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In vitro digestibilty of ryegrass supplemented with hay, corn, or soybean hulls

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IN VITRO DIGESTIBILTY OF RYEGRASS SUPPLEMENTED WITH HAY, CORN, OR SOYBEAN HULLS

By

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An *in vitro* continuous culture rumen fermentation experiment was conducted to evaluate digestibility of annual ryegrass either fed alone or annual ryegrass supplemented with hay, corn, or soybean hulls. Nutrient disappearance of feedstuffs offered were not different ($P > 0.05$) as a percentage of the diet however there were differences ($P < 0.05$) in amounts of individual nutrients digested for each treatment. Ammonia-N concentrations of culture samples were less ($P < 0.05$) for vessels fed corn as a supplement however there was no difference ($P > 0.05$) among vessels fed either ryegrass alone or supplemented with hay or soybean hulls. This may indicate a more efficient use of available nutrients from annual ryegrass when corn was supplemented.

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TABLE OF CONTENTS

ACKNOWLEDGEMENTS ii

LIST OF TABLES iv

LIST OF FIGURES v

CHAPTER

I. INTRODUCTION 1

II. LITERATURE REVIEW 7

Annual Ryegrass and Grazing 7
Corn Supplementation 14
Soybean Hull Supplementation 17
Hay Supplementation 21

III. MATERIALS AND METHODS 23

Ryegrass Collection and Feedstuff Preparation 23
Rumen Fluid Collection 24
Continuous Culture Apparatus and Sample Collection 24
Laboratory Analysis 27
Statistical Analysis 28

IV. RESULTS AND DISCUSSION 29

V. CONCLUSION 38

LITERATURE CITED 40

LIST OF TABLES

TABLE Page

1. Ingredients and concentrations of buffer solutions used for *in vitro* continuous culture rumen fermenter feeding ryegrass either alone or supplemented with hay, corn, or soybean hulls.....26

2. Nutrient composition of feedstuffs used for *in vitro* continuous culture rumen fermentation trials.....31

3. Nutrient disappearance of feedstuffs offered during *in vitro* continuous culture rumen fermentation trials 34

LIST OF FIGURES

FIGURE Page

1. Ruminal ammonia-N assay results presented in mg/dL for each culture sample time and treatment 36

CHAPTER I

INTRODUCTION

Finishing diets formulated for cattle are designed to be cost effective relative to animal performance. Ruminant diets containing large amounts of forage as their primary feed source may not gain or produce as much as an animal receiving grain. Data suggest more time is needed for cattle consuming a forage-based finishing diet to reach appropriate weight. Additionally, a decrease in desirable carcass characteristics once the animal has reached weight for slaughter has been reported in animals finished on a forage-based diet (Bidner et al., 1986). A niche market exists for animals consuming only forage; however, this method of production can cause increased costs due to the amount of time needed for animals to reach market weight (Bennett et al., 1995, Moore et al., 1999, Ryan et al., 2006). Cattle consuming finishing diets with greater amounts of concentrate may also experience decreases in production due to digestive disturbances such as acidosis. However, when allowing cattle to graze grass, several factors must also be considered to ensure each animal receives adequate nutrients to meet their requirements. Animals grazing in the same pasture may have different requirements due to their stage of production, current weight, and genetic variation even within the same breed. Each animal will consume slightly differing amounts of forage and supplements

depending on how and when they are available, especially if the nutrients are from grazing pasture (Kunkle et al., 2000). Studies have been conducted to determine the most effective balance of forage and concentrate of a forage based diet, however, few data exist examining cattle grazing annual ryegrass (Lolium multiflorum) supplemented with energy and (or) protein sources (Anderson et al., 1988; Richards et al., 2006; Roberts et al., 2009).

When cattle consume lush forages as their only energy and protein source, intake of available nutrients may not be adequate to meet desired rates of animal performance, especially for intensely managed production animals (Moore et al., 1999). Supplementation of additional energy and fiber sources may slow down rate of passage and allow more time for bacterial populations in the rumen to utilize nutrients that would otherwise not be digested in the small intestine (Rude et al., 2002). Additionally, cattle grazing lush forages may not effectively utilize N resources from the grass thus losing N to the environment in the form of urea (in urine). Providing energy in the form of a supplement can allow for more effective capture of N in the rumen (Rude et al., 2002).

There is much data comparing various energy supplements fed to ruminants grazing lush pasture, but little research has been reported on ruminants grazing L. multiflorum supplemented with additional fiber or energy sources either *in vivo* or *in vitro* (Miller et al., 2001). Grazing and metabolism trials have been conducted to determine the effect of supplementation to cattle consuming various species of pasture grasses. Ruminants consuming lush pasture that contain large amounts of readily digestible CP may not effectively utilize all of the available nutrients. Capture of N by ruminant microbial populations is critical for the animal to receive maximum benefits of

grazing lush forage. Without existing energetic substrates, CP degraded in the rumen is absorbed as NH_3 and will be excreted in the urine (Elizalde et al., 1999a). Miller et al. (2001) stated that limited supplies of energy in the rumen when grazing lush forage resulted in increased ruminal ammonia; therefore, dietary protein was not efficiently assimilated into microbial protein rather it was absorbed across the rumen wall and effectively lost by hepatic conversion into urea. Further, forage protein was wasted when excess ammonia flow out of the rumen environment occured, thus fermentable energy was insufficient to support microbial growth required to utilize the available excess degraded protein from the forage source (Beever et al., 1986; Broderick et al., 1992). Providing additional fermentable energy in the form of grain may allow for a more efficient microbial capture of N as the forage CP is degraded in the rumen (Rude et al., 2002). However, grain supplementation has been shown to decrease fiber and other nutrient digestibility of fresh forage (Caton and Dhuyvetter, 1997). This decrease in digestibility of the forage does not necessarily mean a decrease in overall digestibility of the diet, rather this could mean large portions of starch digestion may shift from the rumen environment to the small intestine (Elizalde et al., 1999a).

Providing supplemental energy in forms other than readily fermentable starches may provide adequate amount of energetic substrates for rumen microbes while minimizing shifts in microbial populations that would diminish positive associative effects. Soybean hulls (SBH) are a by-product of soybean processing and are obtained from the fibrous coat that surrounds the seed. Hulls have little value for human food or industrial use, but make a suitable feedstuff for ruminants. The increased amount of digestible cellulose (from 40 to 50 %) and relatively reduced amount of lignin (between 2

and 3%) may make SBH supplementation more advantageous for cattle grazing lush pasture (Hsu et al., 1987; Ipharraguerre and Clark, 2003). Additionally, ruminal pH was less affected by supplementation of SBH compared to changes in ruminal pH when corn was supplemented to a forage-based diet and was attributable to the fibrous nature of SBH (Klopfenstein and Owen, 1988).

While energetic supplements, such as SBH or corn, provide substrates for more efficient capture of N and may increase the feeding value of annual ryegrass, these supplements may not affect ruminal rate of passage to the degree that hay supplementation may affect ruminal rate of passage. Feeding moderate quality hay (bermudagrass hay) to cattle grazing lush ryegrass pasture may result in increased time the ingesta remains in the digestive tract thus allowing more efficient utilization of the forages (Rude et al., 2002).

The combination of available energy and N sources is the key for maximizing efficiency of the animal and utilizing available nutrients from L. multiflorum. This ability to provide the proper concurrent nutrients is known as nutrient synchrony. Hersom (2008) described nutrient synchrony as a parallel occurrence of nutrients that the animal consumes or that is present in the diet and rumen environment thereby supplying energy and N sources simultaneously leading to an increase or optimization of microbial efficiency. Hersom (2008) further states that an increase in microbial efficiency should translate into increased animal performance. To understand the synchrony of nutrients supplied one must know the nutritional composition and amount of nutrients that will be supplied to the rumen environment. For grazing ruminants this can be difficult to ascertain because of the difficulty to accurately determine intake amounts of grazed

forage (Bargo et al., 2003a). Continuous culture fermentation systems have been used to collect *in vitro* data from parameters that may be difficult to measure *in vivo* especially when animals are in a grazing trial rather than a confined metabolism trial (Bargo et al., 2003b; de Veth and Kolver, 2001; Hindle et al., 2005). These *in vitro* techniques have been developed to provide a means to study ruminal microbial fermentation in a way more similar to *in vivo* fermentation rather than a simple closed vessel system (Hoover et al., 1976). Conventional continuous culture systems consist of a glass vessel or flask that simulates the rumen environment with flows of solids and liquids both to and from the site of fermentation. In addition to dual flow systems, there are single flow systems that implement a more controlled flow out of the vessel rather than a constant flow.

Feedstuffs may be fed in a chopped, ground, or pelleted form to the culture and then effects resulting from these diets in the form of microbial fermentation characteristics can be quantified and studied. Once the rumen kinetics of the forage itself is understood *in vitro* supplements can be included in a ration in efforts to increase overall nutrient utilization. Digestion behavior of the overall diet can be studied to determine nutrient synchrony or combination of feedstuffs that will be the most advantageous for the desired production of the target species. Associative effects may be positive or negative depending on the supplement-forage interactions that occur in the rumen environment. Associative effects observed between the forage and grain components of diets may actually be detrimental for efficient utilization of nutrients from either grain or forage (Dixon and Stockdale, 1999). Hersom (2008) stated that measurements associated with nutrient synchrony include: ruminal pH, total VFA production and individual VFA concentration, ammonia concentration, microbial yield,

microbial protein, and microbial efficiency. These parameters and their responses can be used to determine the positive or negative response overall and especially measurements of efficient production such as carcass quality and weight gain. Continuous culture fermentation experiments designed to simulate the rumen environment allow researchers to accurately study these parameters that may otherwise be difficult to obtain *in vivo*, therefore the objective of the current experiment was to examine the ruminal effects of feeding annual ryegrass (L. multiflorum) either alone or with supplemental hay, corn, or soybean hulls using continuous culture rumen fermentation techniques.

CHAPTER II

LITERATURE REVIEW

Annual Ryegrass and Grazing

Ryegrass is one of the most important pasture and turf grasses in the world (Hannaway et al., 1999). Ryegrasses are utilized on all continents that posses desired temperate regions for production and it is often a key forage specie in countries with intensive livestock grazing year round (Jung et al., 1996). Annual ryegrass or "Italian" ryegrass is native to southern Europe and was first cultivated in Italy (Piper, 1924). Annual ryegrass has been used in production systems in France as early as 1818 and was introduced to England by 1830 (Piper, 1924). There are two predominant species of ryegrass grown in the United States and dozens of cultivars of both species are commercially available including numerous cold tolerant cultivars. Perennial ryegrass (Lolium perenne) is poorly adapted to the southeastern U.S. with warmer year round temperatures. Perennial ryegrass species have a low tolerance for hot summers and cold winters of the continental United States and will not usually persist long as an adapted grass (Blount et al., 2000). Annual ryegrass (Lolium multiflorum) is a very palatable, high quality and productive cool-season forage that is well adapted to the climate of the southeastern United States (Ball et al., 2002). Environmental factors such as rainfall and ambient temperature will greatly affect growth of L. multiflorum, although once

established the grass will perform well if environmental conditions are satisfied. An estimated one million hectares of annual ryegrass is grown in the southeastern United States alone (Blount et al., 2000). Furthermore, L. multiflorum has the advantage of a long growing season, rapid propagation and growth during autumn months, greater yields compared to other cool-season forages, tolerance to a wide range of environmental conditions as well as utility with different grazing practices and compatibility with other cool-season grasses (Tucker et al., 2001). Under desirable environmental conditions, annual ryegrass has even been shown to behave as a short-term perennial or biennial (Blount et al., 2000). Adequate rainfall and generally mild winters are required for successful growth and utilization of L. multiflorum, again, making it very suitable for the southeastern United States (Blount et al., 2000). Annual ryegrass is often used in management systems with other cool-season grasses to economically extend the grazing season and it can be grazed very close to the ground without detrimental effects on grass re-growth (Jung et al., 1996). Dry matter digestibility (*in vivo*) of L. multiflorum is greater than 65% (Blount et al., 2000). Annual ryegrass also has a relatively large amount of crude protein, 17.9 % DM basis, when compared to other lush forages, and CP concentrations can often exceed 20 % (Mooso et al., 1990). Concentrations of NDF and ADF remain relatively small during this time averaging less than 40 % and 20 % respectively (Mooso et al., 1990). Grazing annual ryegrass throughout winter months is possible because of the plant's ability to withstand cold temperatures (Hoveland et al., 1991, Kallenbach et al., 2003, Keatinge et al., 1980). Additionally, L. multiflorum lacks a true dormancy stage allowing for growth to occur during mild periods in autumn and winter when average temperatures are less than 6 °C (Cherney and Robinson, 1985,

Kallenbach et al., 2003, Keatinge et al., 1980). However, desirable characteristics that make L. multiflorum well suited for grazing may also pose a problem. Annual ryegrass is very palatable leading to the potential for livestock to over consume this forage. Due to the increased amount of water and thereby decreased DM of the leaf, the rate of passage for ruminants can be very fast. Dry matter content has been determined to average 22.6 %, DM basis (Jurgens, 2002). Additionally, L. multiflorum contains large amounts of water-soluble carbohydrates (i.e. fructose, glucose, xylose, sucrose, and fructans) that are most often concentrated in the stem of the grass (Jung et al., 1976, McGrath, 1988, Tucker et al., 2001, Wilman et al., 1992). These readily available carbohydrates make ryegrass an especially valuable forage for grazing ruminants (Tucker et al., 2001). Stocker cattle gains have been reported to be as fast as 0.5 to 1.5 kg per head per day (Sladden and Bransby, 1992). However, complete utilization of the available nutrients will not be realized if the rate of passage exceeds the amount of time needed in the rumen environment or small intestine to effectively digest the nutrients.

Studies have been conducted measuring the effects of various supplements to cattle grazing either annual ryegrass or perennial ryegrass. Researchers have examined the effects of protein supplementation to early-weaned calves grazing ryegrass pasture (Vendramini and Arthington, 2008), grazing stocker cattle on annual ryegrass or ryegrass-mixed (bermudagrass or dallisgrass overseeded with L. multiflorum) pastures (Beck et al., 2005, Coffey et al., 2002) and dairy cows grazing annual ryegrass during lactation (Tucker et al., 2001). Stockpiled annual ryegrass has also been investigated for grazing through winter months when quality forages may otherwise be limited (Kallenbach et al., 2003). Perennial ryegrass may be more cost effective for producers to

maintain than annual ryegrass. Perennial ryegrass is used in areas outside of the southeastern United States where pastures of annual ryegrass species are not effectively established. Grazing and feeding trials have been conducted with L. perenne and mixed pastures (white clover) to determine the nutritive value of ruminants grazing these forages (Cammell et al., 1986, Ulyatt et al., 1988).

Vendramini and Arthington (2008) investigated the effects of supplementation on performance of early-weaned calves grazing cool or warm season pasture. They determined that CP supplementation to calves grazing annual ryegrass had little or no effect on ADG during the trial period. However, early-weaned calves grazing annual ryegrass and receiving 1% BW concentrate had greater ADG than calves grazing bahiagrass pastures receiving 2% BW supplemental concentrate. Furthermore, the researchers concluded that annual ryegrass had greater nutritive value than bahiagrass resulting in increased performance of the calves, but given the low DM concentrations of annual ryegrass it is unlikely that early-weaned calves can consume enough nutrients through grazing alone to meet their requirements (Vendramini and Arthington, 2008, Vendramini et al., 2006).

In addition to research conducted with early-weaned calves, performance of stocker cattle grazing L. multiflorum has been investigated as well. Beck et al. (2005) studied the performance of stocker cattle grazing cool-season annual grass mixtures (oats, cereal rye, annual ryegrass, and winter wheat) to compare BW gains and economic performance of these grasses individually or combinations of these grasses. During a multi-year grazing study animals were allowed to graze pastures seeded with oats (Avena sativa), cereal rye (Secale cereal), annual ryegrass (L. multiflorum) or soft-winter wheat

(Triticum aestivum) or combinations of these cool-season grasses. Although there were year variations due to climatic conditions, annual ryegrass and cereal rye mixed treatments performed better by BW% when compared to other treatments. In the second year of the study annual ryegrass and cereal rye mixed pastures produced 34% more BW gains per hectare. During the final year of the trial, pastures containing annual ryegrass alone or in combination with cereal rye produced 43% more gain per hectare than other treatments. Researchers reported that on a cost basis using annual ryegrass alone or in mixtures may still be a risk because of the variability of L. multiflorum performance due to environmental conditions.

Winter annual forages such as L. multiflorum can be used in tandem with existing warm season perennial grasses to extend the grazing system and improve efficiency of land use for producers (Coffey et al., 2002). Cool-season forages interseeded into warm-season forage pasture may not grow to full potential, but the propensity to increase efficiency of land use along with improving gains relative to animals consuming only dormant forages during winter can be advantageous (Coffey et al., 2002, Moyer and Coffey, 2000). Coffey et al. (2002) compared growth performance of stocker calves grazing sod-seeded annual ryegrass pastures in combination with warm-season grasses. Authors discovered grazing annual ryegrass alone or in combination with rye or wheat may provide adequate winter grazing especially for fall-weaned calves. Furthermore, this may reduce intake of other feedstuff supplements such as hay or grain while also allowing producers to graze fall-weaned calves through the winter and increasing weight gain for sale in the spring (Coffey et al., 2002).

The utilization of lush cool-season forages is not limited to beef cattle production. In the dairy industry, producers manage herds to maximize efficiency and production while minimizing costs. The market price of supplemental feedstuffs can influence the management practices and supplemental feedstuffs that a producer will implement. Grazing pastures is an important tool because of the reduced cost of nutrients delivered to the animal when compared to feeding concentrates, hay or even a total mixed ration (Tucker et al., 2001). When dairy cattle are switched to an intensive grazing situation to utilize available lush pasture both economic and environmental advantages are often discovered (Holden et al., 1994). Additionally, feeding supplemental energy to grazing cows can be beneficial for improving utilization of degradable protein found in L. multiflorum as well as increasing N capture by ruminant microbes (Tucker et al., 2001). Tucker et al. (2001) further reported that the cost of supplements as well as the increased cost of handling and supplying energy does not surpass the value of added animal performance.

When cattle graze very lush pasture containing large amounts of CP, the utilization of pasture protein can be decreased due to rapid fermentation and degradation in the rumen leading to increased nutrient flow out of the rumen (Bach et al., 1999, Lopez et al., 1991). Moreover, significant amounts of NH_3 may be absorbed through the ruminal wall and thus not incorporated into microbial protein (Bach et al., 1999, Siddons et al., 1985). In attempt to further understand the ruminal kinetics leading to inefficient microbial synthesis when lush forage is consumed, ryegrass digestion has been investigated using continuous culture rumen fermentation techniques (de Veth and Kolver, 2001). de Veth and Kolver (2001) designed an *in vitro* experiment to establish

ruminal pH required for optimal pasture digestion and microbial protein when large amounts of readily fermentable carbohydrates or lush pasture are fed as a basal diet. Researchers harvested fresh ryegrass from pastures during spring growth periods and stored it in a freezer. Grass was then freeze-dried and ground in preparation to be fed to ruminal microbial cultures *in vitro*. Ruminal pH was maintained at four different values of 5.4, 5.8, 6.2, and 6.6 to determine the optimal pH for digestion of lush pasture. Authors found that optimal digestion of pasture DM *in vitro* occurred at pH of 6.35 and the largest reduction in pasture DM digestibility occurred when pH was below 5.8. Additionally, ammonia concentration increased when pH increased from 5.8 to 6.6 and was most likely due to increased deamination of dietary N found in the grass (de Veth and Kolver, 2001). Furthermore, microbial N flow increased quadratically as pH increased from 5.4 to 6.6 and researchers determined microbial N flow to the duodenum was optimized at pH of 6.13. Bach et al. (1999) reported increased NH_3N flow in a single flow continuous culture apparatus when only pasture (25 % red clover, 25 % alfalfa, and 50 % orchardgrass) was fed compared to when corn or soybean hulls were supplemented with pasture. Moreover, nonammonia N flow out of the rumen increased significantly when supplements were added to the diet compared to the treatment only receiving grass (Bach et al., 1999). The greatest amount of microbial N as a percent of microbial DM was found to be the pasture only treatment. These data along, with annual ryegrass experiments conducted *in vivo* elicit the need for further research to predict the feeding value of lush pasture with or without energetic supplementation through continuous culture rumen fermentation techniques.

Corn Supplementation

When ruminants consume lush pasture with large concentrations of CP and sufficient energy is not present for ruminal microbial synthesis, a large portion of CP is degraded in the rumen and may be absorbed as NH_3 (Elizalde et al., 1999a). This NH_3 is converted to urea and excreted in urine, thus large amounts of N is lost and not captured by the animal to be used for production (Pond, 2005). Supplementing corn to grazing ruminants to determine the positive or negative associative effects has been extensively studied (Brake et al., 1989, Chase and Hibberd, 1987, Elizalde et al., 1999a, b, Faulkner et al., 1994, Galloway Dl et al., 1993, Grigsby et al., 1993, Hersom, 2008, Hess et al., 1994, Jones et al., 1988, Karnezos et al., 1994, Moore et al., 1999, Pavan and Duckett, 2008, Roberts et al., 2009, Stokes et al., 1988). Elizalde et al. (1999b) found that when cracked corn was supplemented to steers that were fed fresh alfalfa, N utilization and digestion was increased compared to steers that were not supplemented. Furthermore, the researchers concluded that corn supplementation increased the amount of escape protein (amino acids) that is available for intestinal absorption. Similar studies have reported increased animal production when a restricted amount of corn was supplemented to grazing diets. Karnezos et al. (1994) examined the response of lambs grazing alfalfa pasture when increasing amounts of corn was supplemented. Authors determined that lambs fed 247g of corn per day gained on average 20% more when compared to lambs receiving no supplemental grain source. Moreover, supplemental corn resulted in increased lamb growth rates as well as production per hectare. It was concluded that these positive responses were the result of a more efficient utilization of the available CP from grazing alfalfa. More recently, Roberts et al. (2009) found that increasing the

amount of grain (corn) in the diet of beef steers grazing L. multiflorum resulted in increased ADG and decreased the amount of days to reach desired market weight. Additionally, steers consumed less forage DM when supplemented thus allowing researchers to increase stocking rates without causing adverse effects on animal production. In these experiments, corn was supplemented in quantities that had little or no observable negative associative effects that could impair rumen function.

Microbial fermentation in the rumen allows for cellulose digestion that would otherwise be inefficient or unlikely in monogastric animal species. However, rumen microbial fermentation of starch-based grains before exposure to digestive enzymes in the small intestine may introduce inefficiencies resulting in poor utilization of this energy source. Rumen fermentation can reduce the energy value of starch from grain by 30 to 50% although this may be accompanied by an increase in post-ruminal digestion of synthesized microbes and absorption in the form of amino-acids (Dixon and Stockdale, 1999). Additionally, as little as 10 to 15% inclusion of readily fermentable carbohydrates in a diet can impair ruminal fiber digestion (Hoover, 1986).

Research has been conducted to determine possible negative associative effects that can occur from feeding increased amounts of concentrate with forage-based diets. Negative associative effects most often occur when grain is fed in large amounts compared to the amount of forage in the ration. The type or amount of forage has less effect on grain digestion than does the change in microbial digestion of forage resulting from supplementation of grain. Often, decreased efficiency from supplementing cereal grains occurs when animals consume reduced quality forages. Negative associative effect that result from inclusion of grain lead to a decreased ability of ruminant microbes

to utilize available energy from forages especially when the forage is of low to moderate digestibility (Dixon and Stockdale, 1999).

Most often, reductions in ruminal pH are observed and cited as the major cause of reduced fiber digestion of forage when grain is supplemented both *in vivo* and *in vitro* (Calsamiglia et al., 2008, Carey et al., 1993, Caton and Dhuyvetter, 1997, Lardy et al., 2004). Cary et al. (1993) reported a decreased overall pH response curve when corn was supplemented to beef steers consuming brome grass hay as a forage source. Total tract DM and OM digestibility was increased with the addition of corn, but steers fed supplemental corn did not maintain NDF digestibilities equivalent to control steers (hay equalized for CP with soybean meal). Lardy et al. (2004) evaluated the effects of increasing grain (barley) concentrations in a forage-based diet fed to beef cattle. Steers fed 2.4 kg of barley (DM basis) per day showed a decrease in ruminal pH while animals fed either .8 or 1.6 kg of barley yielded no pH response. Overall digestibility of the diet was found to be increased as barley supplementation increased, however the utilization of forage in the diet decreased as measured by intake and ADF digestibility. When Calsamiglia et al., (2008) compared feeding a concentrate-based diet (90 % corn; 10 % hay) to a forage-based diet (60 % hay; 40 % corn) *in vitro,* they found many negative effects that the high concentrate diet had on pH and total digestion of the diet. Total OM and NDF digestion were reduced as a result of declining pH. Ammonia-N concentration was also decreased as a result of declining pH and observed in the concentrate-based diet. Overall the efficiency of microbial synthesis was greatly reduced as a result of decreased ruminal pH. Microbial protein synthesis was found to also be further diminished when microbes were fed the concentrate-based diet compared to the forage-based diet. Other

data suggest decreased ruminal pH when starch is supplemented may not always be an effect of additional carbohydrate fermentation in the rumen. Mould and Orskov (1983) fed sheep large amounts of concentrate and were able to raise ruminal pH with bicarbonate buffer, but DM digestion (*in situ*) was still less than that of the control group fed only forage suggesting ruminal pH was not the complete cause for negative associative effects of the diet.

Corn supplementation will affect intake and digestibility of a forage-based diet if fed in great concentrations. Reduction of forage intake and digestibility may be overcome by increased intake and digestibility of the overall as a result of corn being supplemented. Decreased ruminal pH seems to be one explanation for the negative associative effects reported (Anderson et al., 1988, Burgwald-Balstad et al., 1995, Cerrato-Sanchez et al., 2007a, b, 2008, DelCurto et al., 1990, Grigsby et al., 1992, Leventini et al., 1990, Russell et al., 1979, Sanson et al., 1990). However, there are most likely other factors that lead to reduction of forage intake and digestibility (Caton and Dhuyvetter, 1997).

Soybean Hull Supplementation

Soybean hulls are a readily available, fibrous by-product of the soybean milling industry. During soybean processing, the seed coat is removed to allow for adequate flaking of the kernel and to crack of the whole soybean into smaller pieces. The seed coats (hulls) that are removed are then ground or pelleted to reduce transportation costs and to make them more suited for storage (Ipharraguerre and Clark, 2003). These ground or pelleted soybean hulls are a starch-free, reduced lignin (1.8 to 2.0 % ADL), increased

fiber (62 to 72 % NDF) feedstuff that is a valuable resource for feeding ruminants, especially beef cattle (Hsu et al., 1987, Martin and Hibberd, 1990). As with most by-product feedstuffs, differences among processor facilities often exist, affecting the nutritive value and nutrient composition leading to possible inaccuracies when formulating a ration using these by-products as a supplement. Factors associated with the variable composition of SBH can range from differences in processing methods to environmental conditions that affect chemical composition of the soybean plant during growth (Drackley, 2000, Ipharraguerre and Clark, 2003). The increased amount of CP found in SBH can be of particular use for ruminant diets because of the amount of this CP that can escape microbial degredation in the rumen and flow directly to the small intestine. Ipharraguerre and Clark (2003) found the CP content of SBH to average 11.8 %, and values ranged from 9.4 % (Anderson et al., 1988) to 19.2 % CP (Batajoo and Shaver, 1998) when SBH were found to contain seed components in addition to the hull thus increasing the starch concentration. Furthermore, the researchers stated the feeding value of SBH is affected by the rate at which they are digested in the rumen and the rate at which they leave the rumen and continue to the lower gastro-intestinal tract. Results from several studies have suggested that ruminal microbes are capable of extensively fermenting SBH at rapid rates (Anderson et al., 1988, Batajoo and Shaver, 1998, DePeters et al., 1997, Grigsby et al., 1992, Highfill et al., 1987, Nocek and Hall, 1984, Pantoja et al., 1994). Defrain et al. (2002) postulated that the rapid passage rate of SBH can be attributed to the small particle size and increased specific gravity relative to other ruminal particles. A large positive associative effect has been observed when SBH were added to forage-based diets and animals were allowed ad libitum access to forage and

concentrate (Ipharraguerre and Clark, 2003). Ipharraguerre and Clark (2003) further concluded that the composition of diet had been influenced *in vivo* thus affecting the feeding value of SBH by changing the ruminal forage mat, causing it to remain in the rumen longer. Conversely, these effects were not observed when animals were consuming a limit-fed diet supplemented with SBH (Ipharraguerre and Clark, 2003).

Supplying readily digestible fiber to ruminant microbes for use as an energetic substrate for microbial synthesis, rather than starch, can reduce shifts in microbial populations otherwise resulting in poor utilization of forage. McDonnell (1982) stated that SBH used as an energy supplement has been shown to have fewer negative associative effects on fiber digestion in the rumen than when corn is supplemented. The addition of SBH to a ruminant diet can provide the desired amount of energy needed while minimizing changes in ruminal fermentation. Soybean hulls contain less readily fermentable starch than corn thus maintaining rumen fibrolytic activity that is advantageous for microbial utilization of forage (Hoover, 1986). The inclusion of SBH can shift microbial fermentation to an increase in total ruminal VFA concentration. When SBH were compared to corn supplemented to a diet consisting of a concentrate along with alfalfa hay (concentrate comprised 48.2 % DM), researchers found that the total VFA concentrations were increased when SBH were supplemented compared to corn (Mansfield and Stern, 1994). Cunningham et al. (1993) found that replacing either high-moisture corn or forage in a diet with SBH resulted in greater overall VFA concentrations when SBH were substituted for concentrate, but little or no response when SBH were substituted for forage. Similarly, Hsu et al. (1987) noted an increase in total VFA concentration when diets fed to sheep were supplemented with SBH rather than

corn. However, these results were observed when sheep were fed a diet consisting of 80 % SBH (as fed basis). Ruminal NH_3N concentrations reported when animals were supplemented with SBH have been conflicting and sometimes variable. In dairy cattle, NH_3N concentration has been found to be increased, decreased, or unchanged by addition of SBH (Cunningham et al., 1993, Ipharraguerre et al., 2002, Sarwar et al., 1992). Furthermore, Ipharraguerre and Clark (2003) stated that the ruminal NH_3N concentration of these experiments maintained concentrations adequate for microbial growth or protein synthesis within the rumen environment.

Studies have been conducted to determine the effects of SBH supplementation, specifically, to beef cattle grazing lush cool season forage. Furthermore, efforts have been made to examine the effect SBH supplementation has on increasing ruminal capture and utilization of dietary N into microbial N. Richards et al. (2006) examined the influence of SBH supplementation on rumen fermentation and digestibility. Researchers fed 0.60 % BW (OM basis) of SBH to steers consuming fresh tall fescue and found ruminal pH was not affected. Forage OM intake was reported to decrease when SBH were added, however total OM intake was increased and total tract OM disappearance increased as well. Additionally, the increase in ruminal OM disappearance that was observed was not accompanied by an increase in ruminal VFA concentration. Total N and microbial N flowing out of the rumen to the duodenum was increased due to SBH supplementation. These data suggest supplementing SBH to steers consuming lush cool-season forage may provide for a more efficient utilization of forage while minimizing negative associative effects resulting from the addition of energetic substrates to the rumen.

Soybean hulls are a soybean processing by-product, however hulls posses' great utility for use in ruminant diets as an energy and fiber source. Supplementation of SBH as an energy source to grazing ruminants has shown favorable ruminal associative effects when compared to corn or other high-starch grain supplementation that can have detrimental effects on ruminal kinetics when supplemented in great amounts. Addition of SBH to cattle grazing lush pasture has been shown to increase N capture in the rumen environment and possibly increase the feeding value of nutrient rich cool season forages by allowing for more efficient ruminal fiber digestion.

Hay Supplementation

Protein and energy supplements, such as corn or soybean hulls, elicit little effect on the rate of passage of digesta in ruminant tracts (Freeman et al., 1992). Nutrient dense fresh pasture can be harvested and utilized to make hay to provide a form of stored forage while also delivering energy in the form of fiber for ruminants. When compared to DMI of lush pasture, animals may receive greater amounts of DM when consuming hay due to the increased DM content of the feedstuff itself. Animal performance may be less than expected when cattle graze lush pasture, such as annual ryegrass, due in part to the readily fermentable nature of ryegrass in the rumen environment. Providing fiber in the form of hay may slow down rate of passage to allow available nutrients to be utilized more effectively (Rude et al., 2002).

Since L. multiflorum is known to be readily fermentable in the rumen, much like increased amounts of carbohydrates, the increased fiber in the form of forage added to the diet may slow rate of passage as it has shown to do when added to diets with increased

starch concentrations. Studies have shown when cattle consume an equal amount of DM in the form of pasture or hay, less DM exits the rumen and is passed into the duodenum when pasture is grazed compared to animals consuming hay (Holden et al., 1994). Furthermore, this data suggests lush pasture is more readily digestible in the rumen than hay and grazing lush pasture may allocate fewer nutrients available to the small intestine than hay due to this rapid fermentation rate. Most research has reported hay supplementation to ruminants grazing lush pasture compared to the same animals supplemented with concentrate. Additionally, data exist where hay was fed as the sole forage source with concentrate supplemented rather than being utilized as a supplement itself (Coffey et al., 2002, Lardy et al., 2004) Therefore, there is little data specifically examining the effects of supplementing a quality hay source (bermudagrass) to ruminants grazing lush pasture, such as annual ryegrass.

CHAPTER III

MATERIALS AND METHODS

Ryegrass Collection and Feedstuff Preparation

Fresh annual ryegrass (Lolium multiflorum) was harvested in the winter of 2007 from non-grazed forage plots at the Joe Bearden Dairy Research Center of Mississippi State University. The freshly clipped grass was then transferred to plastic bags and stored in a freezer at -20 ºC. In order to lyophilize the grass, grab samples from the entire amount of harvested grass were packaged into breathable wire-screen bags and stored in a freezer at -80 ºC. Ryegrass samples were then lyophilized for 24 to 48 hours in a Labconco Freezone® 6L Benchtop Freeze Dry System (Labconco Corporation, Kansas City, MO) to preserve the grass and prevent nutrient loss until initiation of the trial. Upon preservation, ryegrass was ground to pass a 2-mm screen in a Thomas Wiley Mill® (Thomas Scientific, Swedesboro, NJ). Hay, corn, and soybean hulls used for the trial were ground to pass a 1-mm screen in a Thomas Wiley Mill® (Thomas Scientific, Swedesboro, NJ).

Rumen Fluid Collection

Rumen inoculum was obtained from two ruminally cannulated steers consuming an ad libitum forage-based diet with bermudagrass hay supplemented. Approximately 4 L of fluid from each of the two animals (8 L total) was collected using an electric medical pump (model 68MOD; C. M. Sorensen Co., Inc.) into an insulated glass jar. Contents were transferred into four insulated containers (2 L each) that had been purged with CO_2 and sealed until inoculum collection. Rumen fluid from each of the two cannulated steers (800 mL from each for a total of 1600 mL) was introduced to each of the four fermentation vessels. Approximately 30 to 50 g of solid matter digesta was added to each of the four fermenters to provide particle-associated microbes to the culture. All of the vessels were supplied with 7 g of ryegrass within 30 minutes of inoculation with ruminal fluid and thus allowed to ferment without interruption until the next day. The day following fluid collection and inoculation was defined as day 1 of the trial. These procedures were then repeated for each of the four separate replications of this experiment.

Continuous Culture Apparatus and Sample Collection

The BioFlo 110® fermentation system (New Brunswick Scientific, New Brunswick, NJ) was used to conduct a semi-continuous culture trial that was replicated four times. A replicate was defined as a 6 day period with days 1 through 3 as an adaptation period and d 4 through 6 as the sampling period. Prior to inoculation with ruminal fluid, vessels were prepared by adding 400 mL of saliva buffer solution and 8 mL of a resazurin solution (as a reducing agent, .25 mg resazurin / mL) producing a final

volume of 2 L in each fermenter. Vessels were continuously flushed with N_2-gas and preheated to 39 ºC. Temperature was maintained by a thermostatically controlled heater system utilizing a water jacket with a hot plate as the heat source. The culture pH was measured by a glass electrode pH meter and held at 6.3 by automatically adding either mineral buffer solution or *2 N* H_2SO_4 as needed via peristaltic pump. A computer controlled a motor driven stirring device to provide constant agitation at 200 rpm. Two different buffer solutions were used (Table 1), a more conventional mineral buffer solution (Buffer 1) was used for the first and third replicate of the trial and a second more concentrated buffer (Buffer 2) was used for the second and fourth replicates of the trial. Buffer 1 was derived from Weller and Pilgrim (1974) and Buffer 2 was obtained from Chung et al. (2006). Buffer 1 was a more conventional in vitro buffer solution containing minerals and urea, whereas Buffer 2 was a newer more concentrated solution.

Fluid volume in each trial was maintained between 2 L and 3 L (depending on addition of buffer or acid to maintain pH) by manual dilution either once or twice daily as needed. All vessels were diluted simultaneously and brought to the same volume (as needed) to minimize effects due to dilution. Effluent that was diluted out of the culture was stored in a plastic jug containing 3 parts *2 N* H2SO4 for every 50 parts effluent in order to prevent any nutrient volatilization and any subsequent fermentation. At the end of each sampling day, a batch sample of 120 mL was transferred from individual effluent was stored in a plastic jug containing 3 parts *2 N* H2SO4 for every 50 parts effluent in order to prevent any nutrient volatilization and any subsequent fermentation.

Table 1. Ingredients and concentrations of buffer solutions used for *in vitro* continuous culture rumen fermenter feeding ryegrass either alone or supplemented with hay, corn, or soybean hulls.

Ingredient	Concentration, g/L H_2O	
	Buffer 1[a]	Buffer 2[b]
Na_2HPO_4 anhydrous	1.955	70.98
$NaHCO_3$	5	42.0035
KCl	0.6	-
$MgSO_4$	0.12	-
$KHCO_3$	1.2	-
Urea	0.4	-

[a] = derived from Weller and Pilgrim (1974)
[b] = derived from Chung et al. (2006)

At the end of each sampling day, a batch sample of 120 mL was transferred from individual effluent containers into small vials for storage at -20 ºC. Trials utilizing the concentrated buffer solution (Buffer 2; replicates 2 and 4) required less dilution than trials using the more conventional mineral buffer solution (Buffer 1). During the second and fourth replicate, effluent samples of 120 mL were obtained directly from the vessel rather than an outflow jug and stored in plastic vials containing 3 parts *2 N* H_2SO_4 for every 50 parts effluent. Ryegrass was fed at a rate of 7 g every 2 h from 0700 to 1700 each day for a total of 42 g $\cdot$ d^{-1} $\cdot$ $vessel^{-1}$ (as fed basis, nutrient composition determined from preliminary lab analysis). Treatments of either hay (HAY = 80 % bermudagrass; 20 % dallisgrass), corn (CORN), soybean hulls (SBH), or no supplement (NONE) were randomly assigned to a vessel for each trial such that all 4 vessels were used for each of the four treatments. Supplements were administered at a rate of approximately 15 % of ryegrass (7 g / d, DM basis) once per day at 0700 for each day of the trial. Additionally, fluid samples of 50 mL were taken from each vessel via pipette on d 4 through 6. Fluid

was stored in plastic vials containing 3 mL *2 N* H_2SO_4 + 2-ethylbutyrate. The first sample was collected at 0700 before any ryegrass or supplements were fed. Subsequent samples were taken at 0, .5, 1, 1.5, 2, 3, 4, 6, 8, 10, and 12 hrs post administration of supplements (0700).

Laboratory Analysis

Effluent samples were used to determine apparent digestibility by subtracting nutrient content determined by proximate analysis of effluent from nutrients found in feedstuffs offered. Effluent and feedstuffs were analyzed for DM, ash, CP (AOAC, 2003), NDF, and ADF (Komarek et al., 1994). To determine DM of feedstuffs samples were weighed into aluminum pans then dried in a 60 ºC forced air oven for 12 h and weighed again to calculate moisture loss. Furthermore, ash content was calculated by burning samples in a muffle furnace (550 ºC for 5 hr) to determine organic matter disappearance. Crude protein analysis was performed by boiling samples in H_2SO_4 for approximately 2 h prior to titration in order to determine N concentration which was used to calculate CP content. Additionally, samples were subjected to NDF and ADF analytical procedures according to Komarek et al. (1994). Effluent samples were subjected to the same laboratory procedures but, calculations were made on a per mL basis rather than by weight.

Culture samples were used to analyze for NH_3N. Samples were frozen at -20 ºC upon collection in small vials (60 mL) containing H_2SO_4 and remained frozen until being thawed for centrifugation (35,000 x *g* at 4 ºC for 20 min). Supernatant was then extracted and used to determine NH_3N concentration of the sample with hypochlorite-phenol

procedure utilizing direct colorimetric methods by spectrophotometer (SpectraMax 340PC^{384} by Molecular Devices, Sunnyvale, CA) (McCullough, 1967).

Statistical Analysis

Preliminary data analysis was performed to determine if there was a block effect due to the different buffer solutions used during the trial. Using the GLM procedures of SAS (2009) it was determined that there was no effect ($P < 0.05$) due to block. Additionally, the GLM procedure of SAS was used to determine if there was a carryover effect from one sampling day to another. It was determined that there was no carryover effect ($P < 0.05$) across sampling days. Nutrient disappearance was analyzed using GLM procedures of SAS, means were separated using Fisher's protected LSD ($P < 0.05$). Ammonia N results were analyzed as a randomized complete block design using Repeated Measures of the Mixed procedures of SAS with the vessel as the experimental unit and replicate as block. Significant means were separated using Tukey's HSD *($P <$ .05).*

CHAPTER IV

RESULTS AND DISCUSSION

Nutrient composition of feedstuffs is presented in Table 2. In general, ryegrass nutrient analysis produced similar data to that reported by (Jurgens, 2002). Ryegrass was subjected to lyophilization in order to preserve nutrients therefore laboratory DM analysis produced data comparable to that of dried ryegrasses. Crude protein and NDF concentrations were found to be similar to those reported by Jurgens (2002), however ADF content was found to be 17.8 % which is considerably less than the concentration reported by Jurgens (2002) of 33 % ADF (DM basis). Additionally, Tucker et al. (2001) reported NDF and ADF values (46.6 % and 23.4 %, DM basis, respectively) that were greater than calculated concentrations of ryegrass in the current study. However, values reported by Tucker et al. (2001) were based of forage samples obtained during an experimental period of 10 weeks thus resulting in variable nutrient concentrations and most probably increased NDF and ADF over time during the sampling period. Conversely, nutrient concentrations more similar to the current findings has also been reported (de Veth and Kolver, 2001). de Veth and Kolver (2001) reported a CP concentration of 18.6 % (DM basis), which is similar to CP concentration of ryegrass (17.28 %) used for the current *in vitro* trial. Additionally, these researchers found NDF and ADF concentrations of annual ryegrass to be 37.9 % and 27.8 % respectively, which

is slightly greater than the current findings (de Veth and Kolver, 2001). Rude et al. (2002) reported decreased overall nutrient composition of L. multiflorum when forage was harvested for use in a metabolism trial compared to nutrient composition of fresh pasture. Researchers concluded that a nutrient decrease may be due to the harvesting method, thus the process of lyophilization and storage of fresh ryegrass in preparation for use *in vitro* may also have negatively impacted nutrient concentration of ryegrass (Rude et al., 2002). Soybean hulls used in the current study were found to contain less CP (9.954 %) when compared to reported average concentration of 12.4 % (Jurgens, 2002). Neutral detergent fiber and acid detergent fiber concentration for ryegrass used in the current study (62.356 % and 45.881 %, respectively) were only slightly less than reported averaged values (67 % and 49 %, respectively) (Jurgens, 2002). Crude protein concentration of bermudagrass hay (HAY) was found to be 12.07 %, which is greater than other reported concentrations (Galloway et al., 1993, Jurgens, 2002). Corn used in the current *in vitro* study was found to contain less CP (7.59 %) and ADF (2.12 %) than reported averages of 10.1 % and 6.1 %, respectively (Jurgens, 2002). However, NDF concentration was considerably greater at 15.09 % compared to the Jurgens (2002) which reported an average of 9.00 % NDF (DM basis).

Nutrient disappearance of feedstuffs *in vitro* is presented in Table 3. Values for each treatment are presented as total grams (disappeared) and as a percentage of the overall diet offered to vessel. There was a difference ($P < 0.05$) of grams digested among treatments for DM, Ash, NDF, and ADF but no difference ($P > 0.05$) was observed when nutrient disappearance was calculated as a percentage of amounts fed.

Table 2. Nutrient composition of feedstuffs used for *in vitro* continuous culture rumen fermentation trials.

	Feedstuff Composition, % DM[1]				
Feedstuffs	DM	CP	Ash	NDF	ADF
Ryegrass	90.41	17.28	10.13	36.17	17.80
SBH	91.51	9.95	5.01	62.36	45.88
Hay[2]	91.93	12.07	9.34	62.56	27.82
Corn	90.46	7.59	1.12	15.09	2.12

[1] = DM (dry matter), CP (crude protein), NDF (neutral detergent fiber), ADF (acid detergent fiber)
[2]= bermudagrass hay

Further, no difference ($P = 0.9988$) was observed for grams CP digested across treatments. Dry matter disappearance (g/d) was decreased when only ryegrass was fed (NONE) compared to when a supplement was administered. Similar results have been observed *in vitro* when a supplement was fed compared to only forage (Bach et al. 1999, Orr 2009). Bach et al. (1999) reported an increase in DM digestibility as a percent of the diet when supplements were administered with pasture compared to when pasture was fed alone to an *in vitro* continuous culture system. The researchers attributed this change in digestibility to an increase in digestible OM content of the diet with the addition of energetic supplements (corn, soybean hulls, or beet pulp). Additionally, similar increases in DM digestibility have been reported *in vivo*. Orr et al. (2007) noted an increase in DM digestibility when steers were fed bermudagrass hay supplemented with either corn or soybean hulls compared to animals only receiving bermudagrass hay. Researchers determined supplementation increased both DM and OM digestibility when low-quality hay was fed. Moreover, DM disappearance of bermudagrass hay was increased by supplementation of either corn or SBH during a similar *in situ* experiment (Nguyen et al., 2007). In the current *in vitro* experiment, DM disappearance of grams digested did not

significantly differ among treatments that included a supplement (SBH, HAY, or CORN). *In vitro* disappearance of ash (g/d) was different ($P < 0.05$) among treatments. This may be due to the variable amount of inorganic constituents of each individual feedstuff given that the percentage of ash digested of the total amount fed for each treatment did not differ significantly. Feedstuff concentration of ash varied from 10.13 % (ryegrass) to only 1.16 % (corn), thus possibly leading to inaccurate or highly variable results when ash disappearance was calculated.

Neutral detergent fiber disappearance (g /d) was different ($P < 0.05$) among treatments ($P<0.0065$). Vessels supplemented with corn did not differ ($P = 0.0879$) from those receiving only ryegrass (NONE) in terms of NDF disappearance of grams per day ($P<0.0879$). However, vessels receiving supplements of either HAY or SBH were different ($P = 0.0065$) from treatments of CORN or NONE. Conversely, Bach et al. (1999) reported a tendency ($P<0.06$) for *in vitro* NDF digestibility (as a % of the diet) to decrease when continuous culture fermenters were fed a pasture mixture (25 % red clover, 25 % alfalfa, and 50 % orchardgrass) supplemented with cracked corn compared to fermenters receiving no supplement.

In the current study, there was not a difference ($P = 0.7985$) between the amount of SBH and HAY treatments disappearing on a daily basis. Additionally, upon nutritional analysis, NDF concentrations of SBH and HAY were found to be similar (62.36 % and 62.56 %, DM basis, respectively). Therefore, this may indicate the fibrous content of SBH resulted in an apparent nutrient digestibility (g / d) similar to HAY, while concurrently increasing energetic substrates for ruminal microbial synthesis. However, this result was not observed when treatments were compared as a percentage of the diet.

During an *in vivo* digestion trial, Orr et al. (2007) reported a significant increase ($P<0.01$) in NDF digestibility (as a % of the diet) when SBH were supplemented to a low-quality hay compared to corn supplementation or hay alone. Furthermore, Nguyen et al. (2007) reported an overall increase in NDF digestibility of a forage-based diet supplemented with SBH. Researchers further stated that supplying a fibrous feedstuff to the rumen should promote fiber-digesting bacterial populations, possibly increasing the fiber digestibility and overall utilization of hay when supplemented with SBH (Nguyen et al. 2007).

Ammonia-N assay results are presented in Figure 1. Fermenters receiving CORN had the least ($P < 0.05$) NH_3N for the duration of the 12 h sampling period. Vessels receiving HAY maintained the greatest NH_3N concentration, although the values did not differ statistically from vessels fed SBH or NONE. There was a difference ($P < 0.05$) between CORN and HAY at each sampling time with CORN having a less NH_3N concentration than HAY. There was also a difference ($P < 0.05$) between NONE and CORN for culture samples taken at 4, 6, and 8 h post feeding of supplement. Ammonia-N concentration between SBH and CORN approached significance ($P = 0.0515$) at 2 h post supplementation; however, this trend did not continue nor was it observed at any other time point.

Table 3. Nutrient disappearance of feedstuffs offered during *in vitro* continuous culture rumen fermentation trials.

	Nutrient disappearance, g / d					**Nutrient disappearance, % of amount fed / d**				
Trt	DM	Ash	CP	NDF	ADF	DM	Ash	CP	NDF	ADF
NONE[1]	36.72[a]	4.15[a]	4.57	11.8[a]	6.36[a]	96.7	97.41	63.05	77.68	85.05
SBH[2]	43.03[b]	4.45[b]	4.63	16.61[b]	10.41[c]	96.04	96.01	57.94	83.72	95.5
HAY[3]	42.98[b]	4.79[c]	5.37	16.33[b]	8.44[b]	95.94	96.7	65.91	82.31	88.38
CORN[4]	41.7[b]	3.96[a]	5.29	13.66[a]	6.63[a]	93.13	91.22	67.63	83.67	86.85
SEM	0.709	0.091	0.879	0.79	0.0387	1.596	2.06	11.221	4.543	4.303
P-Value[5]	0.0001	0.0002	0.9988	0.0065	0.0001	0.8043	0.5137	0.9995	0.7818	0.6482

[1] = ryegrass only; no supplement (42 g ryegrass / d, DM basis)
[2] = ryegrass + soybean hulls (42 g ryegrass + 7 g soybean hulls / d, DM basis)
[3] = ryegrass + bermudagrass hay (42 g ryegrass + 7 g hay / d, DM basis)
[4] = ryegrass + corn (42 g ryegrass + 7 g corn / d, DM basis)
[5] = means separated within column when $P < 0.05$

The largest decrease in NH_3N concentration of CORN occurred at 8 h post supplementation. Although SBH and NONE NH_3N concentration decreased from 6 h to 8 h, CORN NH_3N decreased more ($P < 0.01$) at this time point. According to these data, it appears that when corn was fed as a supplement to annual ryegrass *in vitro* microbial populations may have utilized the available energy substrates, provided by supplying a concentrate to the culture, more efficiently thus reducing excess NH_3 in the culture fluid by using NH_3 to assimilate microbial protein. Excess NH_3 produced from degradation of dietary protein and not incorporated into microbial protein normally could be absorbed through the rumen wall and excreted as urea in urine or perhaps recycled in the saliva and returned to the rumen as a source of NPN (Van Soest, 1994). However, during *in vitro* continuous culture fermentation there is no absorption of nutrients across the rumen wall that would occur *in vivo*; therefore, NH_3 concentrations measured from sampled culture may be an indication of microbial efficiency. Conversely, this may not be the most effective method to describe ruminal protein degradation or efficiency of microbial protein synthesis. Broderick (1992) stated that NH_3 accumulation as an index of degradation can be confounded by the fact that microbes may utilize NH_3 during growth and synthesis, thereby reducing estimates of true rate of protein degradation. Similar *in vitro* continuous culture experiments, that included some form of energetic supplement to lush forage, have produced ruminal NH_3N values numerically less than the ones found for the current *in vitro* study (Bach et al., 1999, Bargo et al., 2003ab, Wales et al., 2009).

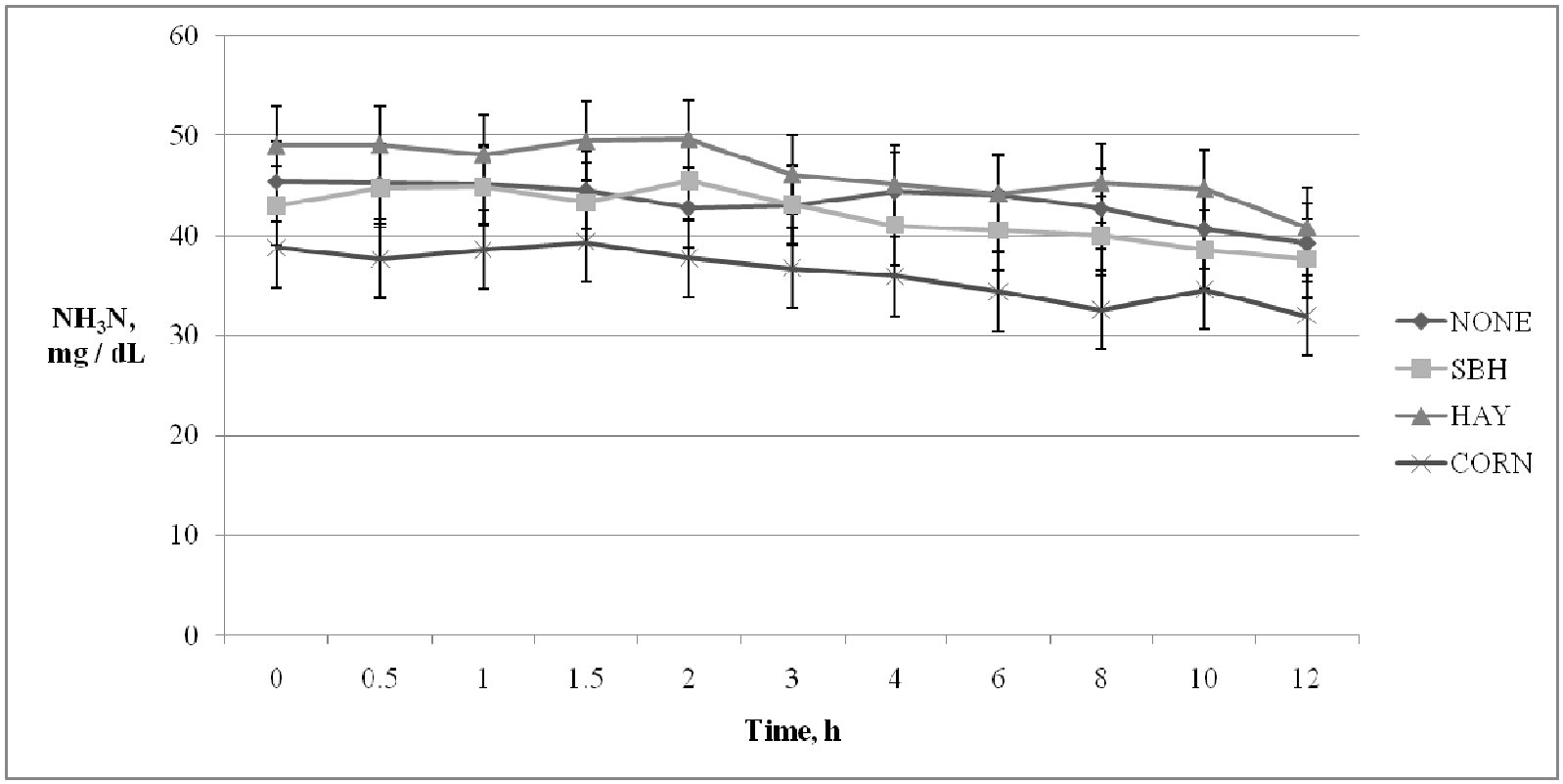

Figure 1. Ruminal ammonia-N assay results presented in mg/dL for each culture sample time and treatment

CORN different ($P < 0.05$) than HAY at each sampling time
NONE, SBH, and HAY were not different ($P > 0.05$) across any sampling time

However, these studies utilized either single or dual-flow continuous culture fermenters with a set dilution rate that was maintained and effluent was also used to determine nutrient and microbial N flow out of the vessel. In the current study, the nature of the apparatus (dilution as needed to maintain volume between 2 and 3 L) along with a set pH value (pH 6.3 measured constantly; buffer or *2 N* H_2SO_4 infused as needed) could explain why the NH_3N concentration values of the culture are numerically greater than those previously reported. Furthermore, similar studies have reported values as a mean over sampling times to examine N flow or total N metabolism; whereas in the current *in vitro* study, NH_3N values are reported for each sampling time during the 12 h period (Bach et al., 1999, Bargo et al., 2003, Wales et al., 2009). Although NH_3N values were greater for the current study, Wales et al. (2009) reported no difference between NH_3N concentrations of cultures maintained with only pasture (perennial ryegrass) or those fed pasture supplemented with 15 % concentrate (barley / steam-flaked corn grain) which is in agreement with the current results. Bargo et al. (2003b) reported a decrease of NH_3N concentration when fermenters were fed concentrate along with pasture. Further, Kolver et al. (1998) reported a decrease of NH_3N concentration from 35.2 to 14.2 mg / dL when starch was supplemented. Although values in the current study are reduced, Bach et al. (1999) also reported a decreased NH_3N concentration (2.1 mg / dL) for vessels fed cracked-corn with pasture when compared with vessels fed only pasture (10.3 mg / dL).

CHAPTER V

CONCLUSION

The use of *in vitro* continuous culture techniques to evaluate feedstuffs for ruminants posses' great value for animal scientists. Parameters that can be easily measured *in vitro* may be difficult to measure *in vivo*. Additionally, *in vitro* continuous culture techniques can be used to study the ecology and kinetics of the rumen environment without invasive procedures being performed on live animals beyond the collection of fluid upon initiation of the experiment. However, *in vitro* experiments are not without their disadvantages as well. Normal rumen functions such as absorption of nutrients across ruminal membranes and recycling of urea back into the rumen environment will not take place by utilizing current *in vitro* techniques. Some of these negative aspects of an unnatural rumen environment sometimes seen *in vitro* can be overcome by adding buffer solutions to simulate saliva flow into the rumen and the timed or automatic release of fluids from the *in vitro* fermenter to simulate digesta flow out of the rumen. As further research is conducted these problems can be addressed and recent research has shown that improved *in vitro* techniques yield results that are useful to advance our understanding of nutrition of the ruminant animal. In the same way research has driven scientists to examine the rumen through various techniques, alternative methods of production and feeding are valuable in providing nutritionists with

information to produce more efficient animals and decrease production costs. The importance of grazing animals on lush forages, such as annual ryegrass, without creating adverse effects on production is helpful for both the producer and the consumer. By increasing efficiency during the production stage, overall cost can be greatly reduced thus providing the consumer with a less expensive product that is still desirable in quality.

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