ABSTRACT

Title of Thesis: NUTRITIONAL AND ENVIRONMENTAL IMPLICATIONS OF STARCH DEGRADABILITY AND NITROGEN FERTILIZATION OF ORCHARDGRASS SILAGE IN TOTAL MIXED RATIONS FED TO LACTATING COWS

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The objectives of this research were to determine the effect of starch and nitrogen (N) availability on microbial protein production and N efficiency, ruminal N efficiency and ammonia, and to assess forage fertilization and grain selection decisions. Diets were incubated *in vitro* batch culture, and fed in a 6 x 6 Latin square *in vivo* digestion trial. Total mixed rations (TMR) contained 50:50 forage:concentrate (dry matter (DM) basis) of second-cutting orchardgrass silage fertilized with 200 (OG200) or 400 (OG400) pounds per acre N, plus concentrate mixes using high to low rumen available starches: barley, corn, and milo. TMR crude protein (CP) was 17% and 18% for *in vivo,* and 20% and 21% for *in vitro* OG200 and OG400 diets, respectively. Synchronous diets were low:low or high:high rumen starch availability:diet N (corn or milo withOG200, and barley with OG400). No effects on ruminal microbial protein synthesis and flow, N flow, or milk production were observed. DM, organic matter (OM) ($P<0.01$), N, and neutral detergent fiber (NDF) ($P<0.02$) total digestibilities

increased with synchronous diets. N digestibility was depressed in diets of low:high rumen starch availability:diet N, due to increased hindgut fermentation adding microbial protein to the feces ($P<0.001$). All OG400 diets had higher fecal N percentage ($P<0.001$). OG400 had higher ruminal ammonia both *in vitro* and *in vivo* ($P<0.05$), and higher total *in vivo* volatile fatty acid (VFA) concentration ($P<0.001$), but rumen pH was stable due to increased recycling of urea. Orchardgrass fertilized at high N can be digested as well as lower N fertilized forages when combined with a rapidly available ruminal starch such as barley, and decrease outputs of fecal DM by up to 401.5 and N by nearly 22 kilograms per year per cow. Crop fertilization and grain selection decisions affect forage composition, rumen fermentation, ration digestibility, and fecal DM and N output.

NUTRITIONAL AND ENVIRONMENTAL IMPLICATIONS OF STARCH DEGRADABILITY AND NITROGEN FERTILIZATION ON ORCHARDGRASS SILAGE IN TOTAL MIXED RATIONS FED TO LACTATING COWS

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INTRODUCTION

As our understanding of nutrient management and environmental sustainability grows, scientists and farmers alike need to be knowledgeable about nutrient balance well beyond the farm gate, including the regional and global impacts of animal operations. The critical need of balancing diets for farm animals has surfaced in the literature at all levels of environmental sustainability research, on a global (Oltjen and Beckett, 1996; Tamminga, 1996), regional (Lanyon, 1992; Freifelder et al., 1998), and farm basis (Meisinger and Thompson, 1996; Jarvis et al., 1996; Kohn et al., 1997). The role of the ruminant animal, and therefore the ruminant nutritionist is critical to advancing the body of knowledge that will make global environmental sustainability possible. The objective of this work is to shed light on the factors that affect nutrient balance with the main-focus of the research presented here being the ecological balance within the stomach of the ruminant itself.

Nutrients of concern for livestock operations include plant nutrients such as nitrogen, phosphorous, and potassium, and air borne pollutants such as ammonia, methane, carbon dioxide, particulate matter and odors (Morse, 1995). Nutrients can be conserved by manipulation of any part of the nutrient cycle: manure, soil, plants, animals, or by manipulation of the entire animal production operation. Kohn et al. (1997) developed a total farm nutrient management model and determined "that improving animal nutritional efficiency would have the greatest proportional impact on total farm (nitrogen) efficiency in most cases" when compared to potential improvements in crop nitrogen uptake or manure nitrogen availability. Sixty-seven to 90% of nitrogen, phosphorous, and potassium ingested from forage by ruminants can

appear in manure (Peterson and Gerrish, 1996; Chalupa et al., 1996), suggesting opportunities for improvement in nutrient utilization and efficiency.

Nitrogen, with its ability to volatilize into the air as ammonia and leach into the groundwater as nitrite or nitrate, is difficult to control and accounts for much of the nutrient loss and pollution in animal operations, particularly manure nitrogen. Overall nitrogen efficiency in ruminant animals is measured by the amount of nitrogen in the diet, compared to the amount of nitrogen captured in animal products such as meat and milk, with the difference constituting a nitrogen loss. Losses of nitrogen from animals can be measured in the form of urea in milk or urine, and fecal nitrogen. Increasing animal nitrogen efficiency could reduce manure (urine and feces, plus bedding material) nitrogen on the farm. Management decisions on the farm on the type and application rates of nitrogen fertilizer on forage crops, and the selection of animal diet ingredients such as grain source, can greatly affect the flow of nitrogen through and out of the farm system due to its volatile nature. Understanding the effects of such decisions, and the science behind them can be used to optimize the whole-farm nitrogen cycle, and ultimately, global sustainability. This work will look at the manipulation of the animal diet, including the fertilization of forage crops and the selection of the grain source to improve ruminal nitrogen efficiency and nitrogen conservation.

The focus of this work is on rumen function and nitrogen efficiency, and how they fit in the context of whole-farm nutrient management decision making. It looks at the potential of the cow to serve as a nutrient management tool, and thus a more efficient converter of human-inedible nutrients such as forage, into human-edible food

through a better understanding of the effect of management changes in forage, fertilization rate, and grain source on the internal rumen environment.

The digestive system of the ruminant is a complex system within a system. The foregut, or rumen, is home to an ecosystem in and of itself, consisting of billions of bacteria, protozoa, and fungi (Van Soest, 1994) living, working and dying together. The rumen is often called the "fermentation vat", as these microbes ferment the food eaten by the animal, either transforming or making available the nutrients it contains. Spent bacteria and protozoa flow from the foregut to the hindgut, and represent the major source of protein to the animal (Van Soest, 1994). The synthesis or growth of these microbes is directly related to the diet consumed by the animal; they are 'what the cow eats'.

The microbes play an important role in the overall efficiency of nitrogen use by the animal. In order for them to utilize available dietary protein nitrogen, there must be an adequate and available supply of energy, or carbohydrate, in the diet. This work evaluates carbohydrate in the form of starch from three different grain sources, barley, corn and milo (sorghum). The overall hypothesis for this research is that ruminal ammonia concentration can be controlled through dietary manipulation of starch and nitrogen sources while maintaining production and increasing animal nitrogen efficiency. This in turn would reduce nitrogen losses in the total farm system, and decrease negative environmental impacts of the farm operation.

The specific objectives of this research are to:

1. Maximize ruminal nitrogen efficiency by manipulation of nitrogen and starch (grain) source to

a. Maximize microbial nitrogen retention (microbial protein synthesis)
b. Minimize ruminal ammonia concentration

2. Assess the impact of management decisions concerning forage fertilization and starch (grain) selection on nitrogen efficiency and performance of animal feeds in the rumen

Chapter 1 is a review of the literature, and examines the importance of ruminant nutrition in the context of global, regional, farm, and animal nutrient balance and sustainability that will drive current and future research. It addresses the global impact of agriculture and the legislative response to environmental concerns, the role of the ruminant in global environmental sustainability, and global implications to animal nutrition strategies. It considers the importance of the whole-farm nutrient management plan as it relates to economics, the environment, and nutrient conservation. The agronomic effects of nitrogen application on forage, ruminant metabolism and microbial protein synthesis, including amino acid delivery to the small intestine are also discussed.

Chapter 2 and 3 summarize an in vitro study and an in vivo digestion trial with lactating cows, respectively. Both studies evaluate the effect of starch sources with different ruminal degradation rates (barley>corn>milo) with orchardgrass silage fertilized at either 200 or 400 pounds per acre of nitrogen on rumen function, including measures of ammonia and volatile fatty acid concentrations, and microbial protein synthesis.

DEDICATION

This work is dedicated to my husband, Stuart, without whom this work would never have been accomplished. His unfailing support and active participation in all phases of the research, as well as his moral and financial support and unwavering encouragement throughout made all the difference. Thank-you for your strength and your patience. This is also dedicated to our girls, Amy and Amanda, who only now are beginning to understand fully the sacrifice they have made. The joy they have brought to my life and the faith they have shown in me is beyond words. Follow your dreams girls, I've got your back. Thank-you too for your strength and patience.

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TABLE OF CONTENTS

INTRODUCTION……...ii

LIST OF TABLES……...x

CHAPTER 1 REVIEW OF THE LITERATURE 1

Agriculture and the Environment..... 1
Global Impact: Societal and Legislative Response 1
Global Sustainability: The Role of the Ruminant..... 2
Global Implications: Farm and Regional Animal Feeding Strategies..... 5
Nutrient Management..... 8
Why Nutrient Management on Farms?..... 8
Economic Viability of Nutrient Management 9
The Nutrient Management Plan and Animal Nutrition 10
Agronomic Considerations: Nitrogen, Animal Nutrition and the Environment 11
Forage Composition: Nitrogen Fertilization and Stage of Maturity..... 11
Implications for Animal Nutrition and the Environment..... 13
Ruminant Metabolism..... 15
Overview..... 15
Ruminant Nitrogen Metabolism 18
The Ruminal Ecosystem..... 23
Microbial Protein Synthesis 30

CHAPTER 2 Orchardgrass *In vitro* Trial……...34

Abstract 34
Introduction 34
Materials and Methods 36
Diets..... 36
Experimental Procedures 36
Batch Culture..... 37
Analytical Procedures..... 37
Statistical Analysis..... 38
Results..... 38
Discussion 39
Conclusion..... 40

CHAPTER 3 Orchardgrass Digestion Trial 41

Abstract 41
Introduction 42
Materials and Methods 44
Diets and Cows..... 44
Experimental Procedures and Sample Collection..... 46

Analytical Procedures 49
Statistical Analysis 52
Results 53
Discussion 57
Conclusion 64

TABLES……66

REFERENCES……..82

LIST OF TABLES

CHAPTER 1 REVIEW OF THE LITERATURE

Table 1-1 Summary of forage composition changes 66
Table 1-2 Types of microbes in the rumen 67
Table 1-3 Roles and end-products of important rumen bacteria 68
Table 1-4 Microbial crude protein in forage dry matter 69
Table 1-5 Summary of energy and nitrogen synchrony experiments 70

CHAPTER 2 *In vitro* BATCH CULTURE STUDY

Table 2-1 Ingredient dry matter percent composition of diet concentrates 71
Table 2-2 Chemical composition of diets 72
Table 2-3 Rumen function measures 73

CHAPTER 3 *In vivo* DIGESTION TRIAL

Table 3-1 Ingredient dry matter percent composition of diet concentrates 74
Table 3-2 Chemical composition of diets 75
Table 3-3 Six by six Latin square design 76
Table 3-4 Intake and digestibility of dry matter and starch 77
Table 3-5 Intake and digestibility of neutral detergent fiber & organic matter 78
Table 3-6 Nitrogen metabolism 79
Table 3-7 Rumen function measures 80
Table 3-8 Body weight, milk production and composition 81

CHAPTER 1

REVIEW OF THE LITERATURE

Agriculture and the Environment

Global Impact: Societal and Legislative Response

The needs of society in the area of environmental and natural resource sustainability reflect a concern for human health as a component of environmental quality. These issues are addressed both in the legislature and through social activism, and are a driving force in current and future research in animal nutrition. Major federal environmental regulations in the United States often serve as the basis for state and regional laws. Federal laws affecting animal agriculture include the Clean Air Act, the Clean Water Act, and the Water Quality Act, among others (Morse, 1995; USDA, 1991). The desired outcome of these regulations is to ensure a safe air and water supply, to limit or eliminate negative effects of human endeavors on delicate ecological systems, and to promote overall sustainability of natural resources.

Assessing the contribution of agriculture to greenhouse gasses and other pollutants is difficult, because they are included in measures of industrial and other sources, and they are from non-point sources. The contributors to global greenhouse gasses from animal operations are methane, carbon dioxide from ruminant animals (Lal et al., 1998; Tamminga, 1996) and nitrous oxide from manure and fertilizer. Overall, agriculture accounts for about 7.3% of total emissions of methane, carbon dioxide and nitrous oxide based on Environmental Protection Agency (EPA) and Department of Energy (DOE) estimates (Lal et al., 1998). Manure and fertilizer are also sources of

nitrogen loss to the environment through leaching and volatilization (Lal et al., 1998; Givens and Rulquin, 2003). Van Horn et al. (1994) stated that in determining losses from manure for fertilizer, nitrogen lost through volatilization and denitrification should be estimated at greater than 50% of the manure nitrogen, with the remaining less than 50% left for crop uptake. Understanding minimum fertilizer needs for forages, and improving ruminal nitrogen efficiency of the diet reduces environmental risks of over-fertilization and excess manure nitrogen in the animal operation (Peyraud and Astigarraga, 1998; Tamminga, 1992).

Global Sustainability: The Role of the Ruminant

Ruminants are unique in that they have two distinct, yet symbiotic metabolic systems, one being the rumen microbial system, and the other the host animal itself. Each system requires the right balance of nutrients for optimal performance, yet the needs of each are different (Chalupa et al., 1996; Van Soest, 1994). Microbes in the rumen have the exclusive ability to convert non-protein nitrogen (peptides, nitrate, and non-essential amino acids) into high-quality protein, while they also are accountable for degrading high-quality protein from the diet. Ruminants have the ability to produce high quality human food from otherwise non-productive land mass, and utilize poor feeds, or feeds not utilized by other animals, including humans (Van Soest, 1994). Understanding the synergy of both the microbial and animal metabolic systems in the ruminant, and improving their combined efficiency for delivering metabolizable nutrients to animal tissues for food production may lead to animal, farm, regional, and global sustainability. This understanding will also help maintain and possibly improve

the unique ruminant metabolic role as global food producer and converter, not competitor (Van Soest, 1994; Oltjen and Beckett, 1996; Dewhurst et al., 2000; Varga and Kolver, 1997; Waldo, 1968).

Oltjen and Beckett (1996) address the concern of ruminants competing for human-edible foods, in particular cereal grains. They state that while ruminants do not require cereal grains, their use in animal diets increases animal production, potentially improving the global conversion efficiency of human-unavailable nutrients, such as forages, to high quality human-edible foods. Additionally, fossil fuel energy required for forage production is lower than that for concentrates (cereal) production (Tamminga, 1996). Oltjen and Beckett (1996) concluded that looking at efficiency of the diet on the global scale, with judicious use of cereal grains, as well as utilization of by-product and other non-competitive food resources for animals will be the most sustainable and practical solution to increasing overall sustainability and high quality food availability for global human consumption. They make the point that to find true global efficiency, the common practice of assigning a single efficiency value to ruminant production must give way to looking at efficiency based on the individual dietary components, and whether or not the components are taken out of the pool of human-edible nutrients.

Oltjen and Beckett (1996) conducted a cost/return analysis (based on Bywater and Baldwin, 1980, and updated based on Baldwin et al., 1992) of different dairy and beef cattle operations, demonstrating an overall increase in food value with ruminant products described below. The net return on a sustainable global basis depends on the type of animal production system. They found, similar to the Kohn et al. (1997)

sensitivity analysis of nitrogen losses from dairy farms, that the efficiency at the global sustainable level was highly dependent on animal diet, and thus, on regional production practices.

One analysis used a 636-kilogram dairy cow producing 8601 kilograms of milk in a standard 305-day lactation on a typical California dairy operation. They calculated 5917 Mcal digestible energy and 256.3 kilograms of digestible protein per year available for human consumption from milk. Subtracting potentially human-edible energy inputs using a cow ration of barley (1555 Mcal) and corn silage (2905 Mcal) yields 4460 Mcal of energy used to produce 5917 Mcal from milk, or 133% efficiency of energy production. Similarly, for human digestible protein production, they calculated potentially human digestible protein inputs from the animal operation using the same cow ration, corn silage (49 kilograms) and barley (40.2 kilograms). Further, they subtracted the value for dry cows (non-lactating, due to give birth) and replacement (new) animals (8.6 kilograms) of digestible protein. This yields 97.8 kilograms of digestible protein used to produce 256.3 kilograms from milk protein plus 13.1 kilograms from cull (sold) cows, for an efficiency of 275%. Changing dietary inputs due to differing effects of season, weather and management, gave even higher efficiencies.

As in the nutrient management plan on the farm, the key is efficiency, though on a global scale. Oltjen and Beckett, (1996) contend that calculations to evaluate the global efficiency of animal operations often incorrectly are based on gross caloric inputs and outputs, without considering the fact that much of the calories consumed by ruminants are not ‘lost’ from humans, instead they are ‘found’. These nutrients are

unavailable (inedible) to humans until the ruminant converts them to edible protein and energy in the form of meat and milk, and thus are a net gain of human nutrient resources. Additionally, the converted nutrients are not only newly available for human consumption; they are of the highest quality in terms of meeting global human nutrient requirements.

Ruminant nutritionists are now focusing on environmental impacts on the farm through nutrient management plans and feeding strategies to improve nutrient efficiency and reduce waste to the environment. As the science moves forward, nutritionists and farmers alike are challenged to maintain environmental sustainability and efficiencies well beyond the farm gate. Careful consideration of the environmental economy of feeds taken out of the human nutrient pool, and possibly looking to the viability and trade-offs of other options such as by-product feeds may become increasingly important in global nutrient efficiency and addressing environmental concerns on the farm, regionally and globally.

Global Implications: Farm and Regional Animal Feeding Strategies

The importance of animal feeding strategies in relation to environmental impact is apparent in the Environmental Protection Agency (EPA) and the United States Department of Agriculture (USDA) National Unified Strategy (USDA/EPA, 1998). It calls for animal feeding operations to have comprehensive nutrient management plans. At the top of the list of key components for such a plan is animal feed management, "Where possible, animal diets and feed should be modified to reduce the amounts of nutrients in manure." (USDA/EPA, 1998). Improving animal nutrient efficiency with

comprehensive feeding strategies that lower nutrient output and environmental impact of manure is a high priority research area identified by the USDA Agricultural Research Service Manure and Byproduct Utilization National Program (USDA ARS, 1998).

Overall nitrogen efficiency in ruminant animals on the farm level is measured by the amount of nitrogen in the diet, compared to the amount of nitrogen captured in animal products such as meat and milk, with the difference constituting a nitrogen loss. The efficiency of animal dietary nutrient utilization is one measure of environmental risk, but must be related to the entire animal operation. For example, the efficiency of nitrogen utilization from corn or grass silage-based diets in dairy cows is low, typically 22 to 28%. Related to this is the fact that animal operations often depend on high inputs of nitrogen fertilizer especially in grass-based systems (Jarvis et al., 1996), intensifying potential negative environmental impacts of the animal operation (Givens and Rulquin, 2003; Jarvis et al., 1995).

Thus, looking at nutritional efficiency alone without understanding the dynamics of the whole-farm operation will not yield the answers needed to reduce negative environmental impacts and maintain economic viability of animal operations. Sustainability of the soil-plant-animal system requires balancing of each component together to limit losses of environmental significant gasses and elements (Tamminga, 1996), including effective management of manure on the farm. An integrated approach looking at the effect of management decisions on the entire animal operation is crucial (Kohn et al., 1997). This is accomplished with a comprehensive farm nutrient management plan that takes all of these factors into consideration.

According to the USDA and EPA, the number of animal units (one animal unit is equivalent to 1000 pounds animal body weight) per dairy farm in the US increased by 93% between 1978 and 1992, while the number of dairy animal feeding operations (AFOs) decreased by over half. In production terms, the USDA and EPA note that the overall number of animal units across all agricultural species in the United States increased about 3% or 4.5 million between 1987 and 1992. This means that animals are consolidated in fewer though larger operations, and has resulted in "the concentration of large quantities of manure and wastewater on farms and in some watersheds" (USDA/EPA, 1998).

> "AFOs can pose a number of risks to water quality and public health because of the amount of animal manure and wastewater they generate. Manure and wastewater from AFOs have the potential to contribute pollutants such as nutrients (e.g., nitrogen, phosphorus), sediment, pathogens, heavy metals, hormones, antibiotics, and ammonia to the environment. Excess nutrients in water can result in or contribute to eutrophication, anoxia (i.e. low levels of dissolved oxygen), and in combination with other circumstances, have been associated with outbreaks of microbes such as *Pfiesteria piscicida*." (USDA/EPA, 1998).

The national goal and performance expectation for animal feeding operations according to the USDA/EPA strategy is for farmers to minimize water pollution from confined animal operations and from manure application on the land through the development of

"technically sound and economically feasible Comprehensive Nutrient Management Plans (CNMPs) to minimize impacts on water quality and health" (USDA/EPA, 1998).

Nutrient Management

Why Nutrient Management on Farms?

Environmental nutrient flow data in the Chesapeake Bay 64,000 square-mile watershed was presented by Boynton, et al. (1995). When the analysis (Boynton et al., 1995) is examined in terms of the amount of nutrients leached into rivers per unit of land, the data favored farms as a major nutrient filter compared to more densely populated regions (Kohn, 1998). Thus, agricultural lands are a vital resource, and effectively managing nutrients on them is a key component in maintaining a healthy, sustainable environment for all.

Following the principles of mass balance, nutrients are neither created nor destroyed. Meisinger and Thompson (1996) explain a critical aspect in management of nutrients is the relative concentration of a given nutrient in a system or sub-unit (sub-system) of a system, since nutrient balance is related to the ability of any one system component to utilize or store it. The sub-units of the animal operation are the animal, manure, soil, and plants. Nutrient management involves nutrients imported into the system such as purchased feed and fertilizer, nutrient conservation within the system, or redistribution of nutrients as exports or losses. Nutrient redistribution can occur within the immediate system or farm operation as in using manure as fertilizer for crop uptake for production of animal feeds, or outside to a more regional or global level as an export of animal or plant products (Lanyon, 1992).

Nutrient conservation is a tool of nutrient management used to maintain, redistribute or reduce nutrients within a system or a sub-unit. The goal of any plan is to maximize overall nutrient efficiency and optimize environmental quality (Meisinger and Thompson, 1996; Goss et al., 1993; Westmoreland Conservation District; Tylutki and Fox, 1997; USDA/EPA. 1998). A simple calculation of the amount of nutrient imported onto the farm, minus losses and exports, expresses the farm nutrient balance, and is the basis of a nutrient management plan. The impact of exported farm nutrients on the regional environment is an integral part of the plan to achieve the societal goal of environmental health and sustainability (Freifelder et al., 1998; Lanyon, 1992, Boynton et al., 1995).

Economic Viability of Nutrient Management

Any nutrient management plan that does not maintain the economic viability of the farm operation is not sustainable (USDA/EPA. 1998; Goss et al., 1993). Farm managers will not adopt practices that will put them out of business. One basic premise of effective plans is that increasing efficiency of use and decreasing losses of nutrients from the system may reduce importation needs, and thus lower overall economic costs of nutrients (Goss et al., 1993; Westmoreland Conservation District). However, implementing new plans and management techniques can be costly initially in terms of labor, facilities, and equipment.

The USDA and EPA outline several programs to assist managers in these initial costs through cost sharing, rental payments for set-aside land, grants, and low interest loans (USDA/EPA, 1998). This financial incentive is important in order to promote

voluntary compliance with the stated goals of the USDA/EPA Unified National Strategy for Animal Feeding Operations (USDA/EPA, 1998). Goss et al. (1993) addresses the importance of financial gains and losses both on and off the farm, and that while this is often the only cost-benefit analysis considered when new environmental laws are debated, the farmer is most concerned with economics on the farm only.

The Nutrient Management Plan and Animal Nutrition

There are many published approaches to developing a nutrient management plan, some more complex and comprehensive than others. The simplest representation of whole-farm nutrient balance is the calculation 'nutrients in' minus 'nutrients out'. Nutrients come into the farm system via livestock or feed or fertilizer purchases and go out via the sale of animals, crops or products, and environmental losses from leaching and volatilization. Meisinger and Thompson (1996) identified four basic steps to nutrient plan development:

1. Define the system and sub-systems
2. Document the (nutrient) inputs and outputs
3. Evaluate potential changes in (nutrient) storage within system components
4. Identify surplus/deficit areas which are
    a. potential (nutrient) loss sites to the environment
    b. potential (nutrient) accumulation/depletions sites (potential monitoring sites)

The USDA/EPA description of a good nutrient management plan states that it contains environmentally sensitive protocols for feed management, manure handling and storage,

land application of manure, land management, record keeping and management of other utilization options (USDA/EPA, 1998).

As stated previously, the potential for improving nitrogen flows in a dairy animal system may be greatest through altering the feeding strategy to improve efficiency (capture of nitrogen in animal products), when compared to the potential improvements through manure, soil or plant management (Kohn et al. 1997). To get a more complete picture of how changes in the diet affect overall nutrient flows in the whole-operation, it is important to look at the feed ingredients as they move through the entire system. Changes in fertilizer application rates on forages intended to feed livestock have implications on ruminal efficiency, in addition to potential fertilizer effects on soil and groundwater. The level of nitrogen application on forage cannot only improve yield, but can alter forage chemical composition, and thus its performance in the rumen (Shingfield et al., 2001; Astigarraga et al., 1994; Valk et al., 1996; Peyraud et al., 1997; Waite, 1970). Further down the management chain of events, decisions on diet formulation and selection of the energy (grain) source are yet other factors that could alter rumen kinetics and nitrogen efficiency (De Visser et al., 1998; Hoover and Stokes, 1991; Petit and Tremblay, 1995; McCarthy et al., 1989).

Agronomic Considerations: Nitrogen, Animal Nutrition and the Environment

Forage Composition: Nitrogen Fertilization and Stage of Maturity

Known to increase both herbage yields and crude protein levels (Peyraud and Astigarraga, 1998; Glenn et al., 1985), nitrogen as a fertilizer is an important component in raising grass forages for dairy cows (Givens and Rulquin, 2003; Journet

and Demarquilly, 1979; Leaver, 1985). Lower forage nitrogen content from reduced nitrogen fertilization increases animal nitrogen efficiency due to the changes in chemical composition of the forage (Shingfield et al., 2001), while increased nitrogen fertilization can increase nitrogen wasted as excess ruminal ammonia converted to urea and excreted in urine and milk. Van Vuuren, et al. (1992) found that ruminal ammonia concentration increased by up to 60% with increased nitrogen fertilization (275 versus 500 kg/ha) on perennial ryegrass (Lolium perenne). Overall grass dry matter utilization by ruminants is inefficient when compared to legumes such as alfalfa, despite a lower lignin fraction in grass. However, efficiency of nitrogen utilization is higher in grass than legumes. Nitrogen fertilization is not required for legumes, and animals generally perform better on them (Glenn and Waldo, 1993). Bertilisson et al. (2001) found higher dry matter intake with clover and clover-grass silage and thus increased nitrogen intake, but overall animal nitrogen efficiency went down when compared to grass alone. Legumes or grasses supplemented with concentrates can increase nitrogen intake and milk production, but nitrogen excretion in waste is also increased (Givens and Rulquin, 2003).

The effects of nitrogen fertilization on the chemical composition of the forage can be somewhat mimicked by reducing the stage of maturity at which it is harvested. As the plant matures, digestibility decreases (MacDonald et al., 1991), after an initial one month period in the spring when digestibility is nearly constant (MacDonald et al., 1991). Digestibility of forages is driven by leaf:stem ratios, the stem more digestible than the leaf in younger plants, while in older plants, the leaf is more digestible (McDonald et al., 1991). Plants fertilized with nitrogen are more succulent, with a

'younger' chemical composition than unfertilized grass of the same age. Stage of maturity and nitrogen fertilization effects from several studies on forage composition and digestibility are summarized in Table 1-1. Looking at these studies, it is apparent that the key to understanding forage utilization lies in changes in cell wall content, due to management decisions regarding nitrogen fertilization, and stage of maturity. Cell wall composition drives the ability of rumen microbes to utilize dietary protein and energy effectively, which in turn drives microbial protein synthesis. As the forage stage of maturity increases, cell wall and lignification of cell wall increases, reducing digestibility.

Implications for Animal Nutrition and the Environment

Farm managers now must add environmental concerns to their decision-making processes, whether dealing with crop or animal management decisions (Chalupa et al., 1996; Valk et al., 1996; Tamminga, 1992; Shingfield et al., 2001). In dairy operations, nitrogen is added to the environment mainly from fertilizer and manure, with additional nitrogen brought into the farm-system in the form of purchased concentrates and protein supplements (Tamminga, 1992). Crop management decisions are inextricably tied to animal nutrition and the environment in real and tangible ways through local and national legislation (Freifelder et al., 1998; Tamminga, 1996; Morse, 1996; Lanyon, 1992).

Freifelder et al. (1998) examined the environmental role of dairy operations and their management decisions on the rural 56,000 hectare Tomales California regional watershed. An initial analysis indicated a steady state where nitrogen inputs from the

atmosphere were roughly equal to the outputs through runoff and groundwater flow, indicating no release or uptake of nitrogen from the system. However, upon a more detailed examination, they found that there must be nitrogen sinks in the system as nitrogen inputs of cows and humans exceeded hydrological outputs by about 2 kilograms per hectare per year. The study of the watershed nitrogen budget included both humans and cattle, with the factors: cattle and human food imports and waste management, and milk export. The contribution of cattle to the budget was affected by the physiological states that alter cattle nutrition, (i.e. age, stage of lactation, and production), and population density. Potential sinks include storage in biomass or soil organic matter, or greater losses due to denitrification than are regained through nitrogen fixation. However, further research is needed to confirm this. Understanding potential nitrogen sinks within a system are important to manage the response of the system to inputs and influences of cattle and humans (Freifelder et al., 1998; Meisinger and Thompson, 1996).

Managers must place a high priority on nitrogen efficiency in decisions concerning forage and feed management, as new environmental regulations require. Legumes have been thought of as environmentally superior since they do not require nitrogen fertilization due to their ability to fix nitrogen. However, efficiency of legume nitrogen use within the animal is generally less than that of grasses despite increased milk production with legumes (Givens and Rulquin, 2003). Legumes and supplemented grass diets are both associated with increased nitrogen intakes as well as increased production, however, as nitrogen intake increases, apparent efficiency of dietary nitrogen retention and milk nitrogen decreases, and is associated with increased fecal

and urinary nitrogen (Givens and Rulquin, 2003; Peyraud and Astigaragga, 1998). Nitrogen fertilizer application on grasses that exceeds plant uptake ability results in runoff and volatilization of nitrogen (Tamminga, 1992; NRC, 2001; Peyraud and Astigarraga, 1998). Thus, the issues of forage production, animal nutrition and production, and environmental concerns seem at odds.

Ruminant Metabolism

Overview

Ruminants have evolved an important ecological niche in their ability to digest feeds that are unusable in humans and convert them to meat and milk (Van Soest, 1994;Oltjen and Beckett, 1996; Dewhurst et al., 2000; Varga and Kolver, 1997). However, their efficiency at transforming these feeds is quite low when considering animal nutrient intake versus product (i.e. meat or milk) nutrient output. Varga and Kolver (1997) reported that only 10 to 35% of energy intake is captured for use by the ruminant animal. Beever and Siddons (1986) state that ruminal nitrogen losses can be up to 30% before digesta reaches the small intestine. The nutrient efficiency is relative, especially when considering the ruminant can digest feeds through fermentation that the human could not utilize at all, converting them into usable, high quality nutrients. Still, with environmental concerns on the rise, it is apparent that such a low conversion rate seen in ruminant digestion could be increased through a better understanding of the mechanisms of ruminant metabolism, and the symbiotic relationship that has evolved between the ruminant and the rumen microbial population.

The ruminant animal gets its sustenance from the products of microbial fermentation in the rumen in the form of volatile fatty acids absorbed across the rumen wall and microbial crude protein. Dietary nutrients escaping rumen fermentation are then subjected to acidic degradation and absorption via the small intestine, which provides energy and amino acids, while hindgut fermentation provides additional volatile fatty acids (Van Soest, 1994; Chalupa et al., 1996; NRC 2001). These routes function together symbiotically for the animal's benefit, however, the nutrient requirements for each route are different and create a challenge in feeding for optimal production and efficiency (Chalupa et al., 1996). Ruminants utilize microbial protein synthesized in the rumen to supply roughly two thirds to three fourths of their amino acid requirements (Satter, 1986; Agricultural and Food Research Council, 1992). Sniffen and Robinson (1987) state as much as 80% of the amino acid requirement comes from microbes.

Fermentation in the rumen is an anaerobic process whereby the microbial population feeds mainly on the host animal's dietary intake of carbohydrates and proteins. Short chain fatty acids for absorption through the rumen wall and microbial cells (microbial protein) are formed for later absorption in the small intestine, and the host animal uses them as energy and protein, respectively. Additional products of fermentation include methane, heat, and ammonia, which represent losses in available energy and nitrogen for the animal (Russell and Hespell, 1981), as well as possible environmental pollutants (Tamminga, 1992). In return, the host is home to numerous species of microbes, and provides them with everything they need, including a constant temperature of 39^{o}C, an outlet for fermentation products toxic to the microbes, constant

buffering, and a supply of urea as a non-protein nitrogen source in saliva or absorbed from the blood directly through the rumen wall (Erdman, 1998, Russell and Hespell, 1981). The types of microbes in the rumen are shown in Table 1-2.

It has been suggested that increasing efficiency of nutrient use in the rumen, i.e. microbial protein synthesis, will reduce nutrient losses that can harm the environment (Tamminga, 1992). Russell and Hespell, (1981) suggested that the type of rumen organisms present control the balance of fermentation products and waste; therefore, the desired ruminal microbes must be cultivated. Varga and Kolver (1997) suggested that one way to accomplish this is through genetic engineering of bacteria to improve fiber digestion, however, this is controversial with consumer acceptance of genetically modified organisms for food production a concern. Additionally, the viability of new organisms added to the rumen ecosystem is not guaranteed.

The complex ruminal ecosystem poses challenges that will only be met by increased understanding of the dynamic interaction between microbes. Some workers looked to by-passing the rumen altogether in favor of small intestinal enzymatic digestion and absorption in the small intestine with "escape" feeds for some of the nutrients, to improve overall nutrient efficiency (Glenn et al., 1977; Serrato-Corona et al. 1997; Knowlton et al., 1998). According to Van Soest (1994), this practice "makes the ruminant more dependent on dietary quality and brings it into competition with non-ruminants." Other workers have sought to understand the animal's amino acid requirements and identify how best to provide them for absorption, whether through microbial fermentation, or by-pass amino acids, or a combined approach (Huhtanen et al., 2002; Lynch et al., 1991; Glenn and Ely, 1984). Still other workers have examined

the idea that optimal nutrient-utilization efficiency lies in timing dietary energy and nitrogen ruminal degradation rates to optimize supply to the microbes, or "synchrony" with mixed results (Casper et al., 1994, 1999; Kolver et al., 1998; Shabi et al., 1998; Hoover and Stokes, 1991; Taniguchi et al., 1994; McCarthy et al., 1989; Casper and Schingoethe, 1989; Van Horn et al., 1985; Petit and Tremblay, 1995). (Also, see Table 1-5.) Before the concepts of cultivation, fermentation supplemented with by-pass nutrients, essential amino acid delivery, or synchrony of substrate degradation theories can be discussed, an understanding of ruminant nitrogen metabolism, the dynamics of ruminal ecosystem and microbial growth (synthesis) is essential.

Ruminant Nitrogen Metabolism

Amino acids generated for absorption in the small intestine by the ruminant come from microbial protein synthesis, protein escaping ruminal fermentation, and from endogenous protein (NRC, 2001; Stern et al., 1994; Van Soest, 1994). For ruminants, microbial protein is considered the highest quality available to meet amino acid requirements (Kalscheur et al., 2000). NRC (2001) protein requirements for dairy cows divide dietary nitrogen fractions into rumen degraded protein (RDP) and rumen undegraded protein (RUP). This approach attempts to characterize the symbiotic relationship of the ruminal ecosystem and the physiologic needs of the ruminant tissues for growth, maintenance, and production.

Ammonia nitrogen from bacterial urease action on dietary or recycled urea and microbial catabolism of amino acids and peptides, is a major rumen available source of nitrogen for microbial protein synthesis (Russell and Hespell, 1981; Kalscheur et al.,

2000). Given the fact that the majority of the host animal's amino acid requirement is met by microbial crude protein, the importance of ammonia nitrogen in the ruminal ecosystem is established. However, excess ammonia in high protein diets is absorbed across the rumen wall and eventually converted to urea by the liver and excreted in the urine. This can have deleterious effects on the environment, as well as represent an economic loss in nitrogen and protein costs to the animal operation (Satter and Slyter, 1974; NRC, 2001; Jonker et al., 1998). Conversely, in low protein diets, endogenous urea is recycled back to the rumen and converted to ammonia for use as a substrate for microbial synthesis, which increases nitrogen efficiency. This can ultimately provide more protein to the small intestines than is in the diet itself (Van Soest, 1994). Satter and Slyter (1974) determined *in vitro* that ammonia nitrogen concentrations above 50 mg per liter of rumen fluid, did not support further increased microbial protein yield, and resulted in an accumulation of ammonia. The start of *in vitro* ammonia accumulation after increasing diet crude protein equivalent to 13% of diet dry matter coincided with reaching the maximum concentration of microbial protein. It can be assumed that similar conditions *in vivo* may result in higher nitrogen concentrations in animal waste.

Considering microbial protein is a major source of amino acids to the ruminant, it is relevant to consider their protein composition. Little has been done to elucidate specific makeup of ruminal microbial protein, but the general nature of proteins and bacterial protein can be considered. Most proteins are classified as glycoproteins, and are covalently associated with carbohydrates. Bacterial cell walls are made up of the glycoprotein, peptidoglycan (Voet and Voet, 1995). Gram-positive bacteria are covered

with teichoic acids, which are glycerol or ribitol polymers linked by phosphodiester bridges. The hydroxyl groups are substituted with d-alanine, glucose, N-acetylglucosamine or other saccharides, and are often terminated in lipopolysaccharides (Voet and Voet, 1995). These amino acid-carbohydrate combinations account for up to 50% of bacterial cell wall dry matter in addition to peptidoglycan. The outer membranes of gram-negative bacteria are a complicated matrix of lipopolysaccharides, proteins, and phospholipids (Voet and Voet, 1995). Therefore, it makes sense that a close interrelationship exists between the availability of proteins, nitrogen and carbohydrates from the diet or as products of fermentation that will be used as substrates for microbial protein synthesis in the rumen. The balance of these available substrates will drive the quantity, quality and efficiency of synthesis of microbial protein.

Intake of nitrogen by ruminants is predominantly from plant sources in the form of forages (leaf and stem protein) and grains (storage or seed protein). Plant nitrogen consists of 60-80% true protein, with the balance as soluble non-protein nitrogen, and a small amount of lignified non-protein nitrogen (Van Soest, 1994). Fresh forage soluble non-protein nitrogen fraction consists of peptides, nitrate, nonessential amino acids, while in fermented feeds such as silage, proteolytic activity during silage fermentation can substantially change the non-protein nitrogen composition. For example, ammonia concentration is increased due to deamination of amino acids during ensiling (MacDonald et al., 1991; Van Soest, 1994).

Rumen fermentation substantially transforms the nutrients fed versus the nutrients ultimately available for absorption in the small intestine. Fermented

carbohydrates, and high quality dietary proteins and non-protein nitrogen are obliterated and replaced with microbial protein and volatile fatty acids (Van Soest, 1994). The resulting amino acid profiles available for absorption in the small intestine will vary based on the diet components. Prior et al., (1981) found that diet composition and digestibility affected ruminal fermentation substrates, and altered the amino acid profile of digesta chyme reaching the duodenum when feeding 100% alfalfa hay versus 10% alfalfa hay plus 90% concentrate. In a recent review, Givens and Rulquin (2003) reported the variation in amino acid profiles of various forages from several studies, and noted that ensiling methods altered their profiles. They go on to report that amino acids in forages and those made into silages, were extensively degraded in the rumen. However, the exact contribution of silages to post-ruminal available protein and amino acids remains unclear.

The efficiency of protein or non-protein nitrogen use for delivering amino acids for absorption and anabolism will depend largely on the availability of energy and nitrogen substrates for microbial protein synthesis, in addition to the amino acid profile of ruminally undegraded protein (Lykos et al., 1997[b]). Too much fermentable energy disrupts rumen function by lowering pH, potentially causing acidosis. Inadequate protein slows fiber digestion and can limit intake. Without adequate fermentable energy, proteolysis followed by deamination of amino acids will occur to provide carbon skeletons for energy anabolism, leaving ammonia as nitrogen waste. Excess ammonia is absorbed through the rumen wall to the blood, which can be toxic. Thus, excess ammonia is carried to the liver and converted to urea for transport back to the rumen via saliva or ruminal resorption, or for excretion in urine or milk. Despite the

established link between available energy and protein and nitrogen metabolism, studies looking at synchronization of carbohydrate and protein have produced mixed results as summarized in NRC (2001) and seen in published reports (Shabi et al., 1998). (Also, see Table 1-5.)

Excess ruminal ammonia enters the blood if the rumen concentration of amino acids and peptides is too high for them to be metabolized as intact amino acids by the rumen microbes. In this situation, rumen available energy is limiting, so some amino acids and peptides are deaminated and the carbon skeletons used for energy by the animal, releasing ammonia. The ammonia diffuses through the rumen wall into the blood, and is carried to the liver, where it is converted to urea. Urea is then filtered out through the kidneys and excreted in the urine (Reynolds, 1992). Urea is a small neutral molecule that can diffuse easily through cell membranes. This results in the concentration of blood urea nitrogen highly correlated to milk urea nitrogen (MUN). Thus, MUN is suggested as a non-invasive tool to measure nitrogen balance in dairy cows (Jonker et al., 1998).

Essential amino acids cannot be synthesized in adequate concentrations by animal tissues to meet needs, and thus are essential dietary components. In the ruminant, the major amino acid source is not from rumen undegraded feed protein, but rather microbial protein. Therefore, determining dietary needs and assessing actual amino acid absorption is complex (NRC, 2001, Van Soest, 1994). Essential amino acids not included in the diet in adequate amounts to meet needs limit animal performance and are termed “limiting”. Studies on limiting amino acids have focused predominantly on lysine and methionine (Schwab, 1996; Armentano, 1994). However,

histadine has also been shown to be limiting in grass-based diets (Huhtanen, et al., 2002; Vanhatalo et al., 1999).

Further research is needed to identify amino acid requirements of the ruminant, which are complex due to the symbiotic relationship between the host animal and the ruminal microbes. Limiting amino acids may vary with changes in diet, altering the amount and possibly the types of amino acids absorbed. Merchen and Satter (1983) reported an increase of nitrogen, amino acid intake, and total amino acid absorption with increasing acid detergent insoluble nitrogen in alfalfa. Changes in intestinal amino acid digestibility with different feeds may also be a factor (Misciattelli, 2001 as reported in Givens and Rulquin, 2003). Attempts (NRC, 1996) have been made to quantify amino acid requirements in beef cattle, but little work on validation of those estimates has been done. This illustrates the need for greater understanding of the ruminal ecosystem and the complex interactions of diet, microbial species, host physiology, and environmental impacts, and the critical role of the ruminant nutritionist in applying that understanding in the future.

The Ruminal Ecosystem

Generally, the current state of research does not adequately address the dynamics of the ruminal ecosystem to make reliable predictions of responses of ruminal microbes, and therefore, the ruminant itself, to dietary manipulations (Dewhurst et al., 2000; Varga and Kolver, 1997; Baldwin et al., 1994; Chase, 1993). The complexity of the relationships between bacteria, protozoa, and fungi, mycoplasma and viruses are not well understood, and the current procedures to examine microbial protein synthesis *in*

vivo are invasive and crude (Dewhurst et al., 2000; Johnson et al., 1998). In addition to limitations in understanding the rumen ecosystem, there is insufficient data on the many different feedstuffs, and the characteristics of their ruminal degradation alone or in combination with other feeds (Dewhurst et al., 2000).

The types of microbes and their relative concentrations in the rumen will vary with changes in the host animal's diet in most cases (Czerkawski, 1986). At the most basic level, there are two general types of microbial populations in the rumen ecosystem. From the nutritional point of view, these are primary (dietary) nutrient fermentors, and secondary nutrient fermentors. Secondary nutrients are the products of the primary nutrient fermentation (Van Soest, 1994). Primary nutrients include dietary fiber (cell wall: cellulose, hemicellulose, pectin, cell wall-bound proteins), proteins, soluble sugars and proteins, and dietary and recycled sources of non-protein nitrogen such as ammonia and urea. The products of primary fermentation that feed the secondary fermentors include formic acid, acetate, propionate, butyrate, ethanol, carbon dioxide, hydrogen, succinate, and lactate. The role and fermentation end products of some important rumen bacteria are listed in Table 1-3 (Russell and Hespell, 1981; Van Soest, 1994). The relative concentrations of these end-products vary with diet, and affect the health and efficiency of ruminal digestion. The general classes of microbes in the ruminal ecosystem that we have some understanding of are the most populous bacteria, the protozoa, and the fungi (Russell and Hespell, 1981; Van Soest, 1994; Sniffen and Robinson, 1987). (See Table 1-2.).

It has been argued that protozoa are not required for proper rumen function, as functioning defaunated rumens have demonstrated. While they are less in number than

ruminal bacteria, they are relatively equal in volume (10-50%) to ruminal bacteria (Van Soest, 1994; Czerkawski, 1986; NRC, 2001). Therefore, it is more likely their role is important and worthy of research (Czerkawski, 1986). They remain in the rumen for long periods, and their products of fermentation are similar to bacteria. Protozoa prey on bacteria, sequestering nutrients in the rumen; and they engulf food particles such as starch, effectively lowering post-ruminal microbial protein supply through predation and competing for substrates, reducing overall microbial efficiency (NRC, 2001; Van Soest, 1994; Sniffen and Robinson, 1987). Protozoa are proteolytic, but unable to utilize ammonia for amino acid synthesis (Van Soest, 1994). While this may seem to be a negative contribution, it has been suggested that the role of the protozoa in the ruminal ecosystem may be that of a metabolic buffer, providing nutrient storage in times of plenty, and nutrient release (lysis) in times of depravation (Czerkawski, 1986). Engulfing starch particles in high concentrate diets may contribute to maintaining ruminal pH, as high concentrate diets with rapidly degradable starch are associated with low pH, with reduced ruminal efficiency, and in the extreme, with acidosis (Van Soest, 1994; Russell and Hespell, 1981).

Protozoa may play a role in maintaining balance in the ruminal ecosystem and may be important to optimizing ruminal fermentation. Defaunated animals given high protein diets released more ammonia than ruminants with protozoa (Czerkawski, 1986), and have shown reduced fiber digestion (Varga and Kolver, 1997). In comparing mixed rumen bacteria and protozoa fed various protein sources, the bacteria consistently produced more ammonia (Nocek and Russell, 1988). Others have found that defaunation decreases ruminal ammonia due to the absence of protozoan deamination of

amino acids (NRC 2001). In a recent review, Dewhurst et al. (2000) noted that efficiency of microbial protein synthesis could be improved by defaunation due to reduced microbial maintenance costs. Before the potential to manipulate protozoa for possible animal and environmental benefits is defined, more research into their role and function in the ruminal ecosystem must be done (Van Soest, 1994; Russell and Hespell, 1981).

Fungi were not discovered in the ruminant stomach until the 1970's. They were difficult to find because fungi firmly attach to the fibrous portion of the diet and were often filtered out in early work (Van Soest, 1994). They can penetrate cell wall with their rhizoids, which resemble roots. The rhizoids secrete a highly soluble cellulase enzyme, and transport nutrients to the main body of the fungi, the sporangium. This enables fungi to access the more soluble nutrients, and results in the eventual disruption of the cell wall making nutrients available to the wider rumen population (Van Soest, 1994; Varga and Kolver, 1997; Sniffen and Robinson, 1987). Fungi are slow growing, make up only 8% of the microbial mass in the rumen (Varga and Kolver, 1997), and therefore do not contribute significantly to microbial protein synthesis. However, their indirect contribution by degrading tougher cell wall components of the diet and making more soluble nutrients available to bacteria is unknown.

Wanderley et al, (1999) conducted an in situ study to examine the extent and kinetics of microbial colonization of forage particles in cows and camels. The animals were ruminally cannulated and were fed 35% of diet dry matter from either dry-rolled or steam-flaked sorghum plus alfalfa hay for the cows; and camels were fed 25% of diet dry matter from barley grain and Rhodes grass hay. Radiolabeled nitrogen (^{15}N) was

incorporated into corn plants via fertilization of the soil it was grown in with labeled nitrogen fertilizer ($(^{15}NH_4)_2 SO_4$). Rumen degradation and nitrogen metabolism was assessed with whole labeled corn incubated in situ. Wanderly et al., (1999) found that rumen bacterial and fungal colonization of forage particles was substantial in both cows and camels, and that colonization "exerts considerable impact on situ estimates of nitrogen degradation". Their results are summarized in Table 1-4. The significance of any interaction or synergy between adhering bacteria and fungi was not described in that study, although the researchers assumed the contribution to colonizing microbes biomass by fungi was only 1-4%. The most recent dairy NRC (NRC 2001), states that little is known about the role of fungi in protein degradation, and considers the role is likely negligible due to their low concentration in ruminal digesta.

Understanding the fermentation characteristics and products of individual microbes will shed much more light on fermentation end product concentrations in the rumen. Perhaps only the painstaking and time-consuming development of individual models for each class or species of microbe (Russell and Hespell, 1981), followed by aggregation of the simpler models (Baldwin et al., 1994) may finally reveal the complete dynamic system of rumen digestion (Russell et al., 1992). Knowing well the individual microbial producers of these end-products, and how they interact within the rumen, in addition to quantifying their numbers, might enable prediction of the ruminal response to any given diet (Russell and Hespell, 1981). Russell et al. (1992) explain that historically, that microbial fermentation in the rumen has been assessed empirically, with the rumen treated as a "black box". However, if ruminant nutrition is to advance beyond continual testing of inexhaustible diet combinations, the details of

the fermentations must be determined (Russell et al., 1992; Russell and Hespell, 1981). The empirical understanding and lack of mechanistic understanding of factors in rumen fermentation are likely factors in the difficulty in explaining of mixed results of numerous experiments attempting to synchronize energy and protein availability to maximize microbial protein synthesis (Table 1-5) (Russell et al. 1992; Dewhurst et al., 2000). This is described in more detail below.

The composition of the microbes that flourish with a particular diet ultimately affect the amounts and types of volatile fatty acids, carbohydrates, amino acids and other nutrients absorbed by the animal (Stern et al., 1994; Van Soest, 1994,). In conjunction with this fact, models of tissue absorption of nutrients, and related physiological events affecting rumen function and nutrient uptake are critical to complete understanding (Baldwin et al., 1994), and would have to be aggregated with the rumen model. This more complete picture will be the forerunner to development of models that can accurately predict rumen function (Baldwin et al., 1994; Sniffen and Robinson, 1987; Russell and Hespell, 1981). As research is designed and models developed, even on the microbial and tissue cellular levels, the global and regional perspective must be incorporated into their use in order for these new animal nutrition decision tools to meet the challenges of economically and environmentally sound and sustainable animal operations in the future.

Attempts to incorporate dynamic, mechanistic models of ruminant fermentation to predict animal responses to a given diet have been published; and two are described briefly here in terms of the types of rumen microbes accounted for in the models. Both are a part of a larger model of total ruminant metabolism, nutrient absorption and

balance. These models are the NC-185 Regional Research Program on Metabolic Relationships in Supply of Nutrients for Lactating Cows (Baldwin et al., 1994) and the Cornell Net Carbohydrate and Protein System (Russell et al., 1992). Neither of these models account for the complete diversity of species known to exist in the rumen, however they do incorporate the characteristics of major niches, or functional groups of microbes. In the NC-185 model, hemicellulolytic and cellulolytic bacteria are handled separately, and the difference in their volatile fatty acid production reflected (Baldwin et al., 1994). In the Cornell model, rumen bacteria are broken down into two groups, those that ferment structural carbohydrates, and those that ferment non-structural carbohydrates. Adjustments are made for the level of protozoa that may proliferate on a given diet and affect the efficiency of microbial protein synthesis, and microbial substrate preferences are accounted for (Russell et al., 1992). Both models incorporate microbial maintenance costs, and rates of degradation of dietary ingredients. Passage rates of dietary components are the most difficult aspect of dynamic modeling in ruminant nutrition (Baldwin et al., 1994). While degradation rate is important, it is affected by the passage rate, which is in turn regulated by the chemical composition of the feed, intake, and processing (Russell et al., 1992). Overall digestion of feed not fermented in the rumen is directly affected by its digestibility in the small intestine, which varies by feedstuff (Russell et al., 1992). Additionally, the historical use of total digestible nutrients to predict rumen microbial synthesis is inaccurate because of variability in the site of digestion, rumen versus post-ruminal, as increased post-ruminal digestion is inversely related to microbial protein synthesis (Russell et al., 1992). In the NC-185 model (Baldwin et al., 1994), the dynamics of water flow with starch and small

particles, as well as dry matter intake are incorporated into the model to attempt to account for passage rates. In the Cornell model (Russell et al., 1992), passage rates are entered manually, based on dietary components and published guidelines to determine the correct rates.

Microbial Protein Synthesis

Dewhurst et al. (2000) in a recent review succinctly related the yield of microbial biomass to substrate availability and to the maintenance costs of microbes, which is directly related to the microbial growth rate. The slower the growth rate, the higher the maintenance cost with a concurrent reduction in microbial cell yield per amount of carbohydrate fermented. Increasing growth rates amount to increasing dilution rates of bacteria, or increased passage of microbes out of the rumen. Increased washout of microbes results in less time in the rumen, and a reduction in maintenance costs of microbes (Robinson et al., 1985). This environment lowers the mean age, death, and predation rates of bacteria, and improves the efficiency of microbial protein synthesis (Van Soest, 1994).

In addition to syntrophic (cross-feeding and symbiotic relationships between classes of microbes), utilization of primary fermentation products (cross-feeding) and predation of bacteria by protozoa, Russell and Hespell (1981) described the factors that determine rumen ecology to include microbial growth rates, substrate affinities and preferences, cell yields and maintenance, tolerance to low pH, cell lysis, properties of the feed, and rumen dilution rates. Similarly, the National Research Council (1985) explained that microbial growth is dependent on substrate availability including pattern

of feeding, composition and rate of degradation of feed, and pH in the rumen, although these were not accounted for in the NRC model (NRC 1985). According to Sniffen and Robinson (1987), factors involved in maximizing microbial protein yield include microbial growth, microbial recycling in the rumen, liquid/particulate passage kinetics, rate/extent of digestion of feed, microbial association with feed, and interaction of bacteria, protozoa, and fungi in the rumen.

Microbial recycling (microbial lysis/death and degradation/utilization by live microbes) in the rumen and liquid/particulate passage kinetics is determined by the time microbes spend in the rumen. This in turn is dependent on rumen volume and passage interaction with particle size (NRC, 1985) and microbial association with feed. Microbes in close association with solids, or those that stay on the border region tend to be retained, while those in free suspension or in the shuttle compartment flow out more rapidly (Sniffen and Robinson, 1987).

Maintaining the proper ruminal environment is critical to optimizing efficiency. Microbes require energy and nitrogen to synthesize microbial protein efficiently. An imbalance in the availability or utilization of these nutrients can cause microbial protein yields to decrease, and potentially damaging nutrient losses to the environment that need to be managed (Tamminga, 1992). If there is a deficiency or under-utilization of crude protein, carbohydrate digestibility can decrease, or inversely, if there is a deficiency of carbohydrate amounts or availability to match dietary protein, excess ruminal ammonia nitrogen is produced and subsequently lost from the system as urea (Kalscheur et al., 2000; Nocek and Russell, 1988). In addition to the balance of energy and nitrogen availability, forage to concentrate ratio and particle size impact microbial

protein yield. Too much concentrate or too small a particle size in the forage reduces the effective fiber content, and promotes lactic acid producing bacteria with readily available starch from grain. Additionally, high concentrate diets lower rumen retention time and rumination thus reducing saliva (ruminal buffer) output, ruminal pH and outflow, which can lead to acidosis, increased propionate and a decreased acetate:propionate ratio, possibly reducing milk fat (NRC, 1989).

The observations of diet responses in the literature will be much more powerful when the microbial and physiological mechanisms that drive them are more fully understood (Baldwin et al., 1994; Russell and Hespell, 1981). Several approaches in the literature manipulate ruminal ecology toward improving nutrient utilization and economic and environmental sustainability of animal operations. These include cultivation of desired microbial species (Russell and Hespell, 1981) manipulation of ruminal fermentation and utilization of by-pass feeds (Glenn et al., 1977; Serrato-Corona et al. 1997; Knowlton et al., 1998), and focusing on delivery of essential amino acids to the small intestine (Huhtanen et al., 2002; Lynch et al., 1991; Glenn and Ely, 1984). Finally, mixed results have been achieved attempting to synchronize energy and protein substrates through dietary manipulation (Casper et al., 1994, 1999; Kolver et al., 1998; Shabi et al., 1998; Hoover and Stokes, 1991; Taniguchi et al., 1994; McCarthy et al., 1989; Casper and Schingoethe, 1989; Van Horn et al., 1985; Petit and Tremblay, 1995). In a review, Dewhurst et al. (2000) summarized the results of several experiments of various approaches testing the synchrony theory (Table 1-5), and found little consistency in attempts to synchronize energy and nitrogen sources for ruminants.

Perhaps the best evidence that synchrony is important is the wide success and acceptance of the total mixed ration (TMR) in the dairy industry. The TMR, on a gross scale, provides nutrients to rumen microbes in a more timely manner than the old days of feeding concentrates and forages separately. The impact the TMR has had on the dairy industry to improve milk production in dairy cows is common knowledge. However, current pressures to decrease environmental impact of dairy cattle operations by manipulating the diet to alter fermentation end products, and thus alter nutrient excretion have so far not succeeded. Attempts to fine-tune the synchronization of nutrients on the microbiological and even the animal tissue levels, go well beyond the gross level represented by the TMR, and seem beyond our current understanding of the variables of feed, of individual animals and their ultimate requirements especially for amino acids, and of the rumen environment itself.

CHAPTER 2

Nutritional and environmental implications of starch degradability and nitrogen fertilization on orchardgrass silage in total mixed rations *in vitro*

Abstract

Efficiency of ruminal nitrogen metabolism in the dairy cow affects whole farm nutrient balance by decreasing losses of ruminal nitrogen as ammonia. The objective of this experiment was to determine the effect of forage fertilization and starch source on ammonia production by ruminal bacteria in batch culture. Diets were formulated to meet National Research Council (1989) requirements for a 700-kilogram cow producing 40 kilograms of milk per day. Diets combined 50% dry matter from orchardgrass second cutting silage fertilized with nitrogen at either 200 (OG200) or 400 (OG400) pounds per acre, and 50% dry matter concentrate containing either 62% barley, corn or milo. Starch degradation rates (k_d) are 24.2, 4.0, and 3.6 for barley, corn and milo, respectively (Tamminga et al., 1990). Diets were incubated using batch culture techniques for up to 48 hours. Ammonia concentration increased with increased fertilization ($P<0.0001$). There was no effect of treatments on pH, molar proportions or total concentration (mM/L) of volatile fatty acids. Ammonia accumulation was lower, and therefore nitrogen use more efficient with reduced nitrogen fertilization.

Introduction

Increasing animal nitrogen efficiency could reduce manure nitrogen on the farm (Kohn et al., 1997). The availability of energy and nitrogen substrate for rumen microbial synthesis will affect the efficiency of protein or non-protein nitrogen use for

delivering amino acids for absorption in the small intestine, in addition to the ruminally undegraded protein amino acid profile (Lykos et al., 1997[b]). Too little energy in the rumen increases ammonia concentration and waste as proteins are used for energy, while too much energy lowers pH and can cause acidosis. Inadequate rumen available protein can slow fiber digestion and limit intake.

In forage grasses grown for dairy cows, nitrogen fertilization increases yield and crude protein concentration, and can reduce forage starch (Peyraud and Astigarraga, 1998; Glenn et al., 1985). Rumen ammonia concentration and nitrogen losses in urine increase as forage crude protein increases, and these losses are potentially harmful to the environment (Van Vuuren, et al., 1992). In contrast, reduced nitrogen fertilization rates lower forage nitrogen while increasing animal nitrogen efficiency due to the changes in chemical composition of the forage (Shingfield et al., 2001).

In the present experiment, orchardgrass silage fertilized from two plots fertilized with different levels of nitrogen was used to alter the nitrogen source, and combined in total mixed rations (TMR) with one of three starch sources (barley, corn, or milo). Starch degradation rates (k_d) are 24.2, 4.0, and 3.6 for barley, corn and milo, respectively (Tamminga et al., 1990). The overall hypothesis for this research is that reducing ruminal ammonia concentration through dietary manipulation of starch and nitrogen sources will enable managers to increase animal nitrogen efficiency. This in turn could reduce nitrogen losses in the total farm system, and decrease negative environmental impacts of the farm operation. The specific objectives of this research were to determine the effect of forage nitrogen and starch (grain) source on ruminal ammonia production in batch culture.

Materials and Methods

Diets

Treatments consisted of diets containing orchardgrass (*Dactylis glomerata*) silages from two plots, one fertilized at 200 (OG200) and the other at 400 (OG400) pounds per acre nitrogen, with three starch sources of varying ruminal degradabilities, barley (B), corn (C), milo (M) in a 2 x 3 factorial arrangement. Silages were second cutting, and ensiled in AgriPac® AST™ silage bags. One silage bag was used for each forage plot. Concentrate mixes were ground through a 6 mm screen. Table 2-1 shows the ingredient composition for concentrate mixes that consisted of 62% of the respective cereal grains, 34% soybean meal and the remainder vitamin and mineral supplements. Six diets containing 50:50 forage:concentrate (dry matter basis) were formulated, three of each starch source with either OG200 or OG400. All six treatment formulations met NRC (1989) requirements for a 700 kg cow producing 40 kg of milk per day of 3.5% milk fat. Diets were isoenergetic at 1.7 Mcal/kg NE_l. Table 2-2 shows chemical composition for all diets. Mixed diets were dried, ground through a 1 mm screen on a Wiley Mill and sub-sampled to be used in the *in vitro* batch culture study.

Experimental Procedures

Two liters of rumen fluid were obtained from one rumen fistulated Holstein cow consuming a diet containing orchardgrass, and a mixture of barley, corn and milo grains, similar to the experimental diets. The rumen fluid was strained through eight layers of cheesecloth, and immediately brought to the laboratory to begin the cultures.

Batch Culture

One gram per vial of each experimental diet dry matter (ground 1 mm) was placed into three 60 mL serum vials (vial 1, vial 2, vial 3), in triplicate (total 9 vials per treatment). One set of three vials had no diets added and served as controls. To initiate the digestion process, 40 mL of a strained rumen fluid:buffer (50:50) plus urea (0.4mM) solution (Slyter, 1990) was added to each vial The vials were then immediately sealed and incubated at 39^{o}C for 48 hours in a shaking (60 rpm) water bath. Aliquots (1mL) for pH, ammonia and VFA analysis were removed after shaking from vial 1 at 0, 2, 4, 6, 8, and 10 hours. Aliquots were removed after shaking from vials 2 and 3 at 24 and 48 hours respectively.

Analytical Procedures

Samples were analyzed for pH (UniFET AMicroprocessor pH/mV/^{o}C with ISFet Sensor, UniFET Inc. San Diego), volatile fatty acids (VFA), and for ammonia concentration. For VFA and ammonia analysis, samples were prepared by first diluting samples with distilled water at a 1:9 ratio. One mL of diluent was pipetted into a microcentrifuge tube, acidified to pH 2 with sulfuric acid, and centrifuged at 15,000 rpm for 10 minutes. The acidified sample (1 mL) was then pipetted into gas chromatography (GC) vials with the addition of an internal VFA standard (2-methylbutyric acid) used for VFA analysis, and then frozen (-20^{o}C). VFA concentrations were determined using an internal standard on a gas chromatograph (model 5890A; Hewlett Packard, Inc. Avondale, PA), equipped with a chromasorb

packed column. Ammonia concentration was determined using the hypochlorite method with a colorimetric microplate reader at 260 nm (Ceres UV900HD).

Statistical Analysis

Experimental units were 60 mL serum vials used for incubation of six diets with either OG200 or OG400 fertilized orchardgrass, and either barley, corn or milo-based concentrates in a 2 x 3 factorial arrangement. All data were statistically analyzed using PROC MIXED least squares means of SAS (SAS Users Guide, 1991). Data were analyzed by analysis of variance model using $Y = \mu + \text{time} + \text{grain} + \text{fertilizer} + (\text{time x grain}) + (\text{time x fertilizer}) + (\text{time x grain x fertilizer}) + \text{error}$, where μ is the grand mean. Treatment means are presented as least squares means, with significance declared at $P<0.05$.

Results

The chemical composition of the total mixed rations is described in Table 2-2. Diets were similar in energy, and crude protein percent, while neutral detergent fiber ranged from 32 to 38 percent. Crude protein content of the diets was similar at 20%, and 21% for the OG200 and OG400 diets respectively, (Table 2-2). Batch culture pH, ammonia and VFA concentrations are shown in Table 2-3. Batch culture pH, molar proportions and total concentration of volatile fatty acids was not different between the treatments. As expected, ammonia concentration was increased ($P<.01$) by OG400 silage due to the increased crude protein content of the forage (Table 2-2). Starch source did not affect ammonia concentration. However, ammonia accumulation

increased over time by fertilization rate and by starch source ($P<0.0001$), and by the interaction of time, fertilization rate and starch source ($P=0.0537$)

Discussion

There was no effect of treatment on rumen pH or volatile fatty acids, either in total concentration (mM/L), or as molar percents. Shabi et al. (1998) report a reduction in total volatile fatty acid concentration in low rumen degradable organic matter and low rumen available crude protein diet ($P<0.01$). In contrast, Petit and Tremblay (1995) compared concentrate mixtures of either soybean and corn or soybean and barley with grass silage and found that total volatile fatty acid production was increased with corn starch compared to barley ($P=0.044$). This could be due to the higher energy density and lower neutral detergent fiber of corn compared to barley, despite barley being more ruminally degradable than corn. This could lead to a more rapid rumen fermentation of corn, but more extensive rumen fermentation of barley. Shabi et al. (1998) report changes in molar percent of acetate in a study of synchronous and asynchronous diets of combinations low or high rumen degradable organic matter and crude protein. The diet of high rumen degradable organic matter and low crude rumen degradable protein yielded the highest molar percent of acetate ($P<0.01$). In contrast, Lykos et al. (1997[a]) reported a linear decline in acetate to propionate ratio with increasing total nonstructural carbohydrates in the diet ($P<0.002$).

Ammonia concentrations were related to fertilization rate alone, and increased with increased nitrogen fertilization. Starch source, and starch source by fertilization rate interaction had no effect on *in vitro* ammonia accumulation. Shabi et al. (1998)

reported increased ruminal ammonia with increased crude protein degradability, however the highest ammonia level on that study was with the combination of low ruminal organic matter availability, and high crude protein availability. Lykos et al. (1997[a]) reported increased ruminal ammonia with decreasing rumen availability of total nonstructural carbohydrates.

Conclusion

Reducing nitrogen fertilization rate on orchardgrass reduced ammonia production, principally by decreasing crude protein content in the forage with reduced nitrogen fertilization. Barley, a rapidly degraded starch source (Tamminga et al., 1990), did not conserve nitrogen through reduced ammonia production when combined with high nitrogen orchardgrass silage, as in other reports. Producers may alter ruminal ammonia production by altering nitrogen application on orchardgrass, which could affect whole farm nitrogen management, and milk production in dairy cattle.

CHAPTER 3

Nutritional and environmental implications of starch degradability and nitrogen fertilization on orchardgrass silage in total mixed rations fed to lactating cows

Abstract

The objectives of this research were to determine the effect of starch and N availability on microbial protein production and N efficiency, ruminal N efficiency and ammonia, and to assess forage fertilization and grain selection decisions. Diets were fed in a 6 x 6 Latin square *in vivo* digestion trial. Total mixed rations (TMR) contained 50:50 forage:concentrate (DM basis) of second-cutting orchardgrass silage fertilized with 200 (OG200) or 400 (OG400) pounds per acre N, plus concentrate mixes using high to low rumen available starches: barley, corn, and milo. TMR crude protein (CP) was 17% and 18% for *in vivo* for OG200 and OG400 diets, respectively. Synchronous diets were low:low or high:high rumen starch availability:diet N (corn or milo withOG200, and barley with OG400). No effects on ruminal microbial protein synthesis and flow, N flow, or milk production were observed. Dry matter, organic matter ($P<0.01$), nitrogen, and neutral detergent fiber ($P<0.02$) total digestibilities increased with synchronous diets. Nitrogen digestibility was depressed in diets of low:high rumen starch availability:diet N, due to increased hindgut fermentation adding microbial protein to the feces ($P<0.001$). All OG400 diets had higher fecal N percentage ($P<0.001$). OG400 had higher ruminal ammonia ($P<0.01$), and higher total VFA concentration ($P<0.001$), but pH was stable due to increased recycling of urea. Orchardgrass fertilized at high N can achieve the higher dry matter, organic matter,

neutral detergent fiber and nitrogen digestibilities of lower fertilized forages when combined with a rapidly available ruminal starch such as barley, and decrease outputs of fecal DM by up to 401.5 and N by nearly 22 kilograms per year per cow. Crop fertilization and grain selection decisions affect forage composition, rumen fermentation, ration digestibility, and fecal DM and N output.

Introduction

Increasing animal nitrogen efficiency could reduce manure nitrogen on the farm. Kohn et al., (1997) developed a total farm nutrient management model and determined that "improving animal nutritional efficiency would have the greatest proportional impact on total farm (nitrogen) efficiency in most cases" compared to potential improvements in crop nitrogen uptake or manure nitrogen availability. Sixty-seven to 90% of forage nitrogen, phosphorous, and potassium ingested by ruminants can appear in manure (Peterson and Gerrish, 1996; Chalupa et al., 1996), suggesting room for improvement in nutrient use and efficiency. Ruminants utilize ruminally synthesized microbial protein to supply roughly 60% to 80% of their amino acid requirements (Sniffen and Robinson 1987; Satter, 1986, Agricultural and Food Research Council, 1992). The efficiency of protein or non-protein nitrogen use for delivering amino acids for absorption in the small intestine depends on energy and nitrogen substrate availability for microbial protein synthesis, in addition to the ruminally undegraded protein amino acid profile (Lykos et al., 1997[b]).

Too much rumen fermentable energy can lower ruminal pH, potentially causing acidosis. Inadequate rumen degradable protein impairs microbial growth, and can slow

fiber digestion and limit intake. With inadequate fermentable energy, proteolysis and deamination of amino acids provides carbon skeletons for energy anabolism, leaving ammonia as nitrogen waste. Excess ammonia is absorbed through the rumen wall to the blood, and converted to urea by the liver. Urea is transported back to the rumen via saliva or ruminal resorption for use by microbes, or for excretion in urine or milk. Urea is a small neutral molecule that can easily diffuse through cell membranes. This results in the concentration of blood urea nitrogen highly correlated to milk urea nitrogen (MUN) (Jonker et al., 1998). Despite the established link between available energy and protein and nitrogen metabolism, studies looking at ruminal carbohydrate and protein synchronization have produced mixed results as is noted in NRC (2001) and seen in published reports (Dewhurst et al., 2000; Table 1-5).

Nitrogen fertilization increases forage yields and crude protein levels (Peyraud and Astigarraga, 1998, Glenn et al., 1985) in grasses used as forages for dairy cows (Givens and Rulquin, 2003; Journet and Demarquilly, 1979; Leaver, 1985). However, higher forage crude protein can increase ruminal ammonia and nitrogen loss in urine, potentially harming the environment (Van Vuuren, et al., 1992). Reduced nitrogen fertilization lowers forage nitrogen while it increases animal nitrogen efficiency due to the changes in chemical composition of the forage (Shingfield et al., 2001).

The overall hypothesis for this research is that ruminal ammonia concentration could be reduced through dietary manipulation of starch and nitrogen sources while maintaining production, and thus will enable managers to increase animal nitrogen efficiency. This in turn could reduce nitrogen losses in the total farm system, and decrease negative environmental impacts of the farm operation. The specific objectives

of this research are to determine the effect of forage nitrogen and starch (grain) source on ruminal ammonia production and microbial nitrogen retention (microbial protein synthesis), and to assess the impact of management decisions concerning forage fertilization and starch (grain) selection on nitrogen efficiency and performance of animal feeds in the rumen. Orchardgrass grown for silage and fertilized at two levels of nitrogen fertilization was used to alter the nitrogen source, and three starch sources (barley, corn, and milo) of varying ruminal degradability were used. Starch degradation rates (k_d) were 24.2, 4.0, and 3.6 for barley, corn and milo, respectively (Tamminga et al., 1990).

Materials and Methods

Diets and Cows

This experiment including all surgical and experimental procedures was conducted under the approval of the USDA Beltsville Area Institutional Animal Care and Use Committee (AUP# GLENN96-008). Treatments consisted of diets containing orchardgrass (*Dactylis glomerata*) silages from two plots fertilized at either 200 (OG200) or 400 (OG400) pounds per acre nitrogen, with three starch sources of varying ruminal degradabilities, barley (B), corn (C), milo (M) in a 2 x 3 factorial arrangement. Silages were second cutting, and ensiled in AgriPac® AST™ silage bags. Table 3-1 shows the ingredient composition for concentrate mixes that consisted of 75% of the respective cereal grains, 21% soybean meal and the remainder vitamin and mineral supplements. Six diets containing 50:50 forage:concentrate (dry matter basis) were formulated, three of each starch source with OG200 and with OG400. All six treatment

formulations met NRC (1989) requirements for a 700 kg cow milking 40 kg per day of 3.5% milk fat. The diets were similar in energy at 1.7 (B, M), and 1.8 (C) Mcal/kg NE_l, and crude protein was 17% for the OG200 diets and 18% for the OG400 diets. Cows were fed ad libitum at 0830 and 2030 hours as a total mixed ration (TMR). Table 3-2 shows chemical composition for all diets.

Five multiparous Holstein cows were fitted with rumen and duodenal cannulae during the dry period a minimum of two weeks prior to parturition, before the onset of the experiment. An additional multiparous Holstein cow had been fitted with ruminal and duodenal cannulae several years prior using identical procedures. Under local anesthetic, cows were fitted with 7.6 cm internal diameter flexible ruminal cannulae, and allowed to heal for two to three weeks. Subsequently under general anesthesia, Komarek-type duodenal T cannulae (ANKOM, Spencerport, NY) were implanted approximately 15 cm posterior to the pylorus. The barrel of the cannula had a 26 mm internal diameter and was 95 mm in length. Cannula in the lumen of the intestine had a 26 mm internal diameter and was 94 mm in length. Surgical procedures were performed by the Veterinary Medical Officer of the Beltsville Agricultural Research Center, USDA, ARS.

Animals were housed in tie stalls with rubber mats bedded with wood shavings. Water was available at all times. Animals averaged 64 days in milk at the initiation of the experiment and were assigned randomly to a 6 x 6 Latin Square arrangement of treatments balanced for carryover effects. The animal surgically altered years prior to the experiment was randomly assigned to column six of the Latin Square, but did not do well in period one. She was replaced starting with period two by a multiparous Holstein

cow fitted six months prior with identical ruminal and duodenal cannulae. The replacement was further into her lactation than the other subjects were (160 days in milk), and further along in her pregnancy. Thus, the replacement was removed from the study at the end of period five to allow her adequate dry off and rest prior to calving. Data for periods two through four were collected for column six of the Latin Square, with periods one and six in the sixth column missing. Diets randomly assigned and balanced for carryover effects in those blocks were the milo diets at both the 200 and 400 per acre N fertilization rates (Table 3-3).

Experimental Procedures and Sample Collection

Each of six experimental periods lasted 21 days. Days 1 – 14 of each period were used for dietary adjustment with samples collected on days 15-21. The start of Period 2 was delayed one week to accommodate the replacement animal, allowing her the full 14 day ration adjustment prior to sampling. The other five subjects thus had one additional week of ration adjustment (21 days total) prior to sampling in Period 2. The start of Period 3 was also delayed one week, resulting in 21 days total ration adjustment for all subjects prior to Period 3 sampling. Body weights were recorded on day 20 of each period. Cows were milked twice a day at 0600 and 1800 hours in a double six herring bone parlor modified to accommodate their duodenal cannulae. Total milk production was recorded automatically in the parlor (Westphalia Dairy Plan V 4.415M FO24036). Milk samples were taken on day 20 at the p.m. milking and on day 21 at the a.m. milking, immediately preserved (D&F Control System 800 Broad Spectrum Microtabs II) then stored (4^{o}C) and shipped over night to Lancaster DHIA for analysis

of fat, protein, lactose, solids not fat (SNF), milk urea nitrogen (MUN), and somatic cell count (SCC).

Grain mixes and silages were sampled daily in equal aliquots, stored additively, covered and refrigerated (4^{o}C). Each collection of aliquots was thoroughly mixed weekly and tested for DM (60 ^{o}C, Hotpack Ovens, Philadelphia). DM values were used to calculate wet weight of ingredients for treatment diets the following week, to maintain consistent forage:concentrate ratio (AOAC, 1984). Dried samples of silages (OG200, OG400) and concentrate mixes (B, C, M) from days 15-20 were saved for later analysis.

Ytterbium (Yb) labeled neutral detergent fiber (NDF) was used to measure DM flow (Ellis & Beever 1984) and dosed on days 10–20 at a rate of 86 g/day, split equally in two closed paper lunch bags placed through the ruminal fistula at feeding (0830 and 2030 hours). Dosing was accomplished by creating a hole in the rumen digesta with the fist, then pushing dose bags down the hole below the digesta surface mat. The gloved hand was rinsed before exiting the fistula. Samples of dry Yb marked NDF used for dosing were collected each sampling period for later Yb analysis.

Total mixed rations and offered and refused treatments (ORTS) were collected in equal aliquots on days 15 – 20, stored additively, covered and refrigerated during the sampling period. Individual treatments were mixed thoroughly prior to collecting aliquots. After the sampling period, each collection of aliquots was thoroughly mixed, sub-sampled, tested for DM (60^{o}C, Hotpack Ovens, Philadelphia), and dried samples saved for later analysis.

Duodenal and fecal grab samples and rumen fluid samples were collected on a staggered schedule during days 18-20, to obtain a sample every two hours post feeding in a 24-hour period, including a zero/24 hour sample at the 0830 feeding. Rumen pH was recorded at each sampling by placing a probe (UniFET AMicroprocessor pH/mV/^{o}C with ISFet Sensor, UniFET Inc. San Diego) directly into the rumen digesta below the fiber mat. Duodenal samples were collected with a duodenal cannula sampling pipe (ANKOM, Spencerport, NY) that diverted all digesta into the sampling cup. Approximately 150 mL of duodenal digesta was collected and then frozen (-25^{o}C). Fecal grab samples were collected in equal aliquots, stored additively, covered and refrigerated (4^{o}C). After the sampling period, the collection of aliquots was thoroughly mixed (Mini-Hobart Model L-800, Hobart Manufacturing, Troy Ohio), tested for DM (60 ^{o}C, Hotpack Ovens, Philadelphia), and dried samples saved for later analysis. Rumen fluid was collected in equal aliquots (Nalgene Mityvac II sampling hand pump) from six rumen locations, two each ventral and caudal from the anterior, middle and posterior regions, mixed and acidified to a pH of two with sulfuric acid (96.1%, 2468-04 Mallinckrodt, Netherlands), then frozen (-25^{o}C).

Rumen bacteria samples were collected at 0, 4, and 8 hours post feeding (0830, 1230, and 1630 hours) by obtaining a rumen grab sample using a sampling cup to obtain equal amounts and to contain liquid and solid, from six rumen locations, two each of ventral and caudal samples from the anterior, middle, and posterior rumen. Rumen grab material was filtered through eight layers of cheesecloth to extract approximately 500 mL of rumen fluid. The remaining fiber was rinsed with 0.9% saline solution at a volume equal to 0.2 times the collected rumen fluid volume. The rinse was added to the

rumen fluid sample. Finally, formaldehyde was added as a preservative for a final concentration of 0.5% formaldehyde. Remaining rumen fiber was returned to the rumen. Volume of rumen fluid collected, and saline and formaldehyde volumes added were recorded. Samples were stored in the laboratory additively during the collection, then mixed well (Corning Magnetic Hot Plate Stirrer PC351) sub-sampled, and frozen (-25^{o}C).

Analytical Procedures

Dried concentrates, silages, total mixed rations (TMR), offered and refused treatments (ORTS), and fecal grab samples were ground to 1 mm on a Wiley mill (Arthur H. Thomas, Philadelphia, PA) prior to chemical analysis. Samples for all analyses were weighed simultaneously for duplicate analyses of DM, ash, neutral detergent fiber (NDF), nitrogen, and starch (Van Soest et al. 1991). DM samples were immediately dried in a forced-air oven at 100^{o}C (Hotpack Ovens, Philadelphia), to a constant weight for an analytical DM content (AOAC, 1984), against which all other assays are calculated. Ash was determined following sample ignition at 500^{o}C for six hours (Sybrone Thermolyne Ashing Oven). NDF was determined using a batch analyzer (ANKOM200 Fiber Analyzer, ANKOM Technology). Total nitrogen was determined by micro-Kjeldahl digestion using automated procedures (Technicon Instruments Corp., Tarrytown, NY). Total starch analysis was completed using a two-stage enzymatic hydrolysis method (Herrera-Saldana et al. 1990) quantifying glucose release with immobilized glucose oxidase-peroxidase (model 2700 select biochemistry analyzer: Yellow Springs Instruments Inc., OH).

Prior to analysis, duodenal samples were thawed and homogenized (Tekmar Tissumizer, Tekmar Co. Cincinnati), and refrigerated overnight to release air bubbles. Individual samples were thoroughly mixed (Corning Magnetic Hotplate Stirrer PC351), and 10 mL was drawn during mixing for each of four composites. Composites were frozen (-25^{o}C) and later lyophilized (Virtis Freeze Dryer, Virtis Co., Gardiner, NY), and ground to 1 mm with a Wiley mill (Arthur H. Thomas, Philadelphia, PA). Assays for DM, ash, neutral detergent fiber (NDF), nitrogen, and starch (Van Soest et al. 1991) were conducted as described above. Freeze-dried, ground duodenal composites and dried, ground fecal composites and Yb marked NDF used for dosing were analyzed for Yb content by wet ashing with nitric acid digestion followed by atomic absorption spectrophotometry analysis for ytterbium. Duodenal and fecal flows (kg/day) were determined using Yb as a marker by dividing the daily dose (intake) of Yb by the amount Yb per kilogram of the sample dry matter (Ellis and Beever, 1984).

Duodenal composites were analyzed for purine content. The procedure used to determine purine (RNA) content is a modification of the analysis of Obisbo and Dehority (1999). Volumes were adjusted to accommodate the small sample size of the rumen bacteria samples. Rumen bacterial samples were thawed, and centrifuged at 12,000 x g for 30 minutes, and supernatant was removed carefully by aspiration. Pellet was washed twice with 0.9% (wt/vol) NaCl solution and dried to a constant weight in a convection oven at 80^{o}C. Dried rumen bacterial samples and ground composite duodenal samples were then analyzed for purine (RNA) content together by period. Samples were weighed for analytical DM and purine analysis simultaneously, including the purine standard (Yeast RNA Sigma #R-6625). Samples were incubated in

Perchloric acid (70%) for 1 hour in a 95^{o}C water bath and removed. Ammonium dihydrogen phosphate (0.0285 M) was added for an additional 15 minute incubation in the 95^{o}C water bath. Supernatant was then filtered (Whatman GF/D fiberglass filter), and 0.5 mL transferred to each of two 15 mL polypropylene copolymer centrifuge tubes. Silver nitrate (0.4 M) and ammonium dihydrogen phosphate (0.2 M) was added, the tubes sealed, mixed thoroughly, and allowed to stand overnight in the refrigerator. Tubes were then centrifuged at 12,000 x g for 25 minutes (Sorvall RC2-B, rotor SM-24 at 10,000 rpm), and supernatant carefully suctioned off. Pellet was then washed with 10.0 mL of precipitation solution (0.4375% perchloric acid and 5% 0.4M silver nitrate in ammonium dihydrogen phosphate solution), and centrifuged and washed again with precipitation solution. Hydrochloric acid (0.05 N) was added, and the standard was diluted to 1/11, 1/21, 1/41, 1/101, and 1/201. The tubes were then covered with marbles and incubated for 30 minutes in a 95^{o}C water bath, removed, sealed and centrifuged at 12,000 x g (Sorvall 10,000 rpm) for 25 minutes. Supernatent was filtered (Whatman 541 paper) and read OD at 260 nm (Beckman DU-600).

Rumen fluid acidified samples were thawed, centrifuged (Sorvall) at 13,000 rpm (20,000 x g) for ten minutes, filtered and stored in scintillation vials prior to analysis for ammonia and volatile fatty acid (VFA) content. Ruminal ammonia nitrogen was determined using automated procedures (Alfa-Laval Bran & Luebbe GTPC System Technicon Auto Analyzer with SC Colorimeter) via the hypochlorite method (Technicon industrial method 339-01; Technicon Instruments Corp.). A subset of the filtered fluid along with an internal VFA standard was added to GC serum vials, frozen

(-25^{o}C), and later thawed for VFA analysis on a gas chromatograph (model 5890A; Hewlett Packard, Inc. Avondale, PA), equipped with a chromasorb packed column.

Statistical Analysis

Experimental design was a 2 x 3 factorial arrangement of treatments in a 6 x 6 Latin square, balanced for carryover effects. Ruminal data (pH, volatile fatty acids, ammonia) were statistically analyzed using the MIXED procedure SAS (SAS Users Guide, 1991). The means over time were analyzed by analysis of variance using the model: Y = μ + grain + fertilizer + (grain x fertilizer) + error, where μ is the grand mean. Grain, fertilizer and treatment were in the class statement. Standard error of the mean (SEM) values were higher for milo diets due to missing data points from dropped cows in the sixth column of the Latin square in periods one and two (Table 3-3) and not included in the ANOVA for ruminal fermentation measures (volatile fatty acids, rumen ammonia, rumen pH). The higher SEM values are those reported in Table 3-7. All other (not ruminal) data included estimates generated in SAS for the missing values in periods one and six, described below.

Estimates were generated using SAS for the missing blocks for all but rumen fermentation samples (volatile fatty acids, rumen ammonia, and rumen pH). Estimates for the two missing values were generated with an iterative procedure, with the 34 observed means (excluding the observed values for the missing milo diets being estimated) used to generate an overall mean, and the period (row) effect determined and removed from individual observations. These corrected means were then used in like manner to determine the cow (column) effect, excluding the milo diets being estimated.

The cow effect was also removed from the individual observations to obtain the true means, with no period or cow effects. The average of the observed adjusted values for the missing diets were deemed to be the missing values, and were used to complete the Latin square with the respective cow and period effect added to determine the final estimated mean for the observation. It was assumed that there were no carryover effects, and that the corrected observed values represented the true population means for the treatments. Thus the complete 36 values for each variable were statistically analyzed using the MIXED procedure SAS (SAS Users Guide, 1991) with the model: Y = μ + period + cow + grain + fertilizer + (grain x fertilizer) + error, where μ is the grand mean, with cow and period in the random statement, and forage year, cow, period, fertilizer, and grain in the class statement. Data are presented as least squares means.

Results

The chemical composition of the total mixed rations is summarized in Table 3-2. Orchardgrass silage fertilized at 200 pounds per acre nitrogen (OG200) was lower in crude protein (13.86%, 15.02%) and higher in dry matter (40.73%, 31.92%) than orchardgrass fertilized at 400 pounds per acre nitrogen (OG400). Crude protein percentage of total mixed rations was lower for OG200 diets than for OG400 diets. Starch content was slightly higher in OG200 versus OG400 diets ($P<0.0482$) and lower in barley diets compared to corn and milo diets ($P<0.0001$). Dietary neutral detergent fiber was 38%, 33%, and 32% of diet dry matter in barley, corn and milo diets,

respectively (P<0.0001). This may explain why the barley diets had lower starch contents.

Intake and digestibility of dry matter is shown in Table 3-4. Dry matter intake, duodenal dry matter flows, apparent and true ruminal digestibilities, and total amount of dry matter digested were not different by treatment. There was an interaction of fertilizer rate and grain for total tract dry matter digestibility where digestibility was increased in the corn and milo diets with OG200 yet it was reduced with those cereal grains in the OG400 diets (P<0.01). Organic matter intake, flows, and rumen and total tract digestibilities followed similar trends (Table 3-5).

Starch intake (Table 3-4) was higher (P=0.02) for OG200 diets compared to OG400 diets, and was followed by increased total tract starch digestion with the OG200 diets versus the OG400 diets (P<0.02) due to increased starch content of the OG200 forage (Table 3-2). There was no effect of fertilization rate on total tract starch digestibility. Starch intake was higher (P<0.001) with corn and milo as compared to barley due to higher diet starch content (Table 3-2). Rumen digestion of starch tended to be reduced (P=0.052) by corn and especially in milo diets, where only 1 kg/day of starch was digested. Rumen starch digestibility averaged 54, 38, and 15% for barley, corn, and milo, respectively across fertilizer treatments as expected. However, total tract digestibility of starch was slightly different by grain source, being reduced by only 1 to 2 percentage units (P=0.02) in the milo treatment compared to barley and corn. This demonstrates the expected shift in the site of digestion for starch, with the majority of starch digestion occurring ruminally in the barley treatments, and conversely, the

majority of total tract starch digestion occurring post-ruminally in the corn and milo treatments.

Due to the higher neutral detergent fiber (NDF) content of the barley diets, NDF intake was greater ($P<0.01$) for barley than corn and milo diets. This was also reflected in the flow of NDF to the duodenum. Amounts of rumen digested NDF tended to be greater in the corn and milo diets while rumen digestibility of NDF was reduced ($P<0.02$) in the barley diets as compared to the corn and milo diets. The amounts of NDF digested in the total tract were greater ($P=0.008$) in the barley diet, but the overall digestibility of NDF was not affected by cereal grain. There were no effects of fertilizer rate on NDF digestion, but there were interactions of fertilizer rate by starch source where the amounts and the digestibility of NDF were highest in the milo diets with low fertilizer rate (OG200), but lowest in the high fertilizer rate (OG400).

Nitrogen metabolism and digestion is summarized in Table 3-6. Nitrogen intake did not differ by treatment, however, fecal nitrogen output increased with decreasing starch degradability in milo, and to a lesser extent, corn ($P=0.0019$), while fecal nitrogen concentration was lower for OG200 compared to OG400 diets (3.22, 3.50, $P=0.0004$) and was lowest for barley diets ($P=0.0001$). Total tract digestibility of nitrogen was greatest for the OG200 diets and the OG400 barley diet ($P=0.027$) as compared to OG400 with corn or milo. There were no effects of fertilizer rate, grain, or fertilizer-grain interaction on duodenal or bacterial nitrogen flow, or bacterial nitrogen efficiency. However, feed nitrogen flow tended to be higher in the corn and milo diets while bacterial nitrogen flows tended to be higher in the barley diets. Ruminal ammonia (Table 3-7) concentration was lower for OG200 versus OG400 ($P<0.0001$).

The percentage of rumen bacterial ribonucleic acid (RNA) in rumen fluid dry matter was higher for OG200 versus OG400 diets (.1267, .1178, P=0.0065).

Rumen pH, ammonia, and volatile fatty acid concentrations are listed in Table 3-7. There was no difference in rumen pH by treatment; however, the mean pH on all diets was low at 5.5. The higher nitrogen fertilization rate increased total volatile fatty acid production comparing OG200 to OG400 (77.79, 82.01, P=0.0002). Increased fertilization rate increased individual molar percentages of volatile fatty acids, except for acetate which had decreased concentration (P<0.0001), and butyrate, which was unaffected by nitrogen fertilization rate (P=0.2427). Acetate percent alone decreased with increasing fertilization for OG200 versus OG400 (67.79, 66.32, P<0.0001), and the acetate to propionate ratio thus decreased with OG200 compared to OG400 (3.51, 3.82, P<0.0001).

Increasing degradability of starch source increased the concentration of acetate for milo, corn and barley, respectively (P<0.0001). Acetate to propionate ratio was highest for barley (P<0.0001), however, it was higher in milo compared to corn (P<0.0001) since the corn diets had significantly higher concentrations of propionate than either barley or milo diets (P<0.0001). Total ruminal volatile fatty acid concentrations, pH, and ammonia levels were not affected by grain selection. Propionate (P=0.0445) and butyrate (P=0.0117) were significantly altered by the interaction of grain and fertilizer rate. Propionate concentration was lowest with barley starch and low nitrogen fertilized silage (OG200), while it was highest for corn starch and high nitrogen fertilized silage(OG400) (P=0.0445).

Milk production and milk composition data are shown in Table 3-8. There were no effects of fertilizer, grain, or fertilizer and grain interaction on either milk production or milk components. However, there was an indication of possible decreased milk fat with decreased starch degradability for barley, corn, and milo (3.77, 3.72, 3.53, P=0.1006), coinciding with the high acetate to propionate ratio found in the barley diets (P<0.0001).

Discussion

Increased nitrogen fertilization increased crude protein and reduced dry matter and starch in orchardgrass, while neutral detergent fiber and organic matter were unchanged (Table 3-2). This was as seen in a study by Peyraud et al. (1997) with fresh perennial ryegrass fertilized at 0 or 80 kilograms per hectare, and with Valk et al. (1996) in their study of fresh grass (*Lolium perenne)* fertilized with nitrogen at either 150, 300, or 450 kilograms per hectare per year. Increased nitrogen fertilization is associated with higher yields and quality forages, especially in terms of crude protein content. However, the efficiency of nitrogen use in animals fed these higher fertilized forages is reduced, causing concern over their environmental impact. This reduced nitrogen digestibility was seen in this study for OG400 diets containing low ruminal available corn or milo concentrates compared to all OG200 diets, however, the OG400 grass combined with highly rumen available barley, was digested equally to the OG200 diets.

Although the forages were different in dry matter and crude protein content, dry matter intake and nitrogen flow rates were not significantly different. However, the

type of grain used in the TOTAL MIXED RATION affected fecal dry matter and nitrogen output, which increased with decreasing ruminal availability of starch in milo on either the low or high nitrogen fertilized orchardgrass. In addition to increasing fecal dry matter, milo diets showed a significant increase in fecal nitrogen concentration, as did diets with higher nitrogen fertilization. This poses manure management challenges, and suggests that ruminal starch was limiting to nitrogen digestion, similar to findings of Kolver et al., (1998). All of the OG400 diets generated higher fecal nitrogen as a percentage of fecal dry matter than the OG200 diets, regardless of starch source. The increase in fecal nitrogen due to milo as the starch source is likely caused by more post-ruminal fermentation and microbial protein synthesis, since very little milo starch was fermented in the rumen. This could pose problems in terms of environmental impact, but may or may not be as threatening to the environment as it seems at first glance. Instead of nitrogen excreted in urine as urea, bacterial nitrogen in the feces resulting from hindgut fermentation may be more stable.

The cause of the significant effect of fertilizer by grain interaction seen in total tract digestibilities of dry matter, organic matter and NDF is unclear; a possible factor is the synchronization or balance of forage nitrogen and rumen available starch for bacterial fermentation. Synchronous diets would be the OG400 with barley (high nitrogen from orchardgrass fertilized at 400 pounds per acre nitrogen and the high rumen availability of barley starch). Also synchronous would be OG200 with either milo or corn (low nitrogen orchardgrass at 200 pounds per acre nitrogen, and low availability for rumen starch with milo or corn). The asynchronous diets would be

OG400 with corn or milo, (high forage nitrogen and low rumen starch availability), and OG200 with barley (low forage nitrogen with high rumen starch availability).

Total tract digestibility of dry matter, organic matter, and NDF were improved with synchronous diets (high:high or low:low nitrogen and ruminal starch degradability), which demonstrates that the balance of nitrogen and energy in the rumen may be more important than absolute amounts. However, these findings differ from the findings of McCarthy, Jr. et al. (1989) where no differences in total tract dry matter digestibility for diets of varying degradabilities of protein (fish meal and soybean meal) and starch (barley and corn) were seen. Similar observations of Kolver et al. (1998) and Shabi et al. (1998) found no differences in the total tract dry matter and NDF digestibility between synchronous and asynchronous diets. There was no effect of fertilization rate on neutral detergent fiber digestion seen in the present study. However, Peyraud et al. (1997) observed increased total digestibility of neutral detergent fiber with perennial ryegrass fertilized at 80 versus 0 kilograms per hectare.

Nitrogen intake and duodenal, bacterial and feed nitrogen flow to the duodenum were the same across all treatments (Table 3-6), similar to results reported by Yang et al. (1997) with barley and corn. In contrast, Overton et al. (1995) found that nitrogen intake and feed nitrogen decreased as barley replaced corn in isonitrogenous diets. There are reports of increased microbial nitrogen flow with barley versus corn (Spicer et al., 1986), while others report no difference (Glenn and Woodward-Greene, 1995). There was no difference in bacterial nitrogen efficiency between treatments or factors, expressed either as bacterial nitrogen per organic matter apparently digested, or per organic matter truly digested, as was seen in the study by Shabi et al. (1998).

Nitrogen digestibility was improved with synchronous diets (high:high or low:low nitrogen and ruminal starch degradability), and as expected, was increased with lower nitrogen fertilization. However, on this study when high fertilization was combined with high rumen available barley starch, nitrogen digestibility was equal to that of the lower fertilized diets. This is due to a combination of the fact that barley was utilized as the major energy source in the rumen and therefore was not limiting to rumen degradable protein digestion; and because on the barley diets, rumen undegraded protein may have been utilized more extensively in the hindgut for energy compared to corn and milo. Additionally, since most of the barley starch was used in the rumen, there was less barley starch available for microbial fermentation in the hindgut, so less microbial protein would appear in the feces, which would depress nitrogen digestibility values. This is supported by the fact that total tract nitrogen digestibilities were similar across treatments accept for the less ruminal available corn and milo with OG400 diets.

Higher fertilized silage depressed starch intake and total tract starch digestion in kilograms per day. This observation simply mirrored the reduction in water-soluble carbohydrates in the orchardgrass with increased fertilization seen in this study, and typically seen in plants with increased fertilization (McDonald et al., 1991). This reduction in forage starch is a likely contributor to reduced nitrogen digestibilities seen in higher fertilized forages. Starch intake was inversely related to starch source rumen degradability, with starch intake highest for milo diets, then corn, then barley, while rumen starch degradability from high to low was barley, corn, and then milo (Table 3-4). This is similar to the degradability values of Tamminga, et al., (1990) who reported starch degradation rates (k_d) of 24.2, 4.0, and 3.6 for barley, corn and milo, respectively.

Starch intake (Table 3-4) was lowest for barley, probably due to its lower starch content (Table 3-2). Increased ruminal degradation of barley starch was followed in turn by reduced duodenal starch flow, and by reduced amounts of total tract starch. Total tract starch digestibility percent was lowest for milo and highest for corn, in concert with the higher energy value of the corn diets. Starch rumen digestibility was highest for barley, followed by corn and then milo, as was expected, which was the opposite of dietary starch content and intake (Table 3-4). Conversely, the grains were reversed for NDF ruminal degradability going from low to high with barley corn and milo, respectively, which was also opposite of dietary NDF content and intake (Table 3-5). This inverse relationship between ruminal digestibility and intake between starch and NDF, suggests that microbes preferentially fermented rumen available starch over NDF in the rumen, with higher rumen availability of starch decreasing NDF rumen digestibility, even as NDF intake increased.

Ruminal pH was not affected by nitrogen fertilization or grain source treatment (Table 3-7). This was similar to observations with high and low nitrogen fertilization levels with Peyraud et al. (1997) and corn and barley diets applied by Yang et al. (1997). Shabi et al. (1998) reported no effects of diet on rumen pH in a two by two-factor study using varying rumen available carbohydrate and protein sources. Taniguchi et al. (1994) found differences in rumen pH due to grain and protein selection ($P<0.05$) ranging from 6.46 with corn and corn gluten meal to 6.64 with barley and soybean meal. Kolver et al. (1998) saw reduced (6.06) pH with synchronous diets. Values for ruminal pH on this study were much lower than all the studies mentioned, which had mean values above six for all treatments, while the present study had a mean

ruminal pH of 5.5. The cause of this low pH is unknown, but it apparently occurred without acidosis, as milk fat (Table 3-8) was relatively normal across treatments.

The unexpected, low mean ruminal pH of 5.5 across all diets seen on this study is of interest. Other rumen fermentation measures were in the normal proportions, though total volatile fatty acid production may have been a bit low, and milk components were unaffected. Although methane production was not measured, it is known that methanogenic bacteria are highly sensitive to low pH, and do not flourish under such conditions. Thus, it is probable reduced methane production occurred on these diets. The ability of animals to thrive and produce on lower ruminal pH could be important in reducing methane production by ruminants. Exploration of potential side effects of such diets, positive or negative, whether affecting the rumen, the animal, or products such as meat or milk, is important. The ability of the microbial population to adapt to various nutritional challenges is the key to understanding how ruminal fermentation can work for the environment while allowing ruminant animals to maximize their role as converters of human inedible nutrients to human edible nutrients.

There was an increase in total rumen volatile fatty acids for the OG400 diets (Table 3-7), without a concurrent reduction in ruminal pH by treatment. This is likely due to increased recycling of urea to the rumen, which acted as a buffer for the OG400 diets. Peyraud et al. (1997), had no difference in ruminal pH and similar increases in total volatile fatty acids with values of 117 and 103 mMol per liter for high and low nitrogen diets, respectively, ($P=0.003$). Yang et al. (1997) reported no change in ruminal pH, and higher total volatile fatty acids for diets of corn (91.3 mMol/L) than of barley (78.4 mMol/L) ($P<0.05$), while Overton, et al. (1995) reported no difference in

total volatile fatty acid concentration with diets of varying ratios of corn and barley. There were no effects of grain source on total volatile fatty acids seen in this study. Individual molar proportions of acids were affected by the both nitrogen fertilization and cereal grain however, indicating that rumen energy metabolism may have been altered by the treatments.

Acetate to propionate ratios were different by starch source, and increased with increased neutral detergent fiber intake, being highest for the barley diets, followed by milo then corn diets. Increased fertilization decreased rumen acetated to propionate ratios. Corn in the diet resulted in increased propionate. These were similar to results reported by Hristov and Broderick (1996) with diets of alfalfa hay, alfalfa silage and corn silage where neutral detergent fiber was highest for the hay, followed by alfalfa silage and corn silage, and the acetate to propionate ratios mirrored the neutral detergent fiber values for each diet, respectively (4.44, 4.17, 3.63, SE=0.04, $P<0.001$). This is expected, as acetate is generally increased with high fiber diets, and propionate is reduced (Van Soest, 1994).

Butyrate was lowest for the barley diets, which could be the result of decreased protozoa. Ushida et al. (1986) reported lower molar proportions of butyrate in defaunated versus faunated sheep. This would coincide with the more readily available barley starch when compared to corn and milo, which would favor fast growing bacteria over slow growing protozoa (Russell and Hespell, 1981). However, in this study, increased ruminal ammonia did not correspond to increased butyrate as was found in the same report for higher protozoa populations (Ushida et al., 1986). Nocek and Russell, (1988) found that protozoa consistently produced more ammonia when

compared to mixed rumen bacteria fed various protein sources. However, the National Research Council (NRC, 2001) notes that it has been found by others that ruminal ammonia is decreased in defaunated rumens since there is no protozoan deamination of proteins.

Conclusion

Ruminal ammonia concentration can be controlled by lowering the nitrogen fertilization rate on orchardgrass grown for silage and fed as a TOTAL MIXED RATION to lactating cows. Increased fertilization rate increases crude protein content of the forage and the TOTAL MIXED RATION, and subsequently, ruminal ammonia concentration. However, it does not follow that urea excretion as milk urea nitrogen will increase with ruminal ammonia, due to urea recycling back to the rumen, and acting as a buffer.

On a gross scale the success of the total mixed ration to increase milk production demonstrates that synchronization of nitrogen and energy is important to rumen microbes. Despite the increased digestibility seen with synchronous diets on this study, attempts to consistently modify the delicate balance of rumen fermentation by balancing dietary nitrogen and energy remains elusive to the scientific community, and therefore it is difficult to make concrete recommendations to farm managers on that point until greater understanding of rumen ecology is achieved.

On the other hand, overall digestibility and fecal nitrogen concentration can be reduced by reducing nitrogen fertilization on orchardgrass, but this may reduce forage yield and quality. This study demonstrated that managers may accommodate higher

nitrogen fertilized grass, and achieve the same nitrogen digestibility as low nitrogen fertilized grass, if it is combined in a total mixed ration with a highly ruminal available starch source such as barley. Environmental impacts of the increased ruminal ammonia that results from such a diet are mitigated if the total crude protein content of the diet is kept below 19%, so that the ruminal ammonia will be preferentially recycled to the rumen, rather than excreted in milk or urine. When compared to diets with high fertilization rates and less ruminal available starch (corn and milo), producers feeding high nitrogen fertilized grass with barley instead of corn or milo, could reduce fecal dry matter by up to 401.5 kilograms per year, and fecal nitrogen output by nearly 22 kilograms per year for each dairy cow. However, these gains must be balanced against potential negative environmental impacts of increased nitrogen fertilization on the forage crop, and the total nitrogen balance of the farm considered. This study showed effects on chemical composition of forage, rumen fermentation, ration digestibility, and amount and nitrogen content of feces based on changes in nitrogen fertilization and selection of starch source for total mixed rations fed to lactating cows that are important in managing nutrients on the farm.

Table 1-1 Summary of studies of changes in forage composition (percent of dry matter) cut at early or late stages of growth and/or fertilized at various rates (kg/ha) of nitrogen.

Forage Type[1]	N Rate kg/ha[11]	Maturity[11]	DM[2]	CP[3]	NDF[4]	ADF[5]	L[6]	WSC[7]	CP Deg[8]	DM Deg[9]	OM Deg[10]	Reference
Dactylis glomerata	80.0	Early	53.8	25.1	39.4	21.4	1.6	7.2	78.0	78.0	---	DeVisser et al., 1998
Dactylis glomerata	80.0	Late	44.2	20.5	46.4	26.2	1.6	3.5	73.0	76.0	---	DeVisser et al., 1998
Dactylis glomerata	NR	Early	92.3	20.2	57.3	28.6	2.5	---	78.0	68.6	---	Balde, et al., 1993
Dactylis glomerata	NR	Late	92.3	12.7	68.1	36.9	3.2	---	69.6	56.1	---	Balde, et al., 1993
Dactylis glomerata	NR	Early	---	28.1	67.6	45.7	7.3	11.6	---	---	---	McDonald et al., 1991
Dactylis glomerata	NR	Late	---	15.6	83.6	58.4	4.9	0.5	---	---	---	McDonald et al., 1991
Lolium perenne	0.0	NR	20.5	10.6	49.6	25.3	2.2	24.6	---	---	---	Peyraud et al., 1997
Lolium perenne	80.0	NR	16.0	15.0	52.8	27.5	2.5	18.0	---	---	---	Peyraud et al., 1997
Lolium perenne	150.0	NR	20.9	14.4	46.8	---	---	17.9	---	---	72.2	DeVisser et al., 1997
Lolium perenne	150.0	NR	17.0	17.1	51.0	24.2	---	11.2	89.8	---	69.4	Valk et al., 1996
Lolium perenne	450.0	NR	19.6	20.0	47.5	---	---	12.9	---	---	72.4	DeVisser et al., 1997
Lolium perenne	450.0	NR	15.9	22.9	52.7	23.3	---	7.9	91.0	---	69.0	Valk et al., 1996
Lolium perenne	NR	Early	18.6	28.0	54.7	36.7	---	4.4	---	62.4	---	McAllan, et al., 1994
Lolium perenne	NR	Late	27.3	18.9	59.9	36.6	---	3.7	---	63.3	---	McAllan, et al., 1994
Lolium perenne	91.0	Early	24.6	15.1	52.0	25.6	2.7	9.9	84.0	---	73.9	Steg et al., 1994
Lolium perenne	91.0	Late	23.7	14.3	52.1	25.5	2.5	10.5	82.0	---	73.2	Steg et al., 1994
Medicago sativa	0.0	Early	92.3	25.2	39.7	29.9	4.8	---	84.8	72.9	---	Balde, et al., 1993
Medicago sativa	0.0	Late	92.4	18.3	50.8	37.7	7.1	---	80.4	61.9	---	Balde, et al., 1993

[1]Summary of studies of perennial ryegrass (Lolium perenne), orchardgrass (Dactylis glomerata), and alfalfa (Medicago sativa). [2]Dry Matter, [3]Crude Protein, [4]Neutral Detergent Fiber, [5]Acid Detergent Fiber, [6]Lignin, [7]Water Soluble Carbohydrates, [8]Crude Protein Degradability, [9]Dry Matter Degradability, [10]Organic Matter Degradability, [11]Not Reported.

Table 1-2. Types of microbes in the rumen.

Type	Description	Number per ml or g
Bacteria	Obligate anaerobes Facultative anaerobes	10^{10} to 10^{11}
Protozoa	Strict anaerobes	10^{5} to 10^{6}
Fungi	Strict anaerobes	10^{7}
Mycoplasma	Parasitic/Unknown	10^{5} to 10^{7}
Viruses (bacteriophages)	Parasitic/Unknown	10^{7}

For comparison, there are 10^{9} humans on the earth. (adapted from Dawson and Van Kessel, 1998)

Table 1-3. Roles and End Products of Important Rumen Bacteria.

Ruminal Niche Occupied	Species	Fermentation Role	Preferred Substrates	End-Products*
Cellulolytic	*Ruminococcus albus*	Primary	Cellulose	C1, C2,ethanol, H_2, CO_2
Hemicellulolytic	*Bacteroides ruminocola*	Primary	Hemicellulose	C1, C2, C3,succinate
Pectinolytic	*Streptococcus bovis*	Primary	Pectin, sugar	C2, ethanol, lactic acid, CO_2
Amylolytic	*Bacteroides amylophilus*	Primary	Amylase	C1, C2, succinate
Ureolytic	*Succinovibrio dextrinosolvens*	Primary, Secondary	Urea, soluble sugars	C1, C2, succinate, lactate
Sugar utilizer	*Lactobacillus vitulinus*	Primary, Secondary	Soluble sugars	lactate
Acid utilizer	*Selenomonas ruminantium*	Secondary Primary	Lactate, succinate, soluble sugars	C2, C3, lactate, H_2, CO_2
Proteolytic	*Butyrvibrio fibrosolvens*	Primary	Cellulose, hemicellulose, starch, pectin, proteins	C1, C2, C4, H_2, CO_2, lactate
Methanogenic	*Methanobacterium ruminantium*	Secondary	H_2	CH_4

C1=Formic Acid, C2=Acetic Acid, C3=Propionic Acid, C4=Butyric Acid, H_2=Hydrogen CO_2.=Carbon Dioxide, CH_4 =Methane
(Adapted from Russell and Hespell, 1981 and Van Soest, 1994).

Table 1-4. Percentage of microbial crude protein in forage dry matter during incubation in cows fed diets of 35% either dry-rolled or steam-flaked sorghum, and in camels fed 25% barley grain[b, c]

Incubation Time (hours)[d]	Microbial Nitrogen (percent of total nitrogen)			
	Whole Corn Cobs	Corn Stalks	Mean	Mean Square Error
2	5.2	9.3	7.2	3
4	16.6	17.2	16.9	3
12	40.0	36.9	38.5	3
24	50.8	49.1	49.9	3
48	55.7	56.0	55.9	3
72	56.4	57.7	57.0	3
Rate of constant degradation (% per hour)[e]	10.4	8.6	9.5	1

[a]Adapted from Wanderley et al. (1999). [b]Cow experiments, values by diets (dry-rolled and steam-flaked) are least squares means of two trials, two cows, two bags per incubation time. [c]Camel experiments, values for whole corn cobs and corn stalks are least squares means of three camels and two bags per incubation time. [d]Incubation time effect was significant ($p<0.01$) in both experiments, with cows and camels; colonization was greater in corn stalks ($p<0.01$) than whole corn cobs with camels. [e]Rate of constant degradation determined by non-linear regression was not different ($p<0.3$) between treatments within cows or camels.

Table 1-5[a]. Summary of data from experiments examining the effect of synchronizing dietary nitrogen and carbohydrate supply on nitrogen utilization or microbial nitrogen (MN) synthesis and efficiency (EMCPS) using different experimental approaches.

Approach / authors[f]	Energy/ nitrogen source	Experiment system	Findings[e]
1. Alteration of Ingredients			
1	Hay and cottonseed hulls plus concentrates	Lactating cows	Synchronization of supplements for rapid fermentation (more degradable starch and protein) gave highest MN flows and EMCPS than asynchronous of slow fermentation synchronous diets.
2	Alfalfa silage + concentrates	Lactating cows	Synchronization of rumen available carbohydrate and protein for rapid degradation gave highest MN flows.
3	Straw + concentrates	In vitro	MN flow and EMCPS higher with a synchronous diet.
4	No information	Lactating cows	Highest MN flow and EMCPS with and asynchronous diet.
2. Same dietary ingredients (changed feeding pattern or pulse dosing into rumen)			
5	Glucose/urea/ trypticase	In vitro	Synchrony lowered ammonia concentrations and fluctuation; no improvement in EMCPS or microbial dry matter; but improved it with a single pulse dose of glucose.
6	Glucose/urea	In vitro	Asynchronous supply of nitrogen and energy yielding substrates only had short-term effects on bacterial growth.
7	Wheat straw/ fishmeal/ molasses[b]	Sheep	Ruminal ammonia lower and more stable with synchrony, but MN flow and EMPS unaffected. Continuous infusion of sugar improved MN flow.
8	Grass/clover-timed concentrate feeding	Lactating cows	Ruminal ammonia concentrations consistently lower with synchronous diet.
9	Grass silage only[c]	Dry cows	No improvement in MN flow with synchronous conditions and sucrose infusion.
10	Grass silage + concentrates[d]	Dry cows	Marked increase in NM flow when malto=dextrin infused synchronously.

[a]Adapted from Dewhurst et al. (2000). [b,c,d]Infusion or pulse dosing of [b]sugar and urea/casein, [c]sucrose or [d]maltodextrin at different times. [f]1. Herrara-Saldana et al. (1990a) 2. Aldrich et al. (1993) 3. Lee et al. (1997) 4. Henderson et al. (1998) 5. Henning et al. (1991)6. Newbold and Rust (1992) 7. Henning et al. (1993) 8. Kolver et al (1998) 9. Kim et al. (1999b) 10. Kim et al. (1999a)

Table 2-1. Ingredient composition of *in vitro* dietary concentrates[1] (percent of DM).

Ingredient	Barley	Corn	Milo
Grain	62.	62.	62.
Soybean meal (48%)	34.	34.	34.
Limestone	1.60	1.60	1.60
Sodium Bicarbonate	1.32	1.32	1.32
Trace mineralized salt Se	0.64	0.64	0.64
Sulfate	0.20	0.20	0.20
Magnesium oxide	0.08	0.08	0.08
Zinc oxide	0.04	0.04	0.04
Vitamin A	0.052	0.052	0.052
Vitamin D	0.028	0.028	0.028
Vitamin E	0.080	0.080	0.080

[1]Treatment diets were 50% orchardgrass silage (200 or 400 pounds per acre nitrogen application, OG200, OG400) and 50% concentrate on a dry matter basis.

Table 2-2. Crude protein, fiber and energy values of dried, ground diets for batch culture, and orchardgrass silages fertilized with either 200 or 400 pounds per acre N, (OG200, OG400). Grain mix for each TMR was one of three formulations of barley, corn, or milo.

Item	OG200 TMR			OG400 TMR				
	Barley[1]	Corn[1]	Milo[1]	Barley[1]	Corn[1]	Milo[1]	OG200	OG400
CP (% DM)	20.2	19.7	20.3	21.4	20.9	21.5	15.2	17.6
NDF (% DM)	35.8	32.7	32.2	36.7	33.6	36.7	56.3	58.0
NE_l Mcal/Kg	1.7	1.8	1.7	1.7	1.8	1.7	1.54	1.54
ME Mcal/Kg	2.7	2.8	2.8	2.7	2.8	2.8	2.4	2.4

[1]TMR consisted of 50% concentrate mix and 50% silage on a dry matter (DM) basis.

Table 2-3. Least squares means for batch culture pH and volatile fatty acid (VFA) concentrations in 6 total mixed rations[1] (TMR) containing silages produced from orchardgrass fertilized at a rate of 200 (OG200) or 400 (OG400) pounds per acre N, and concentrate mixes containing barley (B), corn (C), or milo (M).

Item	TMR[1]						Control	SEM[2]	Effect[3] P =		
	OG200			OG400					Fertilizer Rate	Grain	Fertilizer X Grain
	B	C	M	B	C	M					
pH	6.25	6.25	6.23	6.26	6.25	6.27	6.76	0.029	0.84	0.76	0.94
Total VFA mM	103.82	103.69	99.43	103.08	105.37	102.84	57.46	3.205	0.82	0.97	0.82
VFA, mol/100											
Acetate	64.38	64.10	63.52	64.03	63.04	63.79	69.41	0.613	0.38	0.97	0.51
Propionate	20.34	20.66	20.54	20.46	20.35	20.55	14.91	0.353	1.00	0.37	0.63
Isobutyrate	0.99	1.04	1.15	1.03	1.28	1.15	1.85	0.117	0.78	0.49	0.87
Butyrate	11.22	11.14	11.22	11.21	11.56	11.06	9.81	0.325	0.36	0.80	0.61
Isovalerate	1.65	1.74	2.18	1.78	2.36	1.92	2.64	0.256	0.48	0.29	0.13
Valerate	1.43	1.36	1.40	1.50	1.42	1.53	1.39	0.055	0.98	0.77	0.98
Acetate:Propionate	3.21	3.15	3.14	3.18	3.14	3.14	4.67	0.091	0.73	0.77	0.91
Total Branched Chain Fatty Acids	2.64	2.78	3.32	2.81	3.64	3.07	4.49	0.287	0.46	0.18	0.14
Ammonia mg/dL	21.3	20.7	20.3	26.1	24.6	25.0	22.7	0.607	<0.01	0.19	0.51

[1] Orchardgrass:grain ratio was 50:50 of the total mixed ration on a dry matter basis.

[2] Standard error of the individual treatment means for fertilizer rate by grain interaction.

[3]Probability that fertilizer rate, grain, fertilizer rate and grain interaction, or time did not influence results for different treatments. The effect had a significant influence on the result when the value of P was < .05.

Table 3-1. Ingredient composition of dietary concentrates (percent of DM).

Ingredient	Barley	Corn	Milo
Grain	74.50	74.50	74.50
Soybean meal (48%)	21.30	21.30	21.30
Potassium-magnesium sulfate	0.54	0.54	0.54
Limestone	1.60	1.60	1.60
Magnesium oxide	0.10	0.10	0.10
Trace mineralized salt	0.82	0.82	0.82
Sodium Bicarbonate	0.95	0.95	0.95
Vitamin A	0.06	0.06	0.06
Vitamin D	0.01	0.01	0.01
Vitamin E	0.11	0.11	0.11

Table 3-2: Chemical composition of total mixed rations (TMR) based on silages produced from orchardgrass fertilized at a rate of 200 (OG200) and 400 (OG400) pounds N per acre and concentrate mixes containing barley (B), corn (C), or milo (M).

Item	OG200 TMR			OG400 TMR				
	Barley[1]	Corn[1]	Milo[1]	Barley[1]	Corn[1]	Milo[1]	OG200 Silage	OG400 Silage
DM	53.71	53.56	55.39	49.22	48.60	49.06	40.73	31.92
OM (% DM)	92.46	92.47	92.53	92.48	92.49	92.40	92.49	92.46
CP (% DM)	17.28	17.24	17.01	17.71	17.66	17.43	13.86	15.02
Starch (% DM)	25.78	30.73	31.38	24.36	30.69	30.03	29.30	28.36
NDF (% DM)	37.55	32.84	32.01	37.87	33.17	32.12	34.14	34.39

[1]TMR consisted of 50% concentrate mix and 50% silage (DM basis).

Table 3-3.Distribution of treatment diets fed in a 6 X 6 Latin Square design, balanced for carryover effects. Crossed out blocks represent data dropped due to problems with animal health. Cows were fed total mixed rations[1] (TMR) based on silages produced from orchardgrass fertilized at a rate of 200 (OG200) and 400 (OG400) pounds N per acre and concentrate mixes containing barley (B), corn (C), or milo (M).

Period	Cow 1	Cow 2	Cow 3	Cow 4	Cow 5	Cow 6
1	OG200B	OG200C	OG200M	OG400B	OG400C	~~OG400M~~
2	OG400M	OG200B	OG200C	OG200M	OG400B	OG400C
3	OG200C	OG200M	OG400B	OG400C	OG400M	OG200B
4	OG400C	OG400M	OG200B	OG200C	OG200M	OG400B
5	OG200M	OG400B	OG400C	OG400M	OG200B	OG200C
6	OG400B	OG400C	OG400M	OG200B	OG200C	~~OG200M~~

[1] TMR consisted of 50% concentrate mix and 50% silage (DM basis)

Table 3-4. Least squares means for intake and digestibility of dry matter and starch in cows fed total mixed rations[1] (TMR) containing silages produced from orchardgrass fertilized at a rate of 200 (OG200) and 400 (OG400) pounds N per acre and concentrate mixes containing barley (B), corn (C), or milo (M).

Item	OG200			OG400			SEM[2]	Effect[3], P =		
	B	C	M	B	C	M		Fertilizer Rate	Grain	Fertilizer X Grain
Dry Matter										
Intake, kg/d	19.6	20.2	21.3	19.5	17.8	20.2	0.97	0.06	0.08	0.32
Duodenal flow, kg/d	16.2	17.8	17.9	16.1	15.4	17.6	1.241	0.16	0.16	0.30
Apparent digested ruminal kg/d	3.4	2.3	3.4	3.5	2.5	2.6	1.050	0.76	0.45	0.81
Apparent ruminal digestibility, %	17.8	10.6	16.0	17.9	13.7	12.9	5.21	0.99	0.33	0.71
Truly ruminally digested, kg/d	5.8	4.3	5.0	5.8	4.3	4.6	1.031	0.83	0.23	0.96
True ruminal digestibility, %	29.8	20.5	23.6	30.0	24.0	22.8	4.93	0.77	0.12	0.84
Fecal, kg/d	7.5	6.8	7.3	6.5	6.6	7.6	0.46	0.19	0.05	0.08
Total tract digested, kg/d	12.2	13.4	14.0	13.0	11.2	12.6	0.86	0.11	0.33	0.08
Total tract digestibility, %	62.1	65.8	65.9	66.8	62.5	62.4	2.08	0.53	0.96	<0.01
Starch										
Intake, kg/d	5.1	6.2	6.6	4.8	5.5	6.1	0.33	0.02	<0.01	0.68
Duodenal flow, kg/d	2.2	3.9	5.6	2.5	3.5	5.1	1.03	0.75	<0.01	0.75
Ruminally digested, kg/d	2.9	2.3	1.1	2.2	1.9	0.9	0.98	0.45	0.05	0.90
Ruminal digestibility, %	59.8	39.3	15.8	48.2	35.8	13.8	16.84	0.48	<0.01	0.87
Fecal, kg/d	0.2	0.2	0.4	0.2	0.1	0.3	0.07	0.65	<0.01	0.57
Total tract digested, kg/d	4.9	6.1	6.2	4.6	5.3	5.8	0.32	0.01	<0.01	0.71
Total tract digestibility, %	96.8	97.7	94.3	95.7	97.5	95.1	1.12	0.82	0.02	0.63

[1] Orchardgrass:grain ratio was 50:50 of the total mixed ration (DM basis).

[2] Standard error of the individual treatment means for fertilizer rate by grain interaction.

[3]Probability that fertilizer rate, grain, or fertilizer rate by grain interaction did not influence results for different treatments. The effect had a significant influence on the result when the value of P was < .05.

Table 3-5. Least squares means for intake and digestibility of neutral detergent fiber (NDF) and organic matter in cows fed total mixed rations[1] (TMR) containing orchardgrass silages fertilized at 200 (OG200) and 400 (OG400) pounds N per acre and concentrate mixes containing barley (B), corn (C), or milo (M).

Item	OG200			OG400			SEM[2]	Effect[3], P =		
	B	C	M	B	C	M		Fertilizer Rate	Grain	Fertilizer X Grain
NDF										
Intake, kg/d	7.4	6.6	6.9	7.4	5.9	6.6	0.32	0.13	<0.01	0.39
Duodenal flow, kg/d	4.8	3.6	3.2	4.4	2.9	3.3	0.44	0.20	<0.01	0.39
Ruminally digested kg/d	2.6	3.0	3.7	3.0	3.0	3.2	0.39	1.00	0.17	0.45
Ruminal digestibility, %	34.7	45.2	54.2	40.8	52.2	49.3	5.49	0.46	0.01	0.35
Fecal, kg/d	4.1	3.5	3.4	3.4	3.2	3.6	0.03	0.14	0.08	0.07
Total tract digested, kg/d	3.3	3.1	3.5	4.0	2.7	3.0	0.32	0.51	<0.01	0.02
Total tract digestibility, %	45.3	46.4	51.3	53.6	44.6	45.5	3.78	0.91	0.19	<0.01
Organic Matter										
Intake, kg/d	18.1	18.6	19.6	18.0	16.4	18.6	0.89	0.05	0.07	0.32
Duodenal flow, kg/d	13.8	15.0	15.4	13.1	12.6	15.0	1.16	0.06	0.06	0.36
Apparent digested ruminal kg/d	4.3	3.6	4.2	4.9	3.8	3.6	0.93	0.92	0.45	0.74
Apparent ruminal digestibility, %	24.4	18.6	21.4	27.6	22.9	19.3	4.93	0.55	0.26	0.66
Truly ruminally digested, kg/d	6.6	5.4	5.7	7.1	5.5	5.5	0.97	0.87	0.22	0.91
True ruminal digestibility, %	36.9	28.8	29.2	40.1	33.5	29.6	5.08	0.40	0.07	0.85
Fecal, kg/d	6.7	6.1	6.5	5.7	5.9	6.8	0.41	0.19	0.04	0.06
Total tract digested, kg/d	11.4	12.5	13.1	12.2	10.5	11.8	0.78	0.10	0.32	0.08
Total tract digestibility, %	63.4	66.7	66.8	68.2	63.4	63.3	2.03	0.53	0.80	<0.01

[1] Orchardgrass:grain ratio was 50:50 of the total mixed ration (DM basis).

[2] Standard error of the individual treatment means for fertilizer rate by grain interaction.

[3]Probability that fertilizer rate, grain, or fertilizer rate by grain interaction did not influence results for different treatments. The effect had a significant influence on the result when the value of P was < .05.

Table 3-6. Least squares means for nitrogen metabolism and digestion in cows fed total mixed rations[1] (TMR) containing silages produced from orchardgrass fertilized at a rate of 200 (OG200) and 400 (OG400) pounds N per acre and concentrate mixes containing barley (B), corn (C), or milo (M).

Item	OG200			OG400			SEM[2]	Effect[3], P =		
	B	C	M	B	C	M		Fertilizer Rate	Grain	Fertilizer X Grain
Nitrogen (N)										
Intake, g/d	543	557	585	553	503	567	28.8	0.25	0.12	0.34
Fecal, g/d	226	223	246	209	245	269	18.6	0.25	<0.01	0.09
Fecal %	3.00	3.29	3.38	3.23	3.71	3.57	0.105	<0.01	<0.01	0.33
Total tract digested, g/d	317	334	339	344	258	298	27.0	0.11	0.31	0.08
Total tract digestibility, %	58.5	59.4	57.8	62.1	51.0	52.5	2.06	0.05	0.03	0.02
Duodenal N Flow										
Duodenal flow, g/d	619	675	610	618	593	630	56.5	0.53	0.92	0.43
Bacterial N Flow g/d	215	178	150	210	165	189	35.0	0.73	0.18	0.56
Feed N, g/d	404	497	459	408	427	441	42.2	0.31	0.23	0.53
Bacterial N Efficiency										
Bacterial N g/kg OM apparent digested	67.5	74.3	56.1	53.1	63.6	56.1	16.3	0.81	0.32	0.48
Bacterial N g/kg OM truly digested	34.5	38.2	27.6	32.3	33.6	33.2	6.34	0.91	0.57	0.58
Rumen Fluid Dry Matter Bacterial RNA %	0.124	0.128	0.127	0.114	0.124	0.116	0.0054	<0.01	0.16	0.58

[1] Orchardgrass:grain ratio was 50:50 of the total mixed ration (DM basis).

[2] Standard error of the individual treatment means for fertilizer rate by grain interaction.

[3]Probability that fertilizer rate, grain, or fertilizer rate by grain interaction did not influence results for different treatments. The effect had a significant influence on the result when the value of P was < .05.

Table 3-7. Least squares means for rumen pH and volatile fatty acid (VFA) concentrations in cows fed total mixed rations[1] (TMR) containing silages produced from orchardgrass fertilized at a rate of 200 (OG200) and 400 (OG400) pounds N per acre and concentrate mixes containing barley (B), corn (C), or milo (M).

Item	OG200			OG400			SEM[2]	Effect[3], P =		
	B	C	M	B	C	M		Fertilizer Rate	Grain	Fertilizer X Grain
pH	5.45	5.48	5.53	5.51	5.48	5.56	0.040	0.28	0.18	0.71
Total VFA mM/L	79.7	76.3	78.7	82.9	81.7	78.9	1.48	<0.01	0.14	0.16
VFA, mol/100										
Acetate	69.0	66.8	67.3	67.2	64.9	66.8	0.34	<0.01	<0.01	0.06
Propionate	17.1	19.2	18.5	18.8	20.6	18.5	0.38	<0.01	<0.01	0.05
Butyrate	10.0	10.3	10.5	10.0	10.5	10.5	0.10	0.24	<0.01	0.01
Valerate	1.0	1.0	0.9	1.1	1.2	1.2	0.04	<0.01	0.59	0.19
Isobutyrate	0.8	0.8	0.9	0.9	1.0	1.0	0.04	<0.01	0.51	0.91
Isovalerate	1.5	1.5	1.5	1.5	1.5	1.7	0.04	<0.01	<0.01	0.01
Acetate:Propionate	4.08	3.58	3.70	3.69	3.24	3.67	0.079	<0.01	<0.01	0.97
Total Branched Chain Fatty Acids	2.38	2.33	2.42	2.45	2.46	2.64	0.067	<0.01	0.07	0.48
Ammonia mg/dL	21.0	20.3	21.7	25.1	27.4	26.5	01.27	<0.01	0.65	0.42

[1] Orchardgrass:grain ratio was 50:50 of the total mixed ration (DM basis).

[2] Standard error of the individual treatment means for fertilizer rate by grain interaction.

[3]Probability that fertilizer rate, grain, or fertilizer rate by grain interaction did not influence results for different treatments. The effect had a significant influence on the result when the value of P was < .05.

Table 3-8. Least squares means for body weight, milk production, and milk composition in cows fed total mixed rations[1] (TMR) containing silages produced from orchardgrass fertilized at a rate of 200 (OG200) and 400 (OG400) pounds N per acre and concentrate mixes containing barley (B), corn (C), or milo (M).

								Effect[3], P =		
	OG200			OG400						
Item	B	C	M	B	C	M	SEM[2]	Fertilizer Rate	Grain	Fertilizer X Grain
Body Weight, kg	613	616	622	610	608	607	26.2	0.01	0.74	0.30
Milk production, kg/d	25.5	27.1	27.3	27.3	26.4	26.8	2.05	0.94	0.75	0.56
Milk fat, %	3.67	3.71	3.53	3.86	3.73	3.53	0.239	0.46	0.10	0.66
Milk protein, %	3.20	3.22	3.20	3.18	3.29	3.28	0.112	0.17	0.25	0.34
Milk solids not fat (SNF) %	8.68	8.69	8.69	8.70	8.76	8.77	0.165	0.15	0.64	0.84
Milk urea nitrogen (MUN), mg/dl	13.4	12.5	13.8	13.6	13.4	14.5	0.74	0.22	0.17	0.87

[1] Orchardgrass:grain ratio was 50:50 of the total mixed ration (DM basis).

[2] Standard error of the individual treatment means for fertilizer rate by grain interaction.

[3]Probability that fertilizer rate, grain, or fertilizer rate by grain interaction did not influence results for different treatments. The effect had a significant influence on the result when the value of P was < .05.

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