease in methane production in cultures receiving a higher proportion of concentrate is consistent with the shift in reducing equivalents towards propionate formation. Microbial yields increased with an increase in dilution rate due primarily to reduced maintenance requirements and enhanced utilization of substrate energy for growth related processes. Changing dilution rates has a profound impact on the growth rate of bacteria and alters the metabolic pathways of fermentation. One might envision an increase in the ruminal dilution rates to remove microbial protein from the rumen before microbial cell turnover can occur. Therefore, modification of ruminal dilution rates via salt or particle size reduction may be an attractive solution to increase microbial protein synthesis available in the lower gut and consequently animal performance. On the other hand, the higher energy production as VFA due to reduced methane production in the high concentrate diets resulted in increased microbial efficiencies. We postulate that increased methane production with increasing dilution rate may be due to changes in the metabolic rates and pathways of fermentation of certain methanogens. There is limited information on metabolic differences between species of ruminal methanogens. It would be necessary to determine the contribution of methanogenesis to microbial growth in the rumen in order for methane production to be used as an index for microbial growth and consequently as an in vitro tool for feed evaluation.

Since a shift to grass-based dairying is seen as a mechanism to increase the sustainability of dairy farms, particularly in the southeast region, perennial grasses have attracted much attention. Its application, however, in practical dairy ration formulation is in its infancy in the USA as far as knowledge related to intensive management for lactating cows is concerned.

At the same time, the need for synchrony of releasing available energy and nitrogen in the rumen has been considered especially important with diets based on grass silage due to inefficient microbial capture of nitrogen. In the second experiment, the feeding of eastern gamagrass as hay or silage to lactating dairy cows was investigated with or without supplementing corn to gamagrass silage-based diets. Ensiling of gamagrass maintained its nutritional value as cows fed silage had better feed efficiency than cows fed hay. Due to the close association of gamagrass N to its neutral detergent insoluble fraction and its slow rate of degradation, MUN concentration was reduced, resulting in higher efficiencies of N utilization in cows fed gamagrass silage compared to cows fed gamagrass hay. Supplemental corn increased total DM intake and improved utilization of dietary N compared with gamagrass alone. Overall lactation performance data suggest that the optimum balance in terms of energy and protein for milk protein production is achieved with gamagrass silage-based diet containing between 28 and 55% of DM as supplemental corn. With gamagrass as the only source of forage, corn may be included at 50% of the diet DM to increase milk yield without any detrimental effects on ruminal fermentation or depression in milk fat. As reflected in improved efficiency of N utilization and in reduced MUN concentration, corn supplementation to gamagrass silage is an effective strategy to increase microbial capture of rumen degradable protein while enhancing the availability of ruminal fermentable energy.

Utilization of grass silage nitrogen largely depends on its conversion into microbial protein in the rumen. The main factors affecting microbial assimilation of degraded nitrogen in the rumen with silage-based diets are the amount of non-protein nitrogen in silage, the dietary source of carbohydrate, and the source and level of dietary nitrogen. Since gamagrass has the unique characteristics of nitrogen storage within the neutral detergent insoluble

fraction, the potential to improve microbial capture of gamagrass nitrogen in hay or silage by supplying supplemental energy was investigated using continuous cultures in the third experiment. Lowering the forage to concentrate ratio with inclusion of medium and high levels of corn supplementation resulted in lower acetate to propionate ratio as was observed in the first experiment. Higher molar proportion of butyrate in cultures receiving gamagrass silage compared to gamagrass hay may be due to a higher concentration of protozoa. Cultures fed gamagrass silage with a low level of supplemental corn had the highest lipogenic to glucogenic VFA ratio ((acetate + butyrate):propionate), which may explain the tendency toward increased milk fat concentration in cows fed a similar diet in the lactation trial (second experiment). Numerically higher efficiency of microbial growth in cultures fed gamagrass silage, compared with gamagrass hay, may partly be related to the slower rate of ammonia-N release from gamagrass. This also supports increased N utilization and decreased MUN concentration in cows fed gamagrass silage compared to gamagrass hay in the lactation trial. Since a large proportion of N in gamagrass is associated with the neutral detergent insoluble fraction, restricted fermentation of the neutral detergent insoluble N fraction in gamagrass during the ensiling process could result in an unusually low concentration of NPN (48.2%) as reported in the lactation trial. This may be the driving factor for the improved microbial efficiency of gamagrass hay. Synchronization of energy and N as a means to improve microbial growth efficiency in gamagrass silage with added corn should consider the role of protozoa. They may prevent rapid decreases in ruminal pH and maintain normal fermentation reactions but may also simultaneously increase microbial maintenance cost. Such microbial transaction could interact with efficiencies of microbial growth. However, microbial N flow increased in cultures offered gamagrass silage with high

level of supplemental corn, which can explain the increased milk protein concentration from cows fed a similar diet in the lactation trial. Therefore, feeding gamagrass silage with a high level of supplemental concentrates can be implemented to increase microbial yield.

In conclusion, the composition of the diet and rate of passage of culture contents can interfere with normal metabolic events altering the complex interaction between microbial growth and substrate utilization. On the other hand, gamagrass has characteristics that make it a potential alternative forage for dairy enterprise.

APPENDIX

Interaction Effects between Dilution Rate and Forage to Concentrate Ratio in Continuous Cultures.

(Data from Chapter 3)

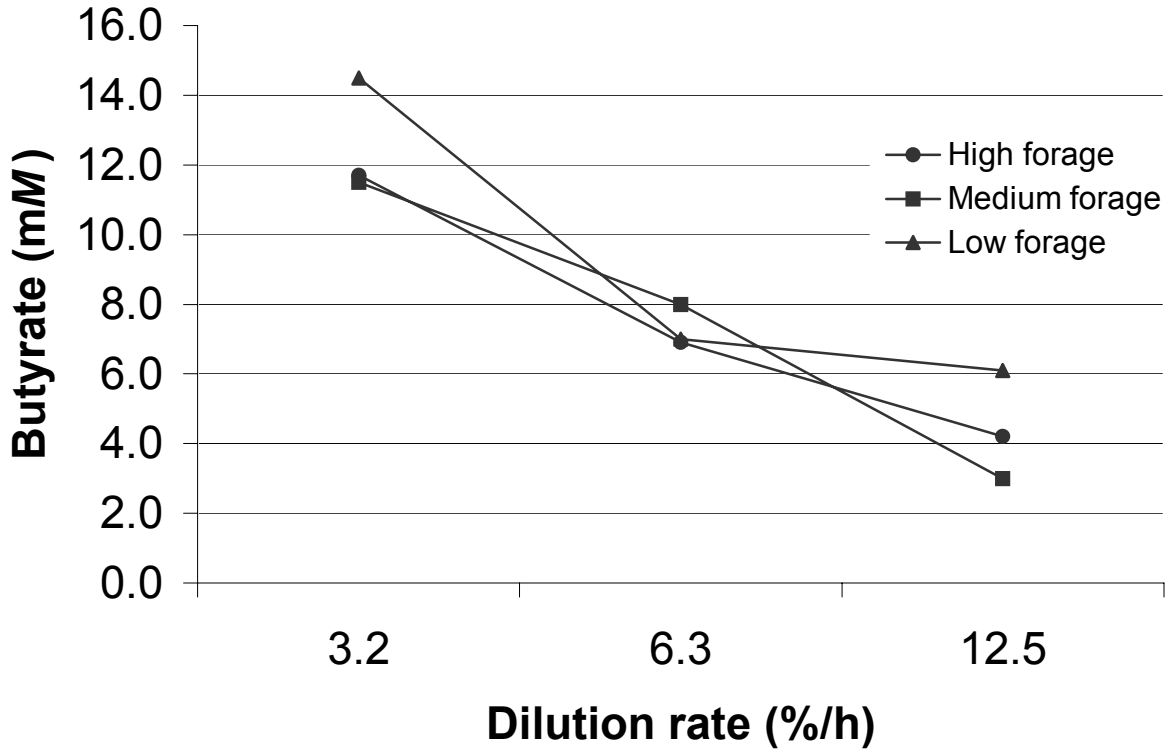

Figure 1. Interaction between dilution rate and forage to concentrate ratio on concentration of butyrate in continuous cultures (High forage = 70% forage and 30% concentrate diet; Medium forage = 50% forage and 50% concentrate diet; Low forage = 30% forage and 70% concentrate diet).

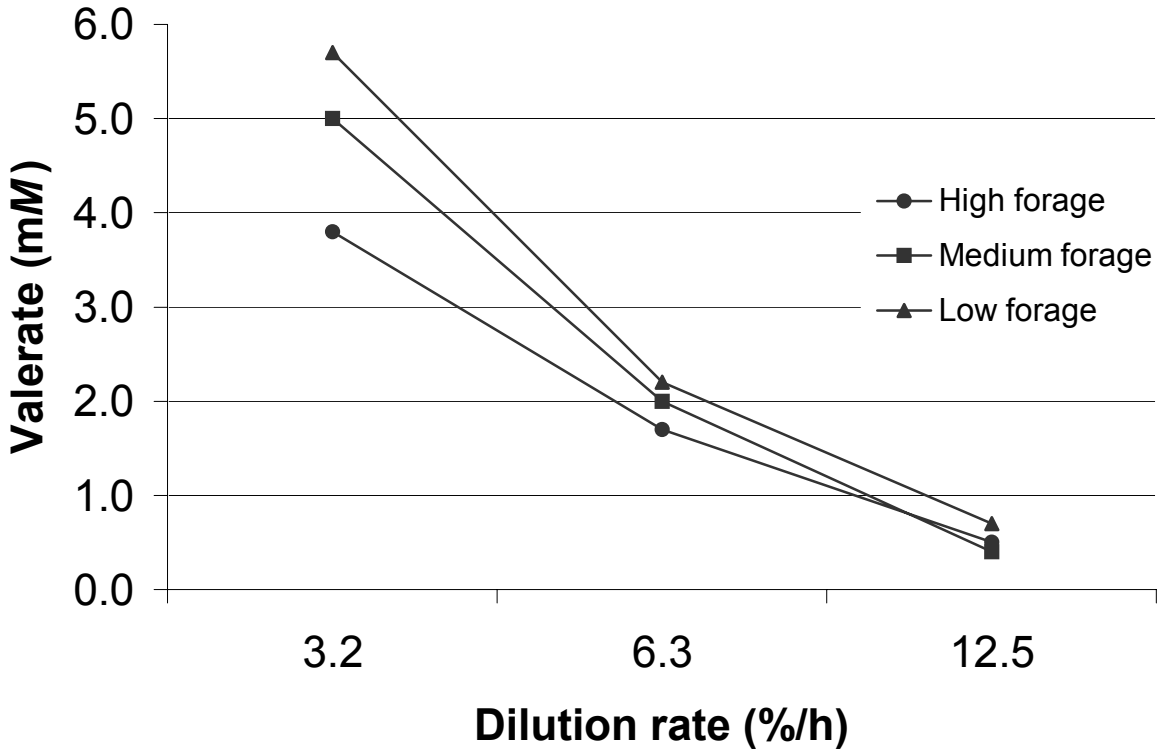

Figure 2. Interaction between dilution rate and forage to concentrate ratio on concentration of valerate in continuous cultures (High forage = 70% forage and 30% concentrate diet; Medium forage = 50% forage and 50% concentrate diet; Low forage = 30% forage and 70% concentrate diet).

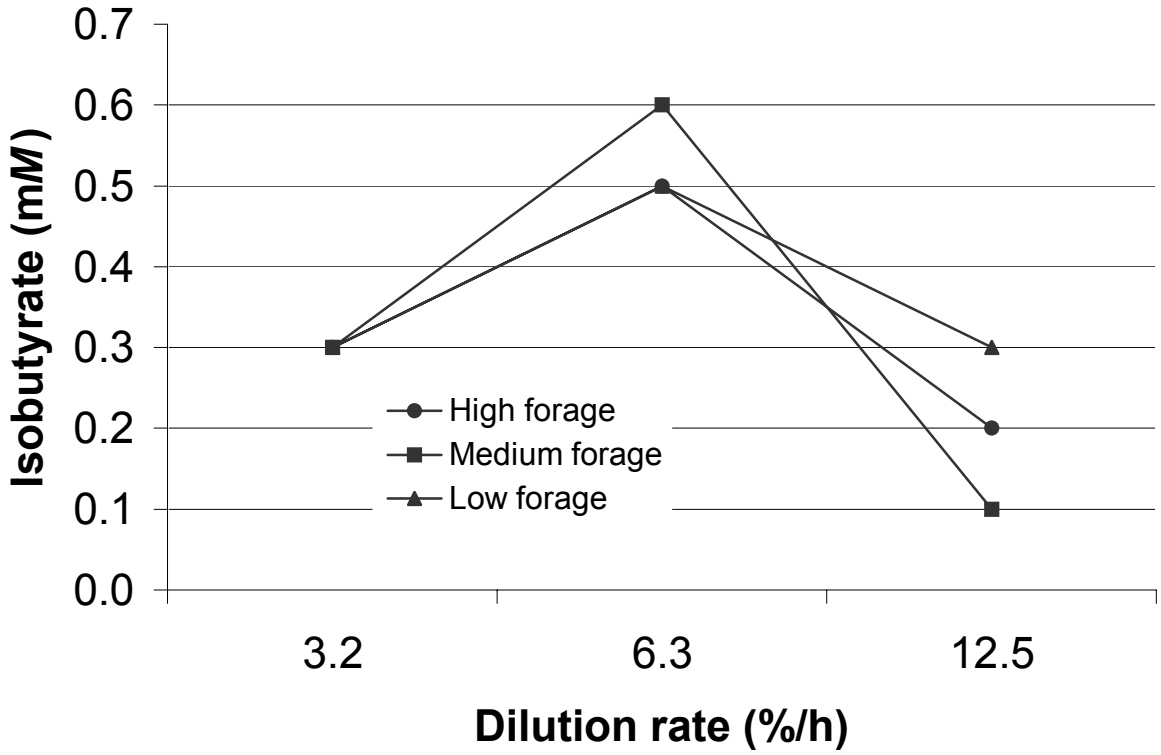

Figure 3. Interaction between dilution rate and forage to concentrate ratio on concentration of isobutyrate in continuous cultures (High forage = 70% forage and 30% concentrate diet; Medium forage = 50% forage and 50% concentrate diet; Low forage = 30% forage and 70% concentrate diet).

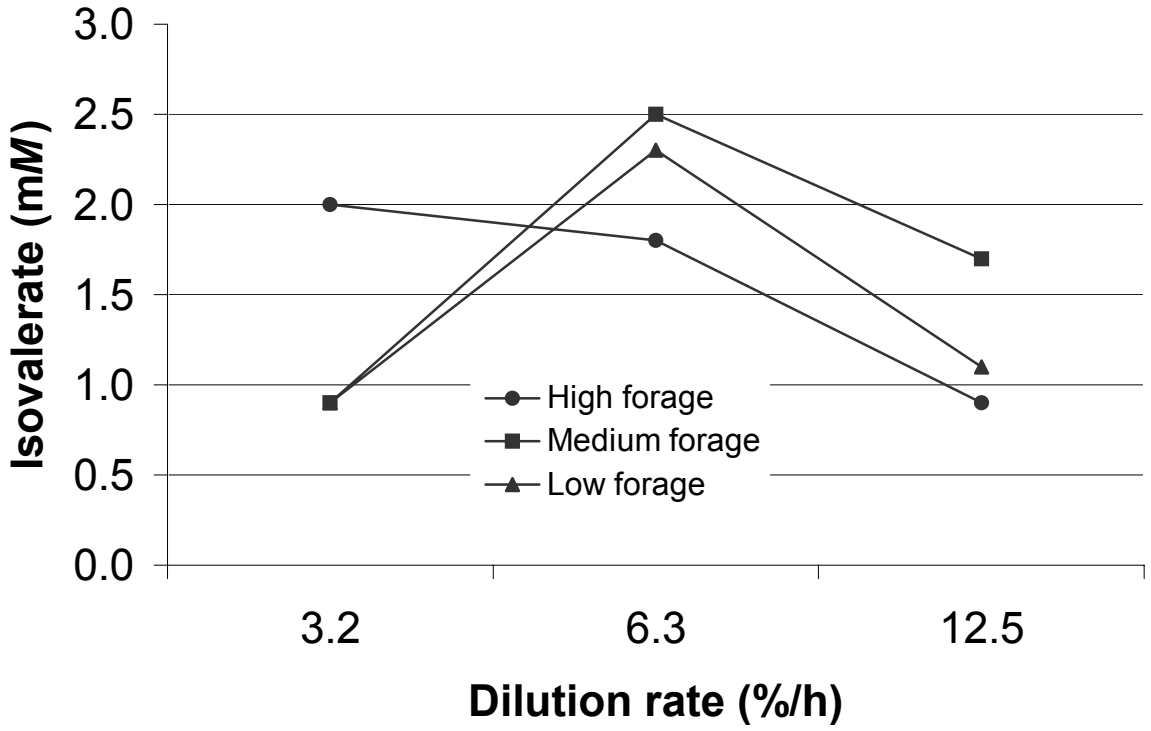

Figure 4. Interaction between dilution rate and forage to concentrate ratio on concentration of isovalerate in continuous cultures (High forage = 70% forage and 30% concentrate diet; Medium forage = 50% forage and 50% concentrate diet; Low forage = 30% forage and 70% concentrate diet).

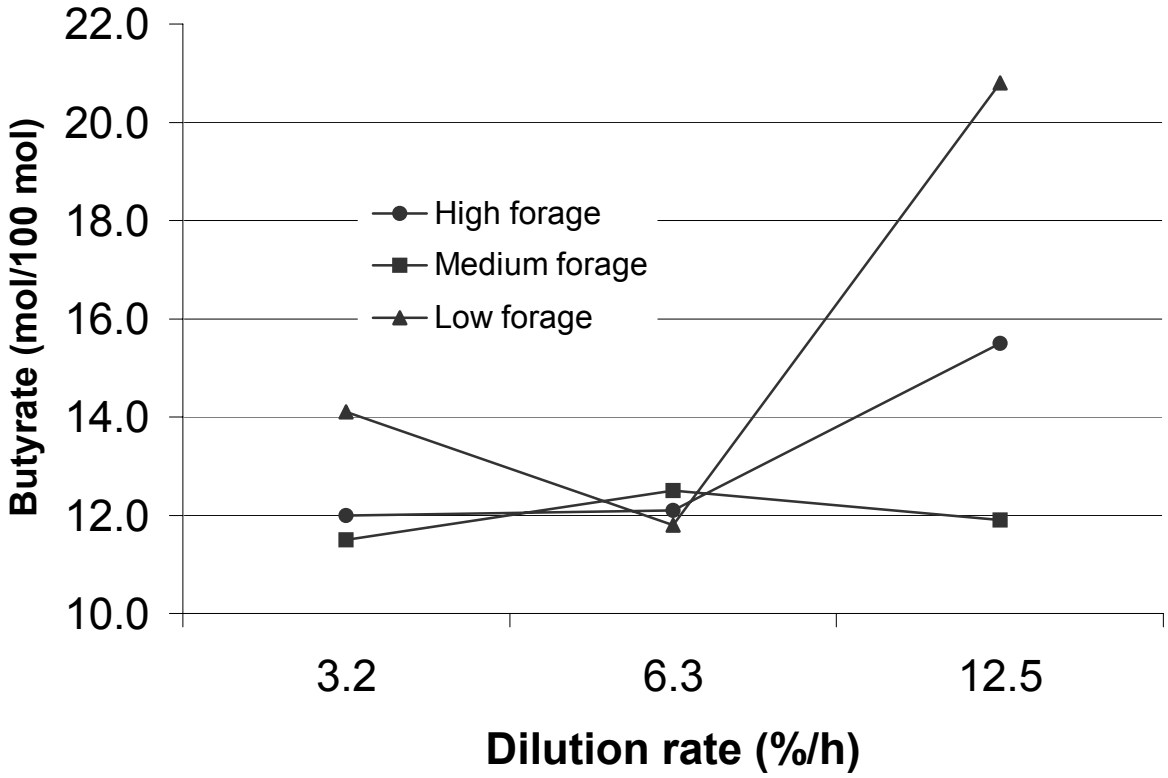

Figure 5. Interaction between dilution rate and forage to concentrate ratio on molar proportion of butyrate in continuous cultures (High forage = 70% forage and 30% concentrate diet; Medium forage = 50% forage and 50% concentrate diet; Low forage = 30% forage and 70% concentrate diet).

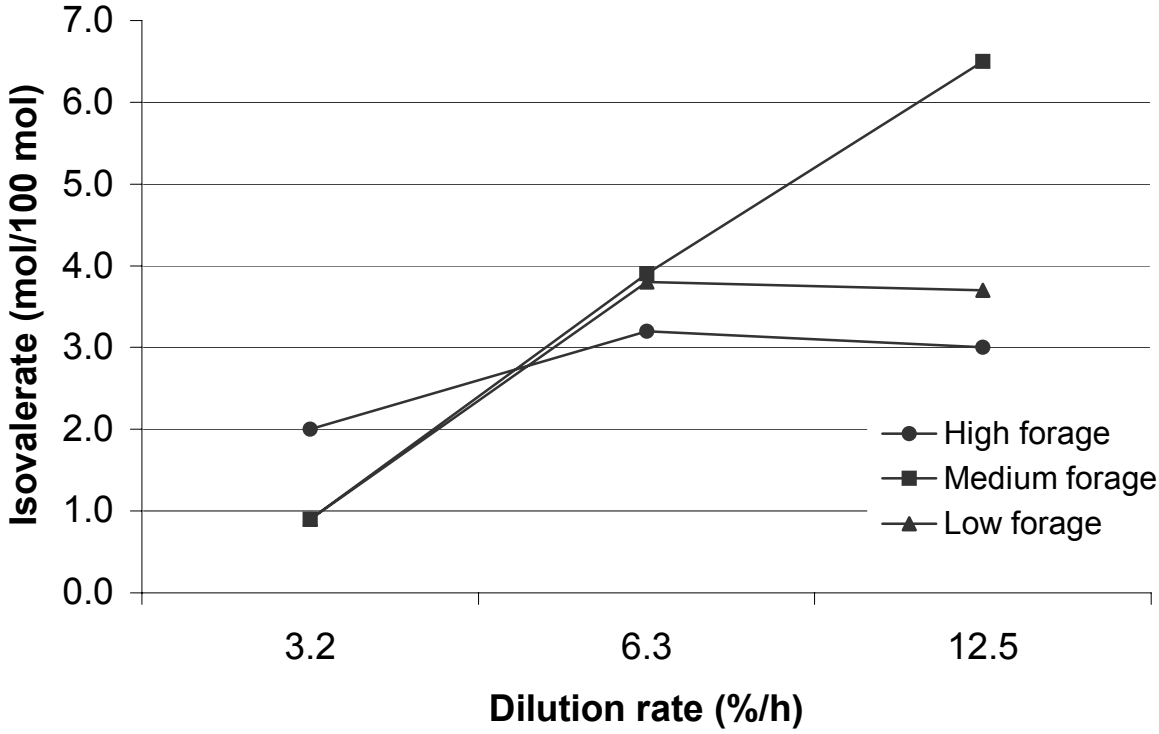

Figure 6. Interaction between dilution rate and forage to concentrate ratio on molar proportion of isovalerate in continuous cultures (High forage = 70% forage and 30% concentrate diet; Medium forage = 50% forage and 50% concentrate diet; Low forage = 30% forage and 70% concentrate diet).

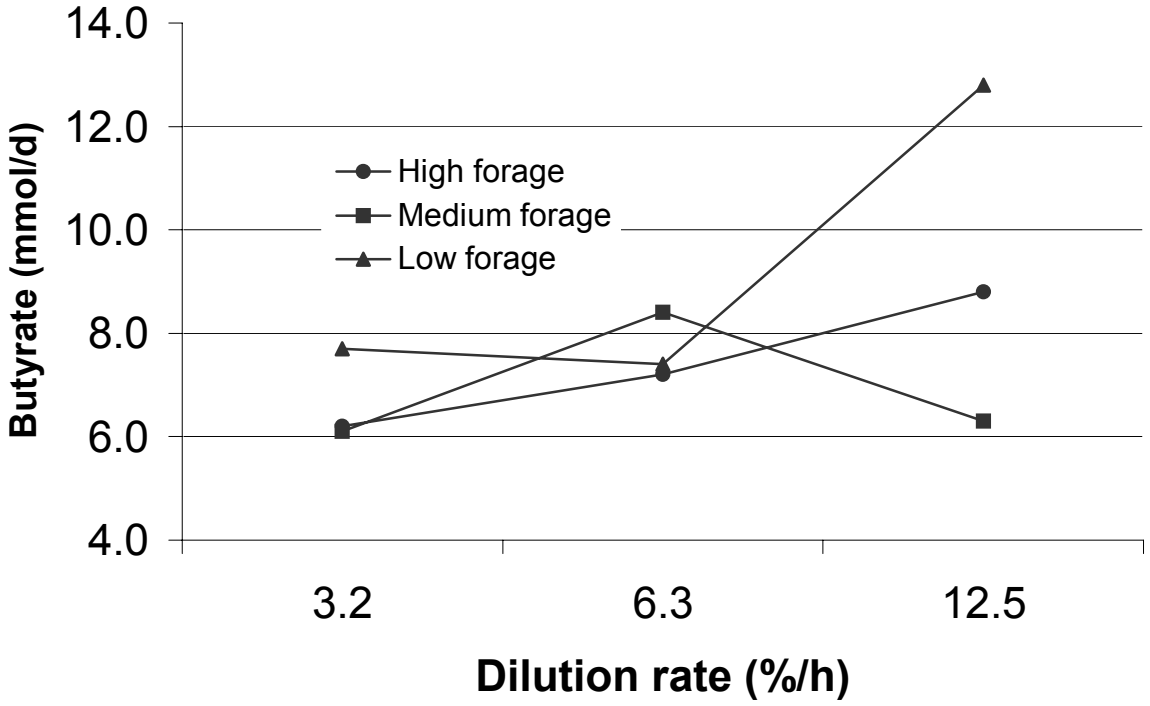

Figure 7. Interaction between dilution rate and forage to concentrate ratio on daily production of butyrate in continuous cultures (High forage = 70% forage and 30% concentrate diet; Medium forage = 50% forage and 50% concentrate diet; Low forage = 30% forage and 70% concentrate diet). Culture samples were taken from corresponding fermentors (nominal volume of 700 ml) and converted to 1 L of ruminal contents.

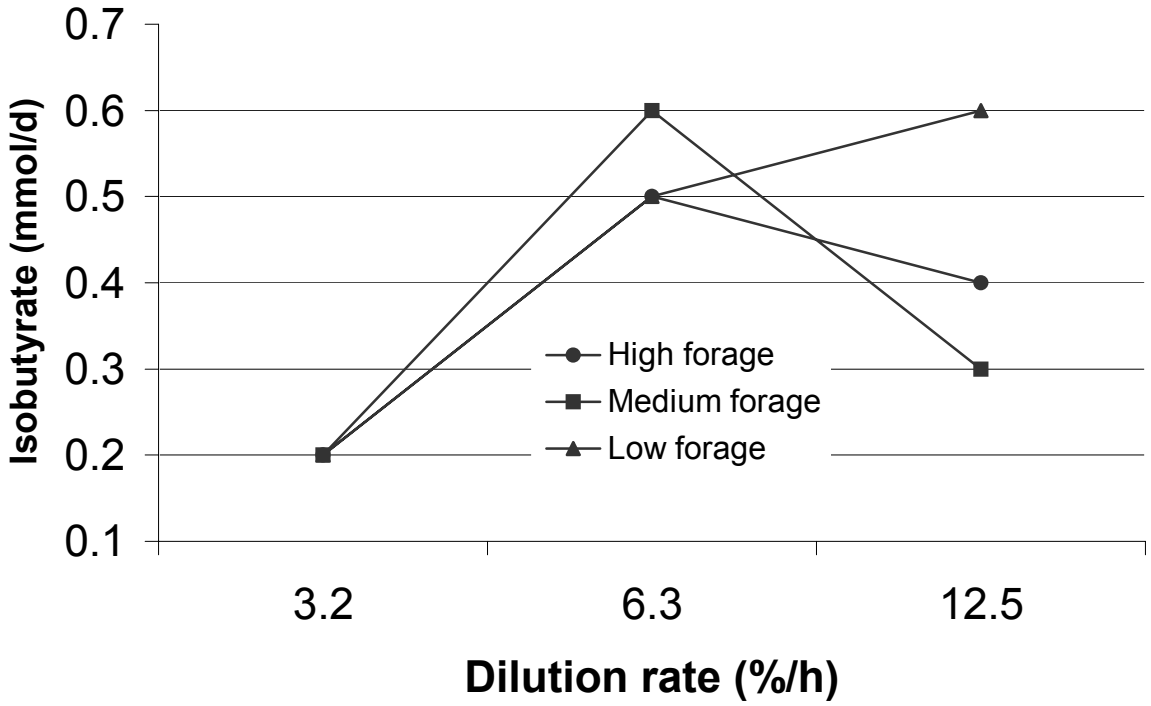

Figure 8. Interaction between dilution rate and forage to concentrate ratio on daily production of isobutyrate in continuous cultures (High forage = 70% forage and 30% concentrate diet; Medium forage = 50% forage and 50% concentrate diet; Low forage = 30% forage and 70% concentrate diet). Culture samples were taken from corresponding fermentors (nominal volume of 700 ml) and converted to 1 L of ruminal contents.

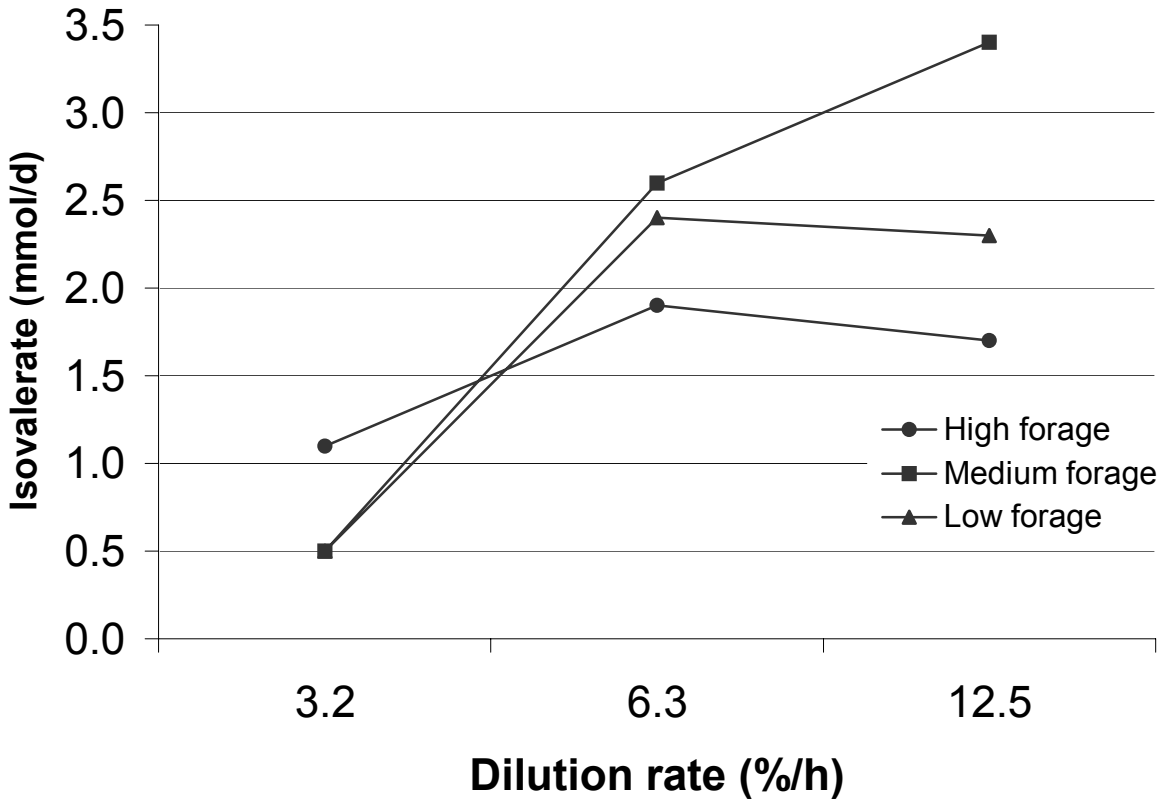

Figure 9. Interaction between dilution rate and forage to concentrate ratio on daily production of isovalerate in continuous cultures (High forage = 70% forage and 30% concentrate diet; Medium forage = 50% forage and 50% concentrate diet; Low forage = 30% forage and 70% concentrate diet). Culture samples were taken from corresponding fermentors (nominal volume of 700 ml) and converted to 1 L of ruminal contents.

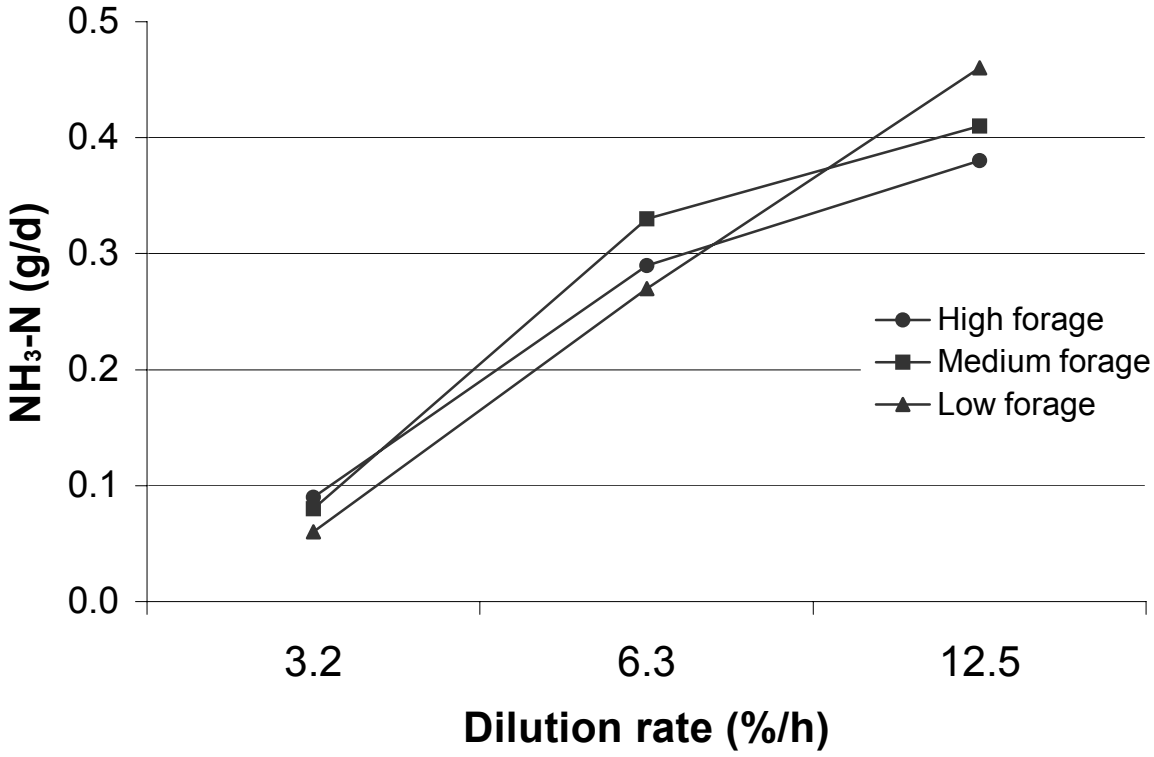

Figure 10. Interaction between dilution rate and forage to concentrate ratio on ammonia-N (NH_3-N) production in continuous cultures (High forage = 70% forage and 30% concentrate diet; Medium forage = 50% forage and 50% concentrate diet; Low forage = 30% forage and 70% concentrate diet).

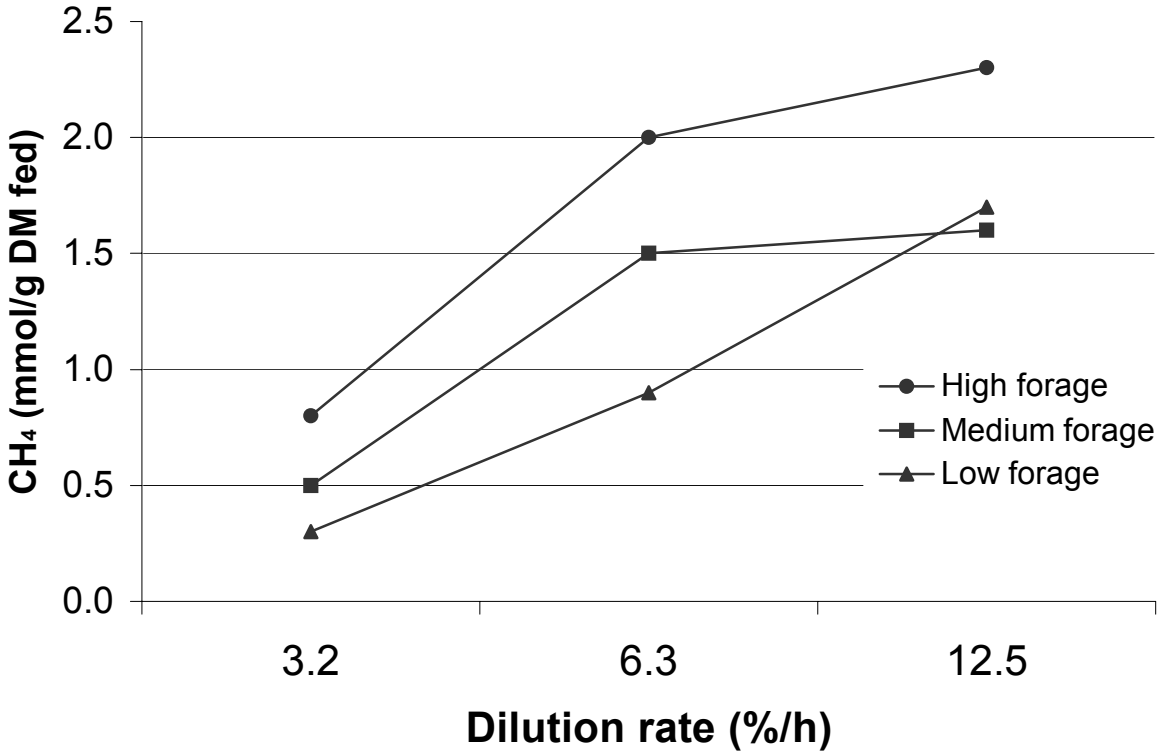

Figure 11. Interaction between dilution rate and forage to concentrate ratio on methane (CH_4) production (per DM fed) calculated from measured concentrations in headspace gas in continuous cultures (High forage = 70% forage and 30% concentrate diet; Medium forage = 50% forage and 50% concentrate diet; Low forage = 30% forage and 70% concentrate diet).

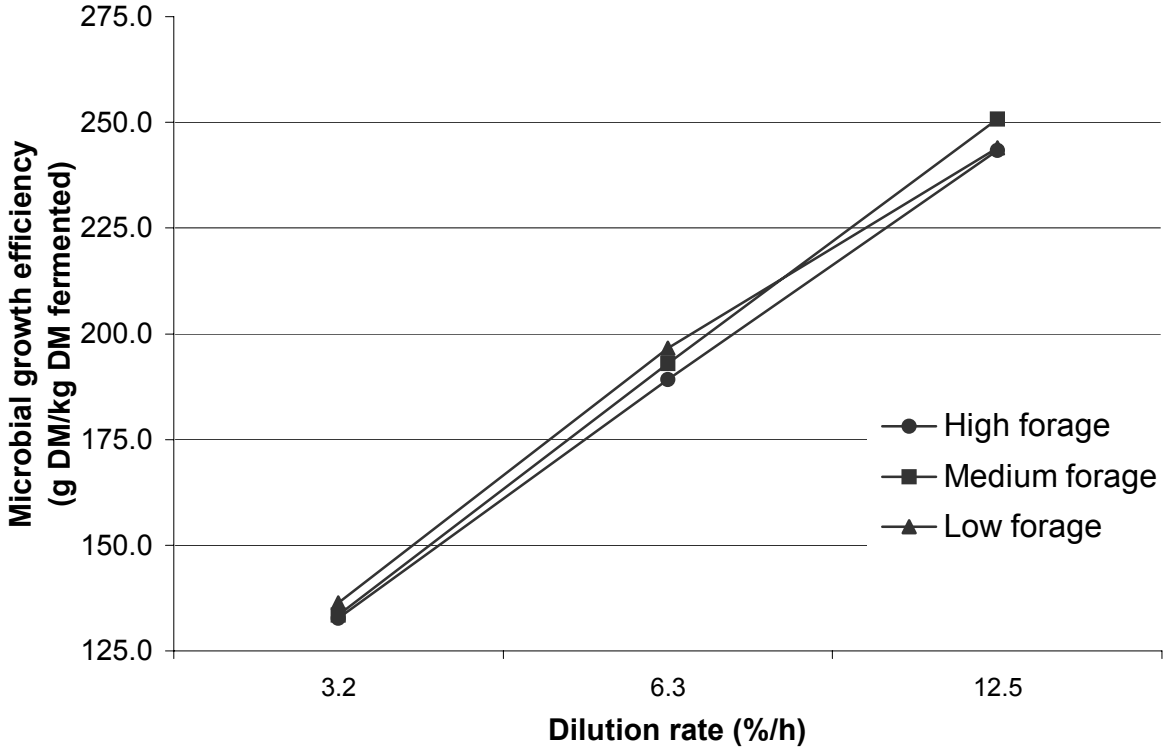

Figure 12. Interaction between dilution rate and forage to concentrate ratio on microbial growth efficiency in continuous cultures (High forage = 70% forage and 30% concentrate diet; Medium forage = 50% forage and 50% concentrate diet; Low forage = 30% forage and 70% concentrate diet).

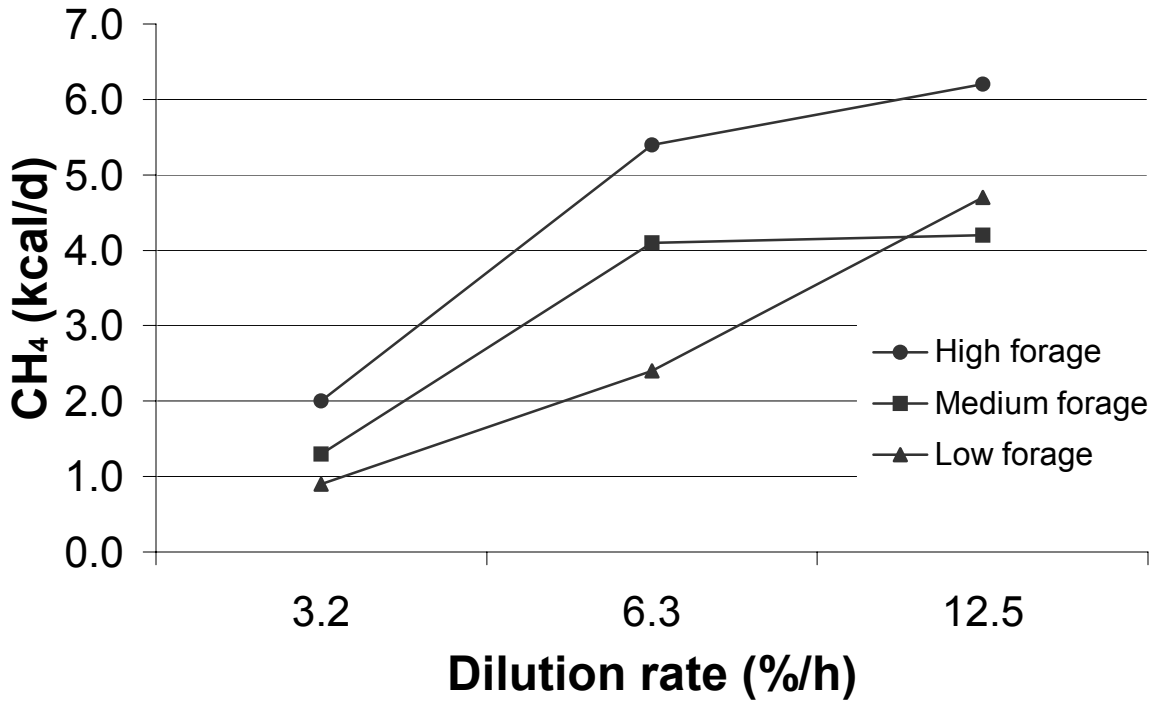

Figure 13. Interaction between dilution rate and forage to concentrate ratio on energetic value of methane (CH_4) calculated from measured concentrations in headspace gas in continuous cultures (High forage = 70% forage and 30% concentrate diet; Medium forage = 50% forage and 50% concentrate diet; Low forage = 30% forage and 70% concentrate diet).

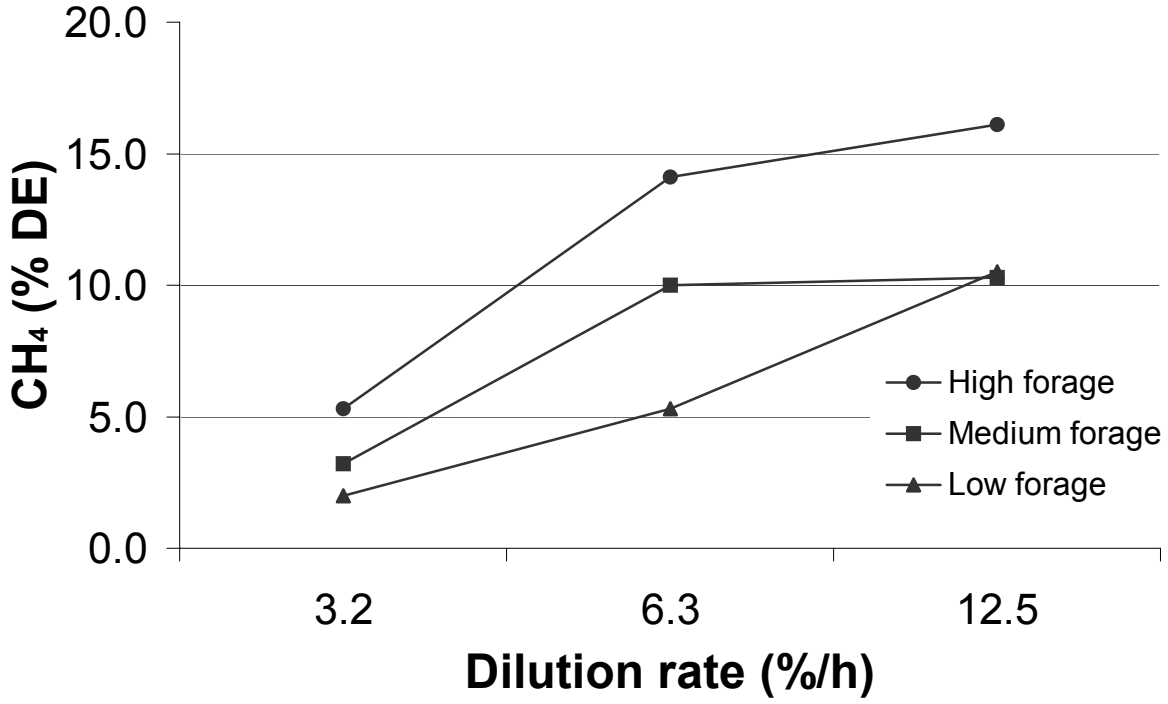

Figure 14. Interaction between dilution rate and forage to concentrate ratio on partitioning of digestible energy (DE) into methane (CH_4) calculated from measured concentrations in headspace gas in continuous cultures (High forage = 70% forage and 30% concentrate diet; Medium forage = 50% forage and 50% concentrate diet; Low forage = 30% forage and 70% concentrate diet).