RUMINAL DEGRADATION OF PROTEIN AND CARBOHYDRATE IN THE DOMESTIC AND WILD RUMINANT

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The undersigned, appointed by the Dean of the Graduate School, have examined the dissertation entitled

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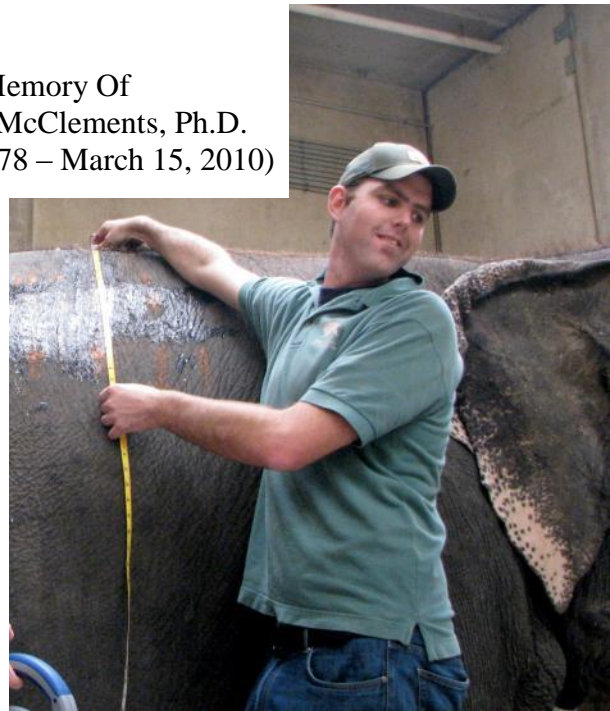
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Professor Mark R. Ellersieck

DEDICATION

I dedicate this dissertation and all the work that went into it to my beautiful loving wife Stephanie Renee Brooks and our son Jonathan David Brooks. Stephanie you have been my support, and Jonathan, you have become my drive to succeed. Everything I ever will be is because of you. I love you both with all that I am.

I would also like to dedicate this work to my colleague, confidante and dear friend Roy McClements. Once upon a time, I met a Giant from a distant land. In frame and in personality, Roy was truly a Giant among all men. You will always be one of my best friends. I will miss your humor and our discussions. I would not be where I am or who I am without having met you. I am forever changed for knowing you, and I am sad for the world because it has lost one of its most unique wonders. You were and will forever be the scientist I aspire to be. I miss you Roy. So long, and thanks for all the fish.

Cheers, Mate!

In Memory Of
Roy Duncan McClements, Ph.D.
(January 13, 1978 – March 15, 2010)

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I Thank You All!

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NOMENCLATURE

A:P	Acetate to Propionate Ratio
AA	Amino Acid
AC	Alfalfa Cubes
ADF	Acid Detergent Fiber
ADG	Average Daily Gain
AL	Alfalfa
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
ATP	Adenosine-5'-Triphosphate
BM	Bloodmeal
BUN	Blood Urea Nitrogen
C	Casein
CAP	Chloramphenicol
CB	Corn Bran
CS	Corn Starch
CGF	Corn Gluten Feed
CNCPS	Cornell Net Carbohydrate and Protein System
CP	Crude Protein
D	Dilution Rate
DC	Dairy Cow
DCI	Dairy Cow Inoculum
DDG	Dried Distiller's Grains
DDGS	Dried Distiller's Grains with Solubles

DL-Met	DL-Methionine
DM	Dry Matter
DMD	Dry Matter Digestibility
DMI	Dry Matter Intake
FAA	Feed Grade Crystalline Amino Acids
FBW	Final Body Weight
G:F	Gain to Feed Ratio
GC	Ground Corn
GER	Gut Entry Rate
GI	Gastrointestinal
GLM	Generalized Linear Model
HS	Hydrazine Sulfate
IBW	Initial Body Weight
k_d	Degradation Rate
k_p	Passage Rate
L-Arg	L-Arginine
L-Lys	L-Lysine
LSD	Least Significant Difference
MD	Mule Deer
MDI	Mule Deer Inoculum
ME	Metabolizable Energy
Met AA	Metabolizable amino acid
MFA	Missouri Farmers Association
MOEFF	Microbial Efficiency
MPS	Microbial Protein Synthesis

N	Nitrogen
NH_3	Ammonia
NH_3-N	Ammonia Nitrogen
NDF	Neutral Detergent Fiber
NLIN	Non-Linear
NPN	Non-Protein Nitrogen
NRC	National Research Council
NSC	Non-Structural Carbohydrate
OM	Organic Matter
OMD	Organic Matter Digestibility
RCBD	Randomized Complete Block Design
RD	Rumen Degradation
RDN	Rumen Degradable Nitrogen
RDP	Rumen Degradable Protein
RDPep	Rumen Degradable Peptide
RUN	Rumen Undegradable Nitrogen
RUP	Rumen Undegradable Protein
SAS	Statistical Analysis Software
SBM	Soybean Meal
SC	Structural Carbohydrate
SDR	Solids Dilution Rate
SH	Soy Hulls
USDA	United States Department of Agriculture
VFA	Volatile Fatty Acid

RUMINAL DEGRADATION OF PROTEIN AND CARBOHYDRATE IN THE DOMESTIC AND WILD RUMINANT

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ABSTRACT

The ability of ruminant animals to generate and utilize high quality microbial protein from lower quality feeds is the latest evolutionary dietary adaptation in mammalian species. To optimize growth and health of the animal in a confined setting, diets need to be formulated to provide balanced levels of rumen degradable energy and nitrogen (RDN) to maximize microbial growth while also providing adequate rumen undegradable protein (RUP) for the animal. To make such formulations, feed degradability needs to be broken down into degradable soluble carbohydrate, structural carbohydrate and protein. The first experiment in this study examined the level of feed-grade crystalline amino acids (AA) required in a diet to provide appreciable levels of limiting AA as RUP. The solubility of these AA in a mixed rumen culture showed dietary inclusion levels would be required at >12% of the diet on a DM basis. The second study conducted looked at the degradability of the starch, neutral detergent fiber (NDF) and protein fractions of common feeds used in a feedlot finishing diet. Degradation rate was measured as the percentage disappearance of the ruminally available nutrient over time. Degradation rates were found to be linear and similar for the feeds tested with the

average rates of degradation being approximately 0.0479 h^{-1} for starch, 0.0292 h^{-1} for NDF and 0.0271 h^{-1} for protein. These rates were then used along with an estimated passage rate of 0.06 h^{-1} to formulate diets with varying levels of rumen degradable protein (RDP) and RDN. Resulting diets were applied to continuous culture fermentations to measure fermentation characteristics and microbial efficiency (MOEFF) with the hypothesis that a diet balanced for the proper amount of RDP to rumen degradable energy would increase efficiency. This study showed MOEFF to be lesser when RDN was limiting and greatest when RDP was provided at less than 100% of requirement. Similar diets with varying RDP and balanced RDN were then applied to crossbred Angus steers to determine effects on growth. The greatest average daily gain (ADG) and feed efficiency was measured when RDP was provided at 115% of the requirement, compared to 95% and 85%. Finally, research was done to determine the *in vitro* digestibility characteristics of commercially available pelleted feeds fed to exotic ruminants. These studies included one low starch/high fiber and two different high starch/low fiber feeds. Both batch culture and continuous culture techniques were employed using dairy cow and mule deer rumen fluid. Both studies confirmed differences exist between fermentation characteristics due to species used as inoculum source. Further, greater fiberolytic activity was seen in mule deer inoculum, and greater proteolytic activity was seen in dairy cow inoculum. Also, decreasing starch in diets increased OM digestibility, likely due to removal of negative associative affects on cellulolytic bacteria.

CHAPTER 1

REVIEW OF LITERATURE

INTRODUCTION

The ability of ruminant animals to generate and utilize high quality microbial protein from lower quality feeds is the latest evolutionary dietary adaptation in mammalian species. To optimize growth and health of the animal in a confined setting, diets need to be formulated to provide balanced levels of rumen degradable energy and nitrogen (RDN) to maximize microbial growth while also providing adequate rumen undegradable protein (RUP) for the animal. To make such formulations, feed degradability needs to be broken down into degradable soluble carbohydrate, structural carbohydrate and protein.

To determine "true" rumen availability of these nutrients, their degradation rates need to be married to ruminal passage rate (Broderick and Clayton, 1992). Feed nutrients have the potential to be fully degraded given enough time in the fermentation media, but many feeds, especially grain-based diets, pass out of the rumen before degradation reaches its full extent. Therefore, by successive calculation of mass of feed multiplied by dilution rate and degradability, the amount of each nutrient available for microbial fermentation can be determined (Sniffen et al., 1992).

Although the exact mechanism controlling particle and liquid dilution rates in the rumen are not known, it has been shown that intake, particle size, saliva production and feed type (browse- vs. grass- vs. grain-based diet) and saliva production from rumination

affect feed retention time in the rumen (Clauss and Lechner-Doll, 2001; Sniffen et al., 1992; Welch, 1982). This paper is not focused on the controls and differences in retention time and dilution rate between animals and diets; however, an excellent review of these aspects has been provided by Mueller (2004).

The purpose of production diets is to maximize growth and minimize cost. Although this holds true for game animal diets, as with deer, the goal of most captive wild animal diets is sustainability and long term health. The most logical way to accomplish this is to mimic natural diets of the target species; however, most zoological institutions do not have the means to provide the animal's natural diet. Instead conventional feeds are used to produce pelleted diets which emulate the nutrient characteristics of the natural diet.

This review will discuss the different aspects controlling nitrogen and carbohydrate degradation by the mixed ruminal microbial populations and the effects this has on microbial efficiency. The review will then continue to discuss how rumen degradable protein (RDP) and rumen degradable nitrogen (RDN) need to be balanced with the rumen available energy to increase production and efficiency, which will also decrease N waste and risk of acidosis. The review will then discuss differences in diets fed to browsing and grazing ruminants, and how both protein and carbohydrate fractions of supplemental diets fed to captive browsing ruminants affect their health.

NITROGEN DEGRADATION AND ASSIMILATION

Nitrogen in Production Systems

One of the most expensive components in livestock feed is crude protein. Mismanagement and animal inefficiency can result in large losses of nitrogen in the urine

and feces. From the 1997 U.S. Census, the USDA estimated 9734.1 million pounds of nitrogen per year is excreted by ruminant livestock. Much of this nitrogen is lost through volatilization soon after excretion, which ends up as a component of acid rain (Kellogg et al., 2000). The remaining waste can be used to fertilize crop lands; however, when manure production exceeds the capacity of the land to utilize this fertilizer, the excess nitrogen runoff fouls surrounding water supplies (Gollehon et al., 2001).

Available Nitrogen in the Rumen

The predominant forms of nitrogen (N) in the rumen are true protein, peptides, individual amino acids (AA), and ammonia (NH_3). Dietary N entering the rumen is able to provide all three of these sources as both immediately soluble feed N and through the degradative processes of the rumen microbes, which will be discussed further below. Peptides and AA are also provided by bacterial and protozoal lysis and can provide up to 45% of the total N flux of the available N pool in the rumen (Koenig et al., 2000). Additionally, NH_3 is recycled into the rumen through saliva and transport through the rumen wall as urea (Van Soest, 1994). Marini & Van Amburgh (2003) used stable isotope infusions of nitrogen labeled urea to determine that the gut entry rate (GER) and rumen wall entry of N remained constant among diets of varying protein levels. This indicates that the kidney and GI tract have a tremendous capacity to salvage nitrogen in the body. They also found microbes sampled from animals on low-nitrogen diets contained more recycled nitrogen. Wickersham et al. (2008b) expanded on this research by examining how the quantity and frequency of nitrogen supplementation in the ruminant diet affects nitrogen recycling mechanisms. They found low-levels of RDP promote recycling efficiency. Microbial nitrogen incorporation was not affected by

frequency of supplementation because the microbes were constantly dependent on recycling for adequate nitrogen status. However at higher levels of supplementation, recycling efficiency decreases when supplemental nitrogen is given less frequently (once every 3 days) because the highly degradable sources were excreted on the first day of supplementation before a deficiency of rumen available nitrogen was produced on days 2 and 3. This left the system in need of recycled nitrogen after much of the excess nitrogen was already excreted. One may infer from these data that decreasing rumen available nitrogen levels such that the microbes are slightly deficient will lead to increased efficiency of nitrogen utilization.

Nitrogen metabolism by microbes

Russell et al. (1992) reviewed the different microbial requirements for protein as outlined by fermentative bacterial type. In this review, bacteria were divided into non-structural carbohydrate (starch and soluble sugars; NSC) fermenting bacteria, and structural carbohydrate (hemicellulose and cellulose: SC) fermenting bacteria. Non-structural carbohydrate fermenters rely mainly on peptides and AA for their main N source, while SC fermenters utilize primarily NH_3. Protozoal populations do not directly utilize NH_3 as a nitrogen source, but they engulf large particles consisting of both carbohydrate and protein along with whole bacteria (Onodera et al., 1977). These protozoa then degrade the bacteria and engulfed substrates for use as peptides and AA, with the majority of their nitrogen coming from bacteria (Bach et al., 2005). Armstead and Ling (1993), using a mixed rumen culture from sheep and ^{14}C labeled peptides and AA, found initial uptake of AA occurs at a faster rate than peptide, which agrees with previous findings by Chen (1987) who concluded peptide uptake is the rate-limiting step

in protein degradation. Armstead and Ling (1993) also calculated peptide contribution to bacterial N requirement to be between 11% to 35%, and AA contribution as 36% to 68% of the N requirement. This study did note rapid metabolism and lack of incorporation of much of the labeled ^{14}C in bacterial mass could mean peptides and AA were also being used for energy rather than as N substrates.

The mechanisms by which N substrates are degraded in the rumen differ according to microbial type (bacteria and protozoa) and substrate (protein, peptides, AA, urea). However, within microbial genre these mechanisms do not vary greatly. Bach et al. (2005), provide a very descriptive graphical representation of the mechanism by which protein is broken down by bacteria. Proteases are secreted by the bacteria to cause extracellular breakdown of proteins into peptides. From there, these peptides and any free AA are engulfed by the bacteria, where a series of dipeptidyl peptidase and tri- and di-peptidases breakdown peptides even further to AA (Wallace, 1996). The resulting peptides and AA can then be directly incorporated into microbial protein, or further acted upon by cytoplasmic deaminase to produce NH_3 and VFA, or CO_2.

Unlike bacteria, protozoa produce intracellular peptidases to degrade engulfed bacterial and dietary protein (Van Soest, 1994). Protozoa also preferentially engulf insoluble proteins. Then, through internal proteases which are not diluted in the rumen fluid, the protozoa are able to degrade these proteins (Jouany, 1996). Upon lysis of the protozoal cell, the once insoluble protein is released as an available form of N for bacteria (Dijkstra et al., 1998). Even though protozoa can make up approximately 50% of the rumen microbial mass, they contribute very little (~ 11%) to the microbial mass entering the duodenum due to their mobility and ability to sequester themselves against the rumen

wall (Russell, 2002; Shabi et al., 2000). This causes microbial efficiency (MOEFF), defined here as grams of microbial N flowing to the duodenum per kilogram of organic matter (OM) truly fermented, to decrease. Studies have shown an increase in MOEFF and microbial N flow to the duodenum in defaunated sheep (Koenig et al., 2000; Meyer et al., 1986). However, total tract digestibility of OM, N, neutral detergent fiber (NDF), and acid detergent fiber (ADF) was reduced (Koenig et al., 2000). This could be attributed to less predation of bacteria by protozoa and less competition for protein sources. However, the lack of degradation furnished by the protozoa prevents solublization of otherwise insoluble components, such as protein and NDF, which then prevents absorption in the lower intestinal tract.

Nitrogen can further be obtained from the diet through additional dietary urea or, as mentioned before, urea recycling into the rumen from the blood. As touched on in the previous section, N absorbed in the small intestine can be transported to the kidneys and liver and converted by the tissues to urea. In times of excess dietary N, this urea is transported to the kidneys and excreted. However, when dietary N is limiting and blood urea N (BUN) concentrations are greater in the plasma than the rumen, the urea crosses the rumen wall where bacteria associated with the rumen wall produce the enzyme urease. Marini et al. (2004) measured urea recycling to the rumen and concluded urease activity is not limiting even at high levels of N recycling or dietary protein. Which is in agreement with previous work by Chalupa et al. (1970); who found that even though urease activity decreases when sheep were fed urea versus soy-protein, the decrease in activity is not detrimental to total urea dissociation. This indicates that when urea enters

the rumen; dissociation to NH_3 is rapid and immediately adds to the NH_3-N pool for use by bacteria.

Methods used to estimate nitrogen degradation rates

Measurement of the degradation rates of protein are difficult using *in vivo* models due to the use of N degradative end-products and the passage rate of the feedstuff from the rumen. This has led to multiple methods of measuring degradation using either *in vitro* batch cultures or *in situ* Dacron bags to measure either end-product appearance or substrate N disappearance, respectively. A review by Stern et al. (1997) discusses the differences between the two techniques and the usefulness of these techniques in estimating true degradation. For the purposes of this review, a brief discussion of specific findings using these techniques will be given as well as the mathematical models applied to them to determine protein degradation.

Due to the uptake of the products of protein degradation by microbes for use as N sources, inhibitors of N uptake have been examined *in vitro* as a way to obtain total degraded N recovery for estimation of protein degradation. Although degradation of protein using hydrazine sulfate (HS) as an inhibitor of N uptake by microbes was not different, the precision of the data was improved. There was also no inhibition of degradation due to waste product accumulation, but these experiments were run for a maximum of 3 to 4 hours (Broderick, 1978). Chloramphenicol (CAP) has been used in addition to HS as an inhibitor of N uptake. Broderick (1987) tested different levels of both HS and CAP and found no improvement in NH_3 recovery with addition of CAP, but there was increasing total N recovery with up to 30 μM CAP. Addition of HS was able to increase NH_3 recovery and total N recovery at 1.0 mM addition, and further improvement

was seen when HS and CAP were combined at these concentrations. The difficulties become that inhibitor concentration and usage give variable results, possibly depending on location, with a lab in Sweden showing 2 mM HS and 90 μM CAP being more effective in NH_3 and total N recovery when compared to the values listed above as determined by a laboratory in Wisconsin (Broderick et al., 2004a). The time frame (< 4 h) of the degradation due to end-product inhibition also limits the usefulness of these inhibitors when determining degradative extent of a feedstuff.

Broderick et al. (2004b) used an *in vitro* assay with N uptake inhibitors to analyze the difference between the use of total N release or NH_3 release for estimating protein degradation. No differences were found in protein degradation rate estimation for alfalfa when using NH_3 release plus AA or using total N release. However, a difference was seen when estimating degradation rate for casein due to rapid degradation to small peptides in this purified readily accessible protein source.

The *in situ* method is a simpler assay and perhaps a more accurate assay for determining *in vivo* degradation rates. Although the fermentation environment is more natural with the animal regulated temperatures, liquid and solid dilution rates, feed agitation, removal of waste products and abundance of microbial enzymes to degrade the feed; the assay still has difficulties imposed with feed particle size, lack of rumination of the feed, and bag pore-size. When comparing the two methods, Borucki Castro et al. (2007) found estimates of rates of degradation from *in situ* experiments were as much as two-fold greater than estimates of the same feeds degraded using *in vitro* methods. However, England et al. (1997) found similar degradabilities between *in situ* and inhibitor *in vitro* techniques when calculating rumen crude protein (CP) availability.

In the same study, England et al. (1997) used Michaelis-Menten calculations to estimate CP degradation rates using the same inhibitor *in vitro* techniques and found these degradation rates to be more rapid with a decreased amount of RUP being supplied to the duodenum. Broderick and Clayton (1992) describe the use of the Michaelis-Menten for predicting protein degradation rates (k_d). They suggest a shorter assay with greater amounts of substrate which allows for reduced end-product inhibition and more accuracy due to the reduced weighing errors. This method estimates protein degradation rate by non-linear regression analysis which is calculated as $k_d = V_{max} : K_m$. This particular assay is only run over a 2 h time period. Like England et al. (1997), Broderick and Clayton (1992) found rates calculated with this method were more rapid than other *in vitro* methods, but they were able to directly calculate a rate without doing any transformation of the data.

The National Research Council (NRC) and Cornell Net Carbohydrate and Protein System (CNCPS) have fractioned N found in feeds into 3 separate components. Nitrogen of a feed is classified as immediately soluble N (A) in the form of NPN including NH_3 and AA, true protein (B), and insoluble N (C; NRC, 2000, 2001). The B fraction is further separated into rapidly degraded true protein (B1), immediately degrading true protein (B2), and slowly degrading true protein (B3; Sniffen et al., 1992). Both systems define rumen degradation (RD) as a combination of both passage rate (k_p) and fractional degradation rate of the feedstuff with RD = $k_d/(k_d + k_p)$. In this equation, passage rate is estimated based on the level of forage versus concentrates in the diet, and the k_d is a specifically measured rate for each particular feed. This fractional degradation rate is determined using an *in vitro* or *in situ* digestion to determine residual N measured either

directly from remaining feed or indirectly from soluble products of degradation. With this information the residual N is plotted as the natural logarithm versus time and curve peeling techniques are applied to determine rates for each fraction (Sniffen et al., 1992).

Similar to the one employed by the NRC and CNCPS, Ørskov and McDonald (1979) arrive at protein disappearance (p) as the final derivation $p = a + b(1-e^{-ct})$, where a and b are similar to fractions A and B from above, c is the k_d of B, and t is time of incubation. However, Batajoo and Shaver (Batajoo and Shaver, 1998) define ruminal availability in what appears to be a combination of both the Ørskov and McDonald and NRC/CNPS models with the formula: [A + B(k_d/(k_d + k_p))], and k_d measured as the percent disappearance of CP over time when measured at 72 h of an *in situ* incubation.

All of the previously described methods and fractionations have led to the need for extensive degradation studies with no one clear method for measuring the degradation rate or rumen availability. These formulations require each feed to have an individual rate of degradation measured, or several rates in the case of the fractionized models. These methods also rely on the type of procedure, type of feed, and length of incubation. This increases complexity of the model used and decreases the overall robustness of the model. This causes great difficulty for feed formulators because these models are trying to accurately and precisely predict protein availability in the rumen. The increasing complexity of the models is not necessarily required when formulating diets for a practical production setting because of the inherent ability of the rumen to adapt to differences in protein availability due the animals tremendous capacity for N recycling.

CARBOHYDRATE DEGRADATION AND ASSIMILATION

Carbohydrates in Production Systems

The ability of ruminant animals to utilize grasses and forages as a means of energy is perhaps both their and our greatest asset. The rumen microbial populations are able to hydrolyze both the nonstructural and structural carbohydrates found in plants and convert them to both microbial protein and absorbable energy in the form of VFA. In 2007, it was estimated that the U.S. has 357 million acres devoted to cropland, 119 million acres devoted to pastureland, and 409 million acres devoted to range/grazing land. Of the 1.9 billion acres of land and water covered by the 48 contiguous states, 45% is devoted strictly to agricultural pursuits and feeding both people and production animals (USDA, 2009). Further, of the croplands devoted to grain production, over 10 billion bushels of corn is produced annually. Fifteen percent of this corn is used to feed approximately 14 million feedlot cattle. With the increasing costs of grains due to their competitive demand by alternative fuel production, increasing feed efficiency by only 1%, at a cost of $0.10/kg of corn grain, would translate into a decrease in total feed costs of $23 million for the feedlot industry (Huntington et al., 2006). Therefore, accurately predicting carbohydrate degradation is essential in formulating diets for the purposes of reducing energy waste, increasing efficiency and decreasing feed costs.

Available energy in the rumen

Dietary energy for use by the microbes enters the rumen primarily in the form of carbohydrates. These carbohydrates can be broken down into the very broad classification of NSC and SC, as explained previously (in: *Nitrogen metabolism by microbes*). Within these two categories, carbohydrates are further classified as free

sugars, pectin and starch, found within the NSC group, and cellulose, hemicellulose, and lignin, found in the SC group (Russell et al., 1992). These carbohydrates classifications constitute niches of microbial energy usage. Although many bacteria can utilize multiple sources of carbohydrates for their energy needs (Russell, 2002), most predominately fall into the NSC fermenters or SC fermenters. Intuitively, the predominance of each niche is dependent upon the prevalence of substrate in the rumen.

Carbohydrate metabolism by microbes

The factors affecting starch digestion and utilization in the rumen have been extensively reviewed in the literature (Harmon et al., 2004; Huntington, 1997; Huntington et al., 2006; Kotarski et al., 1992). In general, amylolytic (starch fermenting) bacteria, first attach to feed particles and secrete endo- and exo-amylases that hydrolyze both α 1-4 and α 1-6 linkages which are binding glucose molecules together to make up amylose and amylopectin. The resulting maltooligomers (disaccharides) are transported into the cell to be broken down into individual hexoses by intracellular maltase and used for the generation of ATP (Kotarski et al., 1992). The impediment to this degradation, when feeding intact grains, is the waxy pericarp covering. This pericarp must be degraded or removed in some manner before the amylolytic bacteria are able to begin degrading the starch rich endosperm of the grain (Huntington, 1997). Further improvements can be made in availability of starch by the processing of the grains. Cracking open the pericarp and/or gelatinizing the starch increases surface area and availability thus increasing enzymatic degradation by bacteria (Horadagoda et al., 2008; Owens et al., 1986). As the amylolytic bacteria metabolize the hexose material, they primarily produce the three carbon molecules propionic and lactic acid (Russell, 2002).

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The implications of over-production of these acids are discussed in a later section of this review (*Calculating for balance*).

Protozoa also utilize primarily starch to meet energy demands. Along with engulfing bacteria for N utilization (see previous section: *Nitrogen metabolism by microbes*), protozoa engulf large starch laden feed particles as energy, preventing its use by bacteria to produce lactic acid. These protozoa slowly metabolize and store the starch, and do not produce significant amounts of lactic acid as a result; this, along with their engulfment of lactic acid producing bacteria, is theorized to provide a partial "buffering" effect on the rumen preventing acid overload (Kotarski et al., 1992).

Cellulolytic (SC fermenting) bacteria rely on cellulose and hemicellulose for their energy supply. Much like the amylolytic bacteria, these bacteria start by attaching themselves to the feed particles, which in degradation models constitutes a lag phase in which little to no degradation occurs (Allen and Mertens, 1988). The bacteria then begin secreting enzymes to degrade the cell wall structural carbohydrates into their hexose oligomers. The cellulases secreted by the bacteria are able to degrade the β 1-4 linkage in the straight glucose chains that makes up cellulose (Krause et al., 2003). Hemicellulase is constructed of a loose matrix constructed of simple sugars including arabinose, galactose and ribose, all interwoven with short chains of xylanose to provide structure to the matrix (Van Soest, 1994). The major end-products produced by cellulolytic bacteria are the VFA acetate and butyrate (Russell et al., 1992). However, unless a diet primarily consists of concentrates or very low quality forage, acetate concentration is greatest of all VFA (Merchen et al., 1986; Rodriguez-Prado et al., 2004).

A major effector of cell wall digestibility is lignification of the cell wall material. The cross-linking that occurs between cell wall carbohydrates and lignin prevents the cellulolytic enzymes from degrading the polysaccharides (Krause et al., 2003). Cell wall digestibility is inversely related to cellulose and hemicellulose digestibility (Jung and Allen, 1995). Structural carbohydrate fermentation can also be limited by rumen pH values less than 6.0 (Cerrato-Sanchez et al., 2007; Mould et al., 1983). Allen and Mertens (1988) suggest the reason for the decrease in fiber digestibility at low pH is that the negatively charged cell walls of bacteria attach to the negatively charged cell walls of the fiber particles forming divalent cation bridges, and when the pH drops, hydrogen ions displace the bacteria and prevent their binding. Russell and Wilson (1996) suggest the reason for the lack of digestion is due to the difference in pH gradient across the bacterial cell wall. As the pH of the extracellular space exceeds the intracellular pH, the proton mode of force across the cell wall decreases and the bacteria are no longer able to take up carbohydrate. When diets are unbalanced to the rate of degradable protein and concentrate-based feeds are fed at high levels in the diet, pH is likely to drop and cause these negative associative effects on fiber digestion, thus decreasing fermentation of all SC in the rumen (Mould et al., 1983).

Methods used to estimate carbohydrate degradation rates

Unlike proteins, there is a great variety of end-products of fermentation for carbohydrates; therefore, estimations of degradability are generally accomplished by measuring the residual starch and NDF after *in situ* digestion. Most assays are conducted over 48 to 72 h. However, starch degradation in the rumen for most feeds is approximately 80 to 100% depending on particle size, and it reaches this extent with a

relatively short retention time in the rumen (between 4 to 8 h; Huntington et al., 2006). Neutral detergent fiber degradation, on the other hand, is a relatively slow process due to the lag phase and low solubility of the cell wall components. Some feeds with greater amounts of NDF will not be fully degraded even with a retention time of 72 h (Allen and Mertens, 1988). In these cases, the feedstuff will likely have passed from the rumen before complete degradation occurs (Hristov et al., 2003).

Similar to the system for fractionating protein, the NRC and CNCPS both divide carbohydrates into various fractions for the purposes of degradation estimates. Carbohydrates in feeds are classified as immediately soluble free sugars (A) rapidly degrading starch (B1), slower degrading available cell wall (B2), and unavailable cell wall (C) which is calculated as lignin ✕ 2.4 (NRC, 2000; Sniffen et al., 1992).

As reviewed in the previous section (*Methods used to estimate nitrogen degradation rates*), Batajoo and Shaver (1998) measured starch rate of degradation and availability using the same method as they did when measuring protein digestion. As did Hoffman et al. (1993), when looking at NDF and starch degradation in a different forages over a range of different maturities. However, most researchers doing digestion of fibrous material account for the lag time, as mentioned earlier (Nocek, 1988; Varga and Hoover, 1983), which is explained in more detail elsewhere (Allen and Mertens, 1988). All digestion assays using this method find wide ranges of digestibilities and degradation rates. This is accounted for in many cases by the inherent differences in proportion of NDF and starch, lignification, maturity, particle size, digestion method, and length of fermentation (Cerneau and Michalet-Doreau, 1991; Offner et al., 2003; Varga and Hoover, 1983). These differences in methods and calculations make comparison between

feedstuffs elusive at best. Standardization of methods is essential for the assessment of feed quality. Further, advancements need to be directed toward determining similarities in macronutrient digestion and not specific differences between individual feedstuffs. Looking at each individual feedstuff leads to a focus on a very small part of the equation while ignoring the bigger picture.

RUMEN AVAILABLE PROTEIN AND CARBOHYDRATE

Nutrient Synchrony

In July 2008, the American Society of Animal Science saw fit to hold a symposium on the importance and viability of the idea of nutrient "synchrony" in beef cattle nutrition. The theory of nutrient synchrony is that diets can be formulated to have synchronous degradation rates of protein and energy components so as to be simultaneously available in the proper proportions required by the microbes. The purpose of which is to improve N capture, as well as MOEFF, and enhance animal performance, while decreasing N waste (Krehbiel et al., 2008).

Hall and Huntington (2008) explained that the concept of nutrient synchrony would require the ability to predict available amounts and fates of all substrates presented to a mixed microbial population. When exploring the meaning of this statement, one begins to realize formulating diets around this complex theory requires knowledge of several internal and external factors and precision in the predictive ability and physiological understanding that is currently beyond our abilities. As mentioned previously, pH, amount of starch fed, plant maturity and fiber source all differ in reported values, and a clear enough picture does not exist to precisely predict their effects on the overall system. Further, the controls over N recycling and passage rate are not well

defined and are essential when determining synchrony in the diet. Reynolds and Kristensen (2008) concluded that diets can be asynchronous in protein supply without any deleterious effects on growth and performance as long as supplemental protein is not provided less than 3 days apart. They also discuss the limitations of predicting N recycling due to the lack of knowledge of controls of urea transport across rumen epithelium and the over prediction of N retention due to the inability to account for all body losses of N. Feeding forage-based diets especially in grazing-based production systems requires a deeper understanding of intake and forage quality to provide accurate prediction and would further require a supplementation program to provide for any nutrient deficiencies (Hersom, 2008). Although controlling intake and nutrient quality is easier in a feedlot scenario, the use of phase-feeding (feeding different dietary components at different times to synchronize degradation) is labor intensive and provides mixed results. Cole and Todd (Cole and Todd, 2008) developed calculations to determine a synchronization index based on passage rates and degradation rates similar to those discussed in both the *Methods to estimate nitrogen digestibility* and *Methods to estimate carbohydrate digestibility* sections of this review. A meta-analysis of several data sets revealed their synchrony index had little, if any, effect on animal performance and negative relationships with feed efficiency and MOEFF. They attribute this to the aforementioned lack of ability to predict N recycling and N capture by the microbes.

When Newbold and Rust (1992) conducted an *in vivo* study looking at varying levels of asynchrony with corn and soybean based diets, they found varying times of addition of N and glucose did not irrevocably impact microbial populations. They propose these varying levels of synchrony may involve transient periods of excesses and

deficiencies of available N. It was shown that microbial populations recovered from these short periods of growth restriction. Richardson et al. (2003) reported limited success using synchronous techniques. They found no differences in growth performance but measured greater energy retention when lamb diets were synchronized versus not. Horadagoda et al. (2008) supplemented ryegrass hay-fed cannulated sheep with different carbohydrate sources and found slower degrading supplemental grains had a synergistic effect on microbial protein synthesis (MPS). However, this study is confounded by the lower than optimal protein concentration of the diet and overall decreased organic matter digestion, likely due to negative associative effects on cellulolytic bacteria. Kim et al. (1999) fed non-lactating dairy cows a basal diet or basal diet plus infusion with sucrose either continuously for 6 h starting either immediately after each feeding (synchronous), or six hours after each feeding (asynchronous). All diets receiving infusions of sucrose saw improvements in MPS, but no differences were found due to synchrony of diets. A study by Henning et al. (1993) looked at pulse-dosing versus continuous infusion of energy and protein in a 2 × 2 factorial design and found no effect on MPS or MOEFF due to synchronization of diets.

Nutrient Balance

A more appropriate theory and term for providing nutrients to the rumen could be "balance." This is a less restrictive model and is based on balancing the available carbohydrate and available protein to meet microbial needs to maximize efficiency. This may sound similar to synchrony, but the "time" aspect is removed and does not require degradation rates to be similar.

A good example of the differences and the confusion in the literature is when fistulated sheep were fed discontinuous diets of ryegrass and varying levels of concentrates, no differences were seen in MPS, but decreased fiber digestibility was seen with increasing concentrate levels (Kaur et al., 2008). However, this study fed ryegrass hay well after concentrates were fed to the animal. Ryegrass hay contained 26% CP and caused ammonia levels to rise from 14 m*M* (adequate ammonia levels ~ 3 m*M*) to 24 m*M*. This high quality hay also contained higher levels of soluble carbohydrates which caused a pH drop below 6.0 to between 5.5 and 5.7 for several hours. The authors attribute differences to asynchrony of the diet. It could be argued this is more likely an unbalanced fermentation resulting in higher degradability of fiber when nutrient proportions are better balanced. What is meant by this is that N is never limited, therefore asynchrony only exists when energy in the diet is lacking in the rumen. When available energy is added into the rumen, the fermentation becomes closer to being balanced not synchronized. This would explain the numerically greater pH and numerically lesser NH_3 observed throughout much of the day at the lowest level of concentrate, even though it had a higher proportion of NSC and CP. The difficulty in interpreting this study is the measurement of MPS is not ruminal synthesis, but total tract synthesis and no mention is made of a measurement of hind gut synthesis or hind gut digestibility; therefore, differences in N retention and microbial measures may be inaccurate.

Similarly, Herrera-Saldana and Huber (1989) miss label synchrony with balance when looking at carbohydrate and protein differences in milk production of lactating dairy cows. Exchanging carbohydrate sources to vary ratios of NDF and starch changes degradability of the feeds, but their study did not measure the rates of degradation and

only assumed synchronization. They also did not measure MPS and cannot adequately determine if synchrony was effective. The diet resulting in greater milk production also had greater amounts of degradable protein and NH_3 available to the animal. This was likely due to greater dry matter intake (DMI).

A simple but elegant study looking at the effects of both high and low amounts of rumen available NSC and high and low amounts of RDP shows when feeding high NSC and RDP, bacterial N passage to the rumen is increased (Aldrich et al., 1993). Further when low NSC were fed with low RDP, MPS was similar to low NSC and high RDP, and high NSC and low RDP had the least amount of MPS. This study shows that maximal MPS is achieved when NSC is balanced with RDP and increasing RDP without also increasing NSC does not improve MPS. This finding is in contradiction with other research which found when RDP is kept constant in the diet, increasing levels of NSC does not increase microbial efficiency, but increasing the ratio of RDP to NSC causes a linear increase in MOEFF (Stokes et al., 1991a; Stokes et al., 1991b). The difference between these studies is the latter two studies looked at microbial efficiency, while the former looked at MPS, which is a factor of MOEFF but is not always directly correlated.

Valkerners et al. (2006) used ruminally and duodenally cannulated bulls to test differences in balance of RDN to fermentable OM. Using different ratios of high and low RDN diets at 2 feedings at 12 h intervals, the treatments consisted of a balanced diet at each meal and two diets with increasing amounts of daily protein fed in the evening meal versus the morning meal. The results showed no differences in retained N, MPS or MOEFF. On a daily basis all animals received similar levels of protein and energy provided to the rumen, which indicated the increased capacity for bulls to recycle-N over

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short periods of time (12 h) and gives credence to the theory of daily balancing of rumen nutrients over synchrony of degradation.

Klevesahl et al. (2003) demonstrated the importance of balance in nutrient availability by infusing starch at two different levels and also infusing casein as a soluble protein source at 7 different levels to determine differences in forage utilization and ruminal characteristics. Intake of OM and NDF displayed a quadratic response to RDP supplementation with intake increasing then decreasing; while RDP supplementation had a positive quadratic response on NDF digestion. Further, increase in starch displayed negative associative effects on fiber digestion, which were negated with increasing supplemental RDP.

Calculating for Balance

Proper calculation of energy to protein balance in the rumen has the ability to decrease N intake and decrease feed costs. If the dietary source and amount of RDP and rumen available energy are properly balanced, current requirements for N intake by high-producing dairy cows can be reduced as much as 600 to 650 g per day without compromising metabolizable amino acid supply to the duodenum, MPS, or milk yield (Ipharraguerre and Clark, 2005; Ipharraguerre et al., 2005).

Hoover and Stokes (Hoover and Stokes, 1991) set the requirement for RDP as high as 14 to 15% DM in the diet. This calculation includes both true protein and NPN to meet microbial requirements. Fu et al. (2001) concluded the RDP requirement of the diet is as low as 12% DM based on a growth study conducted with Angus steers and varying levels of RDP. An *in vitro* continuous culture study done in the same study found, with

adequate NH_3-N (> 1.2 mM), the requirement for rumen degradable true protein can be as low as 3.5% DM without any negative effects on MOEFF.

Most diet formulations for ruminants report a requirement exists for physically effective fiber (NRC, 2000; Stone, 2004; Sudweeks et al., 1981), use of no-roughage diets in beef cattle diets has been successfully implemented (Golden et al., 2008; Pugh, 2007). This particular type of diet formulation operates on the theory of balance. By balancing RDP and rumen available energy, MOEFF can be maximized and waste products responsible for the negative side effects can be minimized.

Rumen carbohydrate and nitrogen availability have been discussed in previous sections of this review, but the balance of these requires a separate component, which is microbial requirements. The requirement for RDP in the NRC (2001) is based off a fixed efficiency of N utilization of 85% of RDP, which leads to the calculation of $1.18 \times RDP \times$ microbial N = amount of rumen degradable N. However, these calculations are based on a MOEFF of 28 g microbial N/kg of OM truly fermented for all bacteria. Bach et al. (2005) proposed a similar calculation only adjusting the formula for RDP requirement, but determined efficiency of microbial N utilization as 69% and MOEFF of 29 g microbial N/kg of OM truly fermented which shifted the formulation to $1.31 \times RDP \times$ microbial N. Any deviation of this would cause their model to deviate from optimal conditions. Meng et al. (1999) looked at the effect of dilution rate (D) on MOEFF and determined MOEFF can be calculated for different types of diets based off dilution rate with the calculations $MOEFF_{SC} = 7.1 + 341.6D - 965.3D^2$, $R^2 = 0.96$; $MOEFF_{NSC} = 1.7 + 368.7D - 586.9D^2$, $R^2 = 0.97$; and $MOEFF_{RDP} = 9.3 + 599.2D - 1445.6D^2$, $R^2 = 0.94$. Using estimated of dilution rate, the calculated MOEFF can be

used, along with the estimation of required NH_3-N and peptide N of 33% and 67% or RDP, respectively (Russell et al., 1992), to determine microbial requirements for NSC, SC and RDP, thus allowing for a diet to be formulated for balance.

Negative Effects of Unbalanced Diets

An imbalance in dietary formulation can have an effect by being formulated to be energy or nitrogen limiting. The effect of limited N causes an inability to sustain microbial growth in the presence of adequate supply of fermentable carbohydrate. This is deemed "energy spilling" (Van Kessel and Russell, 1996). Microbes compensate for excess degradable carbohydrate supply by increasing VFA and lactic acid production, which leads to decrease in ruminal pH and can lead to what is known as subacute to acute ruminal acidosis depending on the severity of the condition (Nagaraja and Titgemeyer, 2007; Owens et al., 1998). The term is called "spilling" because these carbon chains, if not absorbed at a fast enough rate, will continue to be degraded to methane (CH_4) and energy will be lost as heat. The study by Henning et al. (1993) described previously (in: *Nutrient synchrony*) found pulse dosing NSC caused an imbalance in the proportions of RDP and energy. This led to an energy spilling effect resulting in lower MOEFF. In his review on starch utilization, Huntington (1997) pointed out no strong correlation exists between starch digestibility and intake. Starch degradation by rumen microbes is so rapid (within 6 h after feeding), there is no limit in the rumen's ability to ferment starch; however, the over production of short-chain fatty acids (VFA and lactic acid) and gasses causes detrimental effects (decreased pH, bloat, keratinized rumen wall, liver abscesses) on the rumen and the animal which, if uncontrolled, leads to death (Stone, 2004).

Conversely, when RDP is in excess of microbial requirements based on the available energy supply, the situation can be deemed N overload. This leads to N sources being catabolized so the microbes can use the carbon backbones of the AA as energy substrates. What occurs here is an increased production of VFA without lactic acid increase (Pugh, 2007) and accumulation of NH_3 in the rumen. When NH_3 concentration in the rumen exceeds that of blood, NH_3 is absorbed across the rumen wall and transported to the liver, leading to an increase in BUN. The higher levels of RDP and subsequent increase in BUN can lead to decreased uterine pH and decreased circulating levels of progesterone, which exacerbates effects of negative energy balance and decreases overall fertility in cattle (Butler, 1998). Tamminga (2006) reviewed further effects of dietary RDP on fertility and found the literature presented several cases where increased RDP increased days to first ovulation in cattle and decreased overall conception rate.

FEEDING THE WILD RUMINANT

Browsers versus Grazers

Perhaps one of the most complete references in regards to the digestive morphologies of different species of ruminants is _The Ruminant Stomach_ by R.R. Hofmann (1973). This particular book focuses primarily on African species, but it does a thorough job of explaining the differences in classification of ruminant species by digestive strategy. Hofmann explains that ruminant animals can be classified as browsers (concentrate selectors), grazers, or an intermediate type feeder. As is implied by the name, grazing ruminants graze in areas with an abundance of grass species; whereas

browsers subsist mainly on forages, stems, and fruits, and intermediate feeders are able to switch between feeding strategies based on availability of feedstuff. Since this book was published, several excellent papers have been published to update and highlight the difference between these groups, and one wishing to explore this topic can find further information there (Clauss and Lechner-Doll, 2001; Clauss et al., 2003; Ditchkoff, 2000; Gordon and Illius, 1994; Robbins et al., 1995).

For the purposes of this review, a few morphological differences outlined in these papers will be highlighted. Grazing ruminants have wider mouths and generally take larger bites than browsing ruminants. They have a larger rumen for their body size, a better defined reticulum, thicker rumen papillae, smaller parotid salivary glands per body size, and their overall digestive tract is longer for their body size. This results in some key physiological differences in digestion of feed with browsing ruminants. Browsers have smaller initial feed particle size, which leads to a lack of stratification in the rumen as is seen in grazing animals, spend more time foraging and less time ruminating, have greater salivary production and faster particle and fluid passage rates due to diet type. In domestic animals, cattle, water buffalo and sheep would be considered grazers, while goats would be classified as intermediate feeders. In the exotic world these classifications know no size, with the smallest ruminant (Günther's dik dik) and the largest ruminant (Giraffe) both being classified as browsers.

Energy Malnutrition in Captive Ruminants

In 2005, the Lincoln Park Zoo hosted the Giraffe Nutrition Workshop in part to determine how diets of captive browsing ruminants can be improved to decrease incidence of malnutrition recently seen in zoo animals. This conference came about due

to recent cases of what has been termed "Peracute Mortality Syndrome," and was originally described by Fowler (1979). This particular syndrome manifests as sudden death in seemingly healthy giraffes. Upon necropsy giraffes exhibit atrophy of body fat stores, ruminal acidosis and loss of dorsal ruminal papillae (Clauss et al., 2002; Potter and Clauss, 2005). Manifestations of acidosis are not unique to giraffe. Captive deer herds have also presented with ruminal acidosis and parakeratosis (Baker et al., 1998; Woolf and Kradel, 1977), and urolithiasis (Woolf et al., 1976).

Acidosis has been extensively reviewed elsewhere (Bramley et al., 2008; Owens et al., 1998; Stone, 2004), but it can occur as both subacute and acute acidosis. As discussed earlier, this condition can be triggered by energy spilling and causes increased VFA production leading to a decrease in pH below 5.4 for extended periods of time. The acid causes ruminal lesions, keratinization, scarring and blunting of the ruminal papillae. During acidotic conditions, *Fusobacterium necrophorum*, a bacteria found on the rumen wall, enters the blood through ruminal lesions and settles in the liver where it feeds off lactate production and causes the abscesses associated with acidosis (Nagaraja and Titgemeyer, 2007). As recently reviewed in a special symposium on acidosis at the Animal Society of Animal Science National Meeting in Denver, CO this year, VFA are transported across the rumen via special bicarbonate transporters (Aschenbach et al., 2010) and any keratinization of these tissues or blunting of papillae would cause decreased absorption. This would cause two problems, loss of energy substrate for the animal (acetate is a major substrate for energy and subcutaneous fat) and a further drop in ruminal pH (due to accumulation of VFA). This would easily explain the symptoms described above.

Wild versus Captive Diets of Browsers

A sample of natural diets of browsing ruminants, such as white-tailed deer and mule deer, shows composition of these diets is much different than what would be expected by most who hear these animals are eating fruits and leaves. Wild fruits are unlike cultivated fruits in that they contain greater amounts of CP, NDF and ADF and lesser amounts of soluble carbohydrates and starch components (Ammar et al., 2008; Dierenfeld et al., 2002; Short, 1971). These fruits, along with stems and leaves in the browsing diets result in high cellulolytic activity, equal to that of grazing ruminants (Robbins et al., 1995). Browsing ruminants also experience greater changes in feedstuffs due to seasonality, with the higher quality food occurring in mid-spring to early summer and declining in quality and abundance throughout the fall and winter (Pearson, 1969; Short, 1971; Short et al., 1966; Torgerson and Pfander, 1971), which also requires them to maintain a tremendous capacity for N recycling (up to 85% of urea production) when protein availability is lowest (Huapeng et al., 1997).

Diets fed to browsing ruminants in captive settings are based off nutritional recommendations for domestic ruminants and, until recently, have included cultivated fruits and vegetables to substitute for wild browse and fruits. As noted earlier, these captive diets are very discordant with the wild diets in that they contain low NDF and high starch and protein (Dierenfeld et al., 2002). The nutrients within these diets are energy-rich with rapidly fermentable carbohydrates and run the risk of causing acidosis in the animal (Hummel et al., 2006; Odongo et al., 2006).

Initial attempts to emulate a wild diet with deer proved initially successful over a short-term, but the diet contained up to 25% NSC and 42% NDF with 22% CP and long

term effects in some animals were rumen atrophy, parakeratosis, and obesity (Baker et al., 1998). Including browse along with a protein and energy supplement fed to captive deer was able to decrease condition loss and increase N retention compared to other all browse diets (Ullrey et al., 1975); however the all browse diets were less than 7% CP and greater than 37% NSC and 46.4% NDF (Ullrey et al., 1971). Adding a protein supplement (25% CP) at 10% of the diet would only increase the diet to 9% CP. Even with the supplement, the deer in this study still lost body condition and were in negative energy balance.

Recent research evaluating diets formulate for captive zoo animals evaluated traditional diets of high soluble carbohydrates and low NDF against diets with greater amounts of NDF. When the higher NDF experimental diet was fed to giraffe, overall starch consumption decreased and time feeding and ruminating increased, which likely increased salivation (Kearney, 2005). When diets formulated based on the information from this study were used in a live growth study using captive mule deer, animals on the low NSC/high NDF diet displayed improved fecal consistency, time spent eating, and time spent ruminant without increasing total intake (McCusker, 2009).

Since increased intake of a highly digestible diet, along with a decreased fluid dilution due to low salivary secretions, increases risk of acidosis. Dietary recommendations for domestic ruminants experiencing subacute ruminal acidosis are to increase physically effective NDF and decrease nonstructural carbohydrates in the diet (Stone, 2004). This practice would limit intake and increase rumination and salivary secretions. In captive browsing ruminants, this would also be consistent with the wild diets described previously.

Increased digestibility leads to greater total VFA production, which has been associated with increased risk of acidosis. However, if similar digestibility levels exist between diets of high fiber versus diets of high starch, the greater VFA production would be washed out of the rumen *in vivo* due to greater salivary production, provided fiber was in the form of physically effective NDF and stimulated rumination. This would allow for improved energy availability post-ruminally and decreased risk of causing ruminal lesions and keratinization.

The Saint Louis Zoo recently hosted a Ruminant Browser Nutrition Workshop as a follow-up to the Giraffe Nutrition Workshop. Based off the studies mentioned above and further input from domestic animal research, new guidelines for browsing ruminant diets have now been implemented. The most recent recommendation now call for NSC less than 10% DM with less than 7% DM being recommended; protein has been set to a minimum of 14% DM; and NDF levels need to be a minimum of 40% DM with smaller species likely needing less to meet maintenance needs (25 to 30% DM; Schmidt and Kendrick, 2009).

CONCLUSIONS

In summary, nitrogen and carbohydrate degradation in the rumen is varied by microbial type, substrate type (i.e. peptide vs. AA, NSC vs. SC) and amounts of degradable material within the feedstuff. Over the years, there have been several different methods for best determining this degradability to calculate the nutrients available in the rumen to meet the requirements for microbial growth. The optimal diet does not need to be synchronized in degradation rate to prevent any deficiencies or excesses in protein or

energy availability; however, these nutrients do need to be provided to the rumen in the proper ratios to improve MOEFF efficiency and growth performance. Any imbalances in the system will lead to negative effects on digestibility, growth and animal health due to over production of short-chain fatty acids and NH_3. This is epitomized in the captive browsing ruminant whose diets over the decades have led to a decrease in body condition and energy reserves to the point of death for several individuals. Recommended diets for both domestic and captive ruminants is to formulate diets balanced in both protein and energy needed for microbial growth based on estimated MOEFF and requirements.

CHAPTER 2

ASSESSMENT OF AMINO ACID SUPPLEMENTATION ON RUMEN MICROBIAL EFFICIENCY AND NITROGEN METABOLISM USING A CONTINUOUS CULTURE SYSTEM

ABSTRACT

Differing levels of feed grade crystalline amino acids (FAA) were evaluated for effects on microbial efficiency (MOEFF) and nitrogen metabolism in the rumen environment. Six treatments were evaluated ($N = 24$, $n = 4$) using a continuous culture system with a fractional dilution rate 0.06. A basal diet of ground corn (57%), soybean meal (35%), and soybean hulls (8%) was fed (50 g/d) with additional amounts of an equal mix of L-Lys, L-Arg, and DL-Met at 0%, 3%, 6%, 12% and 18% (FAA). A 6% bloodmeal (BM) diet was fed as a positive control. After 4 d acclimation, ammonia and pH of fermenters were analyzed 1 h before and 4 h after feeding for 3 days. Nitrogen, OM and purines were analyzed for diets, bacteria, and effluent to calculate MOEFF. Ammonia concentration rose as FAA supplementation in diets increased ($P < 0.01$). MOEFF tended to be greater for 6% and 12% FAA and BM diets ($P = 0.10$) and least for 3% and 18% FAA. An increase was measured in total bacterial amino acid (AA) composition, but ratio of AA in bacteria remained similar with the exception of Arg, Asp, and Orn ($P < 0.03$). Escape of AA from fermenters was greatest ($P < 0.01$) in 0% FAA and BM diets, leading to the conclusion that FAA supplementation does not supply a viable source of RUP to the animal. Rumen undegradable nitrogen is greatest in 0% and

3% FAA; this along with increased ammonia levels lead to the conclusion that supplemental FAA provided excess nitrogen which did not result in a direct improvement of MOEFF. Our data agreed with previously published data concluding AA degradation rate was more rapid than AA outflow at feasible feeding levels.

Keywords: Continuous Culture, Amino Acids, Microbial Efficiency, Ruminant, Nitrogen

INTRODUCTION

Nutritional requirements for amino acids (AA) in monogastrics are well established (Fuller et al., 1989; Kim et al., 2009; Mack et al., 1999). Direct uptake of AA in the small intestine allows for the addition of feed-grade crystalline AA to compensate dietary deficiencies in AA profile thus increasing production characteristics and decreasing nitrogen (N) excretion (Aletor et al., 2000; Gatel and Grosjean, 1992; Wu et al., 2007). Ruminant production would be expected to respond similarly, however, post-ruminal RUP supply to the small intestine must be achieved.

Dietary methionine and arginine derived from soybean meal, corn gluten meal and bloodmeal are more extensively degraded in the rumen than the other essential AA (Titgemeyer et al., 1989). Further, bacterial methionine and lysine reaching the small intestine have been designated as limiting AA for growing cattle (Richardson and Hatfield, 1978). Abomasal infusion of L- Methionine or L-Lysine increased N retention in steers and decreased urinary waste (Awawdeh et al., 2006).

By supplementing these specific amino acids to the animal in their synthetic free-form, the animal and microbes would be supplied with a balanced amino acid profile and would not lose efficiency due to deamination and urea synthesis. The purpose of this experiment was to study microbial efficiency (MOEFF) and nitrogen metabolism in the rumen environment when differing levels of free crystalline amino acids were added to the diet. Our hypothesis was that elevated levels of free AA in the diet would result in increased outflow of AA from ruminal fermentation.

MATERIALS AND METHODS

Amino Acid Continuous Culture

A basal diet composed of corn (57%), soybean hulls (35%), and soybean meal (8%) ground to pass through 3-mm screen in a Wiley mill (Arthur H. Thomas Company, Philadelphia, PA) was mixed with an equal mix of feed grade crystalline L-Lysine (L-Lys), L-Arginine (L-Arg), and DL-Methionine (DL-Met) at a total of 3, 6, 12, and 18% additional amino acids based on a 50 g/d basal diet (Table 1). Additional negative (basal diet only with no added amino acids) and positive (basal diet + 6% whole bloodmeal) control diets were used for comparison.

The use of animals in this experiment was approved by the University of Missouri Animal Care and Use Committee. Rumen fluid was obtained from a ruminally fistulated multiparous lactating Holstein cow provided *ad libitum* access to a lactation diet (24.5% corn silage, 12.6% alfalfa hay, 15.3% alfalfa haylage, 47.7% concentrate and 19% CP, 24% ADF and 41% NDF) formulated to meet nutritional requirements (NRC, 2001), and housed in free-stall facilities at the University of Missouri-Columbia Foremost Dairy Research Center. Dairy cow rumen fluid was transported from the farm to the lab (estimated travel time 10 min.), strained through four layers of cheese cloth and diluted with McDougall's artificial saliva in a 1:4 dilution of rumen fluid to buffer.

Twenty-four single-flow effluent continuous culture fermenter polycarbonate vessels (Nalgene, Rochester, NY) were inoculated and maintained as described by Meng et al. (1999). Inoculum was added to each fermenter up to the effluent overflow port (approximately 1460 mL). Fermenters were continuously flushed with CO_2 gas, stirred with magnetic stir plates, and immersed in a water bath maintained at 39°C using

thermostatically controlled heaters (model 730, Fisher Scientific, Pittsburgh, PA). High buffer capacity solution modified by Slyter (1990) from McDougall's artificial saliva (McDougall, 1948), containing 107.5 mg urea-N/L and 250 mg cysteine-HCL/L was continuously infused into fermenters using peristaltic pumps (Masterflex model 7520-10, Cole Parmer Instrument Co., Chicago, IL). Fermenter dilution rates were held constant at $6\% \pm 0.2\% \cdot h^{-1}$ for all treatments. Effluent flowed into collection vessels immersed in ice-cooled water. Fermenters were randomly assigned to one of the six treatments ($n = 4$) and fed half the daily ration at 0900 h and 1900 h. The experiment was conducted over a 7-day period, with 4 days adaptation and 3 days sampling.

Sampling

One liter subsamples were taken from effluent collected over the previous 24 h and stored at 4˚C. These samples were composited for each fermenter over the three day period for later analysis. On the last day of sampling, fermenter contents were blended and strained through two layers of cheese cloth and stored at 4˚C until analyzed. Further, 1 hour before feeding and 4 hours after feeding, pH was measured and a 5 mL sample were taken directly from the fermenter and immediately frozen at -20˚C. These samples were later composited for each fermenter over the three day period for each sampling time and analyzed for ammonia and VFA.

Lab Analysis

Blended fermenter samples were centrifuged at 1,000 x *g* for 5 min at 4°C to remove feed particles. Supernatant was re-centrifuged at 27,000 x *g* for 30 min. The resultant pellet was washed once using 0.9% (wt/vol) saline solution then again using deionized distilled H_2O. The final pellet, containing bacteria, was transferred to plastic

cups using deionized distilled water, lyophilized at 10°C (Genesis, Virtis, Gardiner, NY), and ground using a mortar and pestle. Subsamples of effluent (500 mL) were lyophilized at 10°C (Genesis, Virtis, Gardiner, NY), and ground using a mortar and pestle.

For analysis, diets were ground through with a Wiley mill to pass through a 1-mm screen. Samples of diet, effluent and fermenter contents were analyzed for dry matter by drying at 105°C for 24 h, organic matter by incineration at 500°C and total nitrogen by combustion analysis (LECO FP-428; LECO Corporation, St. Joseph, MI). Samples of effluent and fermenter pellets were analyzed for purine content using the procedure of Zinn and Owens (1986) to determine microbial nitrogen, which was used with OM digested to determine microbial efficiency (MOEFF; g microbial nitrogen outflow/kg OM truly digested). Sample ammonia concentration (m*M*) was determined colorimetrically (DU-65 spectrophotometer; Beckman, Palo Alto, CA) with the hypochlorite-phenol procedure of Broderick and Kang (1980). Sample VFA concentration (m*M*) was determined using gas chromatography (Model 3400, Varian, Palo Alto, CA) following procedures outlined by Salanitro and Muirhead (1975). Samples of diet and bacteria were sent to the University of Missouri Experimental Station Chemistry Lab for total amino acid analysis (AOAC, 2010).

Statistical Analysis

All statistical analyses were performed by analysis of variance (ANOVA) using the Proc GLM procedure in SAS® version 9.2 (SAS Institute Inc., Cary, NC). The data were analyzed as a randomized complete block design (RCBD) with 6 treatments. When the *F*-test was significant ($P \leq 0.05$), means separation was performed using Fisher's Least Significant Difference (LSD).

RESULTS

Fermentation Characteristics

Ammonia concentrations rose as the free AA supplementation in the diets increased ($P < 0.01$, Figure 1). Microbial efficiency tended to be lesser ($P = 0.10$) when supplemented with 3% and 18% additional free AA (Table 2), decreasing efficiency compared to basal diet alone (0%). Microbial efficiency tended to be equally greatest in 6% BM, 6% free AA, and 12% free AA supplementation (~ 21%). The same pattern of among treatments was significantly different ($P = 0.03$) for grams of effluent bacterial nitrogen produced daily. The proportion of OM truly digested was not different ($P = 0.11$) in all treatments (54 − 59%). Culture pH taken 1 hour before feeding was not different (6.7 − 6.8; $P = 0.58$). However, 4 h after feeding, pH remained stable (6.83) for 18% supplemental free AA while all other levels decreased ($P < 0.01$) to lower ranges (6.6 − 6.7). Total RUN was greatest ($P < 0.01$) at 0% and 3% supplementation of free AA, and similarly lesser at 6%, 12% and 18% supplementation of free AA and 6% BM. Proportion of dietary arginine, methionine, and lysine by-pass amino acid supply to the effluent was greatest ($P < 0.01$) in the basal diet followed by 6% BM addition and decreased as supplementation of free AA increased.

Short-Chain Fatty Acids

Addition of 3%, 6% and 12% free AA resulted in the greatest production of total VFA, followed by 6% BM addition and 0% additional free AA. Supplementation of 18% free AA resulted in the least concentration of VFA before and after feeding ($P \leq 0.01$). Proportion of acetate in total VFA was least for 18% before and after feeding and

increased as additional AA decreased in the diet ($P < 0.01$). Propionate production was unaffected by treatment ($P > 0.20$). This resulted in a tendency for lower acetate:propionate ratio in 18% additional free amino acid before ($P = 0.10$) and after ($P = 0.06$) feeding. Proportion of butyrate in VFA was greatest for 18% addition and decreased with decreasing additional amino acids ($P < 0.01$). Branched-chain VFA (isobutyrate and isovalerate) were greatest in 6% BM addition, and decreased as additional free amino acids were added to the diets ($P < 0.01$) for both sampling times. Conversely, valerate proportion increased as AA supplementation increased ($P < 0.01$). Lactate production was greatest at 6% BM addition, 0%, 3% and 6% free AA addition and least in 18% free AA addition before ($P = 0.02$) and after ($P = 0.01$) feeding.

Bacterial Amino Acid Composition

Total amino acid concentration in bacteria was greater when AA were supplemented in the diets and were least in bacteria fed 6% BM diet ($P < 0.01$) with AA accounting for 47 to 54 % of bacterial mass. As a result, increase of mass of individual AA mirrored total amino acid. When bacterial profile was evaluated for each individual AA as a percent of total AA instead of bacterial mass, the only differences occurred in the amounts of arginine, aspartate and ornithine. At greater supplementation of AA (12% and 16%) arginine increased compared to all other treatments, replacing aspartate and ornithine, which decreased ($P \leq 0.03$).

DISCUSSION

Differences in RUN agree with the trends observed for MOEFF, the nitrogen in the 0% and 3% suggests a lack of rumen degradable nitrogen compared to fermentable

energy to maximize growth and increase microbial digestion of plant-bound protein. Conversely, the decreased MOEFF and increased RUN in 18% free AA supplementation suggests an excess of rumen degradable nitrogen (RDN) to fermentable energy, which caused microbes to expend more energy dealing with this excess and also caused increased NH_3 levels, both being detrimental to microbial growth.

The culture pH drop measured between the -1 h sampling and 4 h sampling was more pronounced with lesser levels of AA inclusion. The range of difference between treatments at the 4 h sampling was within only 0.1 pH unit, suggesting the excess ammonia production could have a slight buffering affect but not enough to be physiologically significant.

Argyle and Baldwin (1989) only saw an increase in bacterial growth when a complete mixture of AA were added to culture, which would agree with our positive control (6% BM); however, contrary to their findings, we also observed increased growth, as measured by bacterial N, where our limiting AA mixture was added at 6% and 12%. Differences between experiments likely occurred due to their use of casein as a base diet, an animal protein with a complete AA profile, and our use of a corn/soybean based diet with an AA profile limiting in lysine, methionine and arginine. The additional AA at 6% and 12% may be only alleviating the deficiencies of an incomplete dietary AA profile.

When energy is limiting, methionine is ultimately converted to propionate by rumen bacteria and protozoa while L-lysine can be converted to acetate and butyrate by rumen bacteria (Onodera, 1993) with both AA producing NH_3. At 18% AA supplementation, lower proportions of acetate are likely due to its preferential use as a

carbon skeleton for AA construction and an increase in butyrate formation. This, along with a numerically greater proportion of propionate, resulted in the tendency for lesser acetate:propionate ratio. Increased branch-chain VFA production in 6% BM is a direct result of branch-chain AA (valine → isobutyrate and leucine → isovalerate) fermentation (Van Soest, 1994). Griswold et al. (1996) found using AA as a protein source in rumen fluid increased isobutyrate, isovalerate and valerate production; however, they supplemented a mixture of amino acids to simulate soy protein and thus would have greater substrate availability for those particular VFA produced.

Greater levels of total AA within bacteria from dietary treatments with increasing levels of supplementation do not counteract the decreased amount of total effluent bacterial nitrogen, which would be needed to maximize bacterial AA supply post-ruminally. When expressed as percent of total amino acid measured, values obtained from bacteria confirm previous findings indicating bacterial profiles in mixed rumen cultures do not differ greatly due to diet (Bergen et al., 1968; Garrett et al., 1987; Merchen and Titgemeyer, 1992; Purser and Buechler, 1966). However, the differences observed in this study indicate possible alterations of bacterial AA related to metabolizing excess ammonia. Microbial aspartate production requires acetate or lactate along with ammonia as the substrates for the carbon skeleton and nitrogen source (Allison, 1969). Diets containing 12% and 18% AA supplementation displayed lesser concentrations of short-chain fatty acids, which could limit assimilation of this AA into bacteria even with excess ammonia. Data presented by Salter et al. (1979) using ^{15}N isotopes, suggest ammonia incorporation into bacteria proceeds in the order of NH_3 → amide → glutamate → aspartic acid and alanine → other amino acids. They also showed

increasing dietary urea leads to an increase in proportion of arginine and decrease in proportion of aspartic acid incorporation into bacteria. Because synthesis of arginine is slower than most amino acids (Salter et al., 1979), it would stand to reason the production of ornithine from L-arginine (Lewis and Emery, 1962), would limit ornithine accumulation in the microbes.

When free L-lysine, methionine and threonine were infused into the rumen by Volden et al. (1998), degradation rates within the first 2 hours of infusion were greater than 100% h^{-1}. With much lower levels of additional AA than used in the current study, they observed a decrease in ruminal degradation with increasing levels of infusion and thus an increase in relative rumen by-pass of the amino acids. This is contrary to our study where inclusion of increasing levels of AA decreased relative dietary rumen by-pass. Their study, however, examined only apparent digestibility and duodenal samples taken were already subject to hydrolysis in the abomasum and could not differentiate between bacterial, dietary, or infused AA, nor did they account for any amino acids produced through microbial assimilation.

Campbell et al. (1997) added free L-lysine and DL-methionine to corn-based diets fed to growing steers and found no differences in pH, ammonia, or VFA concentrations. Measured VFA concentrations in their study were similar to our levels of supplementation, but lesser than our 6% and 12% AA supplementation diets. Similar to our study, Campbell et al. saw a quadratic response in fermentation characteristics (acetate production, AA degradation and bacterial nitrogen) with the greater and lesser levels of supplementation producing lesser values than the intermediate levels. However, they attributed lack of responses in ammonia, pH and total VFA production to low levels

of inclusion of the supplemental free AA, concluding ruminal degradation and uptake across the rumen wall, along with AA and ammonia uptake by the microbes was far too great to overcome passage rate. They speculated greater levels would be necessary to elicit the desired response of increasing dietary by-pass of AA.

Our data corroborated previously published data that suggested AA degradation rate was more rapid than AA outflow at feasible feeding levels (Chalupa, 1976; Kung Jr. and Rode, 1996). Supplementing free amino acids does not appear to increase post-ruminal dietary amino acid flow, except at high levels of inclusion. This would not be an economically feasible approach. Free AA supplementation in the diet does not appear to supply peptides required to maximize MOEFF. This appears to be due to the preferential uptake of protein and peptides by the microbes, as demonstrated by bloodmeal, while the free AA were instead being broken down into NH_3. This would hold with prior research that shows the incorporation of nitrogen into the microbes from a mixed rumen culture being fed a grain based diet follows the preference of peptides>AA>NH_3 (Carro and Miller, 1999). Similar to Griswold et al. (Griswold et al., 1996) our data leads us to concluded peptide N is needed to maximize MOEFF.

CONCLUSIONS

Feeding free AA did not provide great enough improvement to MOEFF or post –ruminal AA flow to make them an economically or physiologically viable protein supplement for ruminants. Formulating diets for AA requirements will require ruminally stable proteins or amino acid products.

Table 2.1. Nutrient composition of diets with varying levels of supplemental free crystalline amino acids for use in continuous culture.

Ingredient (g/d As-fed)	Level of Dietary Amino Acid Supplementation[1]					
	0%	6% BM	3%	6%	12%	18%
Corn	28.50	28.50	28.50	28.50	28.50	28.50
Soybean Hulls	17.50	17.50	17.50	17.50	17.50	17.50
Soybean Meal	4.00	4.00	4.00	4.00	4.00	4.00
Whole Bloodmeal	-	2.70	-	-	-	-
L-Arginine	-	-	0.45	0.90	1.80	2.70
L-Lysine	-	-	0.45	0.90	1.80	2.70
DL-Methionine	-	-	0.45	0.90	1.80	2.70
Total Diet	50.00	53.00	51.00	53.00	55.00	58.00
Nutrient Composition						
Dry Matter (%)	90.3	86.0	89.0	93.0	94.3	93.8
Organic Matter (% DM)	96.9	97.1	97.0	97.1	97.2	97.3
Crude Protein (% DM)	12.6	19.4	16.0	17.5	23.6	30.3
Dietary Amino Acids (% DM)						
Alanine	0.68	1.26	0.69	0.62	0.59	0.55
Arginine	0.79	1.15	1.58	2.75	4.27	5.67
Aspartate	1.21	2.07	1.20	1.11	1.06	0.98
Cysteine	0.21	0.27	0.22	0.20	0.19	0.19
Glutamate	2.13	2.93	2.08	1.90	1.90	1.75
Glycine	0.73	1.07	0.75	0.68	0.66	0.61
Histadine	0.35	0.86	0.35	0.32	0.31	0.29
Hydroxylysine	0.03	0.02	0.03	0.03	0.02	0.03
Hydroxyproline	0.20	0.17	0.19	0.16	0.17	0.16
Isoleucine	0.50	0.59	0.52	0.51	0.50	0.45
Lanthionine	0.00	0.00	0.00	0.00	0.00	0.00
Leucine	1.07	2.06	1.09	0.99	0.93	0.85
Lysine	0.73	1.37	1.35	2.42	3.73	4.77
Methionine	0.21	0.28	1.17	2.16	3.27	5.40
Ornithine	0.01	0.01	0.01	0.02	0.02	0.02
Phenylalanine	0.58	1.06	0.57	0.53	0.50	0.46
Proline	0.79	1.08	0.79	0.70	0.68	0.65
Serine	0.64	0.96	0.62	0.54	0.50	0.51
Taurine	0.08	0.07	0.10	0.09	0.08	0.06
Threonine	0.49	0.71	0.48	0.43	0.41	0.39
Tryptophan	0.16	0.19	0.18	0.13	0.12	0.12
Tyrosine	0.51	0.66	0.48	0.43	0.45	0.42
Valine	0.61	1.22	0.62	0.58	0.56	0.50
Total Amino Acids	12.69	20.06	15.05	17.29	20.92	24.84

[1] Dietary treatments: 0% = 50 g/d basal diet (- control); 6% BM = 50 g/d basal diet + 2.7 g/d whole bloodmeal (+ control); 3%, 6%, 12% 18% = 50 g/d basal diet + 1.35, 2.70, 5.50 and 8.10 g/d of an equal mix consisting of feed grade crystalline L-Arg, L-Lys, DL-Met, respectively.

Table 2.2. Fermentation characteristics and rumen by-pass amino acids when varying levels of supplemental free crystalline amino acids are fed to continuous culture fermenters.

Item	Level of Dietary Amino Acid Supplementation[1]						SE	P-value
	0%	6% BM	3%	6%	12%	18%		
	(n=4)	(n=4)	(n=4)	(n=4)	(n=4)	(n=4)		
MOEFF[2]	17.53	21.05	14.99	20.78	21.47	15.01	2.04	0.10
Bacterial nitrogen (g/d)	0.48^{ab}	0.63^a	0.40^b	0.63^a	0.62^a	0.46^{ab}	0.06	0.04
OM digested (%)[3]	56.41	58.53	53.87	57.93	54.49	54.08	1.41	0.11
pH -1 h[4]	6.73	6.80	6.73	6.69	6.75	6.82	0.06	0.58
pH 4 h[4]	6.68^{bc}	6.69^{bc}	6.68^{bc}	6.63^c	6.74^b	6.83^a	0.03	<0.01
RUN (% Dietary Nitrogen)	74.88^a	56.62^b	73.71^a	57.12^b	56.77^b	63.01^{ab}	4.08	<0.01
Rumen By-pass Amino Acids (% Dietary Amino Acid)								
Arginine	34.67^a	27.66^b	21.22^c	7.86^d	5.99^e	5.27^f	0.99	<0.01
Methionine	66.37^a	46.57^b	21.18^c	12.99^d	19.94^c	19.55^c	1.86	<0.01
Lysine	93.87^a	64.99^b	71.53^b	39.49^c	31.83^c	14.49^d	2.91	<0.01

[1] Dietary treatments: 0% = 50 g/d basal diet (- control); 6% BM = 50 g/d basal diet + 2.7 g/d whole bloodmeal (+ control); 3%, 6%, 12% 18% = 50 g/d basal diet + 1.35, 2.70, 5.50 and 8.10 g/d of an equal mix consisting of feed grade crystalline L-Arg, L-Lys, DL-Met, respectively.
[2] Microbial Efficiency = g effluent bacterial N/ kg OM truly digested
[3] Organic matter truly digested (%)
[4] pH taken 1 hour before feeding (-1 h) or 4 hours after feeding (4 h)
[abcd] Means with no superscripts in common within the same row are statistically significant.

Table 2.3. Volatile fatty acid (VFA) and lactate concentrations when varying levels of supplemental free crystalline amino acids are fed to continuous culture fermenters.

Fermenter VFA -1 h[2]	Level of Dietary Amino Acid Supplementation[1]						SE	P-value
	0%	6% BM	3%	6%	12%	18%		
Total VFA (mM)[3]	85.96[bc]	86.88[bc]	92.51[ab]	95.49[ab]	102.38[a]	82.49[c]	3.34	<0.01
Lactate (mM)	0.37[a]	0.32[ab]	0.36[a]	0.37[a]	0.26[b]	0.23[b]	0.03	0.02
Acetate:Propionate Ratio	3.41	3.57	3.46	3.67	3.52	2.71	0.23	0.10
			mol/100 mol					
Acetate	59.63[a]	60.22[a]	59.98[a]	59.22[a]	57.40[a]	52.51[b]	1.06	<0.01
Propionate	17.57	17.23	17.47	16.11	16.50	19.86	1.07	0.24
Isobutyrate	0.49[ab]	0.59[a]	0.47[bc]	0.47[bc]	0.39[cd]	0.34[d]	0.03	<0.01
Butyrate	19.94[bc]	19.34[c]	19.49[c]	21.31[bc]	22.03[b]	24.51[a]	0.73	<0.01
Isovalerate	0.81[b]	0.95[a]	0.79[b]	0.76[bc]	0.66[cd]	0.57[d]	0.04	<0.01
Valerate	1.56[d]	1.67[d]	1.82[cd]	2.14[bc]	3.02[a]	2.22[b]	0.12	<0.01
Fermenter VFA 4 h[2]								
Total VFA (mM)[3]	91.01[bc]	92.51[bc]	95.00[abc]	106.46[a]	103.03[ab]	83.44[c]	4.21	0.01
Lactate (mM)	0.76[a]	0.71[ab]	0.59[abc]	0.71[a]	0.52[bc]	0.41[c]	0.06	0.01
Acetate:Propionate Ratio	3.54	3.43	3.65	3.61	3.46	2.61	0.24	0.06
			mol/100 mol					
Acetate	60.38[ab]	60.17[ab]	60.75[a]	59.26[ab]	57.46[b]	52.11[c]	1.03	<0.01
Propionate	17.17	17.87	16.69	16.52	17.12	20.28	1.09	0.21
Isobutyrate	0.50[ab]	0.56[a]	0.46[ab]	0.44[bc]	0.36[cd]	0.29[d]	0.03	<0.01
Butyrate	19.60[bcd]	18.79[d]	19.36[cd]	20.82[bc]	21.56[b]	24.63[a]	0.68	<0.01
Isovalerate	0.78[b]	0.91[a]	0.77[b]	0.72[bc]	0.63[cd]	0.53[d]	0.04	<0.01
Valerate	1.57[d]	1.70[cd]	1.97[bc]	2.23[b]	2.87[a]	2.16[b]	0.11	<0.01

[1] Dietary treatments: 0% = 50 g/d basal diet (- control); 6% BM = 50 g/d basal diet + 2.7 g/d whole bloodmeal (+ control); 3%, 6%, 12% 18% = 50 g/d basal diet + 1.35, 2.70, 5.50 and 8.10 g/d of an equal mix consisting of feed grade crystalline L-Arg, L-Lys, DL-Met, respectively.

[2] Samples were taken 1 h before the morning feeding (-1 h) or 4 h after the morning feeding (4 h)

[3] Total VFA = Acetate + Propionate + Isobutyrate+ Butyrate+ Isovalerate + Valerate

[abcd] Means with no superscripts in common within the same row are statistically significant.

Table 2.4. Amino acid profiles of bacteria grown in continuous culture fermenters fed varying levels of supplemental free crystalline amino acids.

Bacterial Amino Acids	Level of Dietary Amino Acid Supplementation[1]						SE	P-value
	0%	6% BM	3%	6%	12%	18%		
Total Amino Acid (g/100 g)	48.92^{cd}	46.85^d	50.40^{bc}	51.08^{bc}	52.45^{ab}	54.35^a	0.97	<0.01
(% Total Amino Acids)								
Alanine	7.23	7.22	7.21	7.24	7.19	7.24	0.02	0.75
Arginine	4.55^c	4.55^c	4.58^c	4.64^{bc}	4.71^{ab}	4.76^a	0.03	<0.01
Aspartate	12.13^{ab}	12.14^{ab}	12.15^{ab}	12.18^a	12.09^{bc}	12.02^c	0.03	0.01
Cysteine	1.11	1.10	1.10	1.08	1.10	1.08	0.02	0.80
Glutamate	12.34	12.32	12.26	12.27	12.29	12.24	0.06	0.88
Glycine	5.54	5.55	5.55	5.51	5.48	5.50	0.05	0.90
Histadine	1.71	1.74	1.73	1.74	1.74	1.77	0.02	0.54
Hydroxylysine	0.01	0.02	0.03	0.02	0.00	0.03	0.01	0.31
Hydroxyproline	-	-	-	-	-	-	-	-
Isoleucine	5.80	5.69	5.76	5.73	5.81	5.91	0.07	0.32
Lanthionine	0.00	0.00	0.08	0.00	0.00	0.00	0.03	0.35
Leucine	7.42	7.44	7.46	7.43	7.51	7.58	0.04	0.14
Lysine	8.91	8.81	8.92	8.97	8.98	9.07	0.08	0.32
Methionine	2.51	2.47	2.45	2.47	2.53	2.55	0.03	0.18
Ornithine	0.35^{ab}	0.41^a	0.34^{ab}	0.35^{ab}	0.30^{bc}	0.25^c	0.03	0.03
Phenylalanine	4.77	4.81	4.83	4.81	4.78	4.74	0.04	0.61
Proline	3.30	3.34	3.30	3.31	3.25	3.26	0.05	0.85
Serine	3.87	3.94	3.91	3.95	3.90	3.80	0.09	0.85
Taurine	0.02	0.01	0.02	0.02	0.02	0.01	0.01	0.48
Threonine	5.71	5.72	5.66	5.66	5.62	5.58	0.08	0.83
Tryptophan	1.26	1.28	1.25	1.22	1.27	1.19	0.03	0.36
Tyrosine	5.05	5.07	5.08	5.06	5.01	4.89	0.04	0.06
Valine	6.41	6.39	6.33	6.35	6.41	6.55	0.06	0.21

[1] Dietary treatments: 0% = 50 g/d basal diet (- control); 6% BM = 50 g/d basal diet + 2.7 g/d whole bloodmeal (+ control); 3%, 6%, 12% 18% = 50 g/d basal diet + 1.35, 2.70, 5.50 and 8.10 g/d of an equal mix consisting of feed grade crystalline L-Arg, L-Lys, DL-Met, respectively.

[abcd] Means with no superscripts in common within the same row are statistically significant.
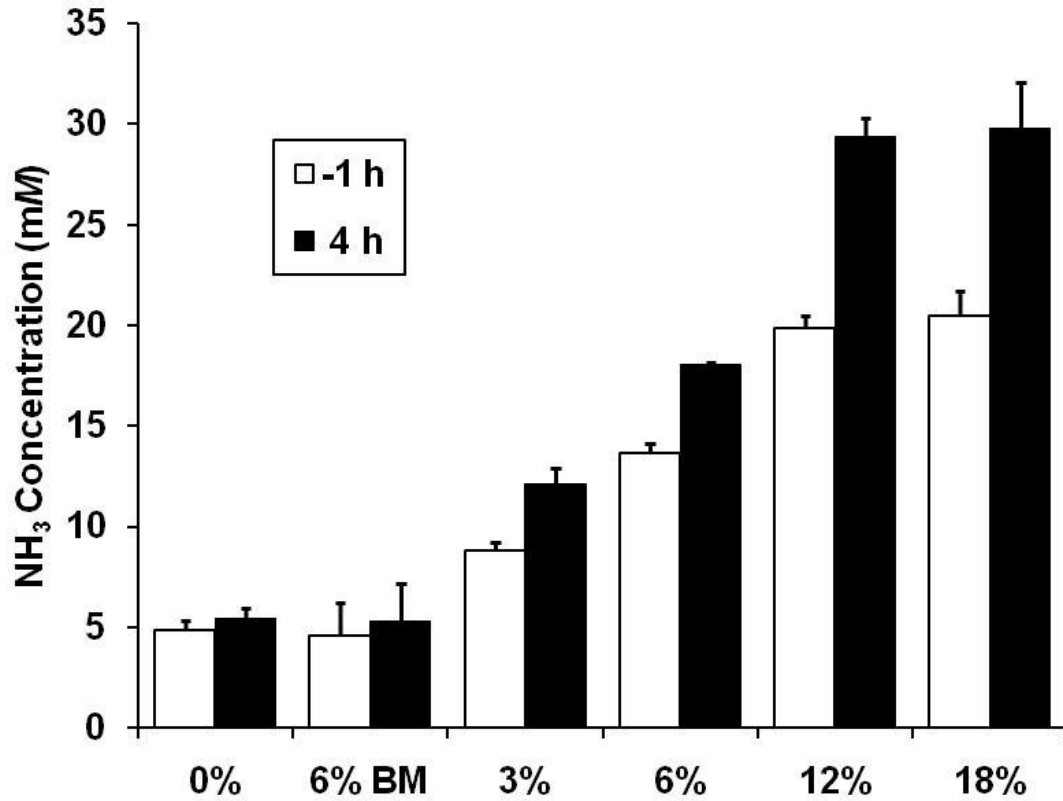
Figure 2.1. Fermenter ammonia concentration taken 1 hour before (□) and 4 hours after (■) feeding when supplementing varying levels of free crystalline amino acids. Dietary treatments: 0% = 50 g/d basal diet (- control); 6% BM = 50 g/d basal diet + 2.7 g/d whole bloodmeal (+ control); 3%, 6%, 12% 18% = 50 g/d basal diet + 1.35, 2.70, 5.50 and 8.10 g/d of an equal mix consisting of feed grade crystalline L-Arg, L-Lys, DL-Met, respectively.

CHAPTER 3

DETERMINATION OF *IN VITRO* PROTEIN (RDP), STARCH AND NEUTRAL DETERGENT FIBER DEGRADATION RATES TO PREDICT RUMINAL PROTEIN AND CARBOHYDRATE AVAILABILITY.

ABSTRACT

To better estimate energy and protein provided to the rumen, it is necessary to know rates at which protein, starch and NDF degrade. Properly formulating diets for protein and energy prevents imbalances which cause acidosis or reduced microbial yield. For protein degradation, two batch culture experiments were performed measuring degradation rates of bloodmeal (BM-A, B, C), dried distiller's grains (DDG), dried distiller's grains with solubles (DDGS), casein (C), soybean meal (SBM-A,B), and corn gluten feed (CGF) using dairy cow rumen fluid. Ammonia release was measured to determine rate of nitrogen degradation (k_d) over a time course of 0 to 48 h. A third batch culture experiment compared degradation rates using a similar method to determine k_d of ruminally degradable starch and NDF of ground corn (GC), corn bran (CB), corn starch (CS), DDG, soy hulls (SH), and ground alfalfa (AL) to predict available energy release in the rumen. Carbohydrate degradation was determined at 0, 4, 8, 12, 16, 24, 36, & 48 h for each feed by measuring total starch and NDF of undigested feed separated via differential centrifugation (1,000 x g, 15 min) and dried at 55°C. Nutrient mass at each time point was calculated and broken-line regression analysis was used to determine time when degradation progressed no further (extent). This time was set as 0% potentially

degradable nutrient remaining and zero-hour was set as 100% potentially degradable nutrient remaining with all other values calculated as a percentage thereof. Data were analyzed to determine homogeneity of slope with the means adjusted to time as a covariate. Pair-wise comparisons for similarity were then done for each feed using a significance level of $P < 0.01$. Slope of the data was indicative of k_d of each nutrient. All R^2 values were > 0.80, and because these data were evaluated as percentages and were corrected for extent of degradation, all intercepts values were similar to 1.0. Protein k_d for BM-A, BM-B, BM-C, C, CGF, DDG, DDGS, SBM-A and SBM-B were 3.56, 2.59, 2.16, 2.25, 2.80, 2.01, 2.39, 3.83 and 2.81, respectively. Starch k_d for AL, CB, CS, DDG, GC and SH were 5.01, 7.33, 4.80, 5.51, 1.94 and 4.14% h^{-1}, respectively. NDF k_d for AL, CB, CS, DDG, GC and SH were 2.38, 2.61, 2.16, 3.36, 4.03 and 2.96% h^{-1}, respectively. Individual differences in nutrient k_d exist between several feedstuffs; however, variation within nutrient are small enough to conclude nutrient degradation is likely physiologically similar, allowing for mean degradation rate to be used in calculating nutrient availability in the rumen.

Keywords: Degradation Rate, NDF, Protein, RDP, Ruminant, Starch

INTRODUCTION

The ruminant animal's symbiotic relationship with its rumen microbial population requires diets to be formulated to meet requirements for both the microbes and the animal. These microbes ferment the carbohydrates in the feed to generate the ATP needed for maintenance and growth. The end products of fermentation come in the form of short-chain fatty acids, which are absorbed across the rumen wall to be used as an energy substrate for the animal. The animal also depends on the higher quality microbial protein leaving the rumen, which the animal digests and uses as amino acids for its own growth and maintenance. The bacteria depend on nitrogen entering the rumen to synthesize amino acids. Non-structural carbohydrate (NSC) fermenting bacteria mainly rely on peptides and free amino acids (AA) for their main source of nitrogen, while structural carbohydrate (SC) fermenting bacteria rely mostly on ammonia (NH_3) for their nitrogen (Russell et al., 1992). Optimizing fermentation yield (microbial growth) and maximizing efficiency of energy capture in the rumen is dependent upon balancing starch and neutral detergent fiber (NDF) degradation with supply of degradable protein and nitrogen. If there is inadequate supply of nitrogen to the rumen, microbial growth suffers as does amino acid supply to the animal. Therefore, the balance of RDP and microbial nitrogen requirement needs to be maintained close to zero. To do this, the amount of RDP and fermentable carbohydrate being fed and their rate of release into soluble fraction of the rumen contents needs to be accurately predicted. The aim of this study was to determine degradation rates (k_d) of protein, starch and NDF in various feedstuffs for use in formulating diets balanced in fermentable energy and protein to maximize microbial growth. We hypothesized rates of protein, starch and NDF degradation were similar

regardless of feedstuff when looking at the potentially degradable fraction of each nutrient.

MATERIALS AND METHODS

In Vitro Feed Degradation

Three separate batch culture experiments were performed at different times for this study; however, all feedstuffs were collected from the University of Missouri feed mill. All samples were dried at 55°C for 24 h and ground through a Wiley mill (Arthur H. Thomas Company, Philadelphia, PA) to pass through a 2-mm screen. All samples were then analyzed for 100% dry matter at 105°C for 24 h and for total N by combustion analysis (LECO FP-428; LECO Corporation, St. Joseph, MI).

The use of animals in this experiment was approved by the University of Missouri Animal Care and Use Committee. For all three experiments, rumen fluid was obtained from two ruminally fistulated multiparous lactating Holstein cows provided *ad libitum* access to a lactation diet (24.5% corn silage, 12.6% alfalfa hay, 15.3% alfalfa haylage, 47.7% concentrate and 19% CP, 24% ADF and 41% NDF) formulated to meet nutritional requirements (NRC, 2001), and housed in free-stall facilities at the University of Missouri-Columbia Foremost Dairy Research Center. Rumen fluid was transported from the farm to the lab (estimated travel time 10 min.), strained through four layers of cheese cloth and diluted with McDougall's buffer (McDougall, 1948) in a 1:4 dilution of rumen fluid to buffer.

Protein Degradation 1

The first experiment evaluated protein degradation in two sources of bloodmeal (BM-B, Porcine Spray-dried Bloodmeal (Missouri Farmers Association (MFA) Inc., Columbia, MO); BM-C, Porcine Flash-dried Red Blood Cells (Hormel Foods Corporation, Austin, MN)), dried distillers grains with solubles (DDGS), and soybean meal (SBM-A). Three grams of each feed were weighed, in triplicate, into 250 mL flasks, which were inoculated with 150 mL of fluid, flushed with CO_2 and closed with a rubber stopper containing a one-valve for to allow for escaping gases. Flasks were placed in a 39˚C shaking water bath and samples were taken at 0, 4, 8, 12, 16, 20, 28, 36, and 44 h via syringe and stored at -20˚C for analysis of ammonia (NH_3) concentration.

Protein Degradation 2

A second experiment evaluated protein degradation in another bloodmeal source (BM-A, Porcine Spray-dried Blood Cells AP 301G), casein (C), corn gluten feed (CGF), dried distillers grains (DDG), and soybean meal (SBM-B). This experiment was conducted as described previously with modifications. Half a gram (0.5 g) of feed was digested in a 50 mL tube containing 25 mL of inoculum. Measurements were conducted in triplicate at every hour point for each feed with tubes sacrificed at 0, 4, 8, 12, 16, 24, 36, and 48 h and stored at -20˚C for analysis of ammonia (NH_3) concentration.

Carbohydrate Degradation

The third experiment evaluated starch and NDF degradation in ground corn (GC), corn bran (CB), gelatinized corn starch (CS), dried distiller's grains (DDG), soy hulls (SH), and ground alfalfa (AL). This experiment used the method previously described in experiment one with the exception that samples were done in triplicate for every hour

point for each feed with flasks sacrificed at 0, 4, 8, 12, 16, 24, 36, and 48 h. Flasks were placed in a cooler at 4°C 10-30 min to stop fermentation. Undigested feed was immediately separated via differential centrifugation (1,000 x g, 15 min at 4°C), dried at 55°C for 2 days, and weighed.

Lab Analysis

Sample ammonia concentration (m*M*) was determined colorimetrically (DU-65 spectrophotometer; Beckman, Palo Alto, CA) with the hypochlorite-phenol procedure of Broderick and Kang (1980). Nitrogen mass was calculated using the concentrations obtained from the ammonia analysis. Nitrogen appearance was used as an indirect measurement of the protein degradation in the feed.

Total starch was determined using the Megazyme – Total Starch Assay Procedure (Megazyme International Ireland Ltd, Bray, Ireland) and NDF was determined with an ANKOM^{200} Fiber Analyzer (ANKOM Technology, Macedon, NY).

Statistical Analysis

All statistical analyses were performed using SAS$^{®}$ version 9.2 (SAS Institute Inc., Cary, NC). For each experiment flask/tube was used as the experimental unit. Broken-line regression analysis using Proc NLIN as described by Robbins et al. (2006) was used to determine at which point each feed reached extent of degradation (no further change in mass) for protein, starch or NDF. The resultant sampling time was set as 0% potentially degradable nutrient remaining and the amount measured at zero-hour was set as 100% potentially degradable nutrient remaining, and all other times preceding this were recalculated as a percentage of degraded nutrient. Percentages were analyzed using Proc GLM analysis of covariance (ANCOVA) to determine the homogeneity of slope

with the means adjusted to time as a covariate. Pair-wise comparisons analyzed between each feed within each nutrient of interest using a significance level of $P < 0.01$. Slope of the data was indicative of degradation rate over time. All R^2 values were ≥ 0.80, and because these data were evaluated as percentages and were corrected for extent of degradation, all intercepts values were similar to 1.0.

RESULTS

Protein Degradation Rates

Protein degradation rate was determined as slope of remaining potentially degradable RDP over time. The R^2 values for all regressions were greater than 0.90 with the exception of DDGS (0.83). The RDP k_d ranged from 0.0201 to 0.0383 h^{-1} with the average rate of protein degradation for all feeds being 0.0271 h^{-1}. Results of pair-wise comparisons between feeds can be found in Table 3.1. The fastest rate of degradation was measured in SBM-A, which was not different ($P = 0.42$) to only BM-A. The slowest rates of degradation were found in DDG and BM-C, which were not different ($P \geq 0.14$) than C. All three blood meal sources had different ($P < 0.01$) rates of degradation. Although both rates measured in soybean meal were on the greater range of all rates measured, they were different ($P < 0.01$). Contrast estimates between animal (BM-A, BM-B, BM-C, and C) and plant (CGF, DDG, DDGS, SBM-A, and SBM-B) based protein sources found no differences ($P = 0.23$). Further contrast estimates between experiment 1 and experiment 2 found no differences ($P = 0.57$). Results from a separate *in situ* degradation study done in our lab measured protein digestion directly found comparable rates of degradation for DDG (0.0208 h^{-1}) and soybean meal (0.244 h^{-1}; M.A. Brooks, unpublished data).

NDF Degradation Rates

Structural carbohydrate degradation rate was determined as slope of remaining potentially degradable NDF over time. The R^2 values for all regressions were greater than 0.86. The rumen degradable NDF k_d ranged from 0.0216 to 0.0403 h^{-1} with the average rate of NDF degradation for all feeds being 0.0292 h^{-1}. Results of pair-wise comparisons between feeds can be found in Table 2. The fastest rate of degradation was measured in GC, which was not different ($P = 0.16$) to DDG. Rate of NDF degradation of DDG was also not different ($P \geq 0.05$) than CB and SH. The slowest rates of degradation were found in CS and AL, which were not different ($P \geq 0.09$) than CB. Rate of degradation of CB was not different than all other feeds ($P \geq 0.05$) except GC ($P < 0.01$).

Starch Degradation Rates

Starch degradation rate was determined as slope of remaining potentially degradable starch over time. The R^2 values for all regressions were greater than 0.80. The rumen degradable starch k_d ranged from 0.0194 to 0.0733 h^{-1} with the average rate of starch degradation for all feeds being 0.0479 h^{-1}. Results of pair-wise comparisons between feeds can be found in Table 3.2. The fastest rate of degradation was measured in CB, which was not different ($P > 0.01$) to only DDG. Rate of starch degradation of AL, CS, DDG and SH were not different ($P > 0.01$). Rate of starch degradation in GC was slower than all other feeds ($P < 0.01$).

DISCUSSION

Ruminant animal nutrition has progressed to the point of evaluating protein based on the amount of rumen degradable protein (RDP) and rumen undegradable protein

(RUP) provided by a feedstuff. This method of fractioning protein based on rumen availability has been adopted by both the dairy and beef industries to help optimize performance and growth of animals (NRC, 2000, 2001).

Measuring protein degradation is complicated by the separation of microbial mass adhering to feed particles. Incomplete removal of microbes from feed particles can artificially decrease true protein degradation. Whereas, measuring ammonia as an indicator of nitrogen degradation provides a much less complex method for determine nitrogen release. However, this method comes with complications in that as protein is broken down to peptides, amino acids and ammonia, microbes utilize these products for continued growth and maintenance (Russell et al., 1992). Other *in vitro* studies have added inhibitors of microbial nitrogen uptake to measure total nitrogen release from proteolysis by the microbes (Broderick, 1978); however, these studies limit microbial growth resulting end-product inhibition, thus they are only recommended for studies consisting of less than four hours (Broderick, 1987; Broderick et al., 2004a). This restriction prevents higher protein feeds from reaching extent, and could prevent accurate rate measurements.

Measuring starch and NDF degradation directly does not have the same difficulties as protein due to the low NDF and starch amounts in microbial mass. Considering the many varied fates of carbohydrate degradation (VFA, CO_2, methane, etc.) in a mixed rumen culture, this direct method is the most ideal for determination of carbohydrate degradation.

In this study, feedstuffs were digested in mixed rumen bacterial culture so protein, starch and NDF degradation were taken to extent, meaning the residence time in the

rumen fluid was long enough to allow for the complete degradation of each nutrient. By measuring this disappearance over time, it was determined at which point this extent was reached. Feeds with lesser amounts of each nutrient reach extent faster than those with greater amounts of each nutrient, thus confounding calculated rates of nutrient degradation between feedstuffs. When these data were corrected for the amount of each potential rumen degradable nutrient, the degradation curves became linear and the plotted slopes became similar. These similar slopes suggest the particular feedstuff is immaterial to the rate of degradation, which is in opposition to the current assumptions (Broderick and Clayton, 1992; Herrera-Saldana and Huber, 1989; Russell et al., 1992; Sniffen et al., 1992). This means, when formulating diets for ruminants, the protein or carbohydrate source is not as important as balancing the diet for rumen degradable portions of protein or carbohydrate.

Previous studies have reported protein degradation rates ranging from 0.015 to 0.355 h^{-1} depending on the method and length of time employed for digestion (Batajoo and Shaver, 1998; Broderick et al., 2004b). These studies measured protein degradation as disappearance of total dietary protein over time, although total degradability is reported, these data still require measurement of individual rates for each feedstuff. Our study found several differences between RDP degradation rates between individual feedstuffs and within individual feedstuffs. However, range of protein degradation rates is likely physiologically similar enough as to allow the mean of the data 0.027 RDP h^{-1}) to be used as a predictive rate of ruminal protein degradation.

Similar to findings for protein degradation, starch and NDF degradation rates have previously shown to vary between feeds (Cerneau and Michalet-Doreau, 1991;

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Hoffman et al., 1993). Cerneau and Michalet-Doreau (1991) also demonstrated differences in degradability and rate occur within feedstuffs when particle size differs. In the current study, particle size within feedstuff was kept similar, but theoretically this only increases surface area and increases the potentially degradable fraction of the feedstuff by decreasing any physical barriers preventing microbial access to starch and would therefore not affect the rate of digestion.

Starch degradation rate measured in several feeds ranged from 0.06 to 0.27 h^{-1} (Batajoo and Shaver, 1998; Herrera-Saldana and Huber, 1989), while NDF degradation was measured in several grasses and legumes of different maturities and found to have degradation rates ranging from 0.02 to 0.27 h^{-1} of rumen degradable NDF (Hoffman et al., 1993; Varga and Hoover, 1983). Differences in rates found by Hoffman et al. are attributed to the wide range of NDF values found in the species of plant measured. The method employed in this study, is designed to adjust for these variations to look only at disappearance of percentage of degradable NDF as determined by taking degradation to extent. Further, Kotarski et al. (1992) believe differences in starch degradation rates may be due to protein association in the endosperm matrix. However, this would mean less starch truly available and apparent rate of degradation would artificially increase due to less available degradable starch. Data obtained in this study would conclude such associations would cause rumen available starch to reach extent faster, but percent of disappearance should remain constant. Differences seen in rates of starch and NDF degradation in ground corn are as of yet unclear. Possibly, as suggested by Hoffman et al. (1993), different rates of degradation do exist between feeds with very high levels or very low levels of a nutrient in that ground corn consists of very little NDF and very high

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levels of starch. If this theory holds true, the question is raised as to why gelatinized corn starch did not show the same type of difference in degradation rate. Perhaps gelatinization removes any inhibitions due to protein complexing, as suggested by Kotarski et al. (1992), which would hold with data presented by Varga and Hoover (1983), who found rates of NDF degradation were similar when feeds were classified by level of protein. Aside from values measured in ground corn, starch and NDF degradation rates appear to be physiologically similar enough to use a mean degradation rate for the calculation of availability of energy for microbial growth.

Determining the rumen degradable portion of these nutrients is essential for proper calculation of the energy available in a feed for degradation by the microbes. Energy in excess of degradable protein leads to energy spilling and methane production along with greater short-chain fatty acid production (Russell et al., 1992). The increase in volatile fatty acids and lactic acid leads to a decrease in ruminal pH and increased risk of subacute to acute ruminal acidosis, resulting in a keratinized rumen and possible death of the animal (Owens et al., 1998). Conversely, excess peptides and nitrogen in the rumen cause a rapid increase in branch-chain fatty acids and NH_3 (Pugh, 2007; Venable, 2004). This increase in rumen NH_3 concentration increases absorption across the rumen wall increasing blood urea nitrogen (BUN). Increased BUN can decrease uterine pH (Butler, 1998) thus increasing days to first ovulation and decreasing overall conception rates (Tamminga, 2006).

Further, knowledge of rumen passage rate is required to determine extent of fermentation of a feed before it leaves the rumen. Currently, ruminal passage rate can range from 0.02 to 0.08 h^{-1}, and is assumed to be slower for forage based diets when

compared to grain based diets (Meng et al., 1999). With current degradation rates, protein and energy, in the form of starch or NDF released over the total residence time in the rumen, can be calculated, and these data can be matched with microbial protein requirement to optimize the ratio of available protein to energy thus maximizing microbial efficiency and reducing negative effects resulting from an energy imbalance.

CONCLUSION

Although individual differences were seen between ruminal degradation rates of starch, NDF and protein in the feedstuffs examined in this study, the variation of degradation rates within nutrient between feedstuffs was small enough to warrant the use of a single rate of degradation for each nutrient when calculating rumen availability. When adjusted for passage rate, the resulting predicted values make possible the balancing of available protein and energy in the rumen required by ruminal microbes to maximize growth and efficiency.

Table 3.1. Degradation rates of rumen degradable protein (RDP) of different protein sources evaluated by *in vitro* digestion.

Feed	$k_{d(Pro)}$[1]	Intercept	R^2	Feeds with differing $k_{d(Pro)}$[1] ($P < 0.01$)
Bloodmeal-A (BM-A)[2]	0.0356	1.0857	0.94	BM-B, BM-C, C, DDG, DDGS
Bloodmeal-B (BM-B)[3]	0.0259	1.0865	0.94	BM-A, BM-C, DDG, SBM-A
Bloodmeal-C (BM-C)[4]	0.0216	1.0540	0.97	BM-A, BM-B, CGF, SBM-A, SBM-B
Casein (C)	0.0225	1.0682	0.98	BM-A, CGF, SBM-A, SBM-B
Corn Gluten Feed (CGF)	0.0280	1.0137	0.94	BM-C, C, DDG, SBM-A
Dried Distillers Grains (DDG)	0.0201	1.0886	0.94	BM-A, BM-B, CGF, SBM-A, SBM-B
Dried Distillers Grains w/ Solubles (DDGS)	0.0239	1.1507	0.83	BM-A, SBM-A
Soybean Meal-A (SBM-A)	0.0383	1.0339	0.90	BM-B, BM-C, C, CGF, DDG, DDGS, SBM-A
Soybean Meal-B (SBM-B)	0.0281	1.0421	0.94	BM-C, C, DDG, SBM-A

[1] $k_{d(Pro)}$ = Degradation rate of rumen degradable protein (RDP) = slope of the degradation curve (% h^{-1})

[2] Porcine Spray-dried Blood Cells AP 301G

[3] Porcine Spray-dried Bloodmeal (Missouri Farmers Association (MFA) Inc., Columbia, MO)

[4] Porcine Flash-dried Red Blood Cells (Hormel Foods Corporation, Austin, MN)

Table 3.2. Degradation rates of rumen degradable neutral detergent fiber (NDF) and starch of different carbohydrate sources evaluated by *in vitro* digestion.

Feed	$k_{d(NDF)}$ [1]	Intercept	R^2	Feeds with differing $k_{d(NDF)}$ [1] ($P < 0.01$)
Alfalfa (AL)	0.0238	1.0088	0.92	DDG, GC, SH
Corn Bran (CB)	0.0261	1.0127	0.97	GC
Corn Starch - Gelatinized (CS)	0.0216	1.0111	0.86	DDG, GC, SH
Dried Distiller's Grain (DDG)	0.0336	0.9944	0.86	AL, CS
Ground Corn (GC)	0.0403	1.0507	0.91	AL, CB, CS, SH
Soy Hulls (SH)	0.0296	0.9963	0.95	AL, CS, GC

Feed	$k_{d(Starch)}$ [2]	Intercept	R^2	Feeds with differing $k_{d(Starch)}$ [2] ($P < 0.01$)
Alfalfa (AL)	0.0501	1.0508	0.82	CB, GC
Corn Bran (CB)	0.0733	1.0142	0.96	AL, CS, GC, SH
Corn Starch - Gelatinized (CS)	0.0480	1.0102	0.99	CB, GC
Dried Distiller's Grain (DDG)	0.0551	1.0000	0.97	GC
Ground Corn (GC)	0.0194	0.9333	0.94	AL, CB, CS, DDG, SH
Soy Hulls (SH)	0.0414	0.9168	0.80	CB, GC

[1] $k_{d(NDF)}$ = Degradation rate of rumen degradable NDF = slope of the degradation curve (% h^{-1})

[2] $k_{d(Starch)}$ = Degradation rate of rumen degradable starch = slope of the degradation curve (% h^{-1})

RUMEN DEGRADABLE PROTEIN (RDP) AND RUMEN DEGRADABLE NITROGEN (RDN) EFFECTS ON MICROBIAL EFFICIENCY IN CONTINUOUS CULTURE AND GROWTH IN CROSSBRED ANGUS STEERS

ABSTRACT

This study was conducted to look at the impact of varying levels of rumen degradable peptide (RDPep) relative to calculated requirement on fermentation characteristics and growth parameters. Initially, a continuous culture experiment was conducted with diets formulated for 60%, 70%, 80% and 100% of required RDPep with -0.2% (% DM) rumen degradable nitrogen (RDN) deficiency and 60% and 100% RDPep with -0.5% RDN deficiency. Twenty-four single-flow fermenters ($n = 6$), were inoculated with rumen fluid obtained from a fistulated Holstein and maintained anaerobically at 39°C with a 0.06 h^{-1} dilution rate. Inadequate RDN decreased organic matter (OM) digestion and post-ruminal microbial N flow while increasing rumen undegradable nitrogen (RUN; $P < 0.01$). Microbial efficiency (MOEFF) was lesser in diets with -0.5% RDN deficiency (3.73 to 11.20 g microbial N/kg OM truly fermented, 60% and 100%, respectively) and greatest when RDPep was less than 100% (~16 to 17 g microbial N/kg OM truly fermented; $P < 0.01$). Total VFA concentrations decreased with -0.5% RDN while increasing levels of lactate were measured ($P < 0.01$). The second experiment was a growth study consisting of four diets with varying levels of RDPep (115, 96, 86, 72 % of required RDPep), all with similar levels of RDN (~ -0.7% RDN, DM basis). Lower

levels of RDN were used in this study to allow for N recycling. Forty-nine yearling crossbred Angus steers (IBW ~ 370 kg), were divided into treatment groups ($n = 12$, with extra steer in 86% RDPep). Each treatment was dividing into three pens with 4 animals per pen. Animals were maintained on treatment for 70 days with pen intake recorded daily and animal weights taken on days 21 and 42 followed by 2-day consecutive weights taken on day 70 and 71 to determine FBW. FBW decreased linearly with decreasing RDPep ($P = 0.05$). Dry matter intake did not differ among treatments (7.77 to 8.51 kg/hd/d; $P = 0.09$). Average daily gain (ADG) did not differ among treatments during the first 42 d ($P > 0.20$). Overall ADG and feed efficiency (G:F) displayed a quadratic effect with greater values occurring with the most and least amounts of RDPep provided (ADG: 2.03 and 1.77 kg/hd/d, G:F: 0.24 and 0.22, respectively; $P = 0.02$). Calculated bacterial metabolizable amino acid (Met AA) passage to the small intestine decreased linearly with decreasing RDPep ($P = 0.01$), while rumen undegradable dietary Met AA did not differ with treatment ($P > 0.01$).

Keywords: Continuous Culture, Peptide, Microbial Efficiency, Ruminant, Nitrogen

INTRODUCTION

Inadequate supply of RDP relative to soluble carbohydrate results in negative associative effects on fiber digestion and lead to energy spilling (Klevesahl et al., 2003; Van Kessel and Russell, 1996). However, when RDP is increased beyond relative levels of readily digestible carbohydrate, ammonia production begins to increase at a rapid rate, which could be detrimental to the animal (Klevesahl et al., 2003). Proper balance of the two fractions is needed to increase microbial growth and yield. If nitrogen is limited, decreased microbial output is likely an effect of a decrease in microbial efficiency (MOEFF; Hoover and Stokes, 1991).

The beef NRC (2000) recommends approximately 60 to 65% of dietary CP be formulated as RDP, and of that approximately 50% needs to be soluble protein. Russell et al. (1992) report RDP provided to a mixed microbial population in the rumen needs to provide approximately 67% of the nitrogen as peptides and amino acids and 33% of the nitrogen as NH_3 to account for nitrogen usage by non-structural carbohydrate (NSC) and structural carbohydrate (SC) fermenting bacteria, respectively. Our lab has previously shown RDP levels recommended for ruminants are higher than required to achieve maximal microbial yield (Fu et al., 2001).

Other studies looking at the utilization of non-protein nitrogen (NPN) versus peptides and amino acid as nitrogen sources have shown increased microbial efficiency when feeding supplemental NPN (Garrett et al., 1987). Further, work by Griswold et al. (2003) demonstrated peptides and amino acids alone, even at high levels, do not degrade enough to maximize microbial efficiency and meet NH_3-N requirements of SC fermenting bacteria.

We propose, when formulating diets, the RDP fraction should be broken down into both the peptide and NH_3-N fractions based on estimated microbial efficiencies to better estimate microbial needs and increase efficiency of nitrogen usage. This study was conducted to look at the impact on fermentation characteristics and growth parameters when levels of rumen degradable peptide (RDPep) relative to calculated requirement vary, while maintaining constant levels of rumen degradable nitrogen.

MATERIALS AND METHODS

RDP Continuous Culture

Rumen degradable protein, peptide and nitrogen requirements were determined by balancing available rumen degradable carbohydrate (energy) to available rumen degradable protein and non-protein nitrogen. Rumen available energy, protein and non-protein nitrogen were calculated hourly using the partial degradation rate (k_d) of each rumen available nutrient (CP, NDF, NSC) along with an estimated solids passage rate (k_p) of 4% h^{-1} and the grams nutrient remaining obtained from the previous hour.

For formulation purposes, RDPep and RDN requirements were calculated as outlined by Russell et al. (1992) using microbial efficiency (MOEFF) estimated using calculations based of work by Meng et al. (1999). Balance of RDP and RDN were calculated by subtracting RDP or RDN provided minus RDP or RDN required, respectively.

Dietary treatments (Table 4.1) were composed of differing levels of corn, soybean meal, and urea. Corn and soybean meal were ground to pass through 3-mm screen in a Wiley mill (Arthur H. Thomas Company, Philadelphia, PA) and urea was ground using a mortar and pestle. Diets 1 was a strictly corn diet consisting of approximately 60% of required RDPep and lacking approximately 0.5 RDN (% DM). Diet 2 was formulated to match the RDPep level of diet 1 and increase RDN balance to -0.2 % DM. Diets 3 to 5 were formulated with increasing levels of rumen degradable peptide (70%, 85%, 100% RDPep required, respectively) while maintaining RDN balance at -0.2 % DM, and diet 6 was formulated to match RDPep value of diet 4 and RDN balance value of diet 1.

The use of animals in this experiment was approved by the University of Missouri Animal Care and Use Committee. Rumen fluid was obtained from a ruminally fistulated multiparous lactating Holstein cow provided *ad libitum* access to a lactation diet (25.2% corn silage, 21.0% ground corn, 13.1% alfalfa haylage, 10.5% vit/min mix, 9.3% alfalfa hay, 7.5% AminoPlus®, 3.8% soybean hulls, 3.7% whole cottonseed, 3.2% soybean meal, 2.8% wet brewer's grain; and 17.0% CP, and 9.0% RDP and 30.9% NDF) formulated to meet nutritional requirements (NRC, 2001), and housed in free-stall facilities at the University of Missouri-Columbia Foremost Dairy Research Center. Rumen fluid was transported from the farm to the lab (estimated travel time 10 min.), strained through four layers of cheese cloth and diluted with McDougall's artificial saliva in a 1:4 dilution of rumen fluid to buffer.

Twenty-four single-flow effluent continuous culture fermenter polycarbonate vessels (Nalgene, Rochester, NY) were inoculated and maintained as described by Meng et al. (1999). Inoculum was added to each fermenter up to the effluent overflow port

(approximately 1460 mL). Fermenters were continuously flushed with CO_2 gas, stirred with magnetic stir plates, and immersed in a water bath maintained at 39°C using thermostatically controlled heaters (model 730, Fisher Scientific, Pittsburgh, PA). High buffer capacity solution modified by Slyter (1990) from McDougall's artificial saliva (McDougall, 1948), containing 107.5 mg urea-N/L and 250 mg cysteine-HCL/L was continuously infused into fermenters using peristaltic pumps (Masterflex model 7520-10, Cole Parmer Instrument Co., Chicago, IL). Fermenter dilution rates were held constant at $6\% \pm 0.2\% \cdot h^{-1}$ for all treatments. Effluent flowed into collection vessels immersed in ice-cooled water. Fermenters were randomly assigned to one of the six treatments ($n = 4$) and fed half the daily ration of 50 g/d at 12 h intervals. The experiment was conducted over a 7-day period, with 4 days adaptation and 3 days sampling.

Sampling

One liter subsamples were taken from effluent collected over the previous 24 h and stored at 4˚C. These samples were composited for each fermenter over the three day period for later analysis. On the last day of sampling, fermenter contents were collected and stored at 4˚C until analyzed. Further, between 1 hour before feeding until 5 hours after feeding hourly pH readings and 2 mL samples were taken directly from the fermenter and immediately frozen at -20˚C. These samples were later composited by hour for each fermenter over the three day period and analyzed for ammonia for all hours and for volatile fatty acid (VFA) at -1 h and 4 h.

Lab Analysis

Fermenter samples were blended for 30 seconds to detach microbes from feed particles then centrifuged at 1000 x *g* for 5 min at 4°C to remove feed particles. Feed

particles were air-dried in a 55° drying oven for 3 days to determine dry weight, which was used with overall intake to determine solids dilution rate (SDR, % h^{-1}). Supernatant was re-centrifuged at 27000 x g for 30 min. The resultant pellet was washed once using 0.9% (wt/vol) saline solution then again using deionized distilled H_2O. The final pellet, containing bacteria, was transferred to plastic cups using deionized distilled water, lyophilized at 10°C (Genesis, Virtis, Gardiner, NY), and ground using a mortar and pestle. Subsamples of effluent (500 mL) were lyophilized at 10°C (Genesis, Virtis, Gardiner, NY), and ground using a mortar and pestle.

For analysis, diets were ground through with a Wiley mill to pass through a 1-mm screen. Samples of diet, effluent and fermenter contents were analyzed for dry matter (DM) by drying at 105°C for 24 h, organic matter (OM) by incineration at 500°C and total N by combustion analysis (LECO FP-428; LECO Corporation, St. Joseph, MI). Samples of effluent and fermenter pellets were analyzed for purine content using the procedure of Zinn and Owens (1986) to determine microbial N, which was used with OM digested to determine microbial efficiency (MOEFF; g microbial N/kg OM truly digested). Sample ammonia concentration (mM) was determined colorimetrically (DU-65 spectrophotometer; Beckman, Palo Alto, CA) with the hypochlorite-phenol procedure of Broderick and Kang (1980). Sample VFA and lactate concentration (mM) was determined using gas chromatography (Model 3400, Varian, Palo Alto, CA) following procedures outlined by Salanitro and Muirhead (1975).

RDP In Vivo Growth Trial

The use of animals in this experiment was approved by the University of Missouri Animal Care and Use Committee. Forty-nine fall-weaned yearling crossbred Angus

steers were obtained for this study. Steers were placed in one of 12 concrete pens and offered *ad libitum* access to water and a receiving diet for 10 d before the feeding period to allow for acclimation to the high concentrate diet. At the start of the experiment, steers 2-day consecutive weights were taken determine initial body weight (IBW). Weights were ordered from smallest to largest and treatments A to D were randomly assigned to every set of four cattle, producing similar average IBW across treatments. The same procedure was repeated within treatment assigning pen numbers 1 to 3 to produce similar average IBW across pens (~370 kg), resulting in 4 treatments with 3 pens per treatment with 4 animals per pen, with the exception of 1 pen with 5 cattle in treatment C.

Diet formulation was as described previously in the RDP continuous culture section. Dietary treatments A, B, C, and D (Table 4.3) consisted of decreasing levels of RDPep (115, 96, 86, and 72 % of required RDPep, respectively) with similar levels of RDN (~ -0.7% RDN, DM basis). All feed ingredients, except corn, were mixed and bagged loose and mixed with the corn as a supplement to be fed once daily at approximately 0900 h. Pen intake was recorded daily, and animal weights were taken on days 21 and 42 to determine production traits for the feeding period, followed by 2-day consecutive weights taken on day 70 and 71 to determine final body weight (FBW). Crude protein, dietary metabolizable energy and metabolizable amino acid requirements and duodenal supply from bacteria and diet were calculated using NRC (2000) values as modified by Mueller (2004).

Statistical analysis

All statistical analyses were performed using SAS® version 9.2 (SAS Institute Inc., Cary, NC). Ammonia and pH data were analyzed using the MIXED procedure with

repeated measures as outlined by Littell et al. (1998). Statistical significance was determined using $P \leq 0.05$ probability level, and LSD lines were calculated to graphically represent differences.

The remaining *in vitro* continuous culture fermentation characteristics were evaluated by ANOVA using Proc GLM. The data were analyzed as a RCBD using 6 treatments with fermenter as the experimental unit. When the *F*-test was significant ($P \leq 0.05$), means separation was performed using Fisher's LSD.

Growth data from the *in vivo* experiment were analyzed as a completely randomized design with pen as experimental unit by ANOVA using Proc GLM. CONTRAST statements were used to evaluate linear and quadratic relationships of the least-squared means between treatments.

RESULTS

Fermentation Characteristics

Estimated SDR for this experiment of 4% h^{-1} was confirmed in the continuous culture (Table 4.1). Although diet 1 SDR was slower than diets 2, 4 and 5 ($P = 0.02$); differences were likely not physiologically different. Actual MOEFF measured in fermenters was lesser when RDN was most lacking (diets 1 and 6) and greatest when RDPep was less than 100 % (diets 2, 3, and 4; $P < 0.01$). Intuitively, grams of bacterial N and percent organic matter digested displayed similar patterns as MOEFF ($P < 0.01$). Percent RUN (Figure 4.1) was lesser in diets 2, 3 and 4 and greatest in diet 1. Ammonia concentrations did not change over time for diets 1 and 6 ($P < 0.05$), but concentrations for diets 2, 3, 4 and 5 quickly rose at feeding and decreased to -1 h levels by 5 h after

feeding. Concentrations for diets 2 and 3 were consistently higher than all other diets followed by diet 4 and then diet 5. Fermenter pH (Figure 4.2) was least for diet 2 followed by diets 3 and 4; however, pH remained similarly greater and did not change for diets 1, 5, or 6 during sampling ($P < 0.05$).

Short-Chain Fatty Acids

Insufficient RDN decreased total VFA production (Table 4.2) both before and after feeding ($P < 0.01$), and after RDN was increased, additional RDPep in the diet increased total VFA production after feeding ($P < 0.01$). Before feeding, lactate concentration was least when both RDPep and RDN were most lacking ($P < 0.01$). Increasing RDPep and keeping RDN lower decreased lactate concentration; however, increasing RDN in the diet had the greatest effect of decreasing lactate levels with diets 2 to 5 being similarly lesser. Although lactate levels increased after feeding in all fermenters, patterns in lactate concentration remained similar to those seen before feeding ($P < 0.01$). Molar proportions of acetate were greater when RDN was most limiting before and after feeding ($P < 0.01$) and decreased as RDPep was added to the diets. Conversely, molar proportions of propionate were least when RDN was most limiting, but only increased with addition of RDPep after feeding ($P < 0.01$). This led to higher acetate:propionate ratios in diets most limiting in RDN before and after feeding. Ratios were not different between treatments with higher RDN levels before feeding but decreased after feeding as RDPep in the diet increased ($P < 0.01$). Molar proportions of butyrate were least when RDN was most limiting, and increased when RDPep and RDN were the least limiting both before and after feeding ($P < 0.01$). Isobutyrate levels were nearly undetectable in almost all treatments both before and after feeding; however,

isovalerate was highest when RDP was greatest regardless of RDN status both before and after feeding ($P \leq 0.03$). Valerate proportions in fermenters were lesser when RDN was most limiting but decreased with increasing RDPep when RDN was least limiting ($P < 0.01$).

Growth Parameters

For the growth trial, IBW and DMI (Table 4.4) did not differ between treatments ($P \geq 0.09$). Final body weight decreased linearly with decreasing RDPep ($P = 0.05$). Average daily gain was not different during the first 42 days of the study; however, overall ADG (0 to 70 d) responded quadratically with diets A and D displaying greater ADG. Feed efficiency (gain to feed ratio; G:F) showed a quadratic effect similar to the one seen in overall ADG ($P < 0.01$). Metabolizable energy consumed daily decreased with decreasing levels of RDPep ($P = 0.05$), but ME consumed per kilogram of body weight gained showed a quadratic effect with lesser amounts consumed for diets A and D. Proportion of metabolizable AA supplied to the lower tract by bacteria decreased quadratically with increasing RDPep ($P < 0.01$) while the proportion provided by the diet increased quadratically ($P < 0.01$). Calculated mass of dietary or total (dietary + bacterial) metabolizable AA (Figure 4.3) was not affected by treatment ($P > 0.10$), but bacterial metabolizable AA mass decreased linearly increasing RDPep. Calculated metabolizable AA supply required showed a quadratic effect ($P = 0.02$) with diets A and D requiring greater amounts to produce the growth rates measured for each treatment group.

DISCUSSION

Microbial Fermentation, Growth and Efficiency

Previous *in vivo* research feeding diets with no roughage (Pugh, 2007) to cannulated animals saw no difference in SDR when feeding increasing levels of RDP or feeding NPN. In our study, a difference in SDR between diets was likely not physiologically significant.

Satter and Slyter (1974) and Slyter et al. (1979) recommend a continuous infusion of urea is needed to maintain NH_3-N concentrations of approximately 1.4 to 3.6 mM to achieve maximal microbial growth. Other studies have confirmed the extent of protein degradation to achieve maximal microbial growth is dependent upon the availability of NH_3-N, especially in the presence of NSC and hemicellulose fermenting bacteria which preferentially scavenge peptides and AA before they can be deaminated to NH_3 for use by SC fermenters (Griswold et al., 2003). In our continuous culture experiment studying the effects of RDPep and RDN on ruminal fermentation, urea was only available through the diet. This caused the rapid increase in NH_3 concentration after feeding, which decreased over 5 h after feeding to levels similar to pre-feeding, which is consistent with other work done with dosing soluble N sources (Henning et al., 1993). The increases in NH_3 between diets agree with data obtained by Arroguy et al. (2004) who say linear increases in NH_3 concentration with increasing urea N addition to RDP. In our study, the balance of RDPep and RDN in diets 2 and 3 was able to maintain NH_3-N concentrations within the recommended concentrations reported to maximize growth and efficiency. Griswold et al. (2003) showed separate effects of urea and RDP addition on OM digestibility, microbial N flow and MOEFF. In all parameters, values increased with urea

infusion into the continuous culture system and with increasing the RDP level from 8 to 11% (DM basis). Our study saw an increase in all parameters with increased RDN balance and increasing proportion of RDPep. The lack of RDN in diets 1 caused a drastic decrease in bacterial N production and bacterial efficiency; however by addition of peptide nitrogen alone was able to increase both microbial growth and efficiency as evidenced by diet 6. Yields and efficiency were further increased when nitrogen was added to diets 2, 3, and 4; however, when RDP was formulated at 100% of requirement, microbial efficiency and yield declined. Although this reason for this is not clear at this time, this may be due to a lack of NH_3-N which, as stated earlier, would cause competition for use of protein-N by NSC and SC fermenting bacteria.

When Pugh (2007) fed no-roughage diets with increasing levels of RDP to cannulated beef steers, no differences were seen in ruminal pH (5.61 to 5.74). The no-roughage diets used in this fermentation displayed similar post-feeding pH with lower level of RDPep and higher levels of RDN. When urea is hydrolyzed by urease, the resulting ammonia has the ability to act as a buffer for ruminal acids due to its ability to accept an electron, producing an ammonium ion. This effect is seen with the initial rise in pH between hours 1 and 2 post-feeding; yet, this appears not to be sufficient to counter act pH levels expressed in diets 2, 3 and 4 after 1 h post-feeding. This is similar to results found by Cameron et al. (1991) who fed starch + urea and produced lower pH values than feeding starch or urea separately. Decreasing pH with increasing ammonia supplementation was seen by Zinn et al. (2003); however, they were unable to explain the exact reason for this effect.

The data suggest pH is increased with increasing proportion of RDPep when feeding isonitrogenous diets. Although diets 4 and 5 had greater total VFA production, which is a major effector of ruminal pH (Stone, 2004), these diets maintained pH levels more conducive to a healthy fermentation. Although diets 1 and 6 produced greater concentrations of lactate, decreased total VFA production likely prevented significant pH changes.

Total VFA production was stimulated by addition of N as urea and increasing levels of RDPep in the diet. The imbalance of available RDN (diets 1 and 6) led to a decrease in overall fermentative activity and energy spilling in the form of lactate production. The increasing peptide levels allowed for an increase in NSC bacteria growth leading to greater degradation of available starch in the diets, which produced greater proportions of propionate. Increasing RDPep also produced greater butyrate and isovalerate via fermentative actions of proteolytic bacteria. Zinn et al. (2003) found similar results with butyrate increasing with decreasing levels of urea in the diet. Decreasing RDPep and increasing urea in the diets led to increases in valerate production. When Chalupa et al. (1970) fed purified diets to sheep with either isolated soy protein or urea as the nitrogen source, no differences were found in acetate, propionate, butyrate or valerate production. Arroquy et al. (2004) displayed a quadratic response for acetate production and a linear response in isovalerate production with increasing supplemental RDP from urea with lowest from steers receiving 100% of supplemental RDP from urea, but no differences were found in propionate, butyrate or valerate production.

In Vivo Growth Parameters

Diets in this experiment were kept isonitrogenous and limiting in total nitrogen. The formulated limiting amount of RDN was based on previous growth data in our lab, which showed no further improvement in growth when RDN was increased beyond a balance of -0.69 % DM (Yi et al., unpublished). This is based on the efficiency of nitrogen recycling to the rumen will make up the difference in required NH_3-N (Kiran and Mutsvangwa, 2007; Wickersham et al., 2008a; Wickersham et al., 2008b).

Zinn et al. (2003) saw a linear effect of ADG when feeding increasing levels of urea when fed a similar length of time as our study, but the diets fed were not isonitrogenous and only supplemented additional urea on a control diet. Further they found no difference in feed efficiency of these different diets (0.20 G:F), but amounts of urea in these diets were much lower than the amounts fed in our study. Similar to our study, Pugh (2007) fed steers with increasing RDP and found a quadratic response to ADG. The quadratic effect on ADG from diet A to D resulted in a linear effect and quadratic trend for FBW. Dry matter intake tended to decrease with decreasing RDP leading to a decrease in metabolizable energy intake. This decreasing intake along with the trend in ADG resulted in a quadratic effect of lower metabolizable energy required for gain in diets A and D along with the quadratic effect in G:F. Calculations of AA supply/requirement led us to conclude increasing RDP increased bacterial nitrogen flow post-ruminally, allowing average daily gain to be increased with similar levels of energy consumption. Although not statistically significant, a similar numerical increase of microbial N flow to the abomasum with increasing RDPep and decreasing urea in the diet was measured by Chizzotti et al. (2008).

These data concur with other research concluding diets need to be formulated for both RDPep and NH_3-N to maximize growth and efficiency (Cecava et al., 1991; Fu et al., 2001). To properly do this, the rate of degradation and passage need to be formulated to achieve a balance in rumen availability of energy and protein (Henning et al., 1993; Hoover and Stokes, 1991).

CONCLUSIONS

Our data suggest diets formulated for a balance of both fermentable carbohydrate and RDPep and N are able to increase microbial yield and efficiency. Properly balanced diets prevent energy spilling, which manifests as excess lactate production and decreased ruminal pH. They decrease excess fecal and urinary N by increasing the efficiency of N utilization. This allows for feeding no-roughage diets without the risk of deleterious effects normally seen with diets high in NSC, while maximizing feed efficiency and average daily gain. Our diets balanced RDPep and N decreased lactate production while increasing OM digestibility and MOEFF. These balanced diets improved calculated post-ruminal metabolizable AA flow, which led to increased ADG and feed efficiency.

Table 4.1. Nutrient composition and fermentation characteristics of diets with varying rumen degradable protein fed to continuous culture fermenters.

Item[1] (% DM)	Dietary Treatments						SE	*P*-value
	1	2	3	4	5	6		
Corn	100.00	97.67	96.43	93.96	91.64	95.60		
Soybean Meal	-	-	1.47	4.40	7.15	4.40		
Urea	-	2.33	2.10	1.64	1.21	-		
Nutrient composition								
Dry Matter (%)	86.53	86.40	87.20	87.73	87.46	87.61		
Organic Matter (% DM)	98.59	98.55	98.57	98.36	98.16	98.39		
NDF (% DM)	8.24	6.55	7.65	7.53	7.70	9.01		
ADF (% DM)	1.82	1.35	1.65	1.75	1.71	2.02		
CP (% DM)	6.87	12.76	12.83	12.95	13.07	8.80		
RDP – CP required (%DM)	5.23	5.11	5.05	4.93	4.81	5.01		
RDP – Peptide required (% DM)	3.75	3.67	3.62	3.54	3.45	3.60		
RDN – NH_3-N required (%DM)	0.24	0.23	0.23	0.22	0.22	0.23		
RDP – Peptide supplied (% DM)	2.13	2.08	2.42	2.95	3.50	2.99		
RDP (% RDP Required)	56.85	56.85	66.71	83.57	101.29	83.11		
RDN Balance (% DM)	-0.48	-0.21	-0.20	-0.19	-0.18	-0.48		
Fermentation Parameter								
SDR (% h^{-1})	3.99[b]	4.17[a]	4.09[ab]	4.17[a]	4.10[a]	4.08[ab]	0.03	0.02
MOEFF[2]	3.73[d]	16.21[ab]	15.71[ab]	17.30[a]	14.71[b]	11.20[c]	0.73	<0.01
Bacterial nitrogen (g/d)	0.09[e]	0.59[ab]	0.54[bc]	0.63[a]	0.49[c]	0.33[d]	0.03	<0.01
OM digested (%)	49.15[d]	73.74[a]	69.10[ab]	74.04[a]	67.38[b]	59.75[c]	2.12	<0.01
RUN (% Dietary Nitrogen)	69.38[a]	23.64[cd]	29.63[bcd]	18.27[d]	31.01[bc]	36.85[b]	4.09	<0.01

[1] Item abbreviations: DM = dry matter, CP = crude protein, MOEFF = microbial efficiency, OM = Organic matter, RDN = rumen degradable nitrogen, RDP = rumen degradable protein, RUN = rumen undegradable nitrogen/dietary by-pass nitrogen, SDR = solids dilution rate.

[2] Microbial Efficiency = g effluent bacterial N/ kg OM truly digested

[abcde] Means with no superscripts in common within the same row are statistically significant.

Table 4.2. Volatile fatty acid (VFA) and lactate concentration of continuous culture fermenters fed diets with varying levels of rumen degradable protein.

Fermenter VFA -1 h[2]	Dietary Treatments[1]						SE	*P*-value
	1	2	3	4	5	6		
Total VFA (m*M*)[3]	61.24[c]	88.37[a]	84.62[ab]	94.67[a]	83.62[ab]	73.48[bc]	4.25	<0.01
Lactate (m*M*)	13.76[a]	2.49[c]	2.06[c]	1.28[c]	1.07[c]	8.82[b]	0.71	<0.01
Acetate:Propionate Ratio	2.78[a]	1.43[c]	1.21[c]	1.10[c]	1.12[c]	2.10[b]	0.13	<0.01
	mol/100 mol							
Acetate	67.76[a]	49.34[c]	45.95[cd]	43.53[d]	41.92[d]	60.06[b]	1.66	<0.01
Propionate	24.52[c]	34.80[ab]	38.59[a]	40.46[a]	37.99[a]	29.29[bc]	1.91	<0.01
Isobutyrate	0.00	0.00	0.00	0.00	0.04	0.00	0.02	0.45
Butyrate	6.95[c]	11.06[b]	11.07[b]	12.12[b]	16.99[a]	9.19[bc]	1.25	<0.01
Isovalerate	0.33[c]	0.26[c]	0.40[bc]	0.60[a]	0.57[ab]	0.40[bc]	0.06	<0.01
Valerate	0.44[d]	4.54[a]	3.99[ab]	3.30[ab]	2.49[bc]	1.07[cd]	0.58	<0.01
Fermenter VFA 4 h[2]								
Total VFA (m*M*)[3]	57.85[c]	81.90[ab]	81.97[ab]	93.84[a]	95.50[a]	72.87[bc]	6.77	<0.01
Lactate (m*M*)	15.28[a]	4.05[b]	3.55[b]	2.58[b]	3.05[b]	13.18[a]	0.81	<0.01
Acetate:Propionate Ratio	3.28[a]	1.77[b]	1.57[bc]	1.26[bc]	0.94[c]	2.64[a]	0.23	<0.01
	mol/100 mol							
Acetate	70.96[a]	53.37[c]	51.32[cd]	46.46[d]	39.14[e]	64.22[b]	1.90	<0.01
Propionate	22.06[e]	30.17[cd]	33.16[bc]	37.21[ab]	42.79[a]	25.94[de]	2.18	<0.01
Isobutyrate	0.00	0.00	0.00	0.02	0.00	0.00	0.01	0.45
Butyrate	6.28[c]	11.20[b]	10.63[b]	12.18[ab]	15.32[a]	8.43[bc]	1.29	<0.01
Isovalerate	0.33[bc]	0.22[c]	0.32[bc]	0.50[a]	0.42[ab]	0.39[ab]	0.05	0.03
Valerate	0.38[d]	5.04[a]	4.57[a]	3.63[ab]	2.34[bc]	1.02[cd]	0.62	<0.01

[1] % RDP supplied/required|RDN Balance (% DM); 1 = 60|-0.5, 2 = 60|-0.2, 3 = 70|-0.2, 4 = 85|-0.2, 5 = 100|-0.2, 6 = 85|-0.5.
[2] Samples were taken 1 h before the morning feeding (-1 h) or 4 h after the morning feeding (4 h)
[3] Total VFA = Acetate + Propionate + Isobutyrate+ Butyrate+ Isovalerate + Valerate
[abcde] Means with no superscripts in common within the same row are statistically significant

Table 4.3. Nutrient composition of diets with varying levels of rumen degradable protein used to evaluate growth performance in crossbred Angus steers.

Item[1] (% DM)	Dietary Treatments			
	A	B	C	D
Corn	86.64	88.24	90.05	92.92
SoyPLUS[2]	4.75	7.37	4.00	0.00
Soybean Meal	4.80	0.00	0.00	0.00
Bloodmeal	1.00	1.41	2.40	3.20
Urea	0.75	0.98	1.22	1.48
KCl	0.20	0.26	0.50	0.55
Limestone	1.44	1.38	1.44	1.48
NaCl	0.19	0.16	0.16	0.15
Trace Mineral Premix[3]	0.09	0.09	0.10	0.10
Vitamin Premix[4]	0.14	0.13	0.13	0.13
Nutrient composition				
Dry Matter (%)	84.68	84.21	84.35	83.94
CP (% DM)	13.68	13.85	14.15	14.06
RDP – CP required (%DM)	4.66	4.73	4.84	4.98
RDP – Peptide required (% DM)	3.34	3.39	3.47	3.57
RDN – NH_3-N required (%DM)	0.21	0.21	0.22	0.23
RDP – Peptide supplied (% DM)	3.86	3.26	2.99	2.60
RDP (% RDP Required)	115.45	96.14	86.19	72.78
RDN Balance (% DM)	-0.69	-0.76	-0.65	-0.63
ME (Mcal/kg DM)	3.09	3.08	3.06	3.04

[1] Item abbreviations: DM = dry matter, CP = crude protein, ME = metabolizable energy, RDP = Rumen degradable protein, RDN = rumen degradable nitrogen.

[2] SoyPLUS (West Central®, Ralston, IA) contained CP 49.84% DM, RUP 60% CP, RDP 40% CP.

[3] Contained (dry-matter basis) 3,006,303 IU of vitamin A, 751,576 IU of vitamin D, 75,158 IU of vitamin E, and 8 g Thiamin/kg.

[4] Contained (dry-matter basis) 2.5% Fe, 2% Mn, 3% Zn, 1% Cu, 100 ppm Se, 500 ppm I, and 100 ppm Co.

Table 4.4. Growth performance and metabolizable amino acid composition for crossbred Angus steers fed varying levels of rumen degradable protein.

Item[2]	Dietary Treatments[1]				SE	*P*-values	
	A	B	C	D		Linear	Quadratic
IBW (kg/hd)	367.45	371.00	372.37	366.77	3.73	0.97	0.25
FBW (kg/hd)	509.78	489.14	485.49	490.96	5.92	0.05	0.06
DMI (kg/hd/d)	8.51	8.18	7.77	8.00	0.22	0.09	0.24
ADG (kg/hd/d)							
0 to 21 d	1.03	0.57	0.58	0.83	0.29	0.67	0.26
0 to 42 d	1.59	1.40	1.32	1.43	0.23	0.59	0.52
0 to 70 d	2.03	1.69	1.62	1.77	0.08	0.05	0.02
Feed Efficiency (G:F)	0.24	0.20	0.20	0.22	0.01	0.12	0.01
ME Consumed (Mcal/d)	26.21	25.12	23.67	24.26	0.69	0.05	0.26
ME (Mcal/kg BW)	12.87	14.88	14.79	13.67	0.48	0.31	0.01
Met AA (g AA/100 g AA)							
Bacterial	64.66	63.08	61.68	61.76	<0.01	<0.01	<0.01
Dietary	35.34	36.92	38.33	38.24	<0.01	<0.01	<0.01

[1] Diets A, B, C, D = 115, 95, 85, 70 % RDP supplied/required.
[2] Item abbreviations: AA = amino acid, ADG = average daily gain, DMI = dry matter intake, FBW = final body weight, G:F = gain:feed ratio, IBW = initial body weight, ME = metabolizable energy, Met AA = metabolizable amino acid.
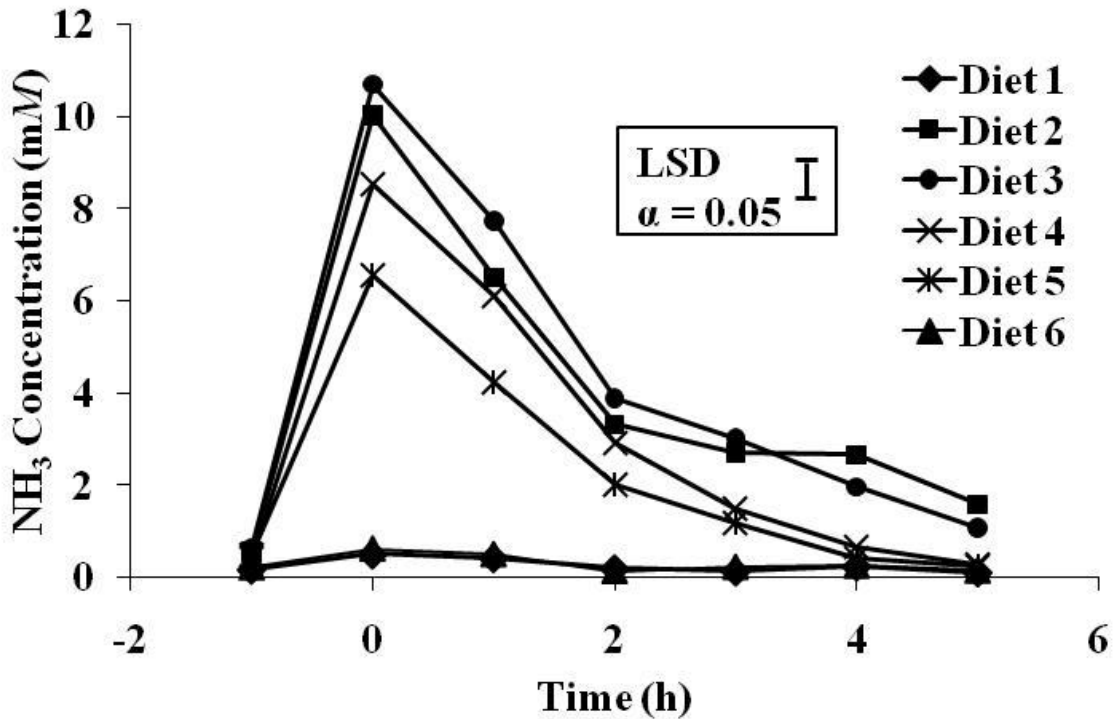
Figure 4.1. Fermenter ammonia (NH_3) concentration measured between 1 hour before (-1 h) and 5 hours after (5 h) feeding from continuous culture fermenters fed diets with varying levels of rumen degradable protein. Dietary treatments are denoted Diet 1 (♦) = 60|-0.5, Diet 2 (■) = 60|-0.2, Diet 3 (●) = 70|-0.2, Diet 4 (✕) = 85|-0.2, Diet 5 (✱) = 100|-0.2, Diet 6 (▲) = 85|-0.5 % RDP supplied/required|RDN Balance (% DM). Urea contribution of feeds (% DM): 1 = 0%, 2 = 2.33%, 3 = 2.10%, 4 = 1.64%, 5 = 1.21%, 6 = 0%.
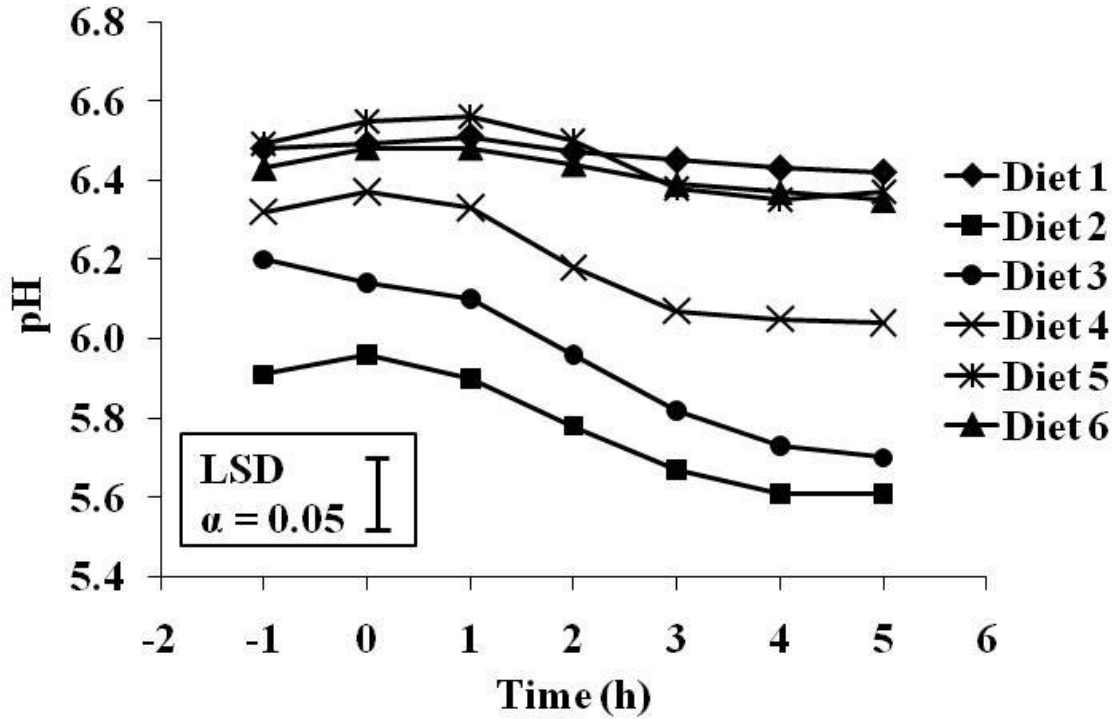
Figure 4.2. Fermenter pH measured between 1 hour before (-1 h) and 5 hours after (5 h) feeding from continuous culture fermenters fed diets with varying levels of rumen degradable protein. Dietary treatments are denoted Diet 1 (♦) = 60|-0.5, Diet 2 (■) = 60|-0.2, Diet 3 (●) = 70|-0.2, Diet 4 (✕) = 85|-0.2, Diet 5 (✱) = 100|-0.2, Diet 6 (▲) = 85|-0.5 % RDP supplied/required|RDN Balance (% DM).
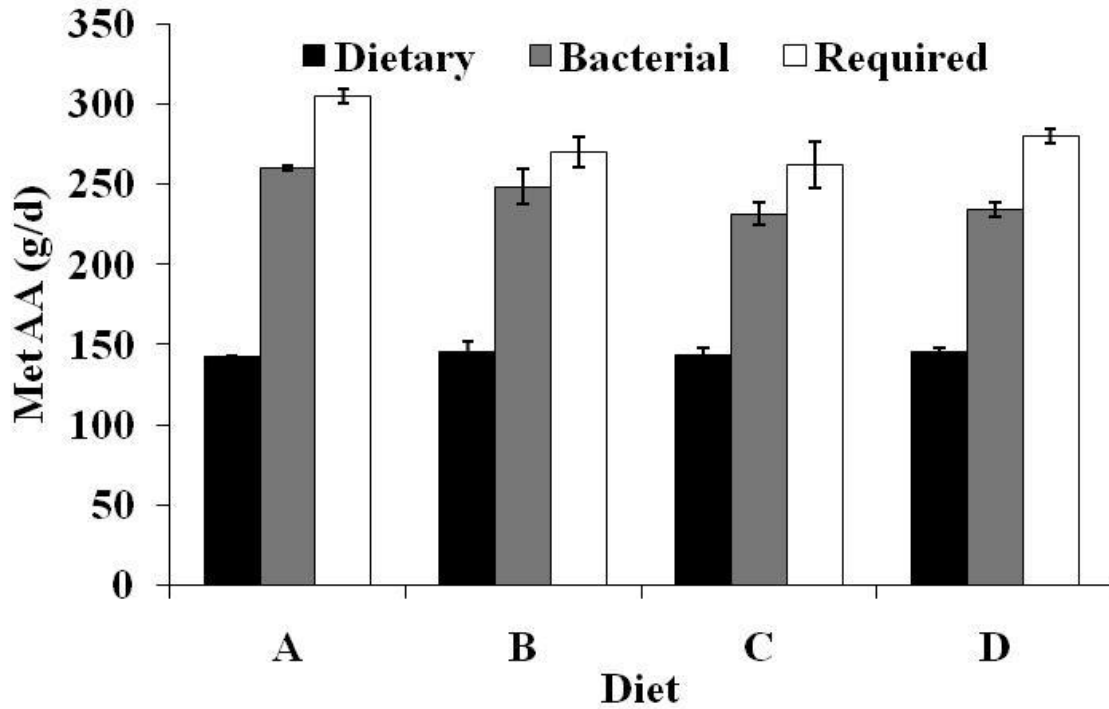
Figure 4.3. Calculated metabolizable amino acid (Met AA) composition and requirement for crossbred Angus steers fed varying levels of rumen degradable protein. Dietary (■), Bacterial (■), and Required (□) g/d of metabolizable amino acids flowing to the duodenum were calculated based on NRC (2000) data. Dietary treatments (A, B, C, D) formulated for 115, 95, 85, 70 % RDP supplied/required, respectively. Data is expressed as least-squared mean values ± SE. Dietary Met AA ($P > 0.10$), Bacterial Met AA (linear, $P = 0.01$), Required Met AA (quadratic, $P = 0.02$), Total Met AA (Bacterial + Dietary, $P > 0.10$).

CHAPTER 5

EFFECT OF VARYING DIETARY STARCH AND FIBER LEVELS AND
INOCULUM SOURCE (MULE DEER VS. DAIRY COW) ON DRY MATTER
DIGESTIBILITY AND VOLATILE FATTY ACID PRODUCTION.

ABSTRACT

Effects of starch and fiber on *in vitro* digestibility and microbial fermentation of three different commercial exotic animal complete feeds using mule deer (MD) or dairy cow (DC) rumen fluid mixed 1:3 with McDougall's buffer were examined. Diets consisted of high starch/low NDF with either alfalfa (diet A) or grain and oilseed byproducts (diet B) and low starch/high NDF (diet C). Six experimental treatments were prepared ($n = 6$; $N = 36$) with 3g of feed and 150mL of inoculum per flask. Flasks were incubated at 39°C with samples taken at 0,1,2,4,6,8,12,16,20,24,36 and 48 h for analysis of ammonia, pH, lactate, and volatile fatty acids (VFA). Dry matter digestibility (DMD) was determined separately over a 48 h period using the same methods. Dry matter digestibility ranged between 44.7 and 74.9%; diet B had both least and greatest values when fermented in MD or DC inoculum, respectively. Initial total VFA concentrations were greater in DC inoculum ($P < 0.01$). Acetate levels rose at faster rates in DC inoculum than MD inoculum for the first 20 h. Over time, acetate fermentation did not differ among diets in DC inoculum until after 24 h when diet C concentration began to rise, and diet A concentration decreased by 48 h. Final concentrations (48 h) of total VFA were not different among diets ($P > 0.05$). Lactate concentrations remained negligible in

DC inoculum, but rose rapidly in MD inoculum only to decline after 12 h. At the same time of lactate decline, propionate levels rose likely due to an increase in lactate fermentation. Acetate:propionate ratios measured for diet C in MD inoculum were greater (~ 5:1), and similar to values found in healthy wild browsing ruminants. Variations between diets became more pronounced in MD inoculum as fermentation progressed. We conclude previous diet and species differences in rumen fluid inoculum contribute to differences when measuring fermentation end products.

INTRODUCTION

Proper nutrient levels required for wild browsing ruminants, are still in question. This has to do with many aspects that have yet to be fully enumerated. However, supplemental diets with over-abundance of rapidly fermentable soluble carbohydrates and lack of slowly fermentable fibrous carbohydrates have been implicated in the occurrence of ruminal lesions, parakeratosis, atrophy and peracute mortality in many browsing ruminants maintained in captivity (Baker et al., 1998; Clauss and Lechner-Doll, 2001; Clauss et al., 2002; Woolf and Kradel, 1977). Continual consumption of such diets by domestic grazing ruminants has been shown to cause lactic acidosis and decreased nutrient absorption (Bramley et al., 2008; Nagaraja and Titgemeyer, 2007). It has been theorized that by increasing the fiber in the form of NDF and decreasing the starch levels, we may be able to combat these negative affects and more accurately simulate a diet that is more reflective of the diet selected by these animals in the wild (Dierenfeld et al., 2002; Kearney, 2005).

Symptoms of acidosis in ruminant animals occur when ruminal pH remains at less than 5.6 for an extended period of time (Owens et al., 1998). Although lactic acid production has been associated with severe cases, total VFA production has been shown to have a greater affect on ruminal pH (Short, 1971) and can lead to initial incidence of subacute ruminal acidosis (Stone, 2004).

In vitro measurement of digestibility and the subsequent ammonia, short-chain fatty acid and microbial production from feedstuffs fermented in rumen inoculum can provide valuable information about nutrient quality provided to animal. Assessment can

also be made as to whether a diet has the potential produce fermentation characteristics associated with an acidotic condition.

The objective of this study was to see how three commercially available wildlife complete feeds with varying starch and fiber levels affect rumen fermentation so that we may be able to better understand how the dietary needs of browsing ruminants differ from those of grazing ruminants.

MATERIALS AND METHODS

Mule Deer And Dairy Cow Batch Culture

All research with mule deer was conducted according to Washington State University's (WSU) Institutional Animal Care and Use Committee protocol #3705. Mule deer (*Odocoileus hemionus*) housed at the Wild Ungulate Facility at Washington State University, were divided into 3 separate groups each containing 3 males and 5 females. Animals were fed a diet of 75% complete feed and 25% alfalfa cubes. Each pen was fed a different complete feed contrasting fiber level or fiber source (forage vs. by-product origin) or level of starch. Dietary ingredients outlined in Table 5.1 and the companion growth study (McCusker, 2009) and nutrient composition is outlined in Table 5.2; in short, diet A was a high starch/low NDF diet with principle fiber sources being alfalfa and wheat middlings, diet B was a high starch/low NDF diet with principle fiber sources coming primarily from grain and oilseed by-products (wheat middlings, oat hulls, soyhulls, canola meal), and diet C was a low starch/high NDF diet with principle fiber sources being soyhulls, beet pulp, oat hulls, and aspen (Table 5.1).

Over three separate sampling periods, ruminal digesta from deer were collected via esophageal tube. Extracted rumen contents were strained on-site through four layers

of cheesecloth and frozen immediately on dry ice. Samples were shipped to the University of Missouri overnight on dry ice and stored at -20°C. After being thawed, rumen fluid samples were pooled within treatment group providing 3 different dietary inoculum sources. Each batch of mule deer rumen fluid was diluted with McDougall's buffer (McDougall, 1948) in a 1:3 dilution of rumen fluid to buffer, making a separate mule deer inoculum (MDI) for each of the dietary treatments as outlined in Table 5.3.

The use of dairy animals in this experiment was approved by the University of Missouri Animal Care and Use Committee. The day the experiment began, rumen fluid was obtained from a ruminally fistulated multiparous lactating Holstein cow to contrast between wild and domestic ruminants. The cow was provided *ad libitum* access to a lactation diet (240 g corn silage, 123 g alfalfa hay, 150 g alfalfa haylage, 467 g concentrate and 190 g CP, 240 g ADF and 410 g NDF/kg DM) formulated to meet nutritional requirements (NRC, 2001), and housed in free-stall facilities at the University of Missouri-Columbia Foremost Dairy Research Center. Dairy cow rumen fluid was transported from the farm to the lab (estimated travel time 10 min.), strained through four layers of cheese cloth, and diluted in the same 1:3 ratio as the MDI to make dairy cow inoculum (DCI).

Diets, A, B, and C and alfalfa cubes, were ground to pass through 3-mm screen in a Wiley mill (Arthur H. Thomas Company, Philadelphia, PA), mixed at a ratio of 75% complete feed and 25% alfalfa cubes.

Six experimental treatments were prepared ($n = 6$; $N = 36$) with 3g of feed and 150mL of inoculum per flask. Experimental design is outline in Table 5.3, with 3 treatments per feed per inoculum source by species. The flasks were placed in a 39°C

shaking water bath, and 3 mL samples were taken of each flask at 0,1,2,4,6,8,12,16,20,24,36 and 48 h.

Lab Analysis

Samples were tested for pH and bacterial growth. Bacterial growth was measured by optical density using a 1:3 dilution of sample to McDougall's buffer and analyzed for absorbance at 600 nm (Newbold and Rust, 1992). Remaining samples were frozen for later analysis of ammonia (NH_3) and volatile fatty acid (VFA) concentrations.

Dry matter digestibility (DMD) was run using the same six experimental treatments (Table 2) and the same ratio of feed:inoculum as the experimental flasks. Feed was weighed into an acetone-rinsed ANKOM cellulose filter and digested anaerobically in a 75 mL flask for the duration of the experiment. Upon termination of the digestion, bags were rinsed and analyzed for NDF DMD using an ANKOM[200] Fiber Analyzer (ANKOM Technology).

For sample analysis diets were ground with a Wiley mill to pass through a 1-mm screen. Samples of diet, effluent and fermenter contents were analyzed for dry matter (DM) by drying at 105°C for 24 h; organic matter (OM) by incineration at 500°C; NDF and ADF using an ANKOM[200] Fiber Analyzer (ANKOM Technology); and total N by combustion analysis (LECO FP-428; LECO Corporation, St. Joseph, MI). Hemicellulose was calculated by difference between NDF and ADF. Sample ammonia concentration (m*M*) was determined colorimetrically (DU-65 spectrophotometer; Beckman, Palo Alto, CA) with the hypochlorite-phenol procedure of Broderick and Kang (1980). Sample VFA and lactic acid concentration (m*M*) was determined using gas chromatography (Model

3400, Varian, Palo Alto, CA) following procedures outlined by Salanitro and Muirhead (1975).

Statistical Analysis

The experiment used a RCBD in a 2 x 3 factorial arrangement to analyze the effect of inoculum source and diet. All statistical analyses were performed using SAS$^{\circledR}$ version 9.2 (SAS Institute Inc., Cary, NC). Mean concentrations of VFA and lactate at 0 and 12 h after feeding, along with DM digestibility, were analyzed by ANOVA using Proc GLM comparing inoculum source dietary treatment and the interaction. When the *F*-test was significant ($P \leq 0.05$), means separation was performed using Fisher's LSD.

Broken-line regression analysis using PROC NLIN as described by Robbins et al. (2006) was used to determine at the length of time required for bacterial growth reach the plateau phase.

Bacterial growth, pH, NH_3, VFA, and lactic acid data were analyzed using the MIXED procedure with repeated measures as outlined by Littell et al. (1998). Means were analyzed for inoculum source, diet, and hour with all interactions thereof. Statistical significance was determined using $P \leq 0.05$ probability level, and LSD lines were calculated to graphically represent differences.

RESULTS

Fermentation Characteristics

Microbial growth curves of diets fermented in DCI did not differ (Figure 5.1). Diet A microbial growth did not differ between inoculum sources. Diet B and diet C fermented in MDI displayed rapid initial growth which remained significantly greater in

diet B throughout the study. Diets fermented in MDI reached maximal growth at 20, 18 and 24 h, respectively. Diets fermented in DCI reached maximal growth at 10, 10 and 12 h, respectively.

All diets remained within physiologically normal pH ranges throughout the duration of the study (Figure 5.2). Diet B in MDI produced a significantly lower pH than all other diets until 16 h, when it rose to a physiologically similar pH. Diet C in DCI steadily dropped in pH to a nadir at least 0.2 pH units lower than all other treatments at 20 h where it remained throughout the study. Conversely, pH measurement in diet C in MDI diverged from the other treatments at 8 h and remained greater than other treatments until the 36 h sampling.

Ammonia concentrations show stark differences between inoculum source (Figure 5.3) with concentrations in DCI diverging to twice that seen in MDI starting at 12 and 16 h sampling. Overall, diet B had greater concentrations for DCI diets and for MDI diets throughout the study, indicating greater protein degradability than both diets A and C. Initially, within respective inoculum sources, diet C NH_3 appearance was lower than diets A and B; however DCI concentration was no longer different by 20 h and, in MDI, concentration rose greater than diet A by 36 h. By the end of fermentation, diet A in MDI had the least concentration of NH_3.

Dry Matter Digestibility

Dry matter digestibility ranged between 44.7 and 74.9% (Table 5.2); diet B had both least and greatest values when fermented in MDI or DCI, respectively. DMD in diets A and C fermented in MDI were not different at approximately 50% digestion; whereas in DCI, diet C (68.0%) was more completely digested than diet A (64.2%).

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Short-Chain Fatty Acids

At 0 h, total VFA concentrations (Table 5.4) were greater when feeds were digested in DCI compared to MDI at the 0 ($P < 0.01$), but were not affected by diet ($P = 0.15$). Acetate to propionate (A:P) ratio was greater in MDI than DCI ($P < 0.01$). This is resultant of greater levels of acetate ($P < 0.01$) and lower levels of propionate ($P < 0.01$) in MDI than DCI. Propionate proportion was also similarly lesser in diets A and C than diet B ($P < 0.01$). Isobutyrate proportion was least in diets A, B, and C fermented in DCI which were similar to diet C in MDI; while diet A in MDI had the greatest percentage ($P = 0.05$). Butyrate, isovalerate and valerate proportions were similarly greater in all diets fermented in DCI with the greatest proportions seen in diets C and B in MDI ($P \leq 0.01$). Lactate concentrations at 0 h were similarly lesser in all diets in DCI ($0.78 - 1.22$ mmol), which were similar to diet A in MDI; however in MDI, diet B (2.69 mmol) and diet C (5.39 mmol) were greater than all other diets ($P < 0.01$).

As shown in Table 5.4, at 12 h total VFA concentrations in diets fermented in DCI were twice that of those fermented in MDI, with diet A and C having the greatest concentrations (97.59 and 93.14 mmol, respectively) when fermented in DCI and the least concentrations (42.25 and 44.01 mmol, respectively) when fermented in MDI. Acetate proportion was greatest in MDI and within inoculum source diet B was greater than diets A and C ($P = 0.02$). Propionate proportion was greatest in DCI, with diet A being greater than diet B or C, but, in MDI, diet B was greater than diets A or C, which were not different than each other ($P < 0.01$). Isobutyrate proportions were greatest in diets A and C in MDI, followed by diet B in MDI, but were not different among diets fermented in DCI ($P < 0.01$). Conversely, butyrate proportions were greatest in diets A

and C in DCI, followed by diet B, but lesser in diets fermented in MDI with diets B and C being similarly least ($P < 0.01$). Isovalerate and valerate proportions were greatest in diet C in DCI, followed by diet A and then diet B in DCI, while the least values were measured in diet C fermented in MDI ($P < 0.01$). Diets A and B fermented in MDI had greater acetate:propionate ratio (10.51 and 10.33) and lactate production (12.83 and 13.38 mmol) than all other diets, followed by diet B in MDI, and all diets in DCI were similarly less ($P \leq 0.02$).

Acetate levels rose at faster rates in DCI than MDI (Figure 5.4) for the first 20 h. As fermentation progressed, acetate fermentation did not differ between diets in DCI until after 24 h when diet C concentration began to rise, and diet A concentration decreased by 48 h. Similarly in MDI, diet C acetate concentration remained numerically greater than diets A and B after 20 h.

In DCI, between 4 and 20 h propionate production was numerically less from diet C compared to diets A and B (Figure 5.5); however, outside of this time, concentrations were indistinguishable from each other. In MDI, propionate production was consistently lower until after 12 h, where diet B concentration quadrupled to similar levels seen in DCI. A slower rise was seen in diet B in MDI between 20 and 48 h to produce levels similar to diets in DCI. Diet C in MDI also rose in concentration after 24 h, but levels remained lower throughout the experiment.

As with propionate, butyrate concentrations in DCI were numerically less from diet C compared to A and B between 4 and 20 h (Figure 5.6). In MDI, diets began to rise in butyrate concentration after 12 h. After 24 h, butyrate concentration significantly rose greater than all diets in both MDI and DCI. Diet A in MDI had concentrations similar to

diets in DCI by 48 h, while concentrations from diet B in MDI remained lower than all other diets in MDI and DCI.

Total VFA concentrations within inoculum source were not significantly different (Figure 5.7); however, between 2 h and 36 h diets fermented in MDI had lower concentrations than diets fermented in DCI. Final concentrations of total VFA were not different between diets.

Lactate concentrations in DCI remained negligible throughout the experiment (Figure 5.8). Lactate concentrations from diets in MDI initially increased rapidly from 0 to 4 h, at which point diet C concentrations slowly decreased to 0 by 36 h. Diets A and B continued to rise to peak at 8 h. Diet B concentrations resolved to negligible levels by 20 h, while diet A concentrations remained at peak levels until 24 h before resolving to levels similar to all diets by 36 h.

Between the 0 and 12 h sampling time, acetate proportion increased in MDI and decreased in DCI, while the opposite was true with propionate proportion (Figure 5.9). As illustrated in Figure 5.9, this shift in VFA production resulted in a doubling in the acetate:propionate ratio in MDI (5.3 to 10.2) and a decrease in the ratio seen in DCI (3.3-2.4). However, after 12 h, shifts in propionate concentration began in MDI diets (Figure 5.5), which, for diets A and B, lowered the A:P ratio to similar levels of those seen in DCI by 48 h.

DISCUSSION

Although rumen fluid for this study was obtained from two different methods (esophageal vs. fistula extraction), previous work by Lodge-Ivey et al. (2009) has shown

no difference exist in bacterial diversity or fermentation products between either sampling method.

Storage and handling were different between the two species inoculum. Generally, fluid is harvested and kept in anaerobic conditions at approximately 39°C for a short amount of time (< 1 h). In this study, mule deer rumen fluid was harvested at different dates and several states away from where the experiment was conducted. The only viable option was to freeze the fluid for storage and transport. Previous work has shown frozen strained rumen fluid will not differ from fresh fluid as long as, upon thawing, it is mixed with buffer and provided at least a 6 h pre-incubation (Luchini et al., 1996a, b). The MDI did not have an adequate pre-incubation time; therefore, this likely increased the lag time seen in the growth curves of the MDI diets. In this experiment, this retarded growth phase caused MDI diets to take twice as long to reach maximal growth as their DCI counterparts. The similarly lower total digestibility of feed in MDI is possibly due to a lack of fermentative activity of the microbes early on in the fermentation.

Species inoculum source, previous diet of the donor, storage and handling of fluid and individual animal variation are all factors which possibly affect the outcome *in vitro* data (Nagy et al., 1967; Urness et al., 1977; Ward, 1971). However, the degree of importance of each factor when trying to obtain useful data has been debated in the literature. Some research has found no difference between domestic and wild rumen fluid (Crawford and Hankinson, 1984; Welch et al., 1983) when looking at dry matter digestibility; however, our data indicate otherwise when looking at fermentation end-

products. Although domestic rumen fluid is a more easily obtainable, it does not adequately simulate the fermentation obtained from an adapted rumen fluid source.

Notably, the NH_3 appearance seen in the DCI was twice the concentration seen in the adapted MDI. Even after microbial growth reached a plateau in MDI, NH_3 concentrations did not begin to increase in a manner similar to DCI. The greater proteolytic activity in DCI, as evidenced by NH_3 appearance, is likely due to the DCI being adapted to a high concentrate lactation diet. In MDI, this could be due to a slower rate of protein breakdown. Yet, it is also likely that the adapted rumen fluid has a greater proportion of fiber fermenting bacteria which exclusively use NH_3 for protein production and are thus constantly removing free NH_3 from the environment. This would tend to line up with the continued bacterial growth seen.

We predicted higher starch would increase rate of fermentation, leading to a concomitant drop in pH due to lactic acid production. However, no treatment caused pH to drop lower than pH 6.3. Diets fermented in MDI remained fairly stable after an initial drop. Peak of acetate and total VFA production occur at the same time pH reaches its lowest point for diet C in DCI. The high digestibility (68%) and lower protein (14%) of diet C in DCI caused an imbalance which led to inefficient microbial production and wastage that resulted in increased VFA production and thus decreased pH. This inverse relationship confirms findings by others which attribute a greater contribution of total VFA to pH even in the absence of lactic acid production (Stone, 2004).

Rapid lactic acid production was seen initially in MDI, but when fermentations neared maximal microbial growth, lactic acid utilizing bacteria began to convert this lactate to other VFA, most notably propionate. At this time, acetate to propionate ratios in

MDI decreased to approximately 2:1 in all diets except diet C which decreased to approximately 5:1. Nagy and Williams (1969) reported similar ratios in wild Pronghorn antelope (~4:1), while Maloiy et al. (1982) reported a range of 4.2:1 to 5.9:1 in wild caught east African ruminants. Further, Short (1971) reported higher acetate to propionate ratios in deer during the early spring (3.9:1 to 4.2:1), which declined during the summer and fall (1.6:1 to 2.2:1). Ranges displayed in this study are well within those measured in healthy wild animals; however, higher ratios correspond with times of the year when animals are in good health and an abundant supply of high quality foods. The lower ratios seen in the literature are associated with times when food is scarce and of low quality and cellulose digestibility and body condition begin to decline (Torgerson and Pfander, 1971).

CONCLUSIONS

Diet digestibility increased in animals adapted to a high concentrate ration. The proteolytic capacity of the domestic ruminants was greater than that of the mule deer in this study. Higher acetate to propionate ratios were measured in diet adapted mule deer rumen fluid, indicating a greater capacity for fiber digestion. Measured ratios in diets containing low starch/high fiber were closer to values found in the literature for healthy wild browsing ruminants. Variations between diets became more pronounced using mule deer rumen fluid as fermentation progressed. This leads us to conclude previous diet and species differences in rumen fluid inoculum do contribute to differences when measuring fermentation end products.

Table 5.1. Dietary ingredients of three commercial exotic ruminant complete feeds with varying starch and fiber levels used to simulate diets fed to mule deer (*Odocoileus hemionus*) for an *in vitro* digestion.

Ingredient (%DM)	Diet A[1]	Diet B[1]	Diet C[1]
Alfalfa	30.42	14.08	-
Apple flavoring	0.30	-	0.30
Brewers dried yeast	1.00	-	1.00
Calcium carbonate	0.72	1.36	0.02
Calcium propionate	0.10	-	0.50
Calcium stearate	-	0.25	-
Canola meal	-	10.00	-
Dicalcium phosphate	0.25	0.01	0.93
DL-methionine	-	0.01	0.24
Dried whey	-	0.01	-
Flaxseed	-	-	1.00
Ground beet pulp	-	-	10.00
Ground corn	18.57	5.00	-
Ground oat hulls	-	15.00	5.75
Ground soybean hulls	-	-	50.00
Ground whole aspen	-	-	6.00
Lactobacillus cultures	-	0.13	-
Lignin sulfonate	-	2.35	1.00
L-lysine	-	0.01	-
Magnesium oxide	0.09	-	0.26
Mixed tocopherols	0.06	0.01	0.06
Molasses	5.00	4.00	6.00
Salt	1.00	0.51	1.00
Sodium sesquicarbonate	-	-	0.40
Soybean hulls	-	9.32	-
Soybean meal (48%)	10.72	0.96	12.70
Soybean oil	1.00	1.56	2.11
Sucrose	0.10	-	0.10
Vitamin/mineral	0.47	0.43	0.63
Wheat middlings	30.20	35.00	-

[1] Diet A = High starch/low NDF (forage fiber basis); Diet B = High starch/low NDF (by-product fiber basis); Diet C = Low starch/high NDF

Table 5.2. Dietary analysis of three commercial exotic ruminant complete feeds, alfalfa cubes and the different dietary treatments with varying starch and fiber levels used to simulate diets fed to mule deer (*Odocoileus hemionus*) for an *in vitro* digestion.

Diet Component (%DM)[1]	Diet A[2]	Diet B[2]	Diet C[2]	Alfalfa Cubes	Diet A + AC[3]	Diet B + AC[3]	Diet C + AC[3]
Dry Matter	90.15	90.97	90.96	91.66	89.96	90.66	90.67
Organic Matter	92.12	92.23	92.28	89.67	91.52	91.73	90.61
Ash	7.88	7.77	7.72	10.33	8.48	8.27	9.39
Protein							
Crude Protein	19.96	18.21	14.94	17.83	19.01	17.93	16.11
Available CP	19.29	17.62	14.04	16.62	18.14	17.13	15.18
Unavailable CP	0.67	0.60	0.87	1.21	0.88	0.80	0.93
Carbohydrates							
Starch	30.53	27.28	9.36	7.49	24.51	22.40	9.14
NDF	27.84	36.28	47.28	48.54	35.03	38.28	46.84
ADF	14.87	16.82	33.44	36.19	21.89	22.04	33.45
Hemicellulose	12.96	19.46	13.85	12.35	13.15	16.23	13.38
DM Digestibility							
Mule Deer Inoculum					52.2[d]	44.7[d]	50.8[d]
Dairy Cow Inoculum					64.2[c]	74.9[a]	68.0[b]

[1] Abbreviations: AC = alfalfa cubes, ADF = acid detergent fiber, DM = dry matter, CP = crude protein, NDF = neutral detergent fiber

[2] Diet A = High starch/low NDF (forage fiber basis); Diet B = (Luchini et al., 1996a)High starch/low NDF (by-product fiber basis); Diet C = Low starch/high NDF

[3] Dietary treatments consist of 75% complete feed and 25% alfalfa cubes.

[abcde] DM digestibility means with no superscripts in common are statistically significant (Inoculum x diet, $P < 0.01$, SEM = 0.82)

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Table 5.3. Experimental design of treatments for digestion of three commercial exotic ruminant complete feeds of varying starch and fiber levels with alfalfa cubes[1] in *in vitro* digestion with rumen fluid from diet adapted mule deer (*Odocoileus hemionus*) or un-adapted lactating dairy cow (*Bos taurus*) to determine dry matter digestibility and volatile fatty acid production.

| Diet[2] | Mule Deer[3] | | | Dairy Cow[4] |
	Inoculum A	Inoculum B	Inoculum C	Inoculum D
Diet A + AC	T1			T4
Diet B + AC		T2		T5
Diet C + AC			T3	T6

[1] Dietary treatments consist of 75% complete feed and 25% alfalfa cubes (AC).

[2] Diet A = High starch/low NDF (forage fiber basis); Diet B = High starch/low NDF (by-product fiber basis); Diet C = Low starch/high NDF

[3] Inoculum letter corresponds to the diet the source animals were being fed (i.e. Inoculum A source came from animals being fed Diet A + AC, Inoculum B animals were fed Diet B + AC, etc.)

[4] Dairy Cow source animal was being fed a high concentrate lactation diet

Table 5.4. Volatile fatty acid (VFA) and lactate production from digestion of three commercial exotic ruminant complete feeds of varying starch and fiber levels with alfalfa cubes[1] using *in vitro* batch culture techniques with Mule deer (*Odocoileus hemionus*) or lactating dairy cow (*Bos taurus*) rumen fluid inoculum.

SCFA 0 h[4]	Mule Deer Inoculum[2]			Dairy Cow Inoculum[2]			SE	P-values[3]		
	Diet A	Diet B	Diet C	Diet A	Diet B	Diet C		Inoc	Diet	Inoc x Diet
Total VFA (mmol)[5]	29.78	30.39	29.19	43.98	41.04	39.30	1.31	<0.01	0.15	0.25
Lactate (mmol)	1.32^c	2.69^b	5.39^a	1.22^{cd}	1.03^{cd}	0.78^d	0.19	<0.01	<0.01	<0.01
Acetate:Propionate Ratio	5.58	4.82	5.51	3.27	3.13	3.37	0.22	<0.01	0.07	0.35
				mol/100 mol						
Acetate	73.99	74.99	75.53	64.61	64.03	64.49	0.65	<0.01	0.54	0.36
Propionate	13.27	15.63	14.09	19.79	20.48	19.12	0.41	<0.01	<0.01	0.10
Isobutyrate	5.91^a	5.06^{ab}	4.68^{bc}	3.63^c	3.76^c	4.43^{bc}	0.40	<0.01	0.67	0.05
Butyrate	5.67^b	3.38^d	4.75^c	9.49^a	9.30^a	9.46^a	0.11	<0.01	<0.01	<0.01
Isovalerate	0.43^b	0.33^c	0.36^c	0.82^a	0.80^a	0.82^a	0.01	<0.01	<0.01	0.01
Valerate	0.73^c	0.60^d	0.60^d	1.66^{ab}	1.63^b	1.69^a	0.02	<0.01	<0.01	<0.01
SCFA 12 h[4]										
Total VFA (mmol)[5]	42.25^d	50.20^c	44.01^{cd}	97.59^a	90.19^b	93.14^{ab}	2.29	<0.01	0.75	<0.01
Lactate (mmol)	12.83^a	7.17^b	13.38^a	0.19^c	0.23^c	0.23^c	0.98	<0.01	<0.01	<0.01
Acetate:Propionate Ratio	10.51^a	9.87^b	10.33^{ab}	2.27^c	2.69^c	2.36^c	0.18	<0.01	0.83	0.02
				mol/100 mol						
Acetate	80.46^b	82.29^a	80.99^b	58.36^d	62.08^c	58.91^d	0.36	<0.01	<0.01	0.02
Propionate	7.67^e	8.34^d	7.89^e	25.67^a	23.12^c	24.95^b	0.13	<0.01	<0.01	<0.01
Isobutyrate	7.73^a	5.93^b	7.88^a	3.65^c	3.90^c	3.72^c	0.32	<0.01	0.02	<0.01
Butyrate	3.48^c	2.68^d	2.76^d	9.67^a	8.51^b	9.66^a	0.05	<0.01	<0.01	<0.01
Isovalerate	0.24^e	0.45^d	0.19^f	0.74^b	0.68^c	0.79^a	0.01	<0.01	<0.01	<0.01
Valerate	0.42^d	0.31^e	0.28^f	1.90^b	1.71^c	1.97^a	0.01	<0.01	<0.01	<0.01

[1] Dietary treatments consist of 75% complete feed and 25% alfalfa cubes.
[2] Diet A = High starch/low NDF (forage fiber basis); Diet B = High starch/low NDF (by-product fiber basis); Diet C = Low starch/high NDF
[3] Inoc = Mule Deer inoculum vs. Dairy Cow inoculum; Diet = A vs. B vs. C; Inoc x Diet = Interaction the main effects
[4] Short-Chain Fatty Acids measured at 0 h and 12 h after fermentation began
[5] Total VFA = Acetate + Propionate + Isobutyrate+ Butyrate+ Isovalerate + Valerate
[abcde] Means with no superscripts in common within the same row are statistically significant
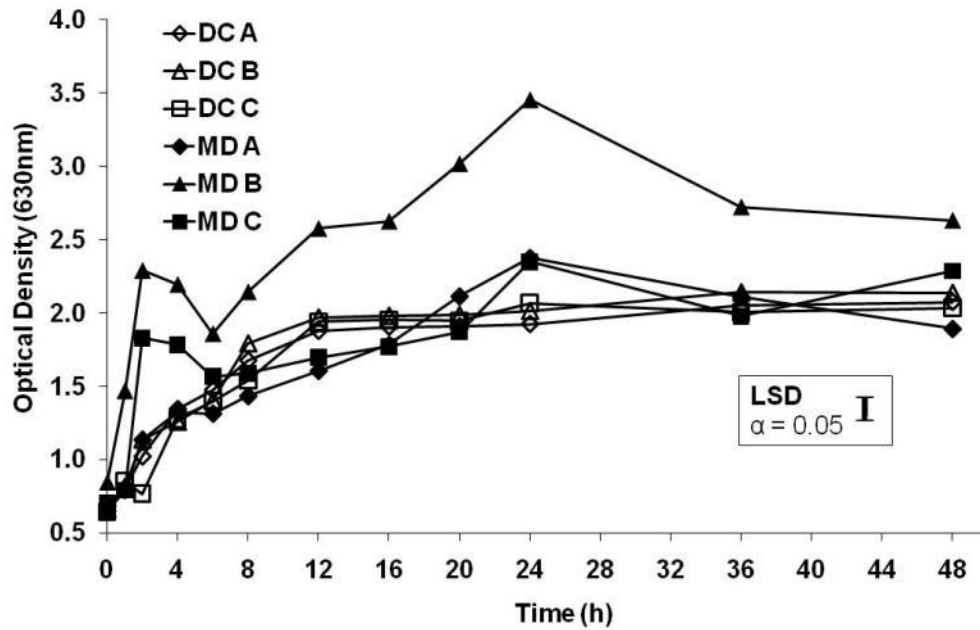
Figure 5.1. Optical density as a measure of bacterial growth of three commercial exotic ruminant complete feeds of varying starch and fiber levels with alfalfa cubes (75/25 mix) using *in vitro* batch culture techniques with mule deer (*Odocoileus hemionus*; closed markers) or lactating dairy cow (*Bos taurus*; open markers) rumen fluid inoculum. Diet A = high starch/low NDF (forage fiber basis; ♦ and ◊); Diet B = high starch/low NDF (by-product fiber basis; ▲ and ∆); Diet C = Low starch/high NDF (■ and □).

Figure 5.2. pH of three commercial exotic ruminant complete feeds of varying starch and fiber levels with alfalfa cubes (75/25 mix) using *in vitro* batch culture techniques with mule deer (*Odocoileus hemionus*; closed markers) or lactating dairy cow (*Bos taurus*; open markers) rumen fluid inoculum. Diet A = high starch/low NDF (forage fiber basis; ♦ and ◊); Diet B = high starch/low NDF (by-product fiber basis; ▲ and △); Diet C = Low starch/high NDF (■ and □).

Figure 5.3. Ammonia (NH$_3$) concentration of three commercial exotic ruminant complete feeds of varying starch and fiber levels with alfalfa cubes (75/25 mix) using *in vitro* batch culture techniques with mule deer (*Odocoileus hemionus*; closed markers) or lactating dairy cow (*Bos taurus*; open markers) rumen fluid inoculum. Diet A = high starch/low NDF (forage fiber basis; ♦ and ◊); Diet B = high starch/low NDF (by-product fiber basis; ▲ and △); Diet C = Low starch/high NDF (■ and □).

Figure 5.4. Acetate production (m*M*) from digestion of three commercial exotic ruminant complete feeds of varying starch and fiber levels with alfalfa cubes (75/25 mix) using *in vitro* batch culture techniques with mule deer (*Odocoileus hemionus*; closed markers) or lactating dairy cow (*Bos taurus*; open markers) rumen fluid inoculum. Diet A = high starch/low NDF (forage fiber basis; ♦ and ◊); Diet B = high starch/low NDF (by-product fiber basis; ▲ and ∆); Diet C = Low starch/high NDF (■ and □).

Figure 5.5. Propionate production (m*M*) from digestion of three commercial exotic ruminant complete feeds of varying starch and fiber levels with alfalfa cubes (75/25 mix) using *in vitro* batch culture techniques with mule deer (*Odocoileus hemionus*; closed markers) or lactating dairy cow (*Bos taurus*; open markers) rumen fluid inoculum. Diet A = high starch/low NDF (forage fiber basis; ♦ and ◊); Diet B = high starch/low NDF (by-product fiber basis; ▲ and △); Diet C = Low starch/high NDF (■ and □).
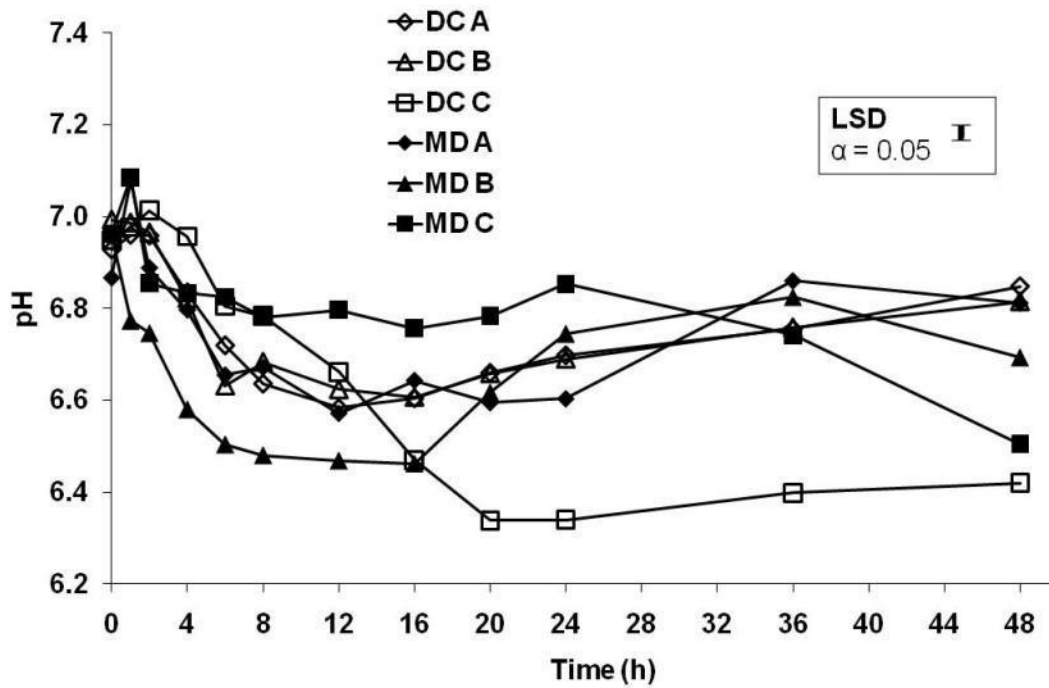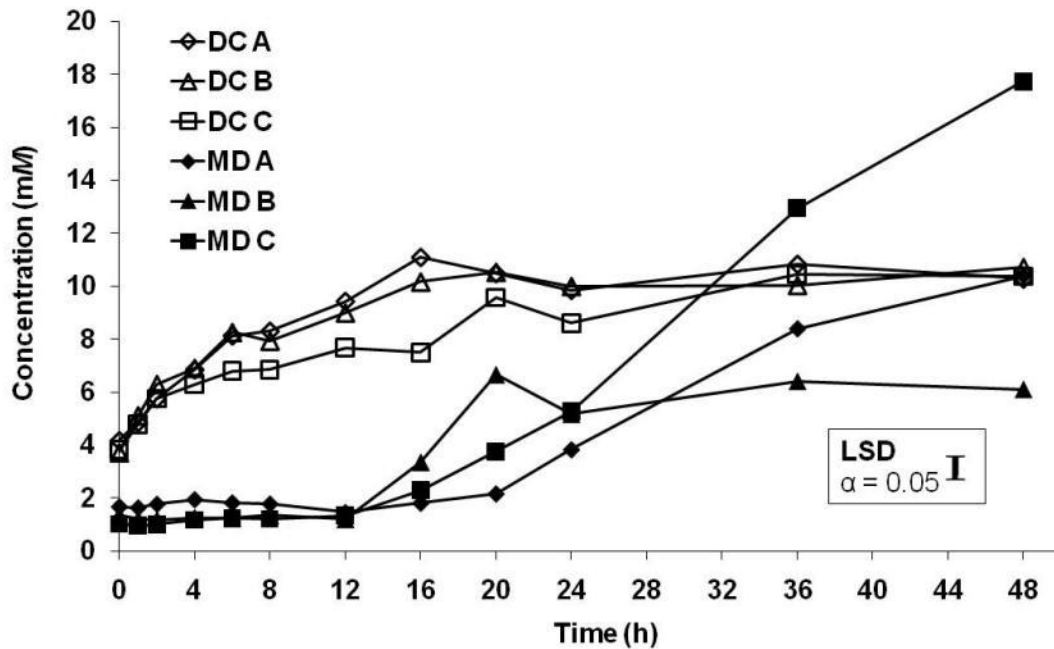
Figure 5.6. Butyrate production (m*M*) from digestion of three commercial exotic ruminant complete feeds of varying starch and fiber levels with alfalfa cubes (75/25 mix) using *in vitro* batch culture techniques with mule deer (*Odocoileus hemionus*; closed markers) or lactating dairy cow (*Bos taurus*; open markers) rumen fluid inoculum. Diet A = high starch/low NDF (forage fiber basis; ♦ and ◊); Diet B = high starch/low NDF (by-product fiber basis; ▲ and ∆); Diet C = Low starch/high NDF (■ and □).
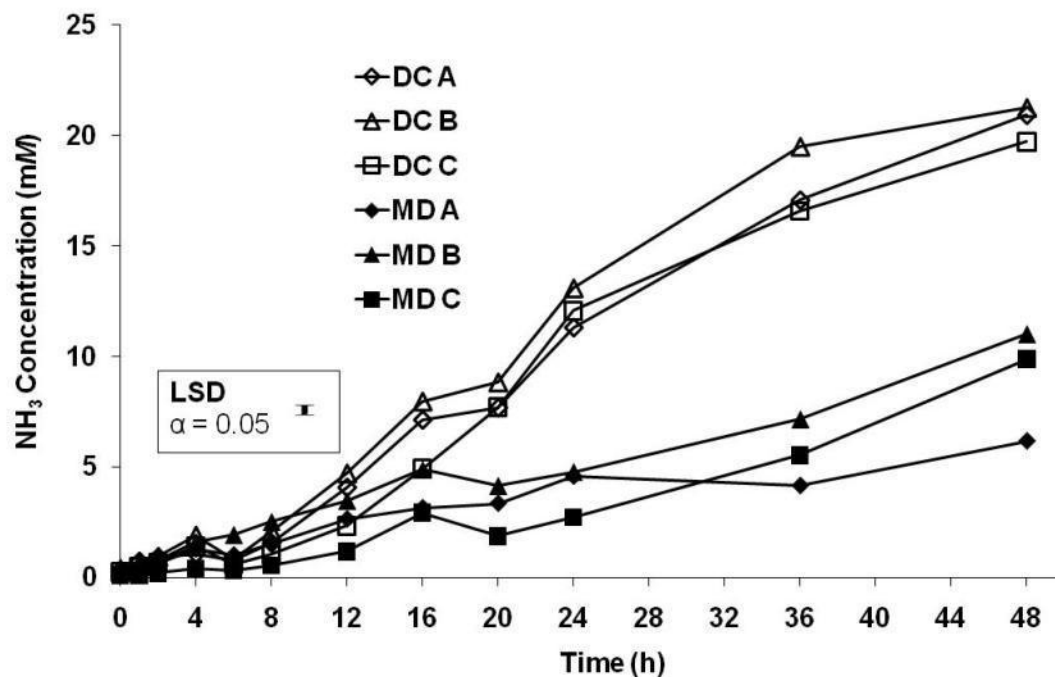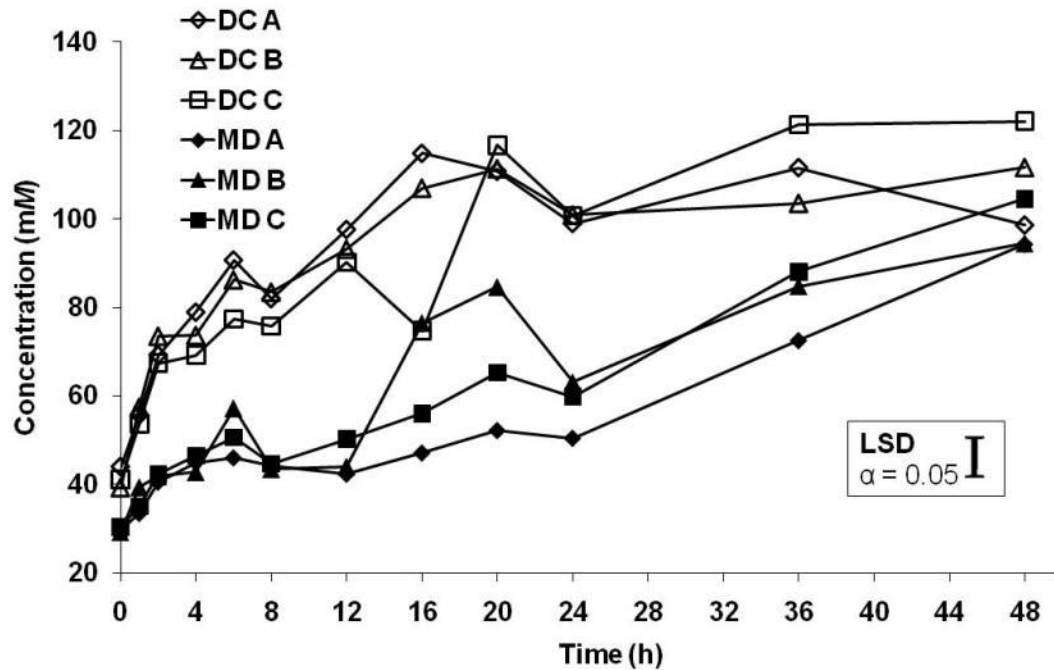
Figure 5.7. Total volatile fatty acid production (m*M*) from digestion of three commercial exotic ruminant complete feeds of varying starch and fiber levels with alfalfa cubes (75/25 mix) using *in vitro* batch culture techniques with mule deer (*Odocoileus hemionus*; closed markers) or lactating dairy cow (*Bos taurus*; open markers) rumen fluid inoculum. Diet A = high starch/low NDF (forage fiber basis; ♦ and ◊); Diet B = high starch/low NDF (by-product fiber basis; ▲ and ∆); Diet C = Low starch/high NDF (■ and □).
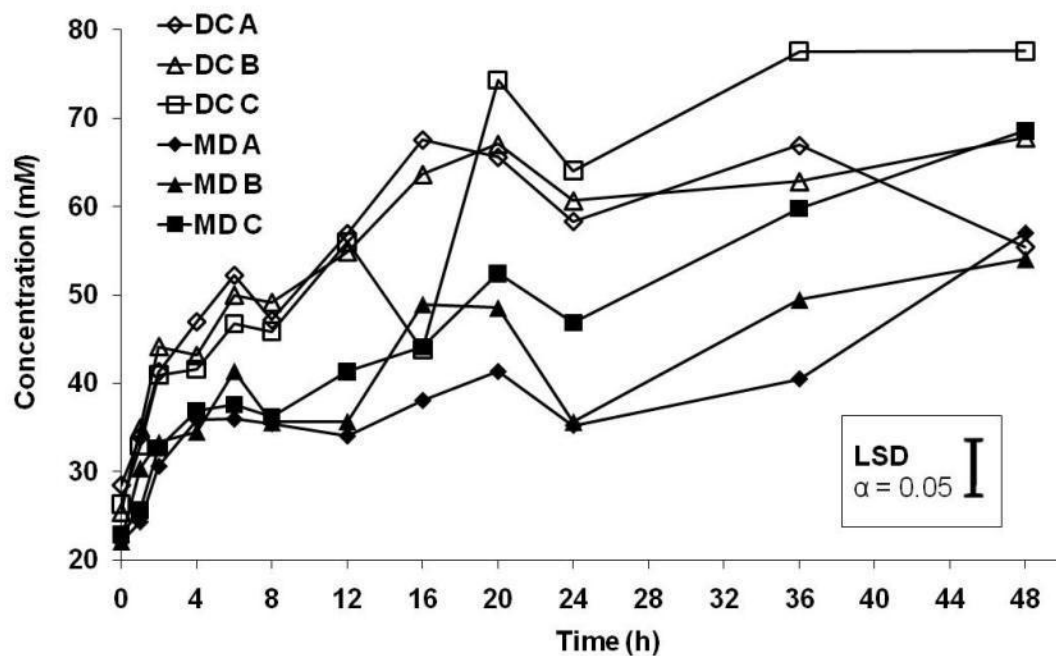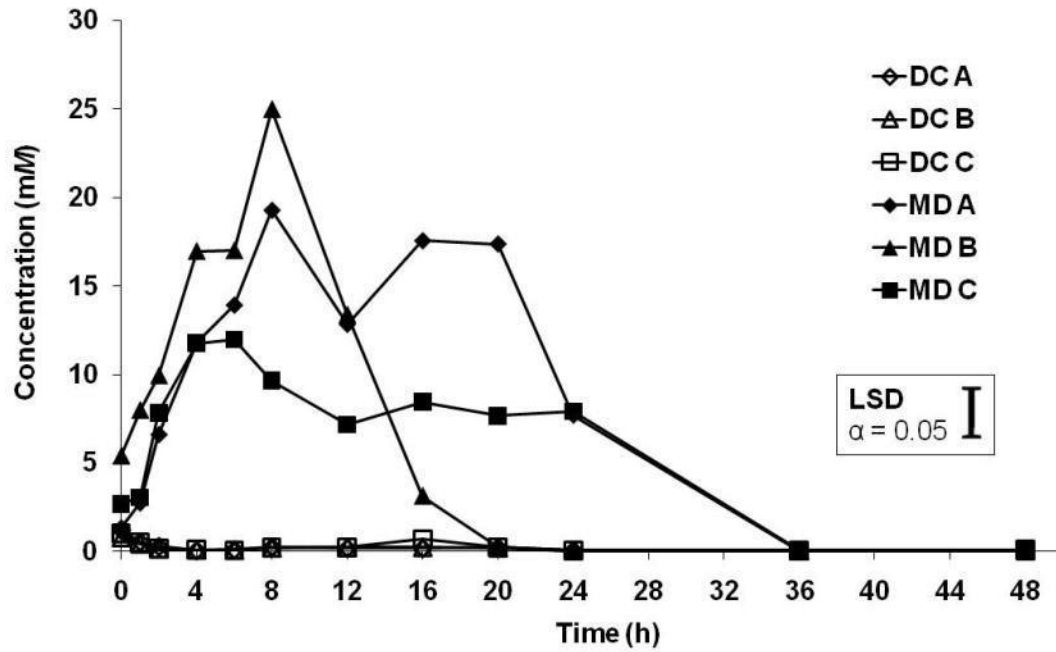
Figure 5.8. Lactate production (m*M*) from digestion of three commercial exotic ruminant complete feeds of varying starch and fiber levels with alfalfa cubes (75/25 mix) using *in vitro* batch culture techniques with mule deer (*Odocoileus hemionus*; closed markers) or lactating dairy cow (*Bos taurus*; open markers) rumen fluid inoculum. Diet A = high starch/low NDF (forage fiber basis; ♦ and ◊); Diet B = high starch/low NDF (by-product fiber basis; ▲ and ∆); Diet C = Low starch/high NDF (■ and □).
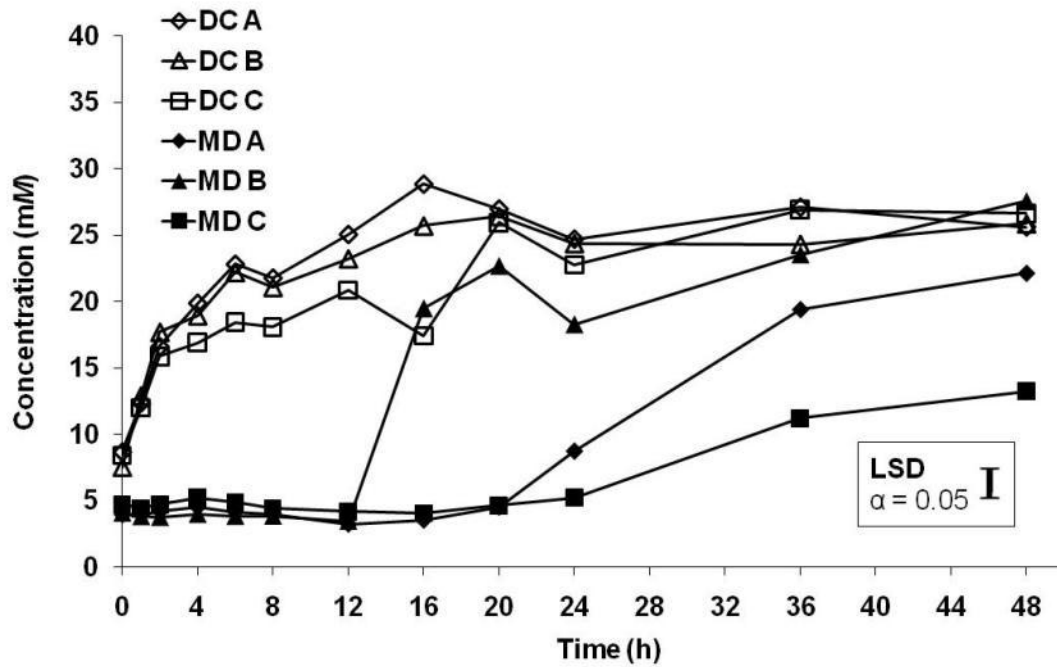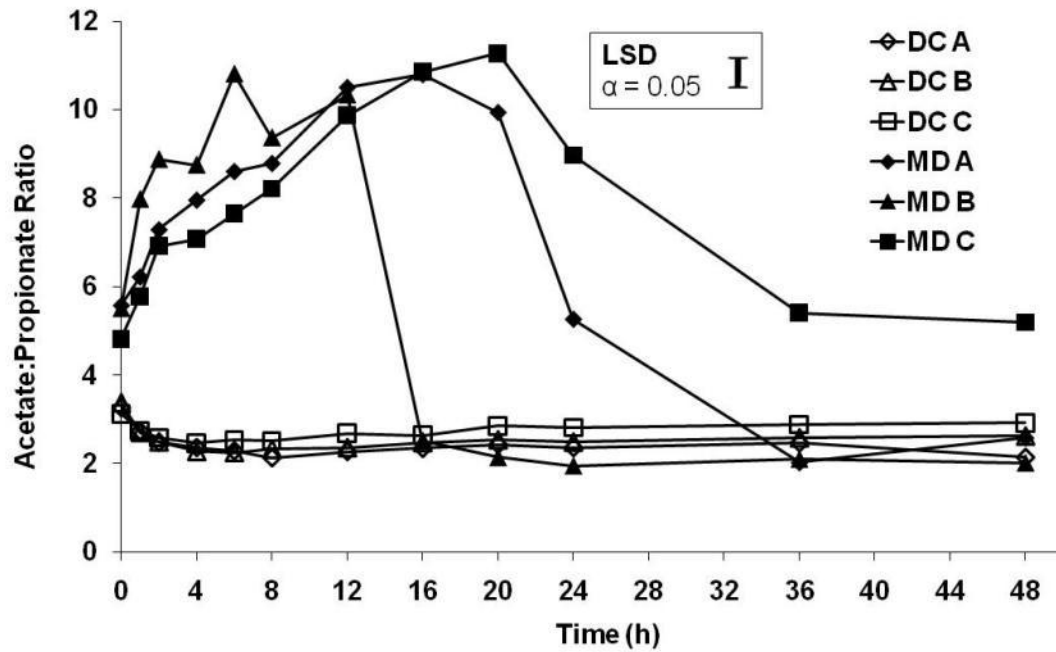
Figure 5.9. Acetate:propionate ratio from digestion of three commercial exotic ruminant complete feeds of varying starch and fiber levels with alfalfa cubes (75/25 mix) using *in vitro* batch culture techniques with mule deer (*Odocoileus hemionus*; closed markers) or lactating dairy cow (*Bos taurus*; open markers) rumen fluid inoculum. Diet A = high starch/low NDF (forage fiber basis; ♦ and ◊); Diet B = high starch/low NDF (by-product fiber basis; ▲ and ∆); Diet C = Low starch/high NDF (■ and □).

CHAPTER 6

EFFECT OF VARYING DIETARY STARCH AND FIBER LEVELS AND INOCULUM SOURCE (MULE DEER VS. DAIRY COW) ON SIMULATED RUMEN FERMENTATION CHARACTERISTICS.

ABSTRACT

This study looked at how starch and fiber affect digestion and microbial fermentation of three different commercial exotic animal complete feeds using mule deer (MD) or dairy cow (DC) rumen fluid. Diets were formulated to provide either high starch/low NDF with either alfalfa (diet A) or grain and oilseed byproducts (diet B) and low starch/high NDF (diet C). Two continuous culture experiments were conducted (MD or DC) with 6 fermenters per treatments. Diets were fed 2x a day over an 8 d period and sampled for ammonia, pH and volatile fatty acids (VFA) before and after feeding on the last 3 days. On day 8, fermenter and effluent contents were collected and analyzed for nitrogen, dry matter digestibility (DMD) and organic matter digestibility (OMD). OMD was greater in MD ($P = 0.02$) and DMD tended to do the same ($P = 0.06$), but there were no differences due to diet ($P > 0.05$). Ammonia concentration was greater in DC ($P < 0.01$), and diets A and B had larger concentrations than diet C ($P < 0.01$). Diet C had lower pH than diet A before feeding ($P < 0.01$) and tended to be lower after feeding ($P = 0.08$). Acetate to propionate ratio was greater in DC than MD before and after feeding ($P < 0.01$), and was greater in diet C than diets A or B ($P < 0.01$). Total VFA production tended to be greater in diets B and C in DC ($P = 0.06$). These data demonstrate rumen

fluid source does have an effect on fermentation. We also conclude fiber source affects fermentation. Increasing fiber level does not negatively affect fermentation of the diet and may increase OMD by removal of negative associative affects by starch on cellulolytic bacteria.

Keywords: *Odocoileus hemionus*, *Bos taurus*, Ruminant, Volatile Fatty Acid, NDF

INTRODUCTION

Several studies examined whether previous diet influenced inoculum source and subsequent fermentation results. Previous studies primarily measured dry matter digestibility (DMD) of various forages, few measured VFA production and most employed *in vitro* "closed" systems (Nagy et al., 1967; Palmer et al., 1976) rather "open" systems, such as *in situ* experiments (Mautz et al., 1976; Person et al., 1980). *In vitro* studies, while widely used and accepted, do not allow measurement of microbial efficiency or fermentation characteristics. Further draw backs have been outlined by Person et al. (1980). They explain these studies limit the physiological data which can be ascertained and applied to the animal. In short, "closed" systems only allow one infusion of fluid and substrate. This limits microbial growth and can cause detrimental effects as waste products, such as VFA and ammonia, accumulate. The subsequent drop in pH is toxic to many rumen microbes and harmful to the host animal. The length fermentation, usually 24 to 48 h, could be overestimating digestibility of a feed because it does not consider passage rate. Passage from the rumen potentially removes feeds before being completely fermented. Continuous culture systems allow for an influx of growth medium and substrate while considering ruminal passage rate. This simulates turnover of the rumen microbial population, and provides a static environment which more accurately imitates daily feeding characteristics of the animal.

Excess dietary starch and lack of dietary fiber have been implicated in mortalities of exotic ruminants. This occurs with grain-based diets, but is also seen in diets with forages high in soluble carbohydrates and low in fiber, such as clover or young alfalfa (Bramley et al., 2008). Acidotic conditions would decrease food consumption and

decreased nutrient absorption (Nagaraja and Titgemeyer, 2007). This is a plausible cause of acidosis and urolithiasis seen in deer (Woolf and Kradel, 1977; Woolf et al., 1976) and peracute mortality syndrome seen in captive giraffe, which demonstrate fat atrophy upon necropsy (Clauss et al., 2002; Potter and Clauss, 2009), due to energy loss and malnutrition. Previous diets have been formulated for captive wild ruminants, but long term effects of feeding diets high in protein (~20%) and soluble carbohydrates (< 20%) have proven unsuccessful (Baker et al., 1998).We would expect fiber-rich diets with lesser amounts of readily fermentable carbohydrates to be more reflective of diet selected by wild browsing ruminants (Dierenfeld et al., 2002; Kearney, 2005). Our objective was to use continuous cultures to determine how complete feeds varying in starch and fiber level would affect rumen fermentation.

MATERIALS AND METHODS

Mule Deer Continuous Culture

All research with mule deer was conducted according to Washington State University's (WSU) Institutional Animal Care and Use Committee protocol #3705. Mule deer (*Odocoileus hemionus*) housed at the Wild Ungulate Facility at Washington State University, were divided into 3 separate groups each containing 3 males and 5 females. Animals were fed a diet of 75% complete feed and 25% alfalfa cubes. Each pen was fed a different complete feed contrasting fiber level or fiber source (forage vs. by-product origin) or level of starch. Dietary ingredients outlined in Table 6.1 and the companion growth study (McCusker, 2009) and nutrient composition is outlined in Table 6.2; in short, diet A was a high starch/low NDF diet with principle fiber sources being alfalfa

and wheat middlings, diet B was a high starch/low NDF diet with principle fiber sources coming primarily from grain and oilseed by-products (wheat middlings, oat hulls, soyhulls, canola meal), and diet C was a low starch/high NDF diet with principle fiber sources being soyhulls, beet pulp, oat hulls, and aspen (Table 6.1). At the termination of the companion growth study three mule deer from each dietary treatment (8 males, 1 female) were sacrificed and rumen contents were collected. Extracted rumen contents were strained on-site through four layers of cheesecloth and frozen immediately on dry ice. Samples were shipped to the University of Missouri overnight on dry ice and stored at -20°C. After being thawed, rumen fluid samples were pooled within treatment group providing 3 different dietary inoculum sources. Each batch of mule deer rumen fluid was diluted with McDougall's artificial saliva in a 1:4 dilution of rumen fluid to buffer, making a separate inoculum for each of the dietary treatments as outlined for mule Deer Inoculum (MDI) in Table 6.3. Eighteen single-flow effluent continuous culture fermenter polycarbonate vessels (Nalgene, Rochester, NY) were inoculated (6 per dietary inoculum source) and maintained as described by Meng et al. (1999). Inoculum was added to each fermenter up to the effluent overflow port (approximately 1460 mL). Fermenters were continuously flushed with CO_2 gas, stirred with magnetic stir plates, and immersed in a water bath maintained at 39°C using thermostatically controlled heaters (model 730, Fisher Scientific, Pittsburgh, PA). High buffer capacity solution modified by Slyter (1990) from McDougall's artificial saliva (McDougall, 1948), containing 107.5 mg urea-N/L and 250 mg cysteine-HCL/L was continuously infused into fermenters using peristaltic pumps (Masterflex model 7520-10, Cole Parmer Instrument Co., Chicago, IL). Fermenter dilution rates were held constant at 6% ± 0.2%·h^{-1} for all treatments. Effluent

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flowed into collection vessels immersed in ice-cooled water. Diets, A, B, and C and alfalfa cubes, were ground to pass through 3-mm screen in a Wiley mill (Arthur H. Thomas Company, Philadelphia, PA), mixed at a ratio of 75% complete feed and 25% alfalfa cubes, and fed to the fermenters twice daily (25 g/feeding) at 12 h intervals ($n =$ 6). The experiment was conducted over an 8-day period, with 5 days adaptation and 3 days sampling.

Dairy Cow Continuous Culture

A second separate continuous culture was run using rumen fluid obtained from two ruminally fistulated multiparous lactating Holstein cows to contrast between wild and domestic ruminants. The use of dairy animals in this experiment was approved by the University of Missouri Animal Care and Use Committee. Cows were provided *ad libitum* access to a lactation diet (20.8% ground corn, 20.5% corn silage, 17.7% alfalfa haylage, 9.3% alfalfa hay, 7.9% AminoPlus®, 6.5% soybean hulls, 5.7% vit/min mix, 4.7% whole cottonseed, 4.7% wet brewer's grain, 2.3% soybean meal; and 17.7% CP, and 9.3% RDP and 31.9% NDF) formulated to meet nutritional requirements (NRC, 2001), and housed in free-stall facilities at the University of Missouri-Columbia Foremost Dairy Research Center. Dairy cow rumen fluid was transported from the farm to the lab (estimated travel time 10 min.), strained through four layers of cheese cloth, and frozen immediately at -20°C. After similar storage time, thawed fluid was diluted in the same 1:4 ratio as the mule deer inoculum and used to inoculate eighteen single-flow effluent continuous culture fermenter as outlined for Dairy Cow Inoculum (DCI) in Table 6.3. All other methods were identical to those described for the mule deer experiment.

Sampling

One liter subsamples were taken from effluent collected over the previous 24 h and stored at 4˚C. These samples were composited for each fermenter over the three day period for later analysis. On the last day of sampling, fermenter contents were blended and strained through two layers of cheese cloth and stored at 4˚C until analyzed. Further, 1 hour before feeding and 4 hours after feeding, pH and a 5 mL sample were taken directly from the fermenter and immediately frozen at -20˚C. These samples were later composited for each fermenter over the three day period for each sampling time and analyzed for ammonia and volatile fatty acid (VFA).

Lab Analysis

Blended fermenter samples were centrifuged at 1000 x g for 5 min at 4°C to remove feed particles. Supernatant was re-centrifuged at 27000 x g for 30 min. The resultant pellet was washed once using 0.9% (wt/vol) saline solution then again using deionized distilled H_2O. The final pellet, containing bacteria, was transferred to plastic cups using deionized distilled water, lyophilized at 10°C (Genesis, Virtis, Gardiner, NY), and ground using a mortar and pestle. Subsamples of effluent (500 mL) were lyophilized at 10°C (Genesis, Virtis, Gardiner, NY), and ground using a mortar and pestle.

For analysis diets were ground through with a Wiley mill to pass through a 1-mm screen. Samples of diet, effluent and fermenter contents were analyzed for dry matter (DM) by drying at 105°C for 24 h; organic matter (OM) by incineration at 500°C; NDF and ADF using an ANKOM[200] Fiber Analyzer (ANKOM Technology); and total N by combustion analysis (LECO FP-428; LECO Corporation, St. Joseph, MI). Hemicellulose was calculated by difference between NDF and ADF. Samples of effluent and fermenter

pellets were analyzed for purine content using the procedure of Zinn and Owens (1986) to determine microbial N, which was used with OM digested to determine microbial efficiency (MOEFF; g microbial N/kg OM truly digested). Sample ammonia concentration (mM) was determined colorimetrically (DU-65 spectrophotometer; Beckman, Palo Alto, CA) with the hypochlorite-phenol procedure of Broderick and Kang (1980). Sample VFA concentration (mM) was determined using gas chromatography (Model 3400, Varian, Palo Alto, CA) following procedures outlined by Salanitro and Muirhead (1975).

Statistical Analysis

The experiment used a RCBD in a 2 x 3 factorial arrangement to analyze the effect of inoculum source and diet. All statistical analyses were performed by ANOVA using Proc GLM in SAS® version 9.2 (SAS Institute Inc., Cary, NC). Least-squared means were compared to determine the main effects of species inoculum source and diet and their interaction. When the F-test was significant ($P \leq 0.05$), means separation was performed using Fisher's LSD.

RESULTS

Fermentation Characteristics

Analysis showed no difference in MOEFF (Table 6.4), bacterial nitrogen production, or rumen undegradable nitrogen (RUN) also known as dietary by-pass nitrogen ($P > 0.10$). DM digestibility over 24 h tended to be greater in MDI when compared to DCI ($P = 0.06$), but no differences were noted due to diet ($P = 0.17$). However, MDI displayed greater OM digestibility than DCI ($P = 0.02$); further, diet C

tended to show greater OM digestibility than diet B, with diet A being similar to both B and C ($P = 0.07$). Ammonia concentration was greater in DCI than MDI both before and after feeding ($P < 0.01$), but dietary differences show diet A and B to be similarly larger than diet C throughout the study ($P < 0.01$), which would agree with the differences in CP levels of the diets. Diet C fermenter pH was significantly lower than diet A before feeding ($P = 0.01$), but only showed a tendency to be lower after feeding ($P = 0.08$) due mainly to a larger drop in pH by diets A and B, most likely due to the greater starch levels in those diets.

Short-Chain Fatty Acids

Before feeding, isobutyrate, a product of amino acid fermentation, was undetectable in MDI and only small amounts were detected in the DCI (Table 6.5). Similarly, molar proportion of isovalerate, another product of amino acid fermentation, was found to be greater in DCI than MDI. Acetate to propionate (A:P) ratio was greater in DCI than MDI ($P < 0.01$). This is resultant of greater levels of acetate ($P < 0.01$) and lower levels of propionate ($P < 0.01$) in DCI than MDI. Lactate tended to be greater in DCI ($P = 0.07$).

Acetate levels in diet C were greater than diets A and B ($P < 0.01$). A:P ratio followed the same pattern, which is expected in a diet with greater amounts of fiber ($P < 0.01$). Diet A and B had similarly high propionate production, however, diet A did not differ from diet C ($P = 0.03$). Diet C also produced a greater amount of lactate than the other two diets ($P < 0.01$).

Molar proportion of butyrate was greatest in diet A fermented in MDI (14.27 %) and similarly lower in all three diets fermented in DCI (7.94 - 9.39 %). Diet B MDI had

similar proportion of butyrate to diet B in DCI and diet C in MDI, which itself is not different than diet A in DCI ($P = 0.04$). Valerate proportion was greatest in diet A in DCI, followed by diet B in DCI. Diet C in DCI was similar to diet B in MDI, which was similar to diet A in MDI. Diet C in MDI had the least proportion of valerate, which was similar to diet A in MDI ($P = 0.02$).

Table 6.5 shows 4 h after feeding acetate levels were unaffected by inoculum source ($P = 0.40$), but molar proportion of propionate, isobutyrate, valerate, and isovalerate were lower in MDI than DCI ($P < 0.01$), as was total VFA concentration ($P = 0.04$), A:P ratio ($P < 0.01$) and lactate concentration ($P < 0.01$).

Diet C had a greater acetate concentration ($P = 0.01$) and lower propionate concentration ($P = 0.04$) than diets A and B; subsequently diet C had a greater A:P ratio ($P < 0.01$). Diet C see also had lower proportion of isobutyrate than diets A or B, which were similar, and diet C had lower proportion of isovalerate than diets A or B, which were dissimilar and greater in diet A ($P < 0.01$).

Butyrate proportion was greatest in diet A in MDI, then diet B in MDI, and all other treatments were similar ($P < 0.01$). Valerate production was greatest in diet A in DCI and least in diet C in MDI, with all other levels falling within a similar range (1.40 − 1.82 %; $P = 0.03$). There was a tendency for greater total VFA production by diet B in DCI than in all diets fermented in MDI and diet A fermented in DCI, but diet B was similar to all diets in both inoculum ($P = 0.07$).

DISCUSSION

One of the disadvantages of working with animals in the field when sampling rumen fluid is the time from harvesting fluid until the time when the digestion begins. Generally, fluid is harvested and kept in anaerobic conditions at approximately 39˚C for a short amount of time (< 1 h). In this study, fluid was harvested at different dates and several states away from where the experiment was conducted. The only viable option was to freeze the fluid for storage and transport. Research has shown as long as rumen fluid is strained before being frozen and, upon thawing, is mixed with buffer and allowed a pre-incubation period of greater than 6 h, no difference is seen between frozen or fresh fluid (Luchini et al., 1996a, b). The 5 day adaptation period allowed for approximately 7 turnovers of total fermenter volume before sampling occurred. A liquid dilution rate (ruminal passage rate) of 6% of the fermenter volume per hour was chosen based off estimations of ruminants feeding on a grain-based diet. Faster passage rates have also been suggested for smaller browsing ruminants whose diet consists more of leaves, twigs, and fruits than grasses (Hofmann, 1973). Browsers in captivity have been shown to ruminate less and allow larger particles to pass out of the omasal opening at a faster rate (Hummel et al., 2008). Browsing ruminants do not have stratified mat of feedstuffs as is seen in domestic animals (Clauss et al., 2006), which was observed in this study upon necropsy of the sacrificed deer.

Rumen fluid inoculum source has been studied for its influence on digestion and fermentation characteristics, with many studies concluding there is no difference between species (Crawford and Hankinson, 1984; Welch et al., 1983) when measuring DMD; however, Ward (1971) and Calibri (2005) concluded feed digestion was different

between species. Further, Palmer et al. (1976) found equations could be applied to domestic animal data to estimate wild species digestibility but would require more sampling to produce accurate equations. Still other studies purport previous diet is more important than inoculum source (Brüggemann et al., 1968; Nagy et al., 1967), while other studies disagree (Crawford and Hankinson, 1984; Welch et al., 1983). Continuous culture systems help adapt rumen fluid to diet before sampling occurs, thus allowing observation of any initial microbial population differences. Inoculum source differences were apparent in many measured characteristics, but few interactions between both source and diet were observed.

Four hours after feeding was chosen as an optimal sampling time because it has shown to exhibit the least pH values during fermentation due to greater amounts of acid production and release of buffers in both the saliva and feed (Owens and Goetsch, 1993).

Greater concentrations of ammonia, branch chain VFA and valerate are consistent with the levels of protein seen in the diets. With diet A having greater amounts of protein, and thus amino acids, it would be expected to have the greatest production of these. Further, differences seen in inoculum are likely due to previous diet of the dairy cow (19% CP). The lactation diet produces a microbial population more efficient in protein degradation. We hypothesize proteolytic bacteria, which were thriving in the dairy cow, maintained a substantial population with experimental diets.

Measured pH values were well within normal ranges seen elsewhere in wild ruminants (Maloiy et al., 1982; Short, 1971). One hour before feeding, pH values, were lower for diet C by 0.05 pH units or less. This could be explained by greater OM digestibility of diet C, resulting in greater lactate and total VFA concentrations combined

with lower ammonia concentrations. However, by 4 h after feeding, these differences had dissipated. Short (1971) has demonstrated total VFA production has the greatest effect on pH when compared to other fermentation characteristics, which would lend credence to this theory.

Greater amounts of fiber in diet C were reflected in production of acetate by cellulolytic bacteria. Although no differences were observed in inoculum within hour point, the change in production of acetate in MDI was more pronounced. This is evidenced by the A:P ratio. Diet C showed the greatest A:P ratio, which is similar to observed values by Short (1971) in mule deer and Maloiy et al. (1982) in African ruminants.

Diets A and B show little differences exist between NDF sources used for pelleted rations. The only observed differences between these diets related to affects which can be attributed to similar dry matter digestion of the diet combined with a greater level of crude protein. Greater NDF level, as observed in diet C, did not negatively affect OM digestibility. In fact, digestibility of diet tended to be greater when compared to greater starch diets. We speculate this is due to negative associative affects seen with high starch diets (Fahey and Berger, 1993).

CONCLUSIONS

Mule deer inoculum showed greater OM digestibility and a tendency for greater DMD. Mule deer inoculum also displayed lower proteolytic activity, greater butyrate production, and greater post-feeding A:P ratios. These data demonstrate differences in microbial populations, which lead us to conclude inoculum source is important. We also

conclude the use of by-products as a fiber source did not negatively affect the fermentation of the diet, and formulating a diet with greater amounts of fiber and lower amounts of starch did not negatively impact fermentation characteristics of the diet. In fact, this tended to increase OM digestibility most likely due to removal of negative associative affects caused by starch on cellulolytic bacteria.

Table 6.1. Dietary ingredients of three commercial exotic ruminant complete feeds with varying starch and fiber levels used to simulate diets fed to mule deer (*Odocoileus hemionus*) for continuous culture fermentation.

Ingredient (%DM)	Diet A[1]	Diet B[1]	Diet C[1]
Alfalfa	30.42	14.08	-
Apple flavoring	0.30	-	0.30
Brewers dried yeast	1.00	-	1.00
Calcium carbonate	0.72	1.36	0.02
Calcium propionate	0.10	-	0.50
Calcium stearate	-	0.25	-
Canola meal	-	10.00	-
Dicalcium phosphate	0.25	0.01	0.93
DL-methionine	-	0.01	0.24
Dried whey	-	0.01	-
Flaxseed	-	-	1.00
Ground beet pulp	-	-	10.00
Ground corn	18.57	5.00	-
Ground oat hulls	-	15.00	5.75
Ground soybean hulls	-	-	50.00
Ground whole aspen	-	-	6.00
Lactobacillus cultures	-	0.13	-
Lignin sulfonate	-	2.35	1.00
L-lysine	-	0.01	-
Magnesium oxide	0.09	-	0.26
Mixed tocopherols	0.06	0.01	0.06
Molasses	5.00	4.00	6.00
Salt	1.00	0.51	1.00
Sodium sesquicarbonate	-	-	0.40
Soybean hulls	-	9.32	-
Soybean meal (48%)	10.72	0.96	12.70
Soybean oil	1.00	1.56	2.11
Sucrose	0.10	-	0.10
Vitamin/mineral	0.47	0.43	0.63
Wheat middlings	30.20	35.00	-

[1] Diet A = High starch/low NDF (forage fiber basis); Diet B = High starch/low NDF (by-product fiber basis); Diet C = Low starch/high NDF

Table 6.2. Dietary analysis of three commercial exotic ruminant complete feeds, alfalfa cubes and the different dietary treatments with varying starch and fiber levels used to simulate diets fed to mule deer (*Odocoileus hemionus*) for continuous culture fermentation.

Diet Component[1] (% on DM basis)	Diet A[2]	Diet B[2]	Diet C[2]	Alfalfa Cubes	Diet A + AC[3]	Diet B + AC[3]	Diet C + AC[3]
Dry Matter	90.15	90.97	90.96	91.66	89.66	89.97	89.99
Organic Matter	92.12	92.21	92.27	89.66	91.41	91.89	91.52
Ash	7.88	7.79	7.73	10.34	8.59	8.11	8.48
Protein							
Crude Protein	19.96	18.21	14.94	17.83	19.01	17.93	16.11
Available CP	19.29	17.61	14.07	16.62	18.14	17.13	15.18
Unavailable CP	0.67	0.60	0.87	1.21	0.88	0.80	0.93
Carbohydrates							
Starch	30.53	27.28	9.36	7.49	24.51	22.40	9.14
NDF	29.96	36.03	48.08	44.04	32.17	37.07	48.46
ADF	16.22	16.86	33.68	34.55	19.74	21.42	34.92
Hemicellulose	13.75	19.18	14.40	9.49	12.43	15.65	13.55

[1] Abbreviations: AC = alfalfa cubes, ADF = acid detergent fiber, DM = dry matter, CP = crude protein, NDF = neutral detergent fiber,
[2] Diet A = High starch/low NDF (forage fiber basis); Diet B = High starch/low NDF (by-product fiber basis); Diet C = Low starch/high NDF
[3] Dietary treatments consist of 75% complete feed and 25% alfalfa cubes.

Table 6.3. Experimental design of treatments for digestion of three commercial exotic ruminant complete feeds of varying starch and fiber levels with alfalfa cubes[1] using a continuous culture system inoculated with diet adapted mule deer (*Odocoileus hemionus*) or un-adapted lactating dairy cow (*Bos taurus*) rumen fluid inoculum to determine fermentation characteristics.

	Mule Deer[3]			Dairy Cow[4]
Diet[2]	Inoculum A	Inoculum B	Inoculum C	Inoculum D
Diet A + AC	T1			T4
Diet B + AC		T2		T5
Diet C + AC			T3	T6

[1] Dietary treatments consist of 75% complete feed and 25% alfalfa cubes.

[2] Diet A = High starch/low NDF (forage fiber basis); Diet B = High starch/low NDF (by-product fiber basis); Diet C = Low starch/high NDF

[3] Inoculum letter corresponds to the diet the source animals were being fed (i.e. Inoculum A source came from animals being fed Diet A + AC, Inoculum B animals were fed Diet B + AC, etc.)

[4] Dairy cow source animal was being fed a high concentrate lactation diet

Table 6.4. Microbial fermentation characteristics from digestion of three commercial exotic ruminant complete feeds of varying starch and fiber levels with alfalfa cubes[1] using a continuous culture system inoculated with mule deer (*Odocoileus hemionus*) or lactating dairy cow (*Bos taurus*) rumen fluid inoculum.

| | Mule Deer Inoculum[2] | | | Dairy Cow Inoculum[2] | | | | P-values[3] | | |
Item	Diet A	Diet B	Diet C	Diet A	Diet B	Diet C	SE	Inoc	Diet	Inoc x Diet
MOEFF[4]	28.22	26.93	23.19	27.04	28.80	23.37	2.48	0.89	0.13	0.83
NH_3 (mM) -1 h[5]	6.43	5.60	2.18	10.47	9.71	6.00	0.40	<0.01	<0.01	0.93
NH_3 (mM) 4 h[5]	5.35	4.29	1.08	10.42	9.01	5.97	0.46	<0.01	<0.01	0.93
DM Digested (%)[6]	57.27	56.96	61.28	54.08	51.26	57.19	2.71	0.06	0.17	0.90
OM Digested (%)[7]	51.00	50.63	55.40	47.41	44.73	50.90	2.32	0.02	0.07	0.88
pH -1 h[8]	6.64	6.62	6.58	6.68	6.64	6.59	0.02	0.27	0.01	0.91
pH 4 h[8]	6.59	6.57	6.55	6.66	6.56	6.58	0.03	0.21	0.08	0.42
Bacterial Nitrogen (g/d)	0.59	0.55	0.53	0.52	0.52	0.48	0.04	0.12	0.45	0.90
RUN (% Dietary Nitrogen)	51.85	56.15	58.46	57.41	61.97	64.40	4.64	0.14	0.34	1.00

[1] Dietary treatments consist of 75% complete feed and 25% alfalfa cubes.
[2] Diet A = High starch/low NDF (forage fiber basis); Diet B = High starch/low NDF (by-product fiber basis); Diet C = Low starch/high NDF
[3] Inoc = Mule Deer inoculum vs. Dairy Cow inoculum; Diet = A vs. B vs. C; Inoc x Diet = Interaction the main effects
[4] Microbial Efficiency = g effluent microbial N/ kg OM truly digested
[5] Ammonia (NH_3) concentration taken 1 hour before feeding (-1 h) or 4 hours after feeding (4 h)
[6] Dry matter digested (%) over 24 h with a dilution rate of 0.06 h^{-1}
[7] Organic matter digested (%)
[8] pH taken 1 hour before feeding (-1 h) or 4 hours after feeding (4 h)
[9] Rumen undegradable nitrogen

Table 6.5. Volatile fatty acid (VFA) and lactate production from digestion of three commercial exotic ruminant complete feeds of varying starch and fiber levels with alfalfa cubes[1] using a continuous culture system with mule deer (*Odocoileus hemionus*) or lactating dairy cow (*Bos taurus*) rumen fluid inoculum.

SCFA -1 h[4]	Mule Deer Inoculum[2]			Dairy Cow Inoculum[2]			SE	P-values[3]		
	Diet A	Diet B	Diet C	Diet A	Diet B	Diet C		Inoc	Diet	Inoc x Diet
Total VFA (mM)[5]	77.73	66.74	73.76	70.65	69.12	83.52	5.95	0.73	0.21	0.38
Lactate (mM)	0.32	0.28	0.65	0.37	0.52	0.82	0.10	0.07	<0.01	0.64
Acetate:Propionate Ratio	2.58	2.52	2.96	3.31	3.17	3.90	0.14	<0.01	<0.01	0.57
				mol/100 mol						
Acetate	60.36	62.15	66.13	67.95	67.21	71.88	0.89	<0.01	<0.01	0.36
Propionate	23.97	25.11	22.59	20.59	21.22	18.45	0.95	<0.01	0.03	0.92
Isobutyrate	0.00	0.00	0.00	0.08	0.03	0.01	0.02	0.02	0.16	0.16
Butyrate	14.27[a]	11.20[b]	10.26[bc]	8.66[cd]	9.39[bcd]	7.94[d]	0.76	<0.01	0.01	0.04
Isovalerate	0.30	0.21	0.13	0.63	0.44	0.33	0.04	<0.01	<0.01	0.21
Valerate	1.09[de]	1.33[cd]	0.90[e]	2.09[a]	1.71[b]	1.39[c]	0.11	<0.01	<0.01	0.02
SCFA 4 h[4]										
Total VFA (mM)[5]	90.53	81.00	89.36	88.14	97.68	95.56	3.95	0.04	0.66	0.07
Lactate (mM)	2.29	2.64	2.63	3.69	4.82	4.86	0.45	<0.01	0.18	0.60
Acetate:Propionate Ratio	4.81	4.52	5.49	3.58	3.39	4.00	0.24	<0.01	<0.01	0.75
				mol/100 mol						
Acetate	66.07	68.77	74.35	68.15	67.49	71.44	1.01	0.40	<0.01	0.06
Propionate	14.00	15.70	13.68	19.16	20.03	17.92	0.77	<0.01	0.04	0.81
Isobutyrate	0.14	0.13	0.04	0.22	0.18	0.11	0.02	<0.01	<0.01	0.57
Butyrate	18.10[a]	13.59[b]	10.86[c]	9.55[c]	10.08[c]	8.60[c]	0.95	<0.01	<0.01	<0.01
Isovalerate	0.29	0.22	0.10	0.60	0.41	0.34	0.04	<0.01	<0.01	0.32
Valerate	1.40[c]	1.60[bc]	0.97[d]	2.32[a]	1.82[b]	1.60[bc]	0.13	<0.01	<0.01	0.03

[1] Dietary treatments consist of 75% complete feed and 25% alfalfa cubes.
[2] Diet A = High starch/low NDF (forage fiber basis); Diet B = High starch/low NDF (by-product fiber basis); Diet C = Low starch/high NDF
[3] Inoc = Mule Deer inoculum vs. Dairy Cow inoculum; Diet = A vs. B vs. C; Inoc x Diet = Interaction the main effects
[4] Short-Chain Fatty Acid samples were taken 1 h before the morning feeding (-1 h) or 4 h after the morning feeding (4 h)
[5] Total VFA = Acetate + Propionate + Isobutyrate+ Butyrate+ Isovalerate + Valerate
[abcde] Means with no superscripts in common within the same row are statistically significant

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VITA

Matthew Alan Brooks was born in Anderson, SC in 1981, and grew up in the town of Pendleton, SC. He is the son of Jeffrey Brooks and Elizabeth Brooks. He has one brother, Major Robert Brooks, USAF. In August of 2005, Matthew married Stephanie Renee Bush, NCC, LPC. Stephanie graduated with her Bachelor of Arts in Psychology from Baylor University, and she went on to get her Master of Science in Counseling from Texas A&M University, Commerce.

Matthew started his undergraduate career in 1999 at Clemson University in the Animal and Veterinary Sciences department. In 2001, he transferred to Cornell University and graduated in 2003 with a Bachelor of Science in Animal Science. After spending two years working at the Fort Worth Zoological Association in their Nutrition department, he began his master's program at Texas A&M University in August of 2005 as a graduate student in the Intercollegiate Faculty of Nutrition. He conducted his research under the direction of Dr. Stephen B. Smith, defended his thesis in October 2007 and was awarded his M.S. in Nutrition in December 2007.

Matthew started his doctoral program at University of Missouri – Columbia in August of 2007 as a graduate student in the Department of Animal Sciences. He conducted his research under the direction of Dr. Monty S. Kerley, defended his dissertation in August 2010, and was awarded his Ph.D. in Animal Sciences with an emphasis in Ruminant Nutrition in December 2010.

Matthew is an Eagle Scout and is a member of the American Society of Animal Scientists, the Nutritional Advisory Group, the Comparative Nutrition Society, Phi Kappa Phi and Alpha Zeta.

Matthew and Stephanie will be welcoming their first child, Jonathan David Brooks, into this world in late October 2010. At which time, Matthew will be pursing his interest in comparative nutrition researching trace mineral bioavailability as a Postdoctoral Fellow at North Carolina State University under the direction of Dr. Jerry Spears.