This is to certify that the

dissertation entitled

Development and initial testing of an improved model for prediction of daily microbial nitrogen flow from the rumen of the dairy cow

presented by

Mary Beth Roe

has been accepted towards fulfillment
of the requirements for

PhD degree in Animal Science

Major professor

Date 1/31/94

IMPROV

DEVELOPMENT AND INITIAL TESTING OF AN IMPROVED MODEL FOR PREDICTION OF DAILY MICROBIAL NITROGEN FLOW FROM THE RUMEN OF THE DAIRY COW

By

Mary Beth Roe

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Animal Science

1994

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ABSTRACT

DEVELOPMENT AND INITIAL TESTING OF AN IMPROVED MODEL FOR PREDICTION OF DAILY MICROBIAL NITROGEN FLOW FROM THE RUMEN OF THE DAIRY COW

By

Mary Beth Roe

A within-day model was developed for prediction of daily ruminal microbial nitrogen flow. Components of this model were: 1. prediction of hourly degradation of true protein, non-protein nitrogen, nonstructural carbohydrate (NSC), and neutral detergent fiber, 2. estimation of hourly rumen pH, 3. adjustment of microbial maintenance requirements according to hourly pH, and 4. adjustment of growth of NSC-degrading bacteria based on hourly degraded true protein: fermented NSC ratio. Predicted individual fermented nutrients, both hourly and daily, were evaluated for prediction of in vivo measured microbial nitrogen flow.

A dataset for development and testing of the within-day model was compiled. Microbial nitrogen flows were determined from fifteen cow studies conducted at several locations (75 observations). Diets were analyzed to fractionate carbohydrate and protein as well as estimate rate of ruminal degradation of each fraction.

Based on comparison of microbial nitrogen flow estimated using the within-day model to in vivo measured microbial nitrogen flow (75 obs.), laboratory effect in combination with within-day model estimates explained 69% of the variation.

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Variation was highly associated with the laboratory in which *in vivo* estimates were obtained (R^2=0.31). Accounting for amounts of individual daily fermented nutrients, especially true protein, NSC, and fat, improved microbial nitrogen flow estimation (R^2=0.86). Further research needs to be conducted to delineate relationships between amounts of individual fermented nutrients and microbial nitrogen flow as well as to more accurately assess rates and extents of nutrient fermentation.

In a separate experiment, daily microbial nitrogen flow from diets varying in carbohydrate and protein availability was estimated as a function of the microbial concentration in the rumen liquids and solids and the pool sizes and rates of passage of each. Differences in daily microbial nitrogen flow (g) per kg dry matter intake of diets comprised of different carbohydrate sources (high-moisture ear corn vs. ground shelled corn) and different protein sources (corn gluten feed vs. soybean meal) were found to be significant ($p<0.05$). The effect of diet on digestion of feeds *in situ* was found to be significant ($p<0.05$).

To my dad

....

Lord for

DEDICATION

To my dad,

..... the man who taught me to work hard, to trust in the Lord for everything, and, of course, to love cows.

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ACKNOWLEDGEMENTS

The author wishes to express her sincere appreciation to Dr. Charles J. Sniffen for encouraging her to go on for a PhD, providing support and enthusiasm as a major professor, and continuing as a mentor after leaving Michigan State.

Gratitude is also expressed to Dr. Mike Allen who was willing to serve as major professor after Charlie, and to the rest of the author's guidance committee: Drs. Werner Bergen, J. Roy Black, and Steven Rust.

Appreciation is extended to the author's parents, Reynolds and Audrey Roe, for their encouragement and prayers.

Special thanks are expressed to the people of Miner Institute for accepting and helping a strange graduate student who seemed to always want others to dig in rumens with her. Thanks go especially to Wanda, Katie, Karen, Dawn, and Donny.

The author would like to thank everyone else who helped her in degree completion and more, especially: Steve Mooney, Bill VanderKooi, Paula Gaynor, Amy Terhune, Dave Main, Dewey Longuski, Jim Liesman, Rich Beverly, Kitty O'Neil, Rick Kohn, Rick Dado, and Katharine Knowlton.

A multitude of thanks go out to the author's two best friends in Michigan: Sara Finton and Elizabeth Wiseman.

Finally, the author thanks the Lord -- for providing opportunities, friends, and strength.

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INTRODUCTION

The development of a microbial flow model which adequately reflects rumen metabolism yet is driven off of easily measured and observed inputs would benefit both the feed industry and the dairy farmer. A more complete understanding of the rumen fermentation, microbial growth, and microbial recycling would enable one to maximize microbial cell production with available feed ingredients and thus, decrease the amount of undegradable intake protein required in the diet, increase the fermentation of fiber in the rumen, and reduce wastage of nitrogen by the animal.

Studies have been undertaken to determine dairy cattle production responses to increases in dietary undegradable intake protein or supplementation of rumen-protected amino acids. However, responses have been inconsistent (Papas et al., 1984, Yang et al., 1986, Schingoethe et al., 1988, Wright and Loerch, 1988). One of the limitations in these studies is that the amount of nitrogen, let alone each amino acid, flowing from the rumen cannot be accurately predicted and thus, it is difficult to determine which amino acids need to be supplemented and to what extent. The microbial, undigested dietary, and endogenous protein all contribute to the amino acid pool flowing out of the rumen.

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Based on an 88 observation dataset, NE_l (mcal/d) was found to be an inadequate predictor of daily microbial nitrogen flow to the duodenum (Cummins et al., 1983, Santos et al., 1984, Prange et al., 1984, Rooke et al., 1985, Stern et al., 1985, Chamberlain et al., 1986, Madsen, 1986, Madsen and Hvelplund, 1988, Kirkpatrick and Kennelly, 1989, Glenn et al., 1989, McCarthy et al., 1989, Robinson and Kennelly, 1990, Sadik et al., 1990). Only 68% of the variation in microbial nitrogen could be explained based on the daily NE_l provided in the diet. Net Energy for Lactation (NE_l) was an especially poor predictor at higher intake levels as might be expected due to larger differences in carbohydrate and protein availability to the microbes with those diets.

In order to improve animal response prediction, computerized models have been developed to mathematically describe and compile scientific data. Models vary in their level of aggregation, ranging from totally empirical to fairly mechanistic, depending on the purpose of the model and the scientific information available to describe the system. Upon model evaluation using an independent database, one can decipher by sensitivity analysis those variables predicted to have the greatest impact on the system, determine the adequacy of their description in the model, and generate ideas for further scientific research to be conducted in order to improve the description of relationships.

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The objective of this work was to develop an improved model for prediction of daily microbial nitrogen flow from the rumen of the dairy cow which was at a high enough level of aggregation to be of practical use on the dairy farm yet sufficiently described the complexity of the rumen ecosystem. It was hypothesized that the prediction of individual nutrients available throughout the day and their relationships to microbial growth, as well as prediction of rumen pH, would allow one to more closely predict rumen microbial nitrogen flow among a variety of diets than one can do using an equation based solely on the total amount of nutrients digested by the animal (NE_l).

The steps involved to meet this objective were: 1.) the development of a within-day model for prediction of microbial nitrogen flow based on previous in vitro and in vivo research, 2.) the compilation of a microbial nitrogen flow database comprised of data from 15 different studies (75 microbial nitrogen flow observations), and 3.) evaluation of the within-day model performance as well as performance of multiple regression equations, using the microbial nitrogen flow database.

Finally, due to the expense and problems associated with microbial nitrogen flow estimation using duodenally cannulated cows, it was necessary to develop a less invasive method requiring only rumen cannulated cows in order to accumulate some of the data incorporated into the microbial nitrogen flow

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database. In a study conducted at Miner Institute, microbial nitrogen flow was measured based on the microbial concentration in the rumen liquids and solids and the pool sizes and rates of passage of each. In this study, the impact of within-day synchrony of carbohydrate and protein availability on microbial nitrogen flow was determined.

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LITERATURE REVIEW

A. Factors Influencing the Flow of Amino Acids to the Duodenum:

1. Microbial Amino Acid Flow:

Mixed culture microbial growth rates vary and microbial recycling in the rumen is not constant (Nocek and Russell, 1988). Microbial nitrogen flow from the rumen is affected by a variety of factors. For these reasons, the use of a microbial crude protein yield constant in models of the rumen system is inaccurate.

Carbohydrate Availability

When the microbial population in the rumen metabolizes nutrients, energy needed to carry out biosynthesis is obtained and cell mass is likely to be increased. As carbohydrate availability increases, more dietary amino acids are incorporated into the microbial pool, less dietary amino acid is used as an energy source, and less nitrogen is wasted as ammonia (Nocek and Russell, 1988). Stern et al.(1978) increased the level of starch in the diet at the expense of cellulose and observed greater microbial growth. Isaacson et

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al.(1975) found that increasing glucose concentration (5.8, 9.9, 12.7, and 25.0 mM) at each of three dilution rates (0.02, 0.06, and 0.12 per h) resulted in increased cell concentration without any affect on yields of cells or products produced per mole of glucose fermented. The amount of ATP generated for growth is determined by the biochemical pathway which is used to catabolize glucose or other substrates (Baldwin and Allison, 1983).

Hungate(1966) suggested that energy is the limiting factor for growth of microbes with a limit of 10 g microbial protein per 100 g of organic matter digested in the rumen. However, one of the major difficulties associated with predicting microbial protein yield from digestible organic matter is in measuring the rumen degradable organic matter (Czerkawski, 1978). Furthermore, this theoretical maximum is based on the assumptions that microbial protein production per kg OM fermented is constant and that all fermented organic matter is used solely for the production of ATP.

Synchronization of Carbohydrate and Protein Availability

It has been shown in continuous culture studies that when the level of nonstructural carbohydrate(NSC) is between 25 and 37% of DM, both energy and degradable protein(DIP) can limit microbial yield (Hoover et al., 1990, Stokes et al., 1991b). Above 37% nonstructural carbohydrate, microbial efficiency is a function of the level of degradable protein in the diet.

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Microbial yield decreased curvilinearly from 34.2 to 10.3 g bacterial N per kg DM digested as the nonstructural carbohydrate / rumen degradable crude protein ratio widened from 1.9 to 8.9 (Hoover, 1987, Stokes et al., 1991b). It was surmised that this inefficiency on the higher NSC:DIP diets was due to energetic uncoupling by the bacteria (Hoover, 1987). Energetic uncoupling is the use of energy for non-growth functions, such as heat production, when growth is limited by nutrients other than carbon. It must be recognized that this work does not take into account differences in actual amounts of fermented NSC but only total NSC in the diet which could vary in fermentability.

The level of degradable protein required to maximize microbial protein yield is still unknown (Hoover, 1988, Hoover and Stokes, 1991). Work based on simply degradable protein intake also does not help in understanding nitrogen stimulation of the bacteria versus amino acid or peptide stimulation of growth. Furthermore, it is difficult to determine the point at which amino acids are used as energy sources for the rumen microbes rather than being primarily used directly for protein synthesis.

Stimulation of microbial growth by ammonia has been studied both <u>in vitro</u> and <u>in situ</u>. Tungstic acid precipitable nitrogen production increased from 35% of maximum when ammonia was provided at 40 g/kg crude protein equivalent to 90% of maximum when 110 g/kg crude protein equivalent was provided <u>in</u>

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vitro but tended to level off at higher levels of ammonia. It was concluded that 50 mg ammonia nitrogen/liter of rumen fluid is enough to support maximum microbial growth (Satter and Slyter, 1974). Work with pure cultures of *B.amylophilus*, *B.ruminicola*, *B.succinogenes*, *B.fibrisolvens*, *M.elsdenii*, *R.albus*, *R.flavefaciens*, and *S.ruminantium* showed that all of these bacteria with the exception of *M.elsdenii* were saturated with ammonia at levels less than 14 mg ammonia nitrogen/liter (Schaefer et al., 1980). Based upon the rate at which barley was fermented *in situ* in sheep fed barley diets supplemented with graded levels of urea, it was concluded that 194 mg ammonia nitrogen/liter of rumen fluid was needed to maximize microbial growth and fermentation (Mehrez et al., 1977). This level of rumen ammonia is about four times greater than that recommended by Satter and Slyter(1974). Unfortunately, however, ammonia required per gram of carbohydrate fermented is difficult to determine from these studies. Based on the work of Russell and Sniffen(1984), it was calculated that 2.47 g ammonia N per 100 gm carbohydrate fermented was the most ammonia nitrogen utilized by the microbes either in the absence or presence of amino acid nitrogen. Odle and Schaefer(1987) found that more ruminal ammonia-nitrogen was needed to optimize the rate of fermentation of ground barley (125 mg/l) than was required for the fermentation of ground corn (61 mg/l). Barley tends to be more rapidly fermented than corn.

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Much in vitro work has been conducted to understand the stimulating effect of amino acids and peptides on growth of the rumen bacteria. Russell et al.(1983) supplied mixed continuous culture incubations with mixed carbohydrate(160 mg/l/h) and supplemented incremental amounts of casein. Ammonia concentrations were maintained at a level which was presumed to be adequate. It was found that there was a significant increase in cell protein synthesis when casein was increased from 0 to 2100 mg/l, with the response to addition of casein being most significant at levels less than 250 mg/l. Ammonia tended to accumulate when casein was provided at levels higher than 250 mg/l.

Research has also been conducted to determine the effect of additions of specific isoacids and/or trypticase on batch culture growth of microbes obtained from a cow fed timothy hay (Russell and Sniffen, 1984). Addition of isovalerate and 2 methyl-butyrate increased mg cell protein / mg OM utilized by 11.2% and 16.4%, respectively. When isovalerate, 2 methyl-butyrate, valerate, and isobutyrate were all added, microbial protein growth was improved by 18.7%. Trypticase supplementation also improved growth by as much as 18.7%. Ammonia was not limiting in any of the incubations.

Maeng and Baldwin(1976) obtained rumen fluid from a cow fed a purified diet containing urea as the sole source of nitrogen and incubated the rumen fluid in vitro with 0, 15, 30, and 45 mg C^{14}-labelled amino acid nitrogen per 100 ml,

replacing urea nitrogen incrementally. Estimated grams of microbial protein per kg digested carbohydrate were 91.2, 124.4, 140.1, and 147.5 for the 0, 15, 30, and 45 mg amino acid-N/100 ml diets, respectively.

Pure continuous culture incubations of B.ruminicola, S.ruminantium, S.bovis, M.elsdenii, and B.fibriosolvens were grown with glucose at .5 g/l and amino acids at 0, .016, .031, .062, .125, .25, and .5 g/l (Cotta and Russell, 1982). Efficiency of conversion of amino acids to bacterial protein was greatest at levels below .031 g/l.

Work conducted in vivo has also indicated that both nitrogen and amino acids are key nutrients besides energy which affect efficiency of microbial growth. Sinclair et al.(1993) devised diets which were equivalent in metabolizable energy (9.5 MJ/kg DM) and degradable intake protein (96 g/kg DM) but were intended to have either a synchronized rate of nitrogen and energy fermentation or an asynchronous rate of fermentation. Microbial N / kg DM intake was 27% higher on the synchronous diet with efficiency of microbial synthesis (g N / kg OM truly degraded) being 13% greater. However, neither of these differences was statistically significant. Herrera-Saldena et al.(1990) conducted a study with cannulated cows in a 4x4 Latin square design with either barley or milo as the primary energy source and either cottonseed meal or brewers dried grain as the protein source. Barley and cottonseed meal were intended to be rapidly fermented while milo and brewers

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dried grain were expected to be slowly fermented. The barley-cottonseed meal diet yielded significantly more microbial N per kg fermented OM than any of the other diets. It was surmised that this result was due to an improvement in synchronization of carbohydrate and protein availability but hourly fermentability of the energy and protein sources was not assessed.

Sulfur, specific peptides or amino acids, and branched chain volatile fatty acids can also limit microbial protein production in the same manner as limited degradable protein can, especially when there is a limited amount of rumen degradable protein in the diet (Bryant et al., 1959, Faichney and White, 1979, Robinson, 1983, Hoover and Miller, 1993).

Nitrogen Absorption and Recycling

Non-protein nitrogen compounds in the rumen accumulate from dietary sources such as urea, from degraded protein compounds, and from recycled nitrogen present in saliva. Excess ammonia beyond what the microbial population requires is absorbed from the rumen into the blood and either excreted or recycled. It is difficult, however, to predict rumen ammonia concentrations at any one time.

Work has not been conducted to predict the amount of dietary protein which is totally degraded to ammonia and isoacids prior to utilization versus that dietary protein incorporated directly as amino acids or peptides into

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microbial protein. It is known, however, that amino acids do accumulate at low levels in rumen fluid (Wright and Hungate, 1967). Peptides have also been found to be converted to microbial protein more efficiently than amino acids (Wright, 1967). Chen et al.(1987a) presented evidence to suggest that the uptake of peptides by the rumen microbes could be a step which limits the rate of protein degradation in the rumen. It was estimated from one study conducted using $^{15}NH_3$ dilution methods that 62% of rumen bacterial nitrogen was incorporated in the form of ammonia, leaving 38% derived directly from peptides and amino acids (Nolan and Stachiw, 1979).

The amount of ammonia absorbed from and recycled back into the rumen must also be understood more fully in order to predict rumen ammonia concentrations. Since it is known that ammonia is preferentially absorbed over the ammonium ion, it is expected that higher ruminal pH levels will result in increased ammonia absorption from the rumen (Russell, personal communication). Hogan(1961) estimated that the transport of ammonia across the rumen wall was three times greater at a pH of 6.5 than at pH 4.5. Roffler and Satter(1975) estimated ruminal ammonia concentrations as being negatively related to the concentration of total digestible nutrients in the ration and positively related to the crude protein content of the diet. Unfortunately, this model does not take into account variations in rumen availability of protein and carbohydrate within feed source in addition to subsequent variations in

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rumen ammonia concentrations throughout the day and thus, is of limited use. The NRC(1985) recommended the use of an equation for the estimation of recycled nitrogen which was derived from data from a study in which cattle were eating 2.5% of their body weight as dry matter. This recycled nitrogen estimation is solely a function of the amount of protein consumed by the animal (Kennedy and Milligan, 1980). Nitrogen conservation by the animal is, however, likely to vary with level of productivity. Furthermore, as mentioned previously, the amount of rumen ammonia utilized by the microbes varies depending upon the energy available for microbial growth and saliva production at any time, making attempts to predict recycled nitrogen and absorption of rumen ammonia based entirely on ration crude protein content inappropriate.

Rumen Available Fat

When the level of fat in the diet exceeds about 5% of the total dry matter, it has been found that fiber digestion decreases due to inhibition of rumen microbial growth (Palmquist and Jenkins, 1980, Maczulak et al., 1981, Boggs et al., 1987).

The rumen microorganisms hydrolyze triglycerides to form glycerol and fatty acids. Glycerol is used as an energy source by organisms such as Anaerovibrio lipolytica, while long chain fatty acids are subjected to biohydrogenation and

are not used as an energy source by the anaerobic rumen bacteria (Emery and Herdt, 1991). A free carboxyl group must be present in order to begin biohydrogenation (Palmquist and Jenkins, 1980). During the process of biohydrogenation, unsaturated fatty acids are first changed from the cis form to the trans form before being completely saturated. Harfoot(1978) suggested that the sequence for biohydrogenation of linoleic acid(18:2 cis-9, cis-12) was through an 18:2 cis-9, trans-11 conjugated diene, to an 18:1 trans-11 monoenoic acid, to 18:0 stearic acid. Accumulation of the trans-11 isomer in the rumen has been noted and it has been suggested that this isomer may be a key intermediate in the conversion process (Harfoot, 1978). The enzymes necessary for biohydrogenation have been found to be present in microbial cells but absent from the cell-free supernatant and washed bacteria (Viviana, 1970). This indicates that the biohydrogenation enzymes are probably extracellular, cell-associated enzymes.

Ruminococcus albus, a gram positive rumen cellulolytic bacteria, has been found to be inhibited by long chain fatty acids. Henderson(1973) measured the growth of R.albus after incubation with capric($C_{10:0}$), lauric(C_{12}:0), myristic($C_{14:0}$), palmitic($C_{16:0}$), stearic($C_{18:0}$), or oleic($C_{18:1}$) acid in concentrations from 0.005 to .5 g/l for 16 hours. Cellobiose (0.4%, w/v) was the only energy source and optical density was used as a measure of growth. All of the long chain fatty

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acids inhibited growth, however, oleic acid had the greatest inhibitory effect by decreasing growth by 80% relative to the control at a concentration of only 0.1 g/l (or .01%). Stearic acid, the saturated equivalent of oleic acid, was much less inhibitory. At .5 g/l (or .05%) of stearic acid, growth was inhibited by only 45% relative to the control. It was hypothesized that the fatty acids adhered to the cell walls of the bacteria and inhibited passage of nutrients but this hypothesis was not tested. It seems significant that cellobiose was used as the energy substrate rather than cellulose and that growth was still limited in the presence of long-chain fatty acids. If long-chain fatty acids only diminish growth by inhibiting the release of extracellular cellulases from the organisms, one would have expected growth not to be limited in this experiment.

Maczulak et al.(1981) analyzed the growth of R.albus in the presence of increasing concentrations of palmitic(C16:0), stearic(C18:0), oleic(cis 9, 18:1), and vaccenic(trans 11, 18:1) acids. Cellobiose was used as the energy substrate and changes in optical density were used to measure growth. When palmitic, stearic, and vaccenic acids made up 0.01% of the culture media, growth relative to the control was inhibited by 12%, 10%, and 19%, respectively. However, when oleic acid was included in the growth media at only .0005%, growth was decreased by 81% relative to the control incubation. The authors suggested that inhibition could be related to the

ability of the long-chain fatty acids to form insoluble soaps in the culture media. The order in which these fatty acids formed complete calcium salts was: stearate > vaccenate > palmitate > oleate. Wu et al.(1991) determined that only 57% of fatty acids fed to dairy cows in the form of calcium soaps were biohydrogenated.

When particulate cellulose was added to the media containing long-chain fatty acids, inhibition was reduced (Maczulak et al. 1981). It is assumed that the cellulose competed with the bacteria for surface lipid adsorption. When Harfoot et al.(1974) incubated mixed rumen bacteria in the presence of long-chain fatty acids, growth was inhibited less when food particles were added to the media.

There is research which indicates that dietary fatty acids are taken up to a significant extent by the rumen microbial population rather than simply remaining adhered to the outside of the bacterial cell walls. Bauchart et al.(1990) analyzed the fatty acid composition of solid-adherent bacteria and liquid-associated bacteria obtained from fistulated cows fed diets containing supplemental lipids. The lipid content of the solid-adherent bacteria was found to be 1.7 - 2.2 times higher than that of the liquid-associated bacteria. It is known that lipids have a tendency to adsorb to solid food particles, therefore, the solid-adherent bacteria would be expected to have greater access to lipids. Lipids were also observed by transmission electron microscopy

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to be in droplets within the cytoplasm of rumen bacteria, rather than solely associated with the cell envelope (Bauchart et al., 1990). Linoleic acid was specifically found in high concentrations within the total free fatty acid of the cell. Triacylglycerols which were not present in the microbes from the control diets, made up 2 - 4% of the total lipids in the bacteria from the cows fed the lipid supplemented diets (Bauchart et al., 1990). Furthermore, as the concentration of unsaturated lipid in the diet increased, there was a proportional decrease in the amount of unsaturated lipid which was biohydrogenated (Bauchart et al. 1990). Perhaps this finding indicates that an upper limit to the rate of unsaturated fatty acid biohydrogenation exists.

Demeyer et al.(1978) incubated mixed rumen microorganisms taken from a sheep with [1-^{14}C]linoleic acid, [U-^{14}C]glucose, or [1-^{14}C]acetate and determined into which lipids the radioactivity was incorporated. Most of the radioactivity was found to be associated with saturated fatty acids present as free fatty acids or incorporated into sterolesters. However, much of the radioactivity was also contained in unsaturated fatty acids of the polar lipids, such as phospholipids and galactolipids. Furthermore, it was concluded that more of the bacterial lipids were made from preformed fatty acids rather than being synthesized de novo.

The lipid fraction of gram-positive organisms in the rumen is comprised mostly of phosphatidylglycerol and

phosphatidylethanolamine, with the acyl chains being primarily odd-numbered branched-chain fatty acids (Harwood and Russell, 1984). The fluidity of the cell membrane is determined by the fatty acids within the membrane phospholipids. Unsaturated fatty acids present in the cis-form are known to pack less tightly than those in the trans-form. Katz and Keeney(1966) found that there were less bacterial unsaturated fatty acids in the trans-form than in the cis-form which were nondialyzable. It was assumed that the nondialyzable fraction consisted of esterified fatty acids. Therefore, they interpreted their results to mean that the structural or polar lipids were primarily made up of cis-unsaturated fatty acids. Published research specifically describing the rumen bacterial cell membrane lipid composition and their fatty acid content has not been found.

The effects of the different lipid classes on rumen bacterial membrane function have not been investigated in much detail. Work has been done with other types of bacteria, such as E.coli, and for the time being one can extrapolate from those results to the rumen bacteria. It is known that the function of the phospholipid bilayer as a barrier is affected by its lipid composition. Unsaturated fatty acids make the membrane more permeable and saturated fatty acids reduce membrane fluidity (Cronan, Jr. et al., 1987). Shorter fatty acid chain length also reduces the barrier function (Cronan, Jr. et al., 1987). Cholesterol is known to be inserted into

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the kink formed by cis-unsaturated fatty acids, increase van der Waals contact, and decrease membrane fluidity (Rawn, 1989). It has been postulated by researchers that the interaction of membrane lipids and proteins affect membrane function but the results are inconclusive (Cronan, Jr. et al., 1987, Rawn, 1989).

Maintenance Energy

The rumen microbes require energy in order to carry out maintenance functions including: motility, turnover of cell macromolecules, active transport, and energetic uncoupling (Hespell and Bryant, 1979). As the maintenance requirement increases, less energy is available for microbial growth. Pure culture studies with individual bacteria have found a range in maintenance requirements from .022 to .187 g glucose / g bacteria / hour (Russell and Baldwin, 1979). The cellulolytic bacteria tend to have lower maintenance requirements than the rest of the rumen bacterial population. For this reason, it should be beneficial to take into account differences in the percentages of different types of microbes present in the rumen each having different maintenance requirements and thus, different overall growth per unit of carbohydrate fermented.

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Turnover Rate

Besides the nutritional requirements of the rumen microbes, other factors which impact microbial growth rate and overall flow must be considered in describing the rumen system. Efficiency of microbial protein yield increases as rate of feed passage through the rumen increases due to reduced cell recycling, decreased mean age of the cells escaping the rumen, and reduced microbial maintenance requirements (Van Soest, 1982, Robinson, 1983). Isaacson et al.(1975) determined that the grams of cells produced per mole of glucose fermented increased from 42 at a dilution rate of .02 h^{-1} to 84 at a dilution rate of .12 h^{-1}. Diets high in concentrate feeds are known to have lower turnover rates (Robinson, 1983), causing an increase in microbial maintenance requirements while at the same time providing more ATP for cell synthesis (Bergen et al., 1980). However, the extent of organic matter digestion is decreased with higher turnover rates lowering the amount of ATP available for use by the microbes (Bergen et al., 1980).

It has been found that when intake is increased on high fiber diets, there is a corresponding increase in NDF turnover rate and a reduction in the ratio of bacterial OM / NDF in the rumen contents (Sniffen and Robinson, 1987). It was suggested that larger particles with more attached microbes were flowing from the rumen at higher intakes. Rode et al.(1985) found that the efficiency of microbial protein synthesis increased

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as the amount of forage in the diet increased or rate of passage of solids increased. Grams bacterial nitrogen per kg of OM truly fermented in the rumen increased from 33.5 on a 24% forage diet to 38.6 when forage was increased to 80% of the diet. Feng et al.(1993) found a decrease in efficiency of microbial protein yield per kg of OM digested with higher NSC and higher degradable fiber diets.

Due to variations in turnover throughout the day, steady state conditions in the rumen cannot be assumed. Chen et al.(1987b) found that when he fed cows twelve times per day, rumen volume and liquid dilution rate were fairly constant throughout the day at 69.7 L (SE=1.6) and .14 h^{-1} (SE=0.005), respectively. However, when cows were only fed once per day, liquid dilution rate was highest two hours after feeding. Rumen volume ranged from 68 to 90 L (78.7 L +-9.5) and liquid dilution rate ranged from .04 to .23 h^{-1} (.13 h^{-1} +-.09) throughout the day when cows were fed only once.

Rumen Protozoal Predation

Rumen protozoa can make up as much as one-half of the microbial mass in the rumen and are known to utilize bacterial protein (Bryant, 1977). Coleman and Sandford (1979) found that the rate at which bacteria were engulfed by protozoa varied from 230 to 2670 bacteria per protozoan per hour. Uptake varied depending on both the type of bacteria and the type of protozoa. For example, Butyrivibrio fibrisolvens was

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digested more rapidly than any of the other bacterial sources investigated. Protozoa are also known to rapidly take up starch granules and thus, have a moderating influence on the rate of ruminal starch fermentation and reduce the incidence of acidotic conditions in the rumen (Russell and Hespell, 1981).

pH

A reduction in ruminal pH, as with high concentrate diets, can diminish microbial yield (Russell et al., 1979, Hoover, 1988). When environmental pH is low, hydrogen ions leak into the cell and the cell must expend energy to remove the hydrogen ions from the cell in order to maintain near neutral intracellular pH (Strobel and Russell, 1986). Thus, the requirement for maintenance energy by the cell is increased. With reduced pH, there is also a tendency for some organisms to switch to a lactate fermentation which yields less ATP per mole of fermented glucose. Furthermore, there is a shift in the bacterial population towards those bacteria, usually-non-cellulolytics, which are more acid tolerant, favor lactate production, and have higher maintenance requirements.

<u>In vitro</u> studies have been conducted in order to quantify the detrimental impact of low environmental pH on bacterial growth. Russell and Dombrowski(1980) investigated the effect of reducing media pH in .25 pH unit increments from pH 6.75 to 4.75 on 10 species of rumen bacteria grown in continuous

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culture. Cellulolytic bacteria, such as R.albus and R.flavefaciens, tended to reduce their growth at pH 6.25 and were totally washed out of the chemostat when the media pH was less than 5.75. Amylolytic bacteria, such as S.ruminantium and B.ruminicola, tended to grow best when pH was about 6.00 and growth was not significantly reduced until pH was less than 5.75. They were not washed out of the vessel until pH reached 5.00. Other species, such as, S.bovis and M.elsdenii, were much more acid tolerant and growth was not significantly reduced until pH 5.00. Pure culture work conducted by Russell et al.(1979) yielded results similar to those of Russell and Dombrowski(1980). M.elsdenii has been reported to ferment 61 to 97% of the lactate produced on high concentrate diets (Counotte et al., 1981).

Mixed rumen bacteria have been grown in carbohydrate-limited batch culture at either an initial pH of 6.7 or 6.0 (Strobel and Russell, 1986). Protein synthesis was decreased 34 to 69% when initial pH was reduced from 6.7 to 6.0. This reduction was accompanied by a decrease in carbohydrate fermentation as well as ATP production and an increase in lactate production. However, the sum of these changes could not totally account for the reduction in bacterial growth observed. For this reason, energy uncoupling by the bacteria was assumed to make up the difference.

Russell(1987) subsequently conducted work with the cellulolytic bacterium, Bacteroides succinogenes, grown under

more acidic conditions. It was found that when pH decreased from 6.9 to 5.7, the difference between intracellular and extracellular pH was maintained using high proton motive force. Below pH 5.7, proton motive force, intracellular pH, as well as the use of cellobiose decreased.

Prediction of ruminal pH is difficult. Researchers have attempted to estimate production of volatile fatty acids because they are known to probably have a great impact on ruminal pH (Morant et al, 1978). Emery et al.(1956) pooled data from four trials with cows fed either primarily concentrate or forage and determined that the millimoles of acids per 100 gm of rumen fluid produced from .45 kg of TDN were, 0.24 to 0.67 acetic acid, 0.02 to 0.26 propionic acid, and 0.04 to 0.21 butyric acid. Bauman et al.(1971) found that propionate production as measured by isotope dilution, varied from 13.3 moles/day on a high fiber diet to 31.0 moles/day on a high grain diet.

The major difficulty in predicting volatile fatty acid production in the rumen arises not so much in the estimation of the stoichiometric parameters but rather in estimating the availability of glucose and other fermentable monosaccharides present in starch, fiber, and protein. Murphy et al.(1982) developed a model which predicted acetate, propionate and butyrate production based on stoichiometric parameters for ruminal fermentation of soluble carbohydrate, starch, hemicellulose, cellulose and protein. Since the fermentation

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patterns of these fractions vary, it was necessary to include them separately within the model. In order to successively apply this type of model on the farm, it is imperative that routine laboratory techniques be developed for determining the carbohydrate fractions and their rates of digestion. It is also quite possible that predictability will be further improved by the addition of other feed fractions, such as protein fractions, to this type of model.

Other factors besides monosaccharide availability should also be considered when predicting volatile fatty acids present in the rumen. Variations in pH can result in shifts in the types of acids produced by the microorganisms as, for example, with S.bovis. Robinson et al.(1986) found decreases in total volatile fatty acid and in D,L-lactic acid production with increased rumen pH. The protozoal population also has an effect. Grummer et al.(1983) fed a diet with 45% dried whole whey to steers and found high butyrate production in the presence of protozoa but high propionate production when the rumen was defaunated. The capacity of feeds to act as buffers varies and impacts ruminal pH. For instance, lignin, pectin and hemicellulose are known to have a greater cation-exchange capacity than compounds such as cellulose and starch (Van Soest, 1982). Ionophores, which are currently included in many dairy heifer rations, are known to increase the molar percentage of propionate produced in the rumen and reduce protein degradation by inhibiting the growth of the gram-

positive bacteria (Van Maanan et al., 1978, Chen and Russell, 1991). Dietary buffers increase ruminal pH and increase the acetate:propionate molar ratio in the rumen (Erdman, 1988). Furthermore, the rate of absorption of volatile fatty acids across the rumen wall may vary. The data of Peters et al.(1992) suggests that with increases in acetic acid production, fractional rate of acetic acid disappearance in the rumen decreases.

Physical characteristics of the feed consumed impact the degree of saliva production for buffering the rumen system. It is estimated that beef and dairy cows produce 108 to 308 L/d of saliva and this is equal to 390 to 1115 g/d of disodium phosphate and 1134 to 3234 g/d of $NaHCO_3$ secretion (Erdman, 1988). Erdman(1988) estimated that a one percentage unit decrease in dietary ADF results in a .0564 unit decrease in rumen pH due primarily to a reduction in chewing and saliva secretion. However, besides fiber content, length and specific gravity of fibrous particles should also be taken into consideration (desBordes and Welch, 1984). Jaster and Murphy(1983) found a reduction in the total chewing time of heifers as forage particle size decreased on diets containing 63% NDF.

Amino Acid Composition of the Microbial Population

It has been concluded by some researchers that the amino acid composition of the microbes flowing out of the rumen is

fairly constant regardless of variations in diet composition (Bergen et al., 1968, Leibholz, 1972, Storm and Orskov, 1983, Hvelplund and Hesselholt, 1987), indicating that flow of microbial amino acids can be predicted with a knowledge of the flow of microbial protein. Researchers have examined bacteria isolated from the rumens of cows consuming four different diets and concluded that no significant differences in amino acid digestibility exist. Digestibility varied between .80 and .91 for individual amino acids (Hvelplund and Hesselholt, 1987).

However, when dietary treatments result in a major change in the dominating bacterial populations in the rumen, significant changes in the composition of the bacteria may be possible. Hvelplund(1986) found a positive linear relationship between the starch and sugar content of the diet and the proportion of amino acid nitrogen in the total nitrogen of isolated bacteria up to about 35% starch and sugar in the diet. Bacteria isolated from diets with more than 35% starch and sugar tended to have a lower proportion of amino acids in the crude protein. Crude protein as a percentage of bacterial dry matter ranged from 39 to 56% with amino acid nitrogen making up about 67% of the crude protein on the 24 diets analyzed. Bergen et al.(1967) found marked differences in the quality of protein from individual strains of rumen bacteria and suspected this to be a result of differences in the digestibility of protein or individual amino acids within

the organisms. Furthermore, it has been found that Gram-negative rumen bacteria are digested to a greater extent by pepsin and pancreatin in vitro procedures than Gram-positive bacteria are (Wallace, 1983). However, at the same time it was recognized that the influence of different proportions of these bacterial types on the digestibility of mixed ruminal bacteria is small (Wallace, 1983).

Variations in the nucleic acid content of rumen bacteria have also been investigated. It has been found that with increases in dilution rate, microbial content of RNA increases (Kjeldgaard, 1967, Bates, 1985). John(1984) found the concentration of RNA in bacteria to be highly correlated with ruminal gas production. The RNA-N:total N ratio of mixed rumen bacteria has also been found to vary with time of sampling in relation to feeding and dietary conditions (Smith and McAllan, 1974). Increases in cell yield may then be slightly offset by a decrease in the proportion of amino acid nitrogen contained in a unit of microbial crude protein. Walker and Nader(1970) found that polysaccharide per unit of DNA varied with time after feeding. Polysaccharide per unit of DNA rapidly increased in the rumen fluid organisms up to four hours after feeding and then slowly declined. From these data it can be hypothesized that the percentage of polysaccharide in the microbes flowing from the rumen on an energy-dense diet may be different from that of organisms on a high fiber diet.

2. Dietary Amino Acid:

A number of factors influence the extent to which dietary amino acids are degraded in the rumen and thus, the types and amounts of dietary amino acids flowing out of the rumen. The degradation rate of a feed varies depending on its proportions of non-protein nitrogen(A), true protein(B), and unavailable protein(C) (Pichard and Van Soest, 1977, Berger, 1986). Each of the true proteins: albumins, globulins, glutelins, and prolamines, differ in rumen solubility and degradability (Sniffen, 1974, Blethen et al., 1990). Feedstuffs are made up of a variety of fractions, each affected differently by pH and thus, possessing various rates of degradation (Broderick and Craig, 1980). The possibility of predicting amino acid degradation of the true protein fractions from predictions of the extent of protein degradation of these fractions has not been thoroughly examined. Susmel et al.(1989) incubated soybean meal, fish meal, dried brewers' grains, and alfalfa silage in polyester bags in the rumen and examined the change in amino acid composition due to fermentation. Methionine was always more highly degradable, threonine was low in degradability, while degradation of the other amino acids was generally dependent on feed type. The distribution of amino acids in the various protein classes (albumins, prolamins, glutelins, and globulins) may be partly responsible for these differences (Tamminga, 1979). The degradation of the total amino acids in soybean meal and alfalfa silage was generally

lower than crude protein degradability. Soluble peptides and the hydrophobicity of these peptides which accumulate in rumen fluid may also need to be accounted for (Chen et al., 1987a, Chen et al. 1987c).

Ruminal residence time also has a significant affect on extent of dietary amino acid degradation (Tamminga, 1979). Rumen models have attempted to predict rate of passage of individual feeds, however, the effect which the entire diet has on rates of feed passage has not been fully addressed in a model (Sniffen et al., 1992). The amino acid degradability of soybean meal present in a high concentrate diet consumed at a low level which passes slowly through the rumen, may be quite different from that of soybean meal present in a diet with a higher level of forage consumed at a high level.

The extent to which dietary amino acid digestion varies, depending on the type and amount of microbes present in the rumen, warrants further study. Rumen fluid proteolytic activity varies from 0.54 to 204 mg casein/hr/ml of rumen fluid as reported in the literature (Krishnamoorthy, 1982). Rumen microbial content however, is the major determinant of proteolytic activity rather than the amount of free protease (Krishnamoorthy, 1982). Furchtenicht and Broderick(1987) found that the rate of casein degradation was highest in a 37% forage diet, intermediate in a 63% forage diet, and lowest in a 100% forage diet. However, others have found that variations in the level of energy in the diet which should

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affect the microbial population, had little effect on dietary amino acid degradability (Russell et al., 1983, Cecava et al., 1988).

Differential use of dietary amino acids by the rumen microbial mixture may also be a factor to be considered. Some researchers have observed differences between amino acids in their rate of assimilation in the rumen (Hvelplund and Hesselholt, 1987, Fugimaki et al., 1989). However, others have not seen variations in amino acid uptake (Broderick et al., 1988). Species such as S.bovis, may be more prominent in the rumens of animals fed large amounts of starch and sugars resulting in use of different amino acids than would be incorporated on a high fiber diet.

3. Endogenous Amino Acid:

Amino acids derived from endogenous sources such as, enzymes, bile, mucus, serum albumin, lymph, and epithelial cells also contribute to the pool to be absorbed from the small intestine (NRC, 1985). Phillipson(1964) estimated that 0.6 g N in the form of bile and 1.5 to 3.0 g N in pancreatic juice entered the duodenum each day in a 40 kg sheep. When the endogenous protein from other sources and from the rest of the intestine were taken into account, it was estimated that a total of 3 to 16 g endogenous amino acid nitrogen entered the small intestine on a daily basis in sheep (Nolan, 1975). Since the presence of food in the duodenum stimulates

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secretion, one could expect proportionally large increases in secretions in a 600 kg cow eating 30 kg of DM per day.

B. Current Procedures for Estimating the Protein and Carbohydrate Fractions and their Degradability:

1. Protein

The rate at which feed protein is degraded in the rumen is complicated by numerous factors which involve the nature of the feed, the individual cow, and the total ration being fed. Crude protein can be fractionated into non-protein nitrogen(NPN), true protein, and unavailable protein, or, the fractions, A, B, and C, respectively (Pichard and Van Soest, 1977). Non-protein nitrogen is rapidly converted into ruminal ammonia while true protein is degraded at a slower rate and the unavailable fraction is not degraded. The extent of protein degradation of a feed will vary depending on its proportions of these fractions and the rate at which the feed passes through the rumen (Balch and Campling, 1965, Tamminga, 1979, Berger, 1986).

True proteins degrade at variable rates (Table 1). Albumins are water soluble while globulins are soluble in dilute salt solutions (Blethen et al., 1990). These high-quality proteins are assumed to be primarily used as a source of amino acids for the rumen microbes unless they are denatured by heat processing. Soy protein contains large

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amounts of albumins and globulins. Prolamines are soluble in alcohol and glutelins solubilize only in dilute alkali (Blethen et al., 1990). These lower quality proteins tend to degrade more slowly in the rumen. Extensin proteins are slowly degradable proteins which are most frequently associated with the plant cell wall (Van Soest, 1982).

Laboratory analysis procedures have been developed which categorize proteins into the fractions: A, B_1, B_2, B_3, B_4, and C (Table 1). These fractions have similar chemical properties and can potentially impact animal performance if varied. Tungstic acid precipitation of true protein can be used to calculate non-protein nitrogen(NPN), fraction A, by difference (Pichard and Van Soest, 1977). Soluble CP, is comprised of NPN in addition to soluble peptides(B_1) and soluble true proteins(B_2). Krishnamoorthy et al.(1982) devised a procedure using borate-phosphate buffer to estimate soluble CP. Tungstic acid precipitation can be used to estimate the rapidly degraded, B_1 and B_2, soluble true protein fractions from the borate-phosphate soluble CP (Sniffen et al., 1992). Perchloric acid can be used to separate the soluble peptides from the soluble CP to estimate the B_1 fraction (Chen et al., 1987a). The intermediately degraded true protein fraction(B_3) and slowly degraded true protein fraction(B_4) can be determined using the neutral detergent procedure (Goering and Van Soest, 1970) without sodium sulfite, filtering the remaining sample on filter paper, and estimating the CP

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remaining in the residue by the Kjeldahl procedure (AOAC, 1990). Crude protein which is not soluble in borate-phosphate buffer but is soluble in neutral detergent is estimated to be the B_3 fraction, while CP which is insoluble in neutral detergent but soluble in acid detergent is estimated to be the B_4 fraction (Pichard and Van Soest, 1977, Sniffen et al., 1992). Unavailable CP (C) remains insoluble after boiling the sample with acid detergent and is assumed to be unavailable to the dairy cow (Pichard and Van Soest, 1977).

Proteins are also classified (NRC, 1989) as either degradable(DIP) or undegradable(UIP) . The degradable protein fraction contains NPN, peptides, and true proteins which are degraded in the rumen. This includes all of fraction A and variable amounts of fractions B_1, B_2, B_3, B_4. Undegradable protein, which is also referred to as escape or bypass protein, is comprised of protein which is not degraded by the microbes but can be either utilized once it arrives in the small intestine or is unavailable to the animal. Small amounts of the B_1 and B_2 fractions, variable amounts of the B_3 and B_4 fractions, and all of fraction C are included in the undegradable fraction.

Table 1. Nitrogen Fractionation Scheme (Pichard and Van Soest, 1977, Blethen et al., 1990, Van Soest, 1982):

	Component	Fraction	
Soluble	NPN	A	Potentially Degraded
	Peptides	B_1	
	Albumins, Globulins	B_2	
Insoluble	Glutelins	B_3	Potentially Undegraded
	Prolamines, Extensins	B_4	
	ADF-CP	C	

Although this system of nitrogen fractionation significantly improves the understanding of protein degradation in the rumen, this chemical separation is not totally adequate. Questions still remain regarding the uniformity of these fractions and consequently, the rate of protein degradation within each fraction (Pichard and Van Soest, 1977, Mahadevan et al., 1980). Enzymatic techniques have been proposed in an attempt to more accurately estimate ruminal protein degradation of feed in an environment which simulates the rumen.

There are two types of enzymatic assays used. The first is an assay to simulate the amount of protein that will break down in the rumen at any timepoint. The concentration of enzyme used must mimic the enzyme concentration, over time, in the rumen. Enzyme must be limiting because it is not in excess in the rumen. Feeds can be incubated for a number of time intervals in order to generate a degradation curve which can be fractionated to describe the rate of degradation of individual protein fractions, or feeds can be incubated for a specific period of time and the amount of rumen degraded

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protein in the feed can be determined based on an assumed rumen residence time or using a regression equation to predict degradability. The second type of assay involves the use of enzyme concentrations 1.5 to 2.5 times higher than those used for a time course measurement in order to degrade that protein expected to be rumen degraded within one hour.

A laboratory procedure is needed for measurement of ruminal protein degradation of feedstuffs. Devising a technique is difficult, however, since the nature of the buffer, type of enzyme, ratio of enzyme to substrate, and properties of the feed sample all have variable effects on protein degradation inside a flask.

Feedstuffs contain a variety of protein fractions, each affected differently by pH and thus, having various rates of degradation (Broderick and Craig, 1980). Bartle et al.(1986) evaluated the effect of buffer pH on degradation of protein sources. Soybean meal protein digestion responded to pH in a quadratic manner with degradation being highest at pH 6-6.5 and lower at pH 5.5 and pH 7. The isoelectric point of soybean protein is approximately pH 5.5 (Berger, 1986). This point at which the protein has no net charge is also the point at which it is least soluble in aqueous solutions (Berger, 1986). Wohlt et al.(1973) increased solubility from 27 to 57% simply by raising the pH of a buffer from 5.5 to 7.5. Extracellular enzymes primarily degrade protein which is in solution. For this reason, it can be concluded that it would

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be most accurate to use a stable buffer with a pH similar to that of the rumen, which is 6.7 (Hungate, 1966) or somewhat lower for high-producing dairy cows, for degradability analysis.

The rate at which a single pure feed protein is degraded in the rumen when enzyme supply is not limiting, depends only on the properties of the protein (Van Soest, 1982). However, in the rumen, many other factors such as, initial lag time required for solubilization and microbial attachment and variations in microbial population and supply of enzyme, create a complex combination of multiple order reactions which break down feed proteins (Krishnamoorthy, 1982, Van Soest, 1982). A constant enzyme / true protein substrate ratio must, therefore, be specified in any commercial laboratory procedure which is intended to mimic the rumen since reactions do not occur at maximum velocity (Roe et al., 1990, Licitra et al., 1993).

Little information has been obtained pertaining to the mechanisms of rumen proteases. Exo- and endopeptidase activity has been found but, the ratio of these in the rumen is unknown. Thus, logically, a commercial enzyme which contains both exo- and endopeptidase activity should be used in any degradability procedure.

Numerous methods are currently available for estimating ruminal protein degradation of feedstuffs. With the <u>in situ</u> (dacron bag) procedure, indigestible bags with a defined pore

size, containing a feedstuff are placed in the rumen of a fistulated animal for various time intervals and the amount of nitrogen removed over time is measured (Nocek, 1985). Problems with this method include: 1. it is not practical for commercial laboratories, 2. it actually measures degradability and solubility rather than only degradability of protein, and 3. microbial contamination can be significant (Wanderly et al., 1993).

The S.griseus method, a modification of a procedure proposed by Pichard and Van Soest(1977), is an in vitro method which incorporates a broad spectrum protease from Streptomyces griseus, at a level of 6.6 units of enzyme/g sample DM, to break the peptide bonds of feedstuffs (Krishnamoorthy et al., 1983). S.griseus has both exo- and endopeptidase activity (Krishnamoorthy, 1982). One-half gram DM sample is incubated in 40 ml of borate-phosphate buffer(pH 7.8-8.0) for 1 hour and then, 10 ml of protease solution(330×10^{-3} units/ml) is added (Krishnamoorthy et al., 1983). It is assumed that all protein which remains insoluble after incubation (18 h for concentrate feeds and 48 h for forages) is ruminally undegradable.

A modification of the S.griseus method using a 1 h incubation period with .5 g of sample incubated in 1 mg enzyme/50 ml of borate-phosphate buffer(7.60 g $NaH_2PO_4 \cdot H_2O$, 13.17 g $Na_2B_4O_7 \cdot 10H_2O$ per liter (pH=8)), and regression equations for prediction of in situ ruminal protein degradation, taking into account the rate of particle

disappearance from the rumen as 0.06/hour, has been recommended in France (INRA, 1989). For single feeds, the equation: Degrad. = $0.36D_1$ + 0.479 + (*) +/- 0.029 (with (*) from +0.154 to -0.184 according to feeds) (r = 0.975) is used, while for compound feeds the equation: Degrad. = 0.870 D_1 + 0.345 +/- 0.025 (r = 0.955) is used, where D_1 equals the proportion of N degraded after 1 h.

With the ficin procedure, protein is broken down by the proteolytic ficin enzyme (Ficus glabrata) in a phosphate buffer with a pH of 6.5 (Poos-Floyd et al., 1985). Ficin is an endopeptidase (Barrett and Salvesen, 1986) and it is recommended for use at a level of 8.24 units/g sample (Poos-Floyd et al., 1985). The insoluble sample remaining after a 1 h enzymatic incubation is analyzed for CP to determine theoretical ruminal CP disappearance.

For the neutral protease with amylase method (Assoumani et al., 1992), .5 g DM sample is first incubated in 25 ml HCl citrate buffer (pH=4.7) and .2 ml Biase (Biocon(U.S.) Inc., Lexington, KY), an enzyme which contains endo-beta glucanase and alpha amylase activities, in order to break down starch and fiber which could potentially inhibit the action of the protease. Then, the remaining sample is incubated with 49.8 ml of sodium citrate buffer and .2 ml Neutrase (Novo Laboratories Inc., Danbury, CT), a neutral protease with endopeptidase activity (Barrett and Salvesen, 1986, Assoumani et al., 1992). It is recommended that 9.6 units of protease/g

sample DM be used (Assoumani et al., 1992).

Some researchers have criticized the use of commercial proteases for estimation of ruminal protein degradation due to the differences in specificity and mode of action between commercial proteases and those present in the rumen. Broderick(1987) proposed an *in vitro* system with strained ruminal liquor and an inhibitor of nitrogen metabolism. Mahadevan et al.(1987) utilized protease from a mixture of rumen microorganisms for degradability analysis. The practicality of these methods for commercial laboratories at this point in time is questionable due to the expense and labor involved.

The reliability with which the *S.griseus* (Krishnamoorthy et al., 1983), ficin (Poos-Floyd et al., 1985), and neutral protease with amylase (Assoumani et al., 1992) methods could be used to predict *in situ* protein degradation of concentrate feeds has been evaluated (Roe et al., 1991). At least 68.5% of the variation in the 18 h *in situ* estimates could be explained by the neutral protease with amylase estimates obtained after 1, 2, 4, 8, and 12 h of incubation. Over 72% of the 18 h *in situ* variation was explained by the results of the 4 h *S.griseus* incubation. Little relationship was found between the ficin results versus the *in situ* results.

After analyzing the results of this study (Roe et al., 1991), it is evident that each of these methods have problems which can cause inaccurate degradability predictions. The

alkaline buffer which is utilized in the S.griseus method is likely to promote the solubilization of glutelins (Blethen et al., 1990). Also, even though Krishnamoorthy et al.(1983) determined the appropriate enzyme concentration required to estimate ruminal degradation for a long 18 h or 48 h incubation, a constant enzyme/protein substrate ratio was not specified. Although Poos-Floyd et al.(1985) did recommend a ratio of substrate to enzyme activity of 3:1 for the ficin method, it was not indicated that any attempt to approximate the proteolytic activity of the rumen was carried out. The neutral protease with amylase procedure did not specify a constant enzyme/protein substrate ratio (Assoumani et al., 1992). Additionally, the neutral protease method is complicated by the sensitivity of the protease to the calcium ion content of the buffer and the viscous nature of the protease which is difficult to accurately pipet and often results in variation between replicates (Roe, 1990).

In order to fully apply new ruminant protein systems, it is desirable to be able to not only predict the quantities of rumen degradable and undegradable protein, but also, to estimate the quantity and rate of digestion of each protein fraction, A, B_1, B_2, B_3, B_4, and C. In the future, by using non-linear regression and multi-pool models, digestion rates of each fraction may be determined. According to Roe et al.(1991), the S.griseus (Krishnamoorthy et al., 1983), ficin (Poos-Floyd et al., 1985), and neutral protease with amylase

(Assoumani et al., 1992) methods did not yield feed protein degradation curves which had a consistent relationship to those generated by the in situ technique. Research to understand more regarding the degradability of individual amino acids within different types of peptides and their presence in the various protein fractions will further improve the efficiency of nutrient utilization in dairy cattle.

2. Carbohydrate

Since it is known that rumen microbial growth is highly dependent upon the availability of carbohydrate, it is imperative that accurate laboratory methods be developed in order to identify uniform carbohydrate fractions and to estimate their rates of rumen degradation (Russell et al., 1983). Many in vivo studies have been conducted to determine the effects of different non-structural carbohydrate levels in the diet, however, due to the non-uniformity of this fraction, it is difficult to make any definite conclusions from these studies (Casper et al., 1990, Aldrich et al., 1993).

Monosaccharides, disaccharides, oligosaccharides, and polysaccharides make up the non-structural component of feeds. Simple sugars tend to be rapidly available while polysaccharides can vary in degradability from fairly rapid, as is the case with steam-flaked wheat, to fairly slow, as is the case with the starch in dry sorghum (Allen, 1991). All free sugars are water soluble. After solubilization they can

be analyzed using direct enzymatic, calorimetric assays such as the glucose oxidase procedure (Sigma, 1990). In order to quantify the starch content of feeds, the starch must first be broken down into glucose which is then analyzed by the glucose oxidase procedure.

Much work has been conducted in an attempt to improve techniques for starch determination in feeds. Kartchner and Theurer(1981) compared starch values obtained using either amyloglucosidase or 0.76 N HCl to degrade starch to glucose units. Amyloglucosidase is known to specifically act on alpha-linked D-glucose polymers and therefore, there is no risk of interference from cellulose breakdown. It was found that with high starch samples, both methods yielded similar results. However, with feeds and feces containing over 17% cellulose, the acid hydrolysis method resulted in higher starch estimates due to cellulose breakdown. Karkalas(1985), realizing the problems of glutinous aggregates, crystalline amylose, and amylose-lipid complexes, proposed the use of 1M NaOH and alpha-amylase as separate pre-treatments to the amyloglucosidase attack on the starch. It has also been suspected that starch may complex with proteins and inhibit amyloglucosidase action. However, when an alkaline protease pre-treatment was conducted, starch values were not increased (O'Neil, personal communication).

The structural carbohydrates include: pectin, cellulose, hemicellulose, and lignin. The detergent system was developed

to quantify the cellulose, hemicellulose, and lignin fractions by sequential analysis. Pectin is not recovered with these procedures. Van Soest et al.(1991) discussed various modifications to the neutral detergent procedure which have been developed primarily in order to better cope with interfering compounds such as starch. Heat stable alpha-amylase is recommended for use in degrading starch on a routine basis and 8 M urea is used on very starchy, low fiber samples in order to remove starch. Urea denatures protein and therefore cannot be used when samples are to be analyzed for the crude protein associated with NDF (Rawn, 1989). Van Soest et al.(1991) recommended a procedure for pectin analysis modified from the procedure of Bucher(1984) which is specific for quantitation of galacturonic acid but not arabans.

The rate of fermentation of each carbohydrate fraction needs to be known in order to predict energy availability over time and extent of fermentation in the rumen. Of the complex carbohydrates, pectin is degraded the most rapidly while starches, cellulose, and hemicellulose are quite variable (Van Soest et al., 1991). In vitro procedures and enzymatic procedures have been developed and primarily used for estimating starch and NDF digestibility of feeds (Goering and Van Soest, 1970, Basery and Campling, 1988, Owen et al., 1991). The in situ procedure has been used for rumen digestibility prediction (Robinson and Kennelly, 1988). In vitro gas production has also been used to determine

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fermentability of feeds (Menke et al., 1979, Pell and Schofield, 1993). It is difficult if not impossible, however, to determine the proportions of gas arising from each of the various carbohydrate fractions being fermented.

C. Measurement of Daily Microbial Nitrogen Flow to the Duodenum:

Currently, most researchers attempting to measure daily microbial nitrogen flow to the duodenum utilize cows with ruminal and duodenal cannulas and infuse indigestible flow markers to measure rate of solid and/or liquid passage. The ratio of bacterial marker to nitrogen in the pure bacterial pellet and in the duodenal digesta is used to calculate bacterial nitrogen flow. Unfortunately, there is little agreement regarding the most appropriate method of obtaining a representative sample of the bacterial population escaping the rumen, the most reliable passage marker, and the most adequate bacterial marker (Broderick and Merchen, 1992, Owens and Hanson, 1992). None of the methods are without problems.

1. Bacterial Isolation

Rumen fluid samples are collected at various intervals around the clock, mixed, and a composite sample is obtained from which a pure bacterial pellet is isolated. Some researchers have used a suction pump placed at different locations in the rumen to pump out rumen fluid samples (Roe,

unpublished method). Others have taken aliquots of rumen contents by hand out of the anterior ventral sac, the dorsal sac, and the posterior ventral sac and composited these samples (Glenn et al., 1989). McCarthy et al.(1989) forced a stoppered bottle down to the reticulo-omasal orifice, allowed the bottle to fill, and stoppered it prior to bringing it out of the rumen. Lykos et al.(1991) isolated the fluid-associated bacteria and the particle-associated bacteria.

The bacterial population flowing out of the rumen is a composite of fluid- and particle-associated bacteria each at many different stages of growth. The percentages of each bacterial type are difficult if not impossible, to estimate. Cecava et al.(1990a) looked at the effect of bacterial source (mixed, fluid-associated, or particle-associated) on the total purine:nitrogen ratio. It was found that the particle-associated bacteria had a significantly lower ratio (1.33) than the fluid-associated bacteria (1.56) with the mixed bacteria tending to have a ratio (1.30) similar to that of the particle-associated bacteria. Merry and McAllan(1983) determined the RNA-N:total N ratio and the diaminopimelic acid N:total N ratios of liquid- and particle-associated bacteria and found both ratios to be higher for the liquid-associated bacteria. One might surmise that this is due to a higher rate of growth in the fluid-associated bacteria. Lykos et al.(1991) found the crude protein content to be higher in the fluid-associated bacteria(45.1%) than in the particle-

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associated bacteria(39.3%).

Bacteria are isolated from the rumen liquid by differential centrifugation (Steinhour et al., 1982). This procedure involves a low-speed centrifugation step to remove feed particles followed by a high-speed centrifugation of the supernatant to obtain a pure bacterial pellet. Unfortunately, however, protozoa, large bacteria, bacteria which are clumped together, and bacteria attached to feed particles are lost during the first centrifugation. Therefore, the bacterial pellet obtained may not actually be representative of the entire microbial population (Stern and Hoover, 1979).

2. Microbial Markers

There are a number of microbial markers currently being used to quantify microbial nitrogen flow from the rumen (Broderick and Merchen, 1992). An ideal marker would be one that is easy to assay, unique to microbial protein, present at a constant ratio with nitrogen regardless of dietary conditions, and biologically stable (Broderick and Merchen, 1992). Microbial nitrogen is calculated based on the marker:nitrogen ratio of the pure sample of mixed rumen bacteria obtained and the amount of marker in duodenal digesta.

Diaminopimelic acid (DAPA) is found in oligopeptides which cross-link with peptidoglycan in bacterial cell walls. There are many problems with the use of DAPA as a marker.

First of all, due to its presence only in the cell wall, smaller cells tend to have a higher DAPA:N ratio than larger cells. Secondly, DAPA has been found in common feed sources. Rahnema and Theurer(1986) estimated bacteria to contain 7.3 mg DAPA nitrogen / g total nitrogen while alfalfa hay, corn, and soybean meal had 3.5, 1.3, and 1.9 mg DAPA nitrogen / g total nitrogen, respectively. Third, rumen protozoa contain DAPA, but only as a result of bacterial engulfment and therefore, contain about 2.4 mg DAPA nitrogen / g total nitrogen (Rahnema and Theurer, 1986). For this reason, DAPA probably underestimates total microbial protein flow at the duodenum (Stern and Hoover, 1979). Finally, the DAPA nitrogen may not adequately reflect microbial recycling in the rumen due to the fact that microbial digestion of proteins in the protoplasm is higher than that of proteins in the cell wall (Broderick and Merchen, 1992). Denholm and Ling(1989) found the proportion of DAPA in duodenal chyme that was not associated with cells to be as high as 71%.

Purines have also been used as an internal marker because of their presence at high levels in rumen bacteria. Cecava et al.(1991) compared undegradable protein estimates calculated using either purines or ^{15}N as bacterial markers. UIP(%DM) for a diet high in soybean meal was 36% and 33% using purine and ^{15}N, respectively. UIP(%DM) for a diet high in corn gluten meal and blood meal was 56 and 52% using purine and ^{15}N, respectively. Due to the closeness of these estimates, it was

concluded that total purines could be used with confidence as a bacterial marker.

Concerns exist regarding the use of total purines as a marker. First, purines are present in dietary ingredients. Titgemeyer et al.(1989) found purine levels of 6.54%, 1.40%, 1.90%, 0.39%, and 6.93% DM in bacteria, soybean meal, corn gluten meal, blood meal, and fish meal, respectively. When purified purines were added to rumens, however, less than 10% could be detected after 45 minutes, indicating that these purines were rapidly used by the rumen bacteria (McAllan and Smith, 1969). Work has not been conducted to estimate rumen degradability of dietary purines in their natural form. Secondly, protozoal purine:nitrogen ratios have been found to be about half that of bacteria. This would lead to an underestimation of protozoal nitrogen flow if it were calculated using the ratio of bacterial purine:nitrogen (Broderick and Merchen, 1992).

Other microbial markers are in use by researchers but to a lessor extent. D-alanine, aminoethyl-phosphonic acid, and ATP have been used as internal markers. Inorganic ^{15}N and ^{35}S have been dosed into the rumen to be incorporated into microbial protein during de novo synthesis (Broderick and Merchen, 1992). These isotope methods do not account for the direct incorporation of dietary amino acids and peptides into microbial protein (Stern and Hoover, 1979). Broderick and Merchen(1992) also suggested a non-invasive method being

developed in which purines present in the urine are used to estimate microbial flow from the rumen.

3. Flow Markers

Markers are fed to or dosed into the rumen as references to estimate flow of digesta out of the rumen. Ideally, a marker must not be absorbed, must have no affect on digestive processes, must flow with the material it is marking, and must be easily and accurately assayed. It is assumed that pool size and dilution rate are constant throughout the day, that passage follows first-order kinetics with no time lag, and that the marker instantaneously and completely mixes with the pool to be marked (Owens and Hanson, 1992).

It is assumed that if the duodenal sample obtained is truly representative of the digesta, any marker which is actually indigestible, should be effective in estimating daily duodenal flow of dry matter (Robinson and Kennelly, 1990). When it is difficult to obtain duodenal samples which contain solutes and particulate matter in the same proportions as the true digesta, as is especially the case with the gutter-type cannula, it is recommended that two markers be used, one for the liquid and one for the solids (Faichney, 1980). If a representative sample is not obtained and the liquids:solids ratio is wrong, a liquid marker is most likely to give an inaccurate estimation of digesta flow because it is assayed as a solid (Faichney, 1980, Robinson and Kennelly, 1990).

Chromium is the most common marker used to estimate microbial flow out of the rumen. It is either pulse-dosed into the rumen as chromium-mordanted fiber, mixed into the TMR as chromium-mordanted fiber, or placed in the rumen as chromic oxide wrapped in filter paper (Uden et al., 1980, McCarthy et al., 1989). After in vitro digestion of chromium-mordanted fiber, 98% of the chromium was found to remain associated with the fiber (Uden et al., 1980). Flow of dry matter at the duodenum(g/day) is calculated by dividing chromium intake(mg/d) by the concentration of chromium at the duodenum(mg/g) (Cecava et al., 1990b).

D. Current Rumen Models for Predicting Microbial Yield:

A variety of models currently exist which are designed to estimate microbial protein flow at the duodenum. Most of the widely used systems are regression equations based on published studies in which microbial protein yield was estimated in vivo (NRC, 1985, INRA, 1989). Each of the models have different levels of aggregation ranging from totally empirical to fairly mechanistic, as determined by the purpose of the model and the information available for use as input to the model (France and Thornley, 1984). It is important that models be developed which adequately reflect rumen metabolism yet use easily measured and observed inputs.

1. NRC(1985,1989) Model

The NRC(1985,1989) microbial nitrogen estimation is calculated based upon the amount of total digestible nutrients(TDN) and degradable intake protein(DIP) in the diet. The amount of microbial nitrogen which can be generated from TDN intake is predicted using a regression equation ((26.13 TDNI(kg/d) - 31.90) R^2=0.77), described in NRC(1985). The amount of microbial nitrogen which can be generated from DIP is then predicted. It is assumed that rumen influx protein is 15% of crude protein intake and that 90% of the rumen available protein is converted to microbial crude protein. The recommendation was made that the lessor of the energy and nitrogen potential be used in computing microbial nitrogen. It is also assumed that 80% of the microbial nitrogen is in the form of amino acids while the other 20% is nucleic acids.

There are numerous problems inherent in the NRC(1985, 1989) model. First of all, TDN is an estimate of the energy available to the animal rather than solely that energy which is digestible by the rumen bacteria (Johnson and Bergen, 1980). Some people have tried to correct for fat and improved the prediction somewhat. Erdman and Komaragiri(1991) compiled a database similar to that of NRC(1985,1989) and computed a regression equation based on NE_l which had an R^2 of only 0.39. Secondly, it is not recognized that the amount of DIP which is needed by the microbes is dependent upon the amount of energy available for the bacteria to use. Recycled nitrogen and

microbial crude protein cannot be a constant percentage of crude protein availability in the rumen because the amount of ammonia and amino acids used by the microbes prior to urea conversion is a function of energy availability in the rumen. Third, it is not biologically correct to have a negative intercept in the TDN equation. Finally, the NRC(1985,1989) equations do not adequately account for variations in rumen degradable fat, synchronization of protein and energy availability, source of degradable nitrogen and isoacid availability, ruminal pH, and washout of the bacteria.

2. INRA (1989) Model

The French PDI (Protein truly Digestible in the small Intestine) system was developed in order to calculate digestible dietary undegraded protein and microbial protein arriving at the duodenum (INRA, 1989). Dietary protein degradability is determined by <u>in situ</u> procedures while fermentable organic matter is calculated as total digestible organic matter minus ether extract, undegraded intake protein, and silage fermentation products. Microbial protein is then assumed to be either 145 g crude protein per kg of fermentable organic matter or 90% of degradable dietary protein, based on regression equations developed using a database containing 405 diets. It is assumed that 80% of microbial nitrogen is in the form of amino acids while 20% is in nucleic acid form.

The limitations of the INRA(1989) model are very similar to those of the NRC(1985,1989) model. First of all, the estimation of fermentable organic matter is very difficult to make due to variations in rate of digestion and rate of passage of feeds. Total fermentable organic matter is also not a uniform fraction, but is made up of multiple carbohydrate and protein fractions each with different rates of ruminal degradation each having a different impact on the microbial pool. Secondly, protein and carbohydrate synchronization in the rumen is not addressed. Finally, the INRA(1989) model does not account for variations in the amino acid and nitrogen making up the DIP fraction, microbial recycling, rumen available fat inhibition of the microbes, washout rate, or ruminal pH.

3. Stokes et al.(1991a) Model

Based on <u>in vitro</u> microbial protein synthesis measurements, Stokes et al.(1991a) developed prediction equations. Dry matter digestion was first calculated as a function of the nonstructural carbohydrate(NSC) and DIP content of the diet. Then, microbial efficiency (g microbial nitrogen / kg digested dry matter) was also determined as a function of the NSC and DIP in the diet. These equations do address synchronization of energy and protein availability somewhat. Unfortunately, however, the equations do not consider the energy derived from fiber or the rate at which

NSC and DIP is available to the microbes. The NSC in barley, for example, is degraded at a much faster rate than that in corn. Furthermore, these equations do not consider the type of DIP, recycling of nitrogen, ruminal pH, rumen degradable fat, or washout rate from the rumen.

4. Danfaer (1990) Model:

Danfaer(1990) converted an existing static model of nutrient digestion and metabolism into a dynamic model. The parameters of the dynamic model were adjusted until it gave the same solutions as the static model. Rate of feed intake and rumen content mass are constant. Carbohydrates are fractionated into sugar, starch, cell wall carbohydrate, glycerol, and fatty acids. Each feed consumed contains assumed proportions of each carbohydrate fraction. Sugar, starch and glycerol are assumed to be totally fermented while 60% of the cell wall carbohydrate is assumed to be fermented. Dietary crude protein is fractionated into unfermentable protein, fermentable protein, ammonium nitrogen, and urea nitrogen. Dietary protein is assumed to be 80% fermentable. The amount of available carbon and nitrogen, volatile fatty acids (mole carbon/mole carbon), and ATP (mole ATP/mole carbon) generated are constant for each nutrient fraction. Synthesis of microbial protein depends on the availability of ATP. Growth per mole of ATP is dependent upon the concentration of amino acids and ammonia in the rumen.

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However, currently, these concentrations are constant at 2.077 and 0.928 mole nitrogen, respectively. The maintenance requirement of the microbial population is not considered.

Although this model is supposedly quite mechanistic and dynamic, predicting moles of ATP generated, volatile fatty acids absorbed, etc., it seems to be of little use for predicting variations in microbial protein yield. No attempt is made to address differences in nutrient digestibility among feedstuffs. Deviations in rate of passage as a result of cow size, dry matter intake, and ration composition are not considered. It is assumed that the ATP generated from each glucose unit digested from a particular fraction is constant with no regard for changes in the fermentation such as the adjustment to greater lactate production. The degree of carbohydrate and protein fermentation synchrony is not considered adequately. Furthermore, this model does not adequately consider the type of DIP, recycling of nitrogen, ruminal pH, or the inhibitory effect of rumen degradable fat.

5. The Cornell Net Carbohydrate and Protein System

The Cornell Net Carbohydrate and Protein System (CNCPS) has recently been described in a series of four articles (Russell et al., 1992, Sniffen et al., 1992, Fox et al., 1992, O'Connor et al., 1993). Carbohydrates are fractionated into sugar(A), starch(B_1), available fiber(B_2), and unavailable fiber(C). Proteins are fractionated into non-protein

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nitrogen(A), rapidly degraded soluble true protein(B_1), intermediately degraded true protein(B_2), slowly degraded true protein(B_3), and unavailable protein(C). Laboratory methods have been described for the quantitation of each fraction in feedstuffs. The proportion of each fraction degraded in the rumen is determined based on the competition between the rate of passage and rate of digestion of each fraction within each feed type.

In the CNCPS, bacteria are classified as either structural carbohydrate(SC) fermenters or nonstructural carbohydrate(NSC) fermenters. Microbial protein yield for each bacterial type is a function of the growth rate that the available carbohydrate can drive (rate of digestion), the bacterial maintenance rate (.05 and .15 g substrate/g bacteria/h for the SC and NSC fermenters, respectively), and the theoretical maximum growth yield (Ymax). Ymax was estimated by Isaacson et al.(1975) to be .50 g bacteria/g carbohydrate/h but this estimate was lowered in the CNCPS to .40 g bacteria/g carbohydrate/h in order to accommodate for the protozoal predation which exists under rumen conditions. When the diet contains less than 20% NDF, bacterial yield is decreased 2.5% for every 1% decrease in NDF in order to account for the growth inhibition under acidic conditions. The yield of NSC fermenting bacteria is enhanced by as much as 18.7% as the ratio of peptides to NSC plus peptides increases from 0% to 14%.

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The Cornell Net Carbohydrate and Protein System seems to be an improvement over other models in existence for predicting microbial protein synthesis, however, it still does not account for all of the variables which have an effect. First of all, the amount of recycled nitrogen flowing into the rumen is determined by the NRC(1985) equation which does not consider energy availability in the rumen. It is also assumed that nitrogen never limits microbial growth. The rates of passage of different feeds are affected only by DM intake, effective NDF, and body weight. Other components of the ration should affect passage of individual feeds, and fractions within feeds also probably have different rates of passage from each other. The amount of protein and carbohydrate in the rumen is predicted, however, the degree of synchronization of carbohydrate and protein availability cannot be determined from the model. The cow is assumed to eat continuously throughout the day. Additionally, all of the factors which affect ruminal pH, such as starch fermentability and hydrogen ion absorption, are not considered as effectors of microbial growth. The decision to decrease Ymax from .50 g bacteria/g carbohydrate/h to .40 g bacteria/g carbohydrate/h to account for the protozoal predation which occurs in the rumen and not the chemostat, was biologically based, however, no studies have been conducted to determine the validity of this estimate. Finally, the degree of uniformity of the protein and carbohydrate fractions in the model is

questionable. These fractions are chemically similar, however, each fraction may contain a combination of protein types (albumins, globulins, prolamins, and glutelins) or starch types (amorphous versus crystalline), each broken down at a different rate by the rumen enzymes.

6. Baldwin et al.(1987) Model

The Baldwin model, a lower level of aggregation model describing whole cow metabolism, has been described in a series of three articles (Baldwin et al., 1987a,b,c). Dietary nutrient fractions used as inputs into the model include: insoluble ash, lignin, soluble ash, lipid, starch, organic acids, lactate, pectin, soluble carbohydrates, hemicellulose, cellulose, insoluble protein, soluble protein, acetate, butyrate, and non-protein nitrogen. Each nutrient enters the rumen in either a large particle pool, small particle pool, or water-soluble pool. Large particles are broken down into small particles. Small particles can escape the rumen or go into the water-soluble pool. Soluble material can either escape the rumen or be fermented. Unfortunately, methods for determining the various nutrient fractions, their rates of digestion, and their presence in the three particle pools have not been described. The uniformity of many of the nutrient fractions is also questionable.

Based on studies conducted with sheep and low-producing cows and biochemical reactions in the laboratory, the rate

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constants of ruminal reactions have been described. It is assumed that the cow eats continuously throughout the day. Rate of rumination is a function of dietary cell wall content. Microbial protein yield for the large particle, small particle and soluble pool bacteria is determined based on the amount of ATP available, energy required for microbial maintenance, and amino acid and ammonia availability. Growth with and without preformed amino acids is calculated. The microbes in the large particle pool cannot escape from the rumen. Microbial maintenance is not adjusted for reductions in ruminal pH.

The lower level of aggregation of the Baldwin model is beneficial for identifying many more of the variables affecting microbial synthesis, however, it also requires one to make many more assumptions based on inadequate knowledge. For example, generating microbial growth from ATP availability requires a complete understanding of the ruminal population, the types of fermentation of each, ruminal pH, and energetic uncoupling. Extrapolation from data from a sheep on a high forage diet to a cow producing 45 kg of milk per day eating a high concentrate diet is difficult. Work also needs to be conducted to develop laboratory methods which fractionate feeds into uniform nutrient fractions with constant rates of degradation before a system such as the Baldwin model can be of any use on the farm.

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Chapter III. Development of a Within-Day Model for the Prediction of Microbial Nitrogen Flow at the Duodenum:

Introduction:

Due to our inability to make cows eat 24 hours per day in normal feeding situations and the fact that we feed many different feeds each containing multiple carbohydrate and protein fractions having various rumen degradation rates, accounting for the within-day fluctuation of carbohydrate and protein availability to the microbes and for variation in the rumen environment should increase the accuracy with which microbial nitrogen flow to the duodenum can be predicted. A within-day model was developed to predict microbial flow from the rumen. Components of this model were: 1. prediction of NDF, NSC, NPN, and true protein available to the microbes each hour, 2. prediction of rumen pH each hour, 3. adjustment of microbial maintenance requirements according to hourly pH, and 4. growth adjustment of nonstructural carbohydrate fermenting bacteria according to the hourly degraded true protein: fermented NSC ratio. The within-day model was developed using data from <u>in vitro</u> and <u>in vivo</u> studies.

A. Prediction of Within-Day Nutrient Availability:

A spreadsheet model was developed for prediction of within-day nutrient availability to the rumen microbes.

Protein Fractionation

Crude protein in each dietary ingredient was fractionated into 4 rumen available crude protein fractions (A, B_1, B_2, and B_3) and one rumen unavailable protein fraction (C) in the model. Crude protein was determined by the Kjeldahl procedure (AOAC, 1990). The procedure of Krishnamoorthy et al.(1982) was used to estimate soluble crude protein. Soluble true protein (B_1) was precipitated using trichloroacetic acid according to the method of Kohn and Allen(1992) and soluble non-protein nitrogen (A) was calculated by difference. Crude protein remaining insoluble after boiling with acid detergent was classified as fraction C (Pichard and Van Soest, 1977). Crude protein insoluble in neutral detergent (with substitution of triethylene glycol for 2-ethoxyethanol and omission of decahydronaphthalene and sodium sulfite) but soluble in acid detergent was classified as the B_3 fraction (Pichard and Van Soest, 1977). The B_2 fraction was estimated by difference as that crude protein which was not soluble in borate-phosphate buffer (Krishnamoorthy et al., 1982) but soluble after refluxing with neutral detergent.

Carbohydrate Fractionation

Carbohydrate in each dietary ingredient was fractionated into "Other NSC" (pectins, beta-glucans, and other non-glucose free sugars), rapidly available starch plus glucose (S_1) (available within 2 h), slowly available starch (S_2), rumen available NDF, and rumen unavailable NDF. "Other NSC" was estimated by difference to be: {100 - [CP + (NDF-NDFCP) + Ash + Fat + Starch + Free Glucose]}. The measurements of the starch plus glucose fractions were based on 2 h and 8 h *in situ* starch disappearance. The amount of S_2 was calculated as the natural antilogarithm of: [(ln(starch remaining at 2h (%DM)) + [2 * [(ln(starch remaining at 2h (%DM)) - ln(starch remaining at 8h (%DM)))/6]]. The amount of S_1 was calculated as the difference between the total amount of starch in the feed and S_2. The measurements of rumen unavailable NDF were obtained by multiplying the lignin content (Goering and Van Soest, 1970) of the feed by the factor 2.4, as derived by Chandler et al.(1980). Available NDF was calculated as: [NDF-NDFCP-(Lignin*2.4)].

NDF was measured using procedures A and B of Van Soest et al. (1991). Heat-stable alpha-amylase (Number A3306; Sigma Chemical Co., St. Louis, MO) was used on a routine basis for all samples (Procedure A) and 8 M urea was also used as a pre-treatment for all very starchy, low fiber samples (Procedure B) prior to boiling in neutral detergent (with substitution of triethylene glycol for 2-ethoxyethanol and omission of

decahydronaphthalene and sodium sulfite).

The following procedure was used for measurement of the free glucose plus starch content of all feeds (Karkalas, 1985, B.A. Lewis, personal communication). Two ml of 1.0 N NaOH was added to 0.15 - 0.2 g of ground sample in a 125 ml Erlenmeyer flask. Twenty-five ml of distilled water and 0.15 ml of glacial acetic acid was then added. Twenty-three ml of distilled water was added plus 50 ul of heat-stable amylase (Number A3306; Sigma Chemical Co., St. Louis, MO). Samples were incubated in an 85°C shaking water bath for 30 minutes. Five ml of acetate buffer (120 ml glacial acetic acid and 164 g sodium acetate (anhydrous) in DH_2O and diluted to 1 liter), 35 ml of distilled water and 10 ml of glucoamylase solution (Sigma Chemical Co., 10 mg/ml filtered through Whatman #541 prior to use) were then added. Samples were then incubated in a 55°C shaking water bath for 2 hours. Samples were filtered through Whatman 541 filter paper and diluted to 250 ml. Two-tenths of a ml of the diluted solution was then assayed for free glucose using glucose oxidase(Sigma, 1990).

For each feed consumed, the amount of each nutrient fraction which was degraded over time was determined based on the competition between its rate of degradation and rate of passage. Rates of degradation for each of the protein fractions in different feeds were estimated using the values currently used by the Cornell Net Carbohydrate and Protein System (Sniffen et al., 1992). For all feeds, the fraction

"Other NSC" was assumed to have a rate of degradation of 20%/h and the starch fraction, S_1, had a K_d of 90%/h. Protein fraction A was assumed to be instantaneously available, although for certain feeds, such as corn silage, this assumption may be in error due to the presence of pre-melanoidins (Bergen, 1984). Rates of S_2 degradation for different feeds were determined according to the amount of starch disappearance from 2 h to 8 h of incubation in situ. The rates of degradation of S_2 were determined using the following equation: [[(ln(starch remaining at 2h (%DM)) - ln(starch remaining at 8h (%DM)))/6]*100]. Rates of available NDF degradation in each feed were determined based on 30 h in vitro degradability (Tilley and Terry, 1963, Goering and Van Soest, 1970). The rates of degradation of available NDF were determined using the following equation: [[(ln 100 - ln(100-((initial available NDF(%DM) - remaining available NDF(%DM) /initial available NDF(%DM))*100)))/30]*100]. A correction was made for the crude protein associated with NDF and for rumen undegradable NDF which was estimated as lignin(%DM) multiplied by the factor 2.4 (Chandler et al., 1980).

The equations of Sauvant and Archimede(1989) were used to predict rates of passage of the nutrient fractions of each feed. Available NDF was assumed to pass according to the rate of passage estimate for forages: $(0.35 + (0.022 * (DMI/(BW)^{.75})) + 0.0002*(\%\ \text{forage in the ration})^2)$, $R = 0.81$. The B_2, B_3, S_1, S_2, and Other NSC fractions were assumed to

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pass according to the rate of passage estimate for concentrates: (-0.424 + 1.45(forage rate of passage)), R = 0.86. The A and B_1 fractions were assumed to pass according to the rate of passage estimate for liquids: (2.45 + (0.025*(DMI/(BW)$^{.75}$))+0.0004*(%forage in the ration)^2),R= 0.79.

The amount of each potentially degradable nutrient fraction in each feed remaining in the rumen at each hour from 0 to 72 hours after ingestion was then calculated based on the competition between rates of degradation and rates of passage using the first-order kinetics equation: $A_t = A_o\ e^{-(Kd+Kp)t}$, where A_t = the amount of nutrient remaining at any time, t, which is potentially degradable, A_o = the initial amount of the nutrient fraction ingested, Kd = the rate of degradation for the fraction of the particular feed, Kp = the rate of passage of the fraction for the particular diet and animal, and t = time after ingestion.

The amount of each nutrient fraction in each feed degraded each hour after ingestion was then calculated as a function of the amount of the nutrient fraction which disappeared each hour and the percentage of the nutrient fraction leaving the potentially degradable pool due to degradation according to the equation: $A_{deg} = [(A_t) - (A_{t+1})] * [Kd / (Kd + Kp)]$, where A_{deg} = the amount degraded at a specific hour, A_t = the amount of nutrient remaining at any time, t, which is potentially degradable, and A_{t+1} = the amount of potentially degradable nutrient remaining at the following

hour. Values were summed to determine total ration non-protein nitrogen (fraction A), true protein (fractions B_1, B_2, and B_3), nonstructural carbohydrate (Other NSC, S_1, and S_2), and NDF fermented by the microbes each hour from the time of ingestion to 72 h after ingestion, assuming at this point that the entire diet was eaten at time 0.

Modeling Meal Patterns Throughout the Day

In order to adequately assess the nutrient status of the microbial population at each hour throughout the day, it is necessary to properly predict the frequency and size of meals consumed by the cow over the 24 hour period. Feeding behavior varies depending on the feeding strategy for the herd, environment surrounding the cow, composition of the diet, palatability, and production level. Freer and Campling(1965) used seven dry, non-pregnant cows to observe eating behavior and reticular motility on many different diets fed either ad libitum or restricted to one daily feeding. Cows ate concentrates faster than forages and ate faster when less food was offered. Heinrichs and Conrad(1987) found that meal length was longer for an alfalfa hay diet than for alfalfa haylage diets and alfalfa pellet diets and that the time between the first and second meal consumed after feeding was longer on the alfalfa pellet diet. Jaster and Murphy(1983) found a diurnal eating behavior pattern when heifers were fed alfalfa hay ad libitum. Less time was also spent eating as

particle size of the hay decreased. Dominance between cows feeding in group situations has also been shown to effect feeding behavior (Mason et al., 1991).

Recently, more feeding behavior work has been conducted using dairy cows eating more typical silage-based diets designed for higher levels of production. Beauchemin et al.(1989) fed a silage-based diet two times per day. An average of ten meals per day were eaten with the following number of chews per meal: 2092, 406, 1452, 268, 1379, 1270, 1156, 1316, 266, 733, showing somewhat of a relationship between number of chews per meal and time of feeding. Vasilatos and Wangsness(1980) fed cows a 60% concentrate, 40% forage total mixed ration ad libitum and found that cows had an average of 12.1 meals per day, each about 21 minutes long. The most eating activity occurred for one hour after each feeding. A diurnal pattern of eating was also seen, perhaps related to the light and dark periods of the day. Dado and Allen(1993) fed early lactation cows a total mixed ration twice per day in stalls. The cows had an average of 11 eating bouts per day. Meal size and frequency gradually decreased with time after feeding. Knowlton (1994) fed diets primarily made up of corn silage, dry rolled corn, and soybean meal ad libitum twice per day and found that cows had an average of 12 meals per day.

For the current model, meal patterns were predicted based on the work of Dado and Allen(1993) and Dado and Allen(In

Press) in which silage-based total mixed diets were fed twice per day. In all of the duodenally cannulated cow studies currently being modeled, cows were not away from feed during milking or at any other time during the intensive collection period so it was not necessary to take that into consideration in the model. Four different meal patterns were modeled in order to account for the different feeding strategies used in the various cow studies from which feed samples were obtained to form the microbial nitrogen database (Chapter IV).

Meal Pattern #1

Cow fed a total mixed diet in equal amounts at 12 h intervals.

% of Total Daily Ration Eaten

At Feeding	-- 15%
2 H Post-Feeding	-- 12%
4 H Post-Feeding	-- 10%
6 H Post-Feeding	-- 8%
9 H Post-Feeding	-- 5%

Meal Pattern #2

Cow fed a total mixed diet at 8 h (30%) and 16 h (70%) intervals.

% of Total Daily Ration Eaten

At Feeding	-- 15%	At Feeding	— 20%
2 H Post-Feeding	-- 7%	2 H Post-Feeding	— 15%
4 H Post-Feeding	-- 5%	4 H Post-Feeding	— 12%
6 H Post-Feeding	-- 3%	6 H Post-Feeding	— 10%
		9 H Post-Feeding	-- 7%
		12 H Post-Feeding	-- 6%

Meal Pattern #3

Cow fed a total mixed diet at 12 h intervals, 33% and 67%.

% of Total Daily Ration Eaten

At Feeding	-- 15%	At Feeding	— 20%
2 H Post-Feeding	-- 8%	2 H Post-Feeding	— 15%
5 H Post-Feeding	-- 6%	4 H Post-Feeding	— 12%
8 H Post-Feeding	-- 4%	6 H Post-Feeding	-- 9%
		8 H Post-Feeding	-- 6%
		10 H Post-Feeding	-- 5%

Meal Pattern #4

Cow fed a mixed diet at 10 h (55%) and 14 h (45%) intervals supplemented with long hay fed at different 10 h (50%) and 14 h (50%) intervals.

% of Mixed Diet Eaten

At Feeding(6AM)	-- 20%	At Feeding(4PM)	— 15%
2 H Post-Feeding	-- 15%	2 H Post-Feeding	— 10%
4 H Post-Feeding	-- 10%	4 H Post-Feeding	-- 8%
6 H Post-Feeding	-- 5%	6 H Post-Feeding	-- 7%
8 H Post-Feeding	-- 5%	10 H Post-Feeding	-- 5%

% of Hay Eaten

At Feeding(3AM)	-- 20%	At Feeding(1PM)	— 20%
2 H Post-Feeding	-- 15%	2 H Post-Feeding	— 15%
3 H Post-Feeding	-- 15%	3 H Post-Feeding	— 15%

The nutrient availability curves previously calculated for the total daily diet were multiplied by the respective factors and started at each meal time. The nutrients available to the microbes each hour were calculated as the sum of nutrients available from each successive meal. Steady state was attained by 48 h. Therefore, the last 24 h of the 72 h which were modeled were assumed to represent the normal rumen, i.e., a rumen that was partially full of fermenting

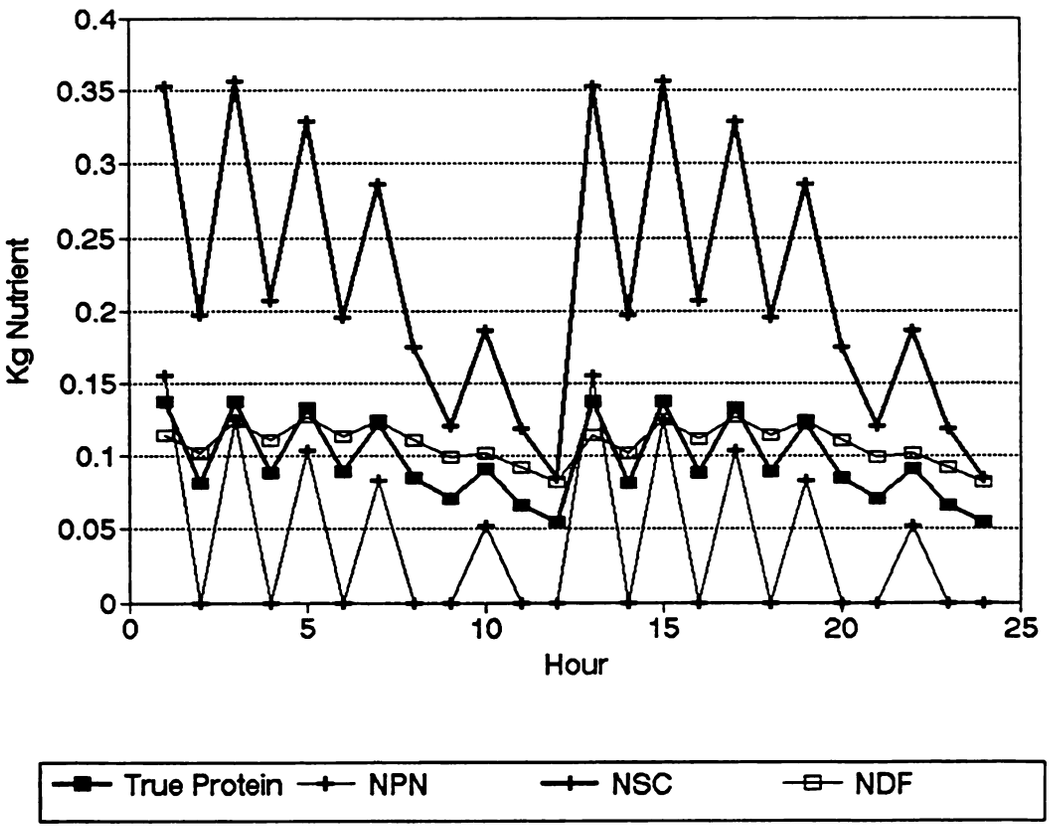
material prior to the start of the 24 h period being modeled.

Figure 1 shows the predicted non-protein nitrogen, true protein, nonstructural carbohydrate, and NDF available to the microbes each hour throughout a 24-h period for a modeled diet (Diet #2, Stokes et al., 1991a). True protein and NDF available each hour tended to remain fairly constant as compared to non-protein nitrogen and nonstructural carbohydrate which tended to peak according to meal pattern. This result was expected due to the differences in rates of degradation of these nutrient fractions. It is recognized that in reality these curves would not have the definite peaks seen but would be much smoother. This result is due to the fact that in the model the cow consumes meals instantaneously on the hour rather than over a period of 10 to 15 minutes. It is assumed, however, that the average hourly availability of nutrients would be similar.

B. Prediction of Within-Day pH Fluctuations in the Rumen:

It has been shown with <u>in vitro</u> studies that when environmental pH is low, hydrogen ions leak into the cell and the cell must expend energy to remove the hydrogen ions in order to maintain near neutral intracellular pH (Russell and Dombrowski, 1980, Strobel and Russell, 1986, Russell, 1987). Furthermore, at lower pH, there is a shift in the bacterial population towards those bacteria which favor lactate production, thus reducing the amount of ATP derived from a

Figure 1. Hourly ruminal nutrient availability in cows consuming Diet #2 in the study of Stokes et al.(1991a) as modeled using the within-day model.



mole of glucose (Strobel and Russell, 1986). In vivo work has shown that within-day variations in ruminal pH can be quite large, depending on patterns of meal consumption (French and Kennelly, 1985).

Prediction of ruminal pH throughout the day is difficult due to problems in the estimation of availability of glucose and other fermentable monosaccharides, shifts in the types of acids produced by bacteria (Robinson et al., 1986), differences in the capacity of feeds to act as buffers (Van Soest, 1982), variations in chewing, rumination, and saliva flow (Jaster and Murphy, 1983, desBordes and Welch, 1984), and differences in rate of absorption of volatile fatty acids across the rumen wall (Peters et al., 1992). More in vitro and in vivo data is needed to adequately describe variations in ruminal pH throughout the day.

Based on within-day carbohydrate and true protein availability in the rumen, variations in rumen pH throughout the day in the current model were predicted. Average rumen pH was determined based on daily fermented NSC and NDF and adjusted hourly based on fermented NSC and true protein.

The average daily pH in the rumen was predicted based on the total NSC and NDF fermented each day according to the equation: (-0.098 NSC fermented(kg) + 0.184 NDF fermented(kg) + 6.191; R^2=0.65). This equation was derived from a 27 observation dataset using stepwise regression procedures (Glenn et al., 1989, McCarthy et al., 1989, Klusmeyer et al.,

1990, Cameron et al., 1991, Klusmeyer et al., 1991a, Klusmeyer et al., 1991b, and Stokes et al., 1991a).

The pH decreasing factor was calculated by multiplying the total kg NSC and true protein available at time, t, by the scaling coefficient, -1.5. The scaling coefficient was determined by comparing the outcome of the model to within-day pH variation previously seen in cow studies (Robinson et al., 1986, Knowlton, 1994). Direct comparison between hourly available nutrients and hourly pH could not be conducted due to insufficient reported hourly pH data.

It was assumed that less saliva would be produced during eating than during rumination, thus, pH would be more likely to increase during periods when the cow was not eating (Cassida and Stokes, 1986, Erdman, 1988, Knowlton, 1994). It was also assumed that more saliva would be generated during meal consumption of long hay than would be on silage-based diets. The pH increasing factor was calculated as: [(total kg NSC and true protein available per day) * (1.5)] * [% of the total pH increasing factor available at time, t]. The % of the total pH increasing factor available at time, t, was estimated according to the predicted meal pattern (Table 2) with less available during eating and more available when not eating.

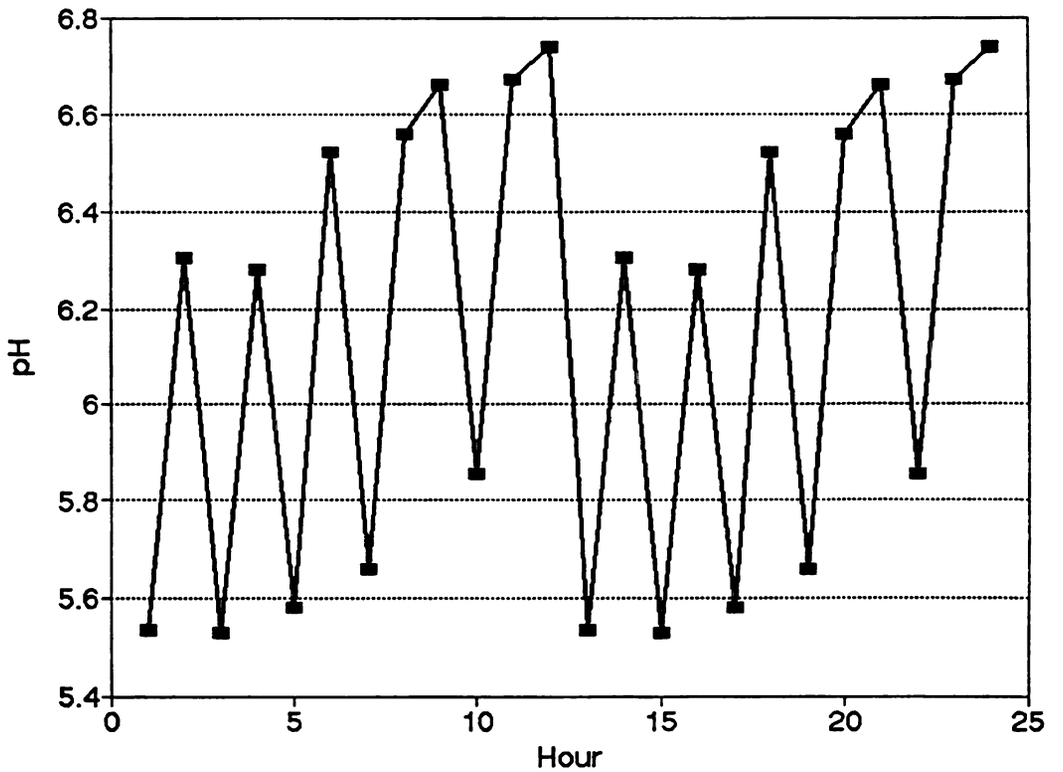
Table 2: Percentage of the total pH increasing factor available at each hour throughout a 24-hour period in the within-day model.

	Meal Pattern			
Hour	#1	#2	#3	#4
1*	1	1	1	5
2	5	5	5	5
3	1	1	1	1
4	5	5	7	5
5	1	1	7	1
6	7	7	1	5
7	1	1	7	1
8	7	7	7	8
9	7	1	1	1
10	1	5	7	5
11	7	1	7	5
12	7	5	7	5
13	1	1	1	1
14	5	7	5	5
15	1	1	1	1
16	5	7	5	5
17	1	7	1	1
18	7	1	5	7
19	1	7	1	7
20	7	7	7	7
21	7	1	1	1
22	1	7	7	5
23	7	7	1	8
24	7	7	7	5

* time of the first feeding of the TMR each day

Figure 2 shows the predicted variation in rumen pH throughout the day from the diet previously modeled in Figure 1 (Diet #2, Stokes et al., 1991a). Unfortunately, effective NDF could not be used in this model due to the fact that feed samples were obtained in ground form. The addition of effective NDF to the model, as well as a more accurate prediction of saliva flow, should significantly improve the prediction of pH (Jaster and Murphy, 1983, Sniffen et al., 1992).

Figure 2. Hourly ruminal pH in cows consuming Diet #2 in the study of Stokes et al.(1991a) as modeled using the within-day model.



Bacteria were divided into two pools in the model: NDF-degrading bacteria and NSC-degrading bacteria. Daily microbial nitrogen yield was calculated for each bacterial type according to the derivation of Pirt(1965) in which overall energy utilization was partitioned into that used for maintenance and that used for growth functions. The efficiency with which the bacteria grow was a function of: maintenance, growth rate, and a theoretical maximum yield.

$$1/Y = m/k + 1/Ymax$$

where: Yield efficiency(Y) = g bacteria/g CHO degraded

Maintenance Rate(m) = g CHO/g bacteria/h

Growth Rate(k) = h^{-1}

Theoretical Maximum Yield(Ymax) = g bacteria/ g CHO/h

The maintenance requirement for the bacteria increased in a stepwise manner as predicted rumen pH declined (Table 3). The initial estimates of maintenance were derived from the pure continuous culture work of Russell and Baldwin(1979) and the stepwise changes in maintenance as a result of pH fluctuations were based on the work of Russell and Dombrowski(1980) in which the percent of maximum growth yield of 10 strains of rumen bacteria grown in pH 4.75 to pH 6.75 media (increasing in .25 pH increments) had been determined.

Table 3: Bacterial maintenance requirements (g CHO/g bacteria/h) modified according to predicted rumen pH in the within-day model.

NSC Bacteria Maintenance:		NDF Bacterial Maintenance:	
Above pH 6.3	0.05	Above pH 6.5	0.02
pH 6.0 - 6.3	0.10	pH 6.25 - 6.5	0.06
pH 5.75 - 6.0	0.12	pH 6.0 - 6.25	0.10
pH 5.5 - 5.75	0.15	Less than pH 6.0	0.50
pH 5.25 - 5.5	0.20		
pH 5.0 - 5.25	0.30		
Less than pH 5.0	1.00		

The growth rate of each bacteria type in the model was a function of the average rate of degradation of the available NDF or NSC (free glucose, starch, and other NSC) consumed by the animal.

The theoretical maximum growth yield in the within-day model was set at .50 g bacteria/ g carbohydrate/h, based on the work of Isaacson et al.(1975) in which experiments were carried out with mixed bacterial cultures with excess amino acid nitrogen, at pH 6.7, and with no protozoa present. Russell et al.(1992) arbitrarily decreased Ymax to .40 g bacteria/g carbohydrate/h in the Cornell Net Carbohydrate and Protein System in an attempt to correct for amino acid limitations, moderate declines in rumen pH, and protozoal predation. However, upon comparison of microbial nitrogen yield estimates of CNCPS to that measured in vivo, it was determined that they were generally too low and that the higher Ymax modified according to rumen pH fluctuations and amino acid availability might be more appropriate. Research data concerning actual Ymax for rumen bacteria is limiting.

Stouthamer(1973) calculated a theoretical Y_{ATP} of 32 g dry weight of cells per mole of ATP, however, this estimate is difficult to incorporate into rumen models due to the problem of predicting ATP production from the various carbohydrate sources and fermentation patterns in the rumen.

Total bacteria were calculated for each hour as the sum of the NSC bacteria and the NDF bacteria. Total bacteria produced per day were summed over the 24 hours. Bacteria were assumed to contain 10% nitrogen (62.5% crude protein) (Russell et al., 1992). It is recognized, however, that large variation in bacterial crude protein content has been found with different diet types due to variations in bacterial growth rates (Hvelplund, 1986). Bucholtz(1972) found bacterial crude protein (%DM) to range from 36.9 to 58.0% with a mean of 49.9%.

Sensitivity of the Model to Predicted Hourly pH Fluctuations:

Ten diverse diets from the database described in Chapter IV were chosen to evaluate the sensitivity of the model to the changes in microbial maintenance requirements imposed due to pH fluctuations (McCarthy et al., 1989, Glenn et al., 1989, Klusmeyer et al., 1990, Robinson and Kennelly, 1990, Klusmeyer et al., 1991a, Klusmeyer et al., 1991b, Stokes et al., 1991a, Cunningham et al., 1991, Garrett and Polan, 1992). Mean daily microbial nitrogen flow calculated from the ten diets in cows at steady state with constant microbial maintenance

requirements (0.05 and 0.15 g carbohydrate/g bacteria/h for the NDF and NSC-degrading bacteria, respectively) and with Ymax set at .50 g bacteria/g carbohydrate/h was compared to mean predicted microbial nitrogen flow when maintenance requirements were modified either according to predicted average daily pH or predicted hourly pH. Differences between all three mean microbial nitrogen flow estimates were significant ($p<0.0001$) (Table 4).

Table 4. Effect of adjustment of microbial maintenance requirements according to rumen pH on mean predicted microbial nitrogen flow for ten diverse diets.

Comparison* #1 vs. #2	Mean#1(SD)	Mean#2(SD)	(p<)	MSE	F-value
Unmod vs. DpH	302.6(98)	287.9(86)	0.0001	27.18	108.53
Unmod vs. HpH	302.6(98)	273.9(81)	0.0001	31.33	79.72
DpH vs. HpH	287.9(86)	273.9(81)	0.0001	26.79	84.41

* Unmod = No maintenance adjustment, DpH = Adjusted for Daily pH, HpH = Adjusted for Hourly pH

As expected (Strobel and Russell, 1986), accounting for variations in pH resulted in significant reductions in predicted microbial nitrogen flow ($p<0.0001$). Accounting for predicted hourly pH fluctuations resulted in greater reduction of predicted microbial nitrogen flow than did accounting only for predicted average daily pH. This result was expected due to the non-linear effect of pH (Russell and Dombrowski, 1980). As pH declines, its incremental effect on microbial maintenance requirements increases.

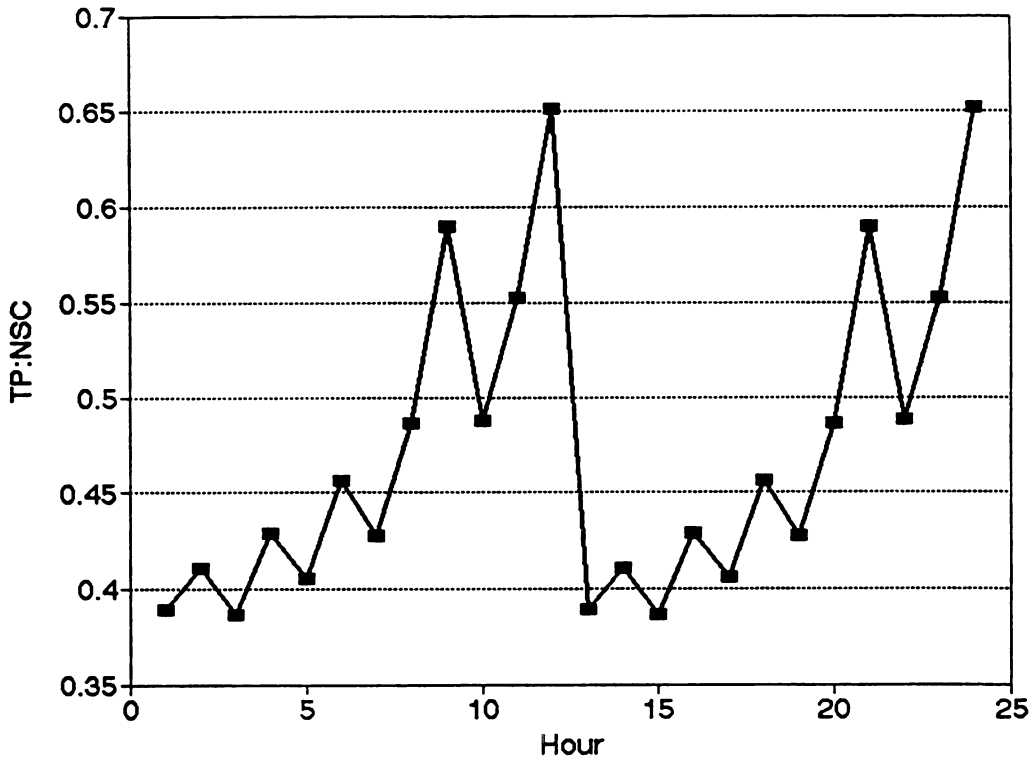
C. Prediction of Within-Day Fluctuations in the Degraded True Protein: Fermented NSC Ratio in the Rumen:

The work of Hoover et al.(1990), Stokes et al.(1991b), Russell et al.(1983) and Russell and Sniffen(1984) has shown that the degraded true protein: fermented NSC ratio has an impact on microbial growth. For this reason, in the model the ratio of true protein degraded each hour to NSC fermented each hour throughout the day was computed. True protein was made up of B_1, B_2, and B_3 protein while NSC was the sum of available free glucose, starch, and Other NSC.

In Figure 3, the degraded true protein: fermented NSC ratio in the rumen throughout a 24-hour period in the cow on the diet modeled in Figure 1 is shown (Diet #2, Stokes et al., 1991a). Due to the more rapid degradation of nonstructural carbohydrate than true protein, the ratio was lower nearer the times of maximum consumption of the total diet.

Microbial mass produced each hour throughout the day was modified for the NSC-fermenting bacteria based on the degraded true protein:fermented NSC ratio in the rumen each hour. In the work of Russell and Sniffen(1984), cell protein per gram of carbohydrate fermented increased as the amino acid: carbohydrate ratio increased. However, the slope of the line decreased when the amino acid:carbohydrate ratio reached 0.062. It was therefore assumed in the model that this was the point at which amino acids were being used primarily as an

Figure 3. Hourly ruminal degraded true protein:fermented NSC in cows consuming Diet #2 in the study of Stokes et al.(1991a) as modeled using the within-day model.

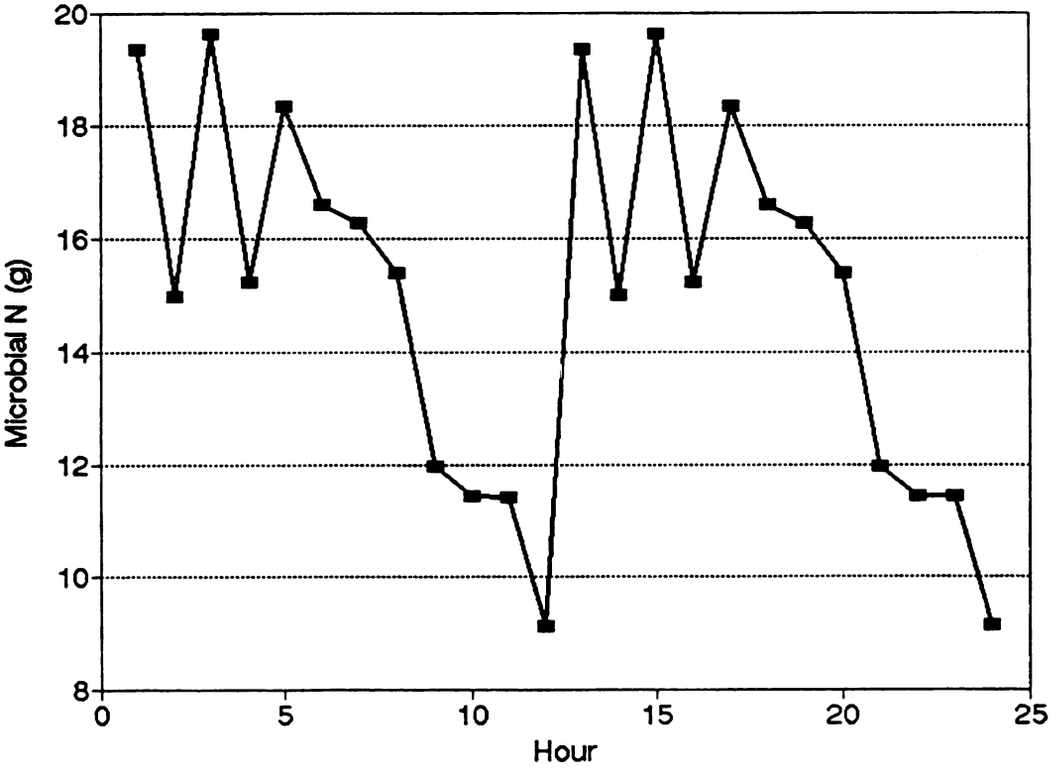


energy source for the bacteria rather than as monomers for the formation of bacterial protein (Hoover and Stokes, 1991). It was assumed that a Ymax of .50 g bacteria/ g carbohydrate/h was attained at this ratio of 0.062 TP:NSC (Isaacson et al., 1975). In the model, when the degraded TP:fermented NSC ratio was 0.062, microbial yield was not modified; it remained at 100%. When the ratio was less than 0.062, microbial yield was less than 100% of baseline, the percentage decreasing from the baseline according to the equation derived from the data of Russell and Sniffen(1984): (298.4178(TP:NSC) + 81.89957), R^2=0.97 (4 obs.). When degraded TP: fermented NSC was greater than 0.062, microbial yield was predicted to be greater than 100% of baseline, the percentage increasing from the baseline according to the equation: (64.03066(TP:NSC) + 97.6924), R^2=0.98 (4 obs.). Figure 4 shows the production of microbial nitrogen (g) each hour throughout a 24-hour period in the cow on the diet modeled in Figure 1 (Diet #2, Stokes et al., 1991a). Although modified by both the predicted hourly pH and the predicted degraded TP: fermented NSC ratio, microbial nitrogen production appears to be primarily driven by the availability of energy throughout the day.

Sensitivity of the Model to Hourly Fluctuations in the Degraded TP: Fermented NSC Ratio:

The same ten diets from the database described in Chapter IV, which were used to evaluate the sensitivity of the model to ruminal pH, were chosen to further evaluate the sensitivity

Figure 4. Hourly microbial nitrogen production (g) in cows consuming Diet #2 in the study of Stokes et al.(1991a) as modeled using the within-day model.



of the model to changes in the degraded TP: fermented NSC ratio (McCarthy et al., 1989, Glenn et al., 1989, Klusmeyer et al., 1990, Robinson and Kennelly, 1990, Klusmeyer et al., 1991a, Klusmeyer et al., 1991b, Stokes et al., 1991a, Cunningham et al., 1991, Garrett and Polan, 1992). Mean daily microbial nitrogen flow calculated from the ten diets in cows at steady state with constant microbial maintenance requirements (0.05 and 0.15 g carbohydrate/g bacteria/h for the NDF and NSC-degrading bacteria, respectively) and with Ymax set at .50 g bacteria/g carbohydrate/h was compared to mean microbial nitrogen flow when the daily or hourly TP:NSC ratio was accounted for, with and without daily or hourly pH as a modifier(Table 5).

Table 5. Effect of adjustment of microbial maintenance requirements according the degraded TP: fermented NSC ratio on mean predicted microbial nitrogen flow for ten diverse diets.

Comparison* #1	#2	Mean#1(SD)	Mean#2(SD)	(p<)	MSE	F-value
Unmod vs.	DTP	302.6(98)	343.2(105)	0.0001	20.93	188.63
Unmod vs.	HTP	302.6(98)	329.9(103)	0.0001	12.31	560.10
DTP vs.	HTP	343.2(105)	329.9(103)	0.0001	9.84	1016.89
Unmod vs.	DpHTP	302.6(98)	319.2(93)	0.0001	35.71	59.51
Unmod vs.	HpHTP	302.6(98)	301.8(88)	0.0001	40.41	44.72
DpH vs.	DpHTP	287.9(86)	319.2(93)	0.0001	13.76	342.29
HpH vs.	HpHTP	273.9(81)	301.8(88)	0.0001	12.45	370.73
DpHTP vs.	HpHTP	319.2(93)	301.8(88)	0.0001	27.92	92.85

* Unmod = No maintenance adjustment, DTP = Adjusted for Daily TP:NSC, HTP = Adjusted for Hourly TP:NSC, DpH = Adjusted for Daily pH, HpH = Adjusted for Hourly pH, DpHTP = Adjusted for Daily pH and TP:NSC, HpHTP = Adjusted for Hourly pH and TP:NSC

Accounting for the effect of variations in the degraded TP: fermented NSC ratio increased predicted microbial nitrogen flow over both those microbial nitrogen flow estimates unmodified by pH and those modified according to predicted ruminal pH ($p<0.0001$). This was expected with this dataset due to the fact that the mean TP:NSC ratio was 0.260 which was higher than 0.062. The combined negative effect of hourly pH and positive effect of the hourly TP:NSC ratio resulted in a very slight decrease in predicted microbial nitrogen flow from the estimate obtained when no changes in microbial maintenance or overall growth rate were considered (Table 5). The net result would be expected to vary greatly depending on diet.

Predicted microbial nitrogen flow was greater when the effect of the TP:NSC ratio was predicted on a daily basis rather than an hourly basis (Table 5). The low TP:NSC ratios predicted following meal consumption, perhaps below 0.062, might not be adequately reflected in mean daily TP:NSC. The effect of low TP:NSC ratios following meal consumption would be compounded by the increased NSC fermentation at these times.

A flow chart of the within-day model is shown in Appendix J.

Chapter IV. Compilation of a Microbial Nitrogen Flow Database

Introduction:

A dataset of sufficient size and diet variation to be useful for developing and testing a model for prediction of microbial nitrogen flow to the small intestine was needed. Due to the time and expense involved in microbial nitrogen flow estimation in vivo, it was necessary to collaborate with other researchers and to use some studies which were not conducted with the sole intent to change microbial nitrogen flows by treatment. The microbial nitrogen flow dataset was comprised of diets from a duodenally cannulated cow study conducted at MSU, thirteen duodenally cannulated cow studies conducted by other researchers, and a study conducted with ruminally cannulated cows at Miner Institute. Diets were analyzed in order to understand the rates and extents of protein and carbohydrate fermentation in the rumens.

A. Description of Studies:

1. MSU Duodenally Cannulated Cow Study:

Four Holstein cows in their second lactation were fed a corn-based diet. Microbial nitrogen flow was determined on all four cows in three different duodenal infusion treatment periods. Thus, twelve microbial nitrogen flow measurements were obtained from the same diet, the only differences in microbial nitrogen flow being those associated with individual cows and variations in dry matter intake. The ingredient and chemical composition of the diet is shown in Table 6.

The experiment began when the cows were 84 days postpartum. The experimental periods were 21 days in length with the last 3 days being the intensive collection periods. Diets were fed ad libitum as a total mixed ration with 50% fed at 0900 h and 50% fed at 2100 h. Co-EDTA was prepared as described by Uden et al.(1980) and used as a passage marker. Co-EDTA was continuously infused (0.04% of dry matter intake per day) into the rumen for the last 5 days of each experimental period. Every 9 hours throughout the 3 day intensive collection period, 500 ml of rumen fluid was obtained using a suction pump connected to a tube with 4 mm diameter holes along its length. At the same time, 1000 ml of duodenal chyme was obtained by inserting a diversion gate into the duodenal cannula (Robinson and Kennelly, 1990). Samples were frozen at -20°C prior to further analysis and composited

by treatment and period for each cow.

Core samples of duodenal chyme were taken from the frozen treatment period composites, freeze-dried, and ground through a 1-mm screen. The dry matter (AOAC, 1990), organic matter (AOAC, 1990), nitrogen (Hach et al., 1987), and total purine content (Cecava, personal communication, adapted from Ushida et al., 1985 and Zinn and Owens, 1986) of the duodenal chyme was determined. The cobalt content of the duodenal chyme was determined by first digesting samples with concentrated nitric acid and perchloric acid followed by flame emission analysis with atomic absorption spectrophotometry (Thermo Jarrell Ash Co., Model Smith/Hieffge 4000, 8E Forge Parkway, Franklin, MA).

One liter of frozen rumen fluid was cored from the larger composite rumen samples for isolation of a pure sample of rumen bacteria. The rumen fluid was thawed and 500 ml of saline(.9%) solution was added. The solution was blended in a Waring blender at low speed for two minutes and then strained through six layers of cheesecloth. The strained rumen fluid was centrifuged at 400xg for 20 minutes, the pellet was discarded, and the supernatant was spun at 20,000xg for 30 minutes. The bacterial pellet was then washed with saline and centrifuged again at 20,000xg for 30 minutes. The bacterial pellet was immediately frozen, freeze-dried, ground with a mortar and pestle, and later analyzed for dry matter (AOAC, 1990), nitrogen (Hach et al., 1987), and total purines

(Cecava, personal communication, adapted from Ushida et al., 1985 and Zinn and Owens, 1986). The procedure used for bacterial isolation was derived from methods described by others (Steinhour et al., 1982, Cecava et al., 1990b, Klusmeyer et al., 1990, Kohn, personal communication).

The procedure used for total purine estimation was as follows: one-half gram of digesta or .2 g bacteria were weighed into a 25-ml screw cap culture tube. $HClO_3$(70%) was added (2.5 ml). The tube was capped and incubated in a 90 to 95°C water bath for 1 h. After 15 minutes, tubes were vortexed and returned to the water bath. Tubes were then removed from the water bath and 17.5 ml of dilute buffer (.0285 M $NH_4H_2PO_4$) were added. Tubes were vortexed again and reinserted back into the water bath for 15 minutes. Tubes were then removed from the water bath and the contents were filtered using a glass filter (Whatman GF/D). One-half ml of filtrate was transferred to a 15 cc centrifuge tube (polypropylene) and .5 ml of .4 M $AgNO_3$ and 9 ml of .2 M $NH_4H_2PO_4$ were added. Tubes were then stoppered, vortexed, covered, and placed in the cooler (5°C) overnight. Tubes were then centrifuged at 25,000xg for 20 min, the supernatant was discarded, 10 ml of pH 2 water was added, and the tubes were centrifuged again. The supernatant was then discarded, 10 ml of .5 N HCl was added, and the tubes were stoppered and vortexed. Stoppers were then placed on top of tubes lightly and the tubes were incubated in a 90 to 95°C water bath for 30

minutes. Tubes were then removed from the water bath, vortexed, and centrifuged as before. The supernatant was then filtered through hardened filter paper (Whatman 541) and read on the spectrophotometer at 260 nm. Pure yeast RNA was used as the standard.

The amount of microbial nitrogen arriving at the duodenum was calculated based on the flow of dry matter and the proportion of the total nitrogen at the duodenum derived from microbes. Flow of DM at the duodenum was calculated by dividing the total Co dosed per day (mg) by the concentration of Co at the duodenum (mg/g DM). Total nitrogen at the duodenum was calculated by multiplying the DM flow (g) by the concentration of nitrogen in the duodenal digesta (%DM). The proportion of total nitrogen at the duodenum supplied by microbes was estimated by dividing the nitrogen:purine ratio of the bacterial isolate by the duodenal chyme nitrogen:purine ratio (Cecava et al., 1990b).

Comparisons of the treatment means were made using the General Linear Model procedure of SAS (SAS, 1982). The model used was: $Y_{ijk} = u + cow_i + period_j + treatment_k + e_{ijk}$. Contrasts made between duodenal infusion treatments were: casein versus lysine, casein versus control, casein versus control and lysine, and control versus lysine. Results of the MSU study are recorded in Appendix H.

2. Duodenally Cannulated Cow Studies of Other Researchers:

Samples of feeds fed in thirteen duodenally cannulated cow studies (51 different diets) conducted by various researchers were obtained for protein and carbohydrate fractionation. An effort was made to gather a dataset as large and diverse as possible which could be used to compare microbial nitrogen yield predicted by the developed model as a function of carbohydrate and protein availability to the microbial nitrogen yield measurement obtained in vivo.

McCarthy et al. (1989)

The purpose of this study was to determine the effect of protein and carbohydrate sources on rumen fermentation and nutrient flow at the duodenum. Four early lactation multiparous Holstein cows with an average weight of 583 kg were used. Treatments were: corn plus soybean meal, corn plus fish meal, barley plus soybean meal, and barley plus fish meal. The ingredient and chemical compositions of the diets are shown in Table 6. Diets were fed as a total mixed ration every 12 hours ad libitum.

Duodenal chyme was collected every 3 hours throughout the last 3 days of each treatment period, with the sampling time moved up one hour every day so that each hour of the 24-hour period was represented. Rumen contents for bacterial isolation were collected near the reticulo-omasal orifice six different times during the last 3 days of each treatment

period. Rumen contents were preserved with formaldehyde and saline and frozen prior to analysis. Bacteria were isolated using the method of Steinhour et al.(1982). Total purines were used as a bacterial marker (Zinn and Owens, 1982). Chromic oxide (10 g), wrapped in filter paper, was dosed into the rumen twice each day on day 7 through day 16 and the chromium concentration of the duodenal digesta was determined for estimation of dry matter flow (Williams et al., 1962).

Klusmeyer et al. (1990)

The purpose of this study was to determine the effect of different sources and amounts of protein on rumen fermentation and nutrient flow to the small intestine. Four multiparous Holstein cows were used in a 4x4 Latin square and fed a 14.5% CP diet with soybean meal, an 11% CP diet with soybean meal, a 14.5% CP diet with corn gluten meal, and an 11% CP diet with corn gluten meal. The ingredient and chemical compositions of the diets are shown in Table 6. Diets were fed as a total mixed ration ad libitum at 12 hour intervals.

Duodenal chyme and rumen fluid were collected and analyzed as in McCarthy et al.(1989) with the exception that six ruminal samples for bacterial isolation were collected near the reticulo-omasal orifice during the last 3 days of each treatment period at 2, 6, and 10 hours after feeding.

Klusmeyer et al. (1991a)

The purpose of this study was to determine the effects of calcium salts of long-chain fatty acids and protein source on rumen fermentation and flow of nutrients to the duodenum. Four multiparous Holstein cows were fed ad libitum diets as a total mixed ration every 12 hours. The treatments imposed were: soybean meal with no fat, soybean meal with fat, fish meal with no fat, and fish meal with fat. The ingredient and chemical compositions of the diets are shown in Table 6.

Duodenal chyme and rumen fluid were collected and analyzed as in McCarthy et al.(1989) with the exception that six ruminal samples for bacterial isolation were collected near the reticulo-omasal orifice during the last 3 days of each treatment period at 1, 3, 5, 7, 9 and 11 hours after feeding and bacteria were isolated from the fresh rumen contents using the method of Steinhour et al.(1982).

Klusmeyer et al. (1991b)

The purpose of this study was to determine the effects of calcium salts of long-chain fatty acids and the proportion of forage in the diet on rumen fermentation and nutrient flow to the duodenum. Four Holstein cows averaging 113 d postpartum at the beginning of the experiment were given four dietary treatments: low (50%) forage with no fat, low (50%) forage with fat, high (67%) forage with no fat, and high (67%) forage with fat. Diets were fed ad libitum as a total mixed ration

every 12 hours. The ingredient and chemical compositions of the diets are shown in Table 6. Sampling and analysis of the rumen digesta and duodenal chyme was conducted in the same manner as that described by Klusmeyer et al.(1991a).

Cameron et al. (1991)

The purpose of this study was to determine the effects of urea and/or starch supplementation to diets containing fish meal on rumen fermentation and nutrient flow to the duodenum. Four midlactation, multiparous Holstein cows were fed dietary treatments supplemented with: no urea and no starch, only urea, only starch, and both urea and starch. Diets were fed ad libitum as a total mixed ration at 12 hour intervals. The ingredient and chemical compositions of the diets are shown in Table 6.

Duodenal chyme and rumen fluid were collected and analyzed as in McCarthy et al.(1989) with the exception that six ruminal samples for bacterial isolation were collected from the reticulum at 1, 3, 5, 7, 9 and 11 hours after feeding.

Stokes et al. (1991a)

The purpose of this study was to determine the effects of different levels of nonstructural carbohydrate and degradable intake protein on rumen fermentation and microbial protein flow to the duodenum. Three Holstein cows, 36 days postpartum

at the beginning of the experiment, were fed diets containing: 38% NSC and 13.2% DIP, 31% NSC and 11.8% DIP, and 24% NSC and 9% DIP. Diets were fed ad libitum as a total mixed ration at 12 hour intervals. Experimental periods were 17 days in length. The ingredient and chemical compositions of the diets are shown in Table 6.

Duodenal digesta samples were collected every 12 h on day 13 through day 16 with a 3 hour advance in collection time each day. Rumen digesta for bacterial isolation were collected on day 16 just before feeding and at 3, 6, 9, 12, 20, 22, and 24 hours after feeding. Rumen samples were strained through four layers of cheesecloth and preserved with saturated mercuric chloride prior to bacterial isolation according to the procedure of Smith and McAllan (1974). Protozoa were collected from rumen contents collected at 3 hours after feeding. DAPA and total purines were used as bacterial markers (Zinn and Owens, 1982, Webster et al., 1990). Ytterbium-labeled diet was dosed into the rumen at each feeding beginning on day 3 of each period. The Yb-content of duodenal digesta was analyzed to estimate dry matter flow.

Glenn et al. (1989)

This study was conducted in order to determine the effects of feeding formaldehyde- and formic acid-treated alfalfa or orchardgrass silages at two intake levels on rumen

fermentation and duodenal flow of nutrients in Holstein steers. Four steers with an average weight of 209 kg were used in a 4x4 Latin square with the dietary treatments including alfalfa or orchardgrass fed at 65 or 90 g DM/kg $BW^{.75}$. Steers were fed individually at 12 h intervals. Experimental periods were 42 days in length. The ingredient and chemical compositions of the diets are shown in Table 6.

Duodenal chyme was sampled on six consecutive days at 0, 2, 4, 6, 8, and 10 hours after both feedings such that samples were taken every 12 hours with the time moved forward by 2 hours each day. Four hours post-feeding on days 38 through 40, rumen contents were taken from the anterior ventral sac, the dorsal sac, and the posterior ventral sac, mixed, and squeezed through four layers of cheesecloth. Rumen bacteria were isolated using the method of Steinhour et al.(1982). Total purines were used as a bacterial marker (Zinn and Owens, 1986). Flow of dry matter at the duodenum was calculated using the method of Faichney(1980) with ytterbium and chromium used as flow markers.

Robinson and Kennelly (1990)

This study was designed to examine the functionality of a duodenal cannula and the adequacy of indigestible markers used to estimate flow at the duodenum. Two multiparous Holstein cows were used in a 2x2 Latin square in which the treatments were: a diet containing more rapidly degradable

protein and a diet containing more slowly available protein. Diets were fed as a mixed ration supplemented with long hay. Experimental periods were 21 days in length. The ingredient and chemical compositions of the diets are shown in Table 6.

Duodenal samples were collected at 1006 h on day 17, 0518 h on day 18, 0030 h on day 19, 1942 h on day 19, and 1454 h on day 20. Samples of rumen digesta were collected at 2200 h on day 17, 1300 h on day 18, and 0400 h on day 19. Bacteria were isolated from fresh rumen fluid that was blended in a Waring blender and strained through six layers of cheesecloth. Filtrate was centrifuged at 1000xg for 10 min and the supernatant was centrifuged at 11,000xg for 20 minutes, washed with saline, and centrifuged again at 11,000xg for 20 minutes. DAPA was used as the marker for microbial nitrogen (Czerkawski, 1974, Russell and Robinson, 1984). Flow of dry matter at the duodenum was estimated using chromium-mordanted cell wall, ytterbium-soaked whole crop oat silage, and cobalt-EDTA as indigestible markers.

Cunningham et al. (1991)

The purpose of this study was to understand more regarding the effect of ration composition on amino acid flow to the duodenum. Four early lactation cows were used in a 4x4 Latin square in which the diets were composed mainly of: 1) corn silage, corn grain, soybean meal and distillers' grains, 2) corn silage, corn grain, whole cottonseed, and soybean

meal, 3) alfalfa hay, corn, and roasted soybeans, and 4) alfalfa hay, corn grain, whole cottonseed, and brewers grains. Diets were fed as a total mixed ration ad libitum at 12 hour intervals. The ingredient and chemical compositions of the diets are shown in Table 6.

The flow of microbial nitrogen to the small intestine was estimated in two ways: first, as the sum of that from the fluid-associated bacteria and the particle-associated bacteria and, second, based on the nitrogen from the fluid-associated bacteria plus only those particle-associated bacteria which were removed by homogenization. Two different estimates of bacterial nitrogen were obtained. Bacterial samples were retrieved from near the reticulo-omasal orifice. Cells were washed with both saline and 50% methanol (Lykos et al., 1991). Chromic oxide was used to estimate dry matter flow at the duodenum. Total purines were used as the bacterial marker.

Virginia Study, Wonsil (1991)

This study was conducted in order to understand more regarding fatty acid biohydrogenation in the rumen and fatty acid digestibility in the small intestine. Determination of microbial nitrogen flow to the duodenum was a secondary objective of the experiment. Two separate 4x4 Latin square trials were carried out using multiparous Holstein cows. Treatment periods were 21 days in length. In the first Latin square, treatments were: control, 3% tallow, 3.5% hydrogenated

tallow, and 4.0% coated tallow. In the second Latin square, treatments were: control, 3% hydrogenated fatty acid, 3% fish oil, and 3% soy oil. All diets were fed ad libitum with 75% of the diet fed at 1400 h and 25% fed at 0600 h. The ingredient and chemical compositions of the diets are shown in Table 6.

Rumen fluid for bacterial pellet isolation was collected on day 21 using a pump equipped with a plastic pipe with 4 mm holes and strained through a stainless steel strainer. The fresh rumen fluid was strained through six layers of cheesecloth and centrifuged at 200xg for 10 minutes. The supernatant was then centrifuged at 35,000xg for 20 minutes to obtain the bacterial pellet. The pellet was washed with distilled water and recentrifuged three times.

Cytosine was used as the bacterial marker. Ruminal and duodenal samples were digested with 70% perchloric acid prior to separation of cytosine using high performance liquid chromatography. A 25 cm Partisil-10 SCX L column was used at room temperature and cytosine was detected at 254 nm and normally eluted at 10 minutes.

Garrett and Polan (1992)

The objective of this experiment was to examine differences in rumen fermentation and flow of nutrients to the duodenum in cows fed diets differing in rumen available carbohydrate and rumen undegradable protein. Treatments

arranged in a 4x4 Latin square were: a primarily corn and soybean meal concentrate, equal amounts of corn and barley supplemented with soybean meal and blood meal or just blood meal, and barley supplemented with soybean meal and blood meal. All animals were in their first lactation and were fed twice per day (35% at 0600 h and 65% at 1400 h). Treatment periods were 21 days in length. The ingredient and chemical compositions of the diets are shown in Table 6.

Rumen bacteria were sampled and harvested in the same manner as was done in the Wonsil study previously described. Chromic oxide and cobalt-EDTA were used as markers of dry matter flow. The cytosine content of the bacteria was used to estimate microbial nitrogen flow.

Glenn, #8601

The purpose of this study was to determine the effects of the type of duodenal cannula used and the stage of lactation of the animal on duodenal nutrient flow in primiparous cows. Two T-cannulated cows and two T-cannulated cows were fed the same diet made up of 50% forage and 50% concentrate at two stages of lactation, six weeks and eleven weeks. Diets were fed ad libitum in equal portions at 12 hour intervals. Periods were 28 days in length. The ingredient and chemical compositions of the diets are shown in Table 6.

Duodenal digesta was sampled on five consecutive days at 0, 2, 4, 6, 8 and 10 h after the AM feeding and 0, 4, and 8 h

after the PM feeding. Ruminal contents were sampled from three locations four hours after feeding on days 16 through 19. Bacterial nitrogen quantitation was completed according to the methods described by Glenn et al.(1989) with total purines as the microbial marker. Ytterbium, chromium, and cobalt were used as markers of dry matter flow and were prepared as described by Glenn et al.(1989) with the following exceptions. NDF from dietary silages was extracted and Yb-marked NDF was made by soaking NDF in Yb solution with a first rinse with .01 M acetate.

Illinois Study, Clark (1991)

This study was conducted in order to examine differences in rumen fermentation and nutrient flow at the duodenum in Holstein cows fed four different primary dietary protein sources: soybean meal, blood meal, fish and blood meal, and corn gluten meal. The ingredient and chemical compositions of the diets are shown in Table 6. Sampling and analysis of the rumen digesta and duodenal chyme was conducted in the same manner as that described by Klusmeyer et al.(1991a).

3. Miner Institute Study:

Two early lactation Holstein cows (60 DIM) and two late lactation Holstein cows (142 DIM) were fed diets designed to vary in synchrony of rumen available carbohydrate and protein availability throughout the day. The ingredient and chemical

compositions of the diets are shown in Table 6. Diets were fed as a total mixed ration ad libitum at 12-h intervals. Microbial nitrogen flow was measured based on the microbial concentration in the rumen liquids and solids and the pool sizes and rates of passage of each as described in Chapter VI.

Table 6. Characteristics of microbial yield studies in which feeds were obtained for nutrient fractionation analyses:

	McCarthy et.al., 1989, J.Dairy Sci. 72:2002			
	Diet #1	Diet #2	Diet #3	Diet #4
Major Forage (26%DM)	Alfl/Gr Sil	Alfl/Gr Sil	Alfl/Gr Sil	Alfl/Gr Sil
Minor Forage (19%DM)	Corn Sil	Corn Sil	Corn Sil	Corn Sil
Major Energy Conc.	GSHCorn	GSHCorn	Barley	Barley
Major Protein Conc.	Fish meal	SBM-48	Fish meal	SBM-48
NDF (%DM)	34.40	30.20	35.60	32.50
NE_l (Mcal/kg)	1.66	1.66	1.65	1.65
CP (%DM)	14.50	15.00	14.60	14.90
DMI (kg/day)	23.30	24.20	20.50	20.90
Microbial N (g/d)	259	294	282	310
Body Wt. (kg)	583.00	583.00	583.00	583.00

	Klusmeyer et.al., 1990, J.Dairy Sci. 73:3526			
	Diet #1	Diet #2	Diet #3	Diet #4
Forage (60%DM)	Corn Silage	Corn Silage	Corn Silage	Corn Silage
Major Energy Conc.	GSHCorn	GSHCorn	GSHCorn	GSHCorn
Major Protein Conc.	SBM	SBM	CornGlutMeal	CornGlutMeal
NDF (%DM)	38.50	37.10	36.90	36.80
NE_l (Mcal/kg)	1.66	1.65	1.66	1.65
CP (%DM)	15.00	11.40	14.50	11.30
DMI (kg/day)	21.80	20.90	20.90	21.60
Microbial N (g/d)	361	344	316	330
Body Wt. (kg)	580.00	580.00	580.00	580.00

Table 6. (cont'd) Characteristics of microbial yield studies in which feeds were obtained for nutrient fractionation analyses:

	Klusmeyer et.al., 1991a, J.Dairy Sci. 74:2206			
	Diet #1	Diet #2	Diet #3	Diet #4
Major Forage (30%DM)	Alfalfa Sil	Alfalfa Sil	Alfalfa Sil	Alfalfa Sil
Minor Forage (20%DM)	Corn Sil	Corn Sil	Corn Sil	Corn Sil
Major Energy Conc.	GSHCorn	GSHCorn	GSHCorn	GSHCorn
Major Protein Conc.	SBM-48	SBM-48	Fish meal	Fish meal
NDF (%DM)	33.30	33.10	33.80	33.00
NE_l (Mcal/kg)	1.67	1.78	1.68	1.78
CP (%DM)	17.80	17.80	18.10	18.00
DMI (kg/day)	25.10	23.80	23.40	22.30
Microbial N (g/d)	340	325	284	303
Body Wt. (kg)	*	*	*	*

	Klusmeyer et.al., 1991b, J.Dairy Sci. 74:2220			
	Diet #1	Diet #2	Diet #3	Diet #4
Major Forage	AlfSil(30%DM)	AlfSil(30%DM)	AlfSil(40%DM)	AlfSil(40%DM)
Minor Forage	CrnSil(20%DM)	CrnSil(20%DM)	CrnSil(27%DM)	CrnSil(27%DM)
Major Energy Conc.	GSHCorn	GSHCorn	GSHCorn	GSHCorn
Major Protein Conc.	SBM-48	SBM-48	SBM-48	SBM-48
NDF (%DM)	30.30	29.80	35.00	34.30
NE_l (Mcal/kg)	1.68	1.80	1.61	1.71
CP (%DM)	18.10	17.60	17.90	18.20
DMI (kg/day)	25.50	24.00	24.70	23.30
Microbial N (g/d)	313	297	336	313
Body Wt. (kg)	*	*	*	*

Table 6. (cont'd) Characteristics of microbial yield studies in which feeds were obtained for nutrient fractionation analyses:

	Cameron et.al., 1991, J.Dairy Sci. 74:1321			
	Diet #1	Diet #2	Diet #3	Diet #4
Major Forage (35%DM)	Alfalfa Sil	Alfalfa Sil	Alfalfa Sil	Alfalfa Sil
Minor Forage	CrnSil(20%DM)	CrnSil(20%DM)	CrnSil(7.5%DM)	CrnSil(7.5%DM)
Major Energy Conc.	GSHCorn	GSHCorn	GSHCrn/Starch	GSHCrn/Starch
Major Protein Conc.	Fish meal	Fish meal	Fish meal	Fish meal
NDF (%DM)	32.70	32.60	27.50	27.40
NE_l (Mcal/kg)	1.61	1.60	1.61	1.61
CP (%DM)	14.90	16.90	14.80	16.80
DMI (kg/day)	23.10	23.00	21.60	21.00
Microbial N (g/d)	357	374	312	376
Body Wt. (kg)	*	*	*	*

	Glenn et.al., 1989, J. Anim Sci. 67:513			
	Diet #1	Diet #2	Diet #3	Diet #4
Major Forage(100%DM)	Alfalfa Sil	Alfalfa Sil	Orchardgrs Sil	Orchardgrs Sil
Major Energy Conc.	---	---	---	---
Major Protein Conc.	---	---	---	---
NDF (%DM)	35.20	35.20	59.40	59.40
NE_l (Mcal/kg)	1.87	1.82	1.57	1.65
CP (%DM)	24.75	24.75	19.88	19.88
Sol CP (%CP)	62.40	62.40	46.40	46.40
DMI (kg/day)	4.64	6.03	4.25	5.51
Microbial N (g/d)	134	175	76	94
Body Wt. (kg)	209.00	209.00	209.00	209.00

Table 6. (cont'd) Characteristics of microbial yield studies in which feeds were obtained for nutrient fractionation analyses:

	Robinson & Kennelly, JDS, 73:3146		Stokes et.al., JDS, 74:871		
	Diet #1	Diet #2	Diet #1	Diet #2	Diet #3
Mjr Forage(29%DM)	OatSil	OatSil	GrHay(21%DM)	CrnSil(34%DM)	GrHay(27%DM)
Mnr Forage(12%DM)	AlfSil	AlfSil	CrnSil(9%DM)	GrHay(13%DM)	CrnSil(15%DM)
Mnr Forage(18%DM)	AlfHay	AlfHay	----	----	WhtStw(15%DM)
Mjr Energy Conc.	Barley	Barley	Grd. Corn	Grd. Corn	Grd. Corn
Mjr Protein Conc.	Canola Ml	Dist.Dr.Grain	Canola Ml	WheatMds/CGM	CornGlutMl
NDF (%DM)	40.20	42.00	27.40	33.10	39.90
NE_l (Mcal/kg)	1.59	1.64	1.70	1.71	1.61
CP (%DM)	17.13	18.14	18.70	18.40	18.10
DMI (kg/day)	19.38	18.29	21.90	21.10	18.30
Microbial N (g/d)	157	177	317	333	202
Body Wt. (kg)	640.00	590.00	591.90	570.30	590.30

	Cunningham et.al., 1991, J.Dairy Sci. 74(Suppl.1):179			
	Diet #1	Diet #2	Diet #3	Diet #4
Major Forage	CrnSil(29%DM)	CrnSil(29%DM)	AlfHay(45%DM)	AlfHay(26%DM)
Minor Forage	MixHay(17%DM)	AlfHay(13%DM)	-------	CrnSil(5%DM)
Major Energy Conc.	GSHCorn	GSHCorn	GSHCorn	GSHCorn
Major Protein Conc.	SBM/Dist.Gr.	WholCot/SBM	Roasted Soy	WholCot/Brewers
NDF (%DM)	33.10	27.20	25.20	29.80
NE_l (Mcal/kg)	1.61	1.65	1.65	1.65
CP (%DM)	16.80	19.60	19.00	19.70
Sol CP (%CP)	26.20	25.80	22.50	22.30
Microbial N #1 (g/d)	429	495	525	500
Microbial N #2 (g/d)	329	408	420	436
DMI (kg/day)	19.80	21.00	21.50	20.20
Body Wt. (kg)	520	520	520	520

Table 6. (cont'd) Characteristics of microbial yield studies in which feeds were obtained for nutrient fractionation analyses:

	Virginia Study/Wonsil, 1991, 1st 4x4			
	Diet #1	Diet #2	Diet #3	Diet #4
Major Forage (38%DM)	Alfalfa Sil	Alfalfa Sil	Alfalfa Sil	Alfalfa Sil
Minor Forage (14%DM)	Corn Silage	Corn Silage	Corn Silage	Corn Silage
Major Energy Conc.	Corn Grn.	Corn Grn.	Corn Grn.	Corn Grn.
Major Protein Conc.	SBM-44	SBM-44	SBM-44	SBM-44
NDF (%DM)	27.75	27.67	27.91	27.91
NE_l (Mcal/kg)	1.85	1.80	1.83	1.69
CP (%DM)	18.00	18.00	18.00	18.00
DMI (kg/day)	24.13	23.76	24.79	24.10
Microbial N (g/d)	229	223	224	233
Body Wt. (kg)	590.8	588.1	593.2	605

	Virginia Study/Wonsil, 1991, 2nd 4x4			
	Diet #1	Diet #2	Diet #3	Diet #4
Major Forage (40%DM)	Alfalfa Sil	Alfalfa Sil	Alfalfa Sil	Alfalfa Sil
Minor Forage (15%DM)	Corn Silage	Corn Silage	Corn Silage	Corn Silage
Major Energy Conc.	Corn Grn.	Corn Grn.	Corn Grn.	Corn Grn.
Major Protein Conc.	SBM-44	SBM-44	SBM-44	SBM-44
NDF (%DM)	28.29	27.29	27.33	27.93
NE_l (Mcal/kg)	1.67	1.78	1.78	1.78
CP (%DM)	18.00	18.00	18.00	18.00
DMI (kg/day)	24.60	22.90	21.40	24.10
Microbial N (g/d)	256	205	182	217
Body Wt. (kg)	579.5	578.6	582.4	577.3

Table 6. (cont'd) Characteristics of microbial yield studies in which feeds were obtained for nutrient fractionation analyses:

Garrett and Polan, 1992, J.D.S.75(Suppl.1):232

	Diet #1	Diet #2	Diet #3	Diet #4
Major Forage (35%DM)	Alfalfa Sil	Alfalfa Sil	Alfalfa Sil	Alfalfa Sil
Minor Forage (14%DM)	Corn Silage	Corn Silage	Corn Silage	Corn Silage
Major Energy Conc.	Cracked Corn	Gr.Barl/Cr.Crn	Gr.Barl/Cr.Crn	Grd Barley
Major Protein Conc.	SBM	SBM/Bld Ml	SBM	SBM/Bld Ml
NDF (%DM)	29.19	30.39	30.92	32.90
NE_l (Mcal/kg)	1.72	1.69	1.69	1.69
CP (%DM)	19.74	20.20	20.07	20.72
DMI (kg/day)	20.50	19.70	19.20	20.30
Microbial N (g/d)	241	276	281	261
Body Wt. (kg)	502	490	478	498

Glenn Study, #8601

	Diet #1	Diet #2
Major Forage(30%DM)	Alfalfa Silage	Alfalfa Silage
Minor Forage(20%DM)	Corn Silage	Corn Silage
Major Energy Conc.	GSHCorn	GSHCorn
Major Protein Conc.	SBM	SBM
NDF (%DM)	25.82	27.39
NE_l (Mcal/kg)	1.72	1.72
CP (%DM)	16.25	16.25
DMI (kg/day)	14.50	16.10
Microbial N (g/d)	240	272
Body Wt. (kg)	523	536

Table 6. (cont'd) Characteristics of microbial yield studies in which feeds were obtained for nutrient fractionation analyses:

	Illinois Study / Clark, 1991			
	Diet #1	Diet #2	Diet #3	Diet #4
Major Forage(48%DM)	Corn Silage	Corn Silage	Corn Silage	Corn Silage
Minor Forage(15%DM)	Alfalfa Sil	Alfalfa Sil	Alfalfa Sil	Alfalfa Sil
Major Energy Conc.	GSHCorn	GSHCorn	GSHCorn	GSHCorn
Major Protein Conc.	SBM	Blood meal	BldMl/FishMl	Corn Glut Ml
NDF (%DM)	33.00	36.00	35.20	32.10
NE_l (Mcal/kg)	1.65	1.63	1.63	1.65
CP (%DM)	17.10	16.60	16.40	16.70
DMI (kg/day)	24.20	24.10	22.50	23.80
Microbial N (g/d)	290	263	208	249
Body Wt. (kg)	*	*	*	*

	MSU Cannulated Cow Study, 1991
	Diet #1
Forage	Corn Silage
Major Energy Conc.	GSHCorn
Major Protein Conc.	SBM
NDF (%DM)	31.30
NE_l (Mcal/kg)	1.63
CP (%DM)	14.70
DMI (kg/day)	20.50
Microbial N (g/d)	215
Body Wt. (kg)	595

Table 6. (cont'd) Characteristics of microbial yield studies in which feeds were obtained for nutrient fractionation analyses:

	Miner Institute Study, 1993					
	Diet A	Diet B	Diet C	Diet D	Diet E	Diet H
Major Forage	CornSil(34%)	CornSil(34%)	CornSil(35%)	CornSil(35%)	GrassSil	GrassSil
Minor Forage	AlfSil(25%)	AlfSil(23%)	AlfSil(25%)	AlfSil(26%)	----	----
Mjr Energy	HMECorn	HMECorn	GSHCorn	GSHCorn	HMECorn	GShCorn
Mjr Protein	SBM	CrnGlutFd	CrnGlutFd	SBM	SBM	SBM
NDF (%DM)	29.7	32.3	31.9	29.6	36.6	37.0
NE_l (Mcal/kg)	1.72	1.72	1.72	1.69	1.47	1.47
CP (%DM)	18.3	16.6	16.4	18.0	16.3	16.4
DMI (kg/day)	21.7	24.9	28.1	28.0	19.4	20.3
MicrobialN(g/d)	311	250	348	438	174	153
Body Wt. (kg)	611	618	663	653	601	601

* Information Unavailable from the Investigator

B. Analysis of Dietary Ingredients from Studies in the Microbial Nitrogen Flow Dataset:

1. MSU Duodenally Cannulated Cow Study:

Materials and Methods:

Protein Fractionation

The A, B_1, B_2, B_3, and C protein fractions of all individual feed ingredients present in the diet were determined. Crude protein was determined by the Kjeldahl procedure (AOAC, 1990). The procedure of Krishnamoorthy et al. (1982) was used to estimate soluble crude protein. Soluble true protein (B_1) was precipitated using trichloroacetic acid according to the method of Kohn and Allen(1992) and soluble non-protein nitrogen (A) was calculated by difference. Crude protein remaining insoluble after boiling with acid detergent (C) was measured (Pichard and Van Soest, 1977). Crude protein insoluble in neutral detergent (with substitution of triethylene glycol for 2-ethoxyethanol and omission of decahydronaphthalene and sodium sulfite) but soluble in acid detergent was classified as the B_3 fraction (Pichard and Van Soest, 1977). The B_2 fraction was estimated by difference as that crude protein which was not soluble in borate-phosphate buffer (Krishnamoorthy et al., 1982) but soluble after refluxing with neutral detergent.

Carbohydrate Fractionation

All individual feed ingredients were analyzed for ADF (Goering and Van Soest, 1970), NDF (Van Soest et al., 1991), lignin (Goering and Van Soest, 1970), and starch plus free glucose (Karkalas, 1985, B.A. Lewis, personal communication). Heat-stable alpha-amylase (Number A3306; Sigma Chemical Co., St. Louis, MO) was used on a routine basis for all samples (Procedure A) and 8 M urea was also used as a pre-treatment for all very starchy, low fiber samples (Procedure B) prior to boiling in neutral detergent (with substitution of triethylene glycol for 2-ethoxyethanol and omission of decahydronaphthalene and sodium sulfite) for the estimation of NDF (Procedures A and B in: Van Soest et al., 1991). Other NSC, including pectins, beta-glucans, and non-glucose sugars, was estimated by difference.

The following procedure was used for estimation of the free glucose plus starch content of all feeds. Two ml of 1.0 N NaOH was added to 0.15 - 0.2 g of ground sample in a 125 ml Erlenmeyer flask. Twenty-five ml of distilled water and 0.15 ml of glacial acetic acid was then added. Twenty-three ml of distilled water was added plus 50 ul of heat-stable amylase (Number A3306; Sigma Chemical Co., St. Louis, MO). Samples were incubated in an 85°C shaking water bath for 30 minutes. Five ml of acetate buffer (120 ml glacial acetic acid and 164 g anhydrous sodium acetate in distilled H_2O and diluted to 1 liter), 35 ml of distilled water and 10 ml of glucoamylase

solution (Sigma Chemical Co., 10 mg/ml filtered through Whatman #541 prior to use) were then added. Samples were then incubated in a 55°C shaking water bath for 2 hours. Samples were filtered through Whatman 541 filter paper and diluted to 250 ml. Two-tenths of a ml of the diluted solution was then assayed for free glucose using glucose oxidase(Sigma, 1990).

Feeds containing more than 30% starch plus glucose were analyzed for 8 h *in vitro* degradable starch plus glucose while feeds containing more than 30% NDF were analyzed for 30 h *in vitro* degradable NDF (Tilley and Terry, 1963, Goering and Van Soest, 1970). The rates of degradation of starch were determined using the following equation: [[(ln 100 - ln(100-((initial starch(%DM) - remaining starch(%DM) / initial starch(%DM))*100)))/8]*100]. The rates of degradation of available fiber were determined in the same manner with a correction made for the crude protein associated with NDF and for rumen undegradable NDF which was estimated as lignin(%DM) multiplied by the factor 2.4 (Chandler et al., 1980).

All dietary ingredients were also analyzed for lipid (AOAC, 1990), dry matter (AOAC, 1990), and organic matter (AOAC, 1990).

In situ DM and CP digestibility

Individual feed ingredients were incubated in dacron bags in the rumen of one of the cows consuming the study diet for 0, 1, 4, 8, 12, 24, and 48 hours. Corn silage was also

incubated for 72 h. Each feed was incubated in 4 bags for each time period in two different runs.

The bags were constructed of 53+-10 micron pore size polyester material with heat-sealed seams (Ankom Products, Spencerport, New York). Each bag contained five grams of sample. After closing the bags with 20 cm nylon clamp ties, 10 cm x 20 cm bag surface area remained, providing approximately 12.5 mg DM / cm^2 surface area. Quadruplicate sets of bags were attached to a 225 gram weight and 1 yard of 80-pound test monofilament fishline (for retrieval), and inserted into the rumen. Sets of bags were inserted in order of decreasing incubation time interval in order to facilitate their removal from the rumen all at the same time.

Prior to ruminal incubation, all of the bags were soaked in lukewarm water for 15 minutes for the purpose of hydrating the samples and removing all water-soluble material. After removal from the rumen, the bags were rinsed with lukewarm water until the rinse water ran clear. They were then placed in a 60°C oven to dry for 48 hours. Sets of bags were soaked and rinsed without ruminal incubation to determine degradation at 0 h within the rumen.

After the bags were dried, the degree of dry matter and crude protein degradation over time was determined. All of the bags with their remaining contents were weighed and the loss in weight as a result of incubation was computed. The bags were emptied and residues were ground through a 1-mm

screen of a cyclone mill (UDY Corporation, Fort Collins, Colorado). Samples were analyzed for their dry matter (AOAC, 1990) and crude protein content (Hach et al., 1987). Rates of B_2 degradation for each concentrate feed were determined according to the amount of 8 h in situ CP disappearance after correction for the A and B_1 fractions. The rates of degradation of B_2 for each concentrate feed were determined using the following equation: [[(ln 100 - ln(100-((initial B_2(%DM) - (initial B_2(%DM) - (total remaining CP at 8 h - (A + B_1))(%DM)) / initial B_2(%DM))*100)))/8]*100].

Results:

Fractionation and in vitro degradability:

The results of the protein and carbohydrate fractionation analyses and in vitro starch and NDF degradability are recorded in Appendices A, B, and C.

In situ rate analysis:

Dry matter degradation (Appendix H) and crude protein degradation (Appendix H) over time were computed for each of the dietary ingredients. Rates of B_2 degradation are recorded in Appendix D.

2. Duodenally Cannulated Cow Studies of Other Researchers:

Materials and Methods:

Protein and carbohydrate fractionation and *in vitro* NDF and starch plus glucose degradability analysis of the feeds fed in the studies of other researchers were carried out as previously described for the MSU cannulated cow study feeds. An attempt was made to obtain individual concentrate mix samples and individual forage samples from all studies, however, this was not always possible. Only samples of the total mixed diets fed could be obtained and analyzed from the studies of McCarthy et al.(1989), Klusmeyer et al.(1990), Stokes et al.(1991), and Glenn #8601. No *in situ* degradability analyses were conducted on the feeds from other researchers.

Results:

The results of the protein and carbohydrate fractionation analyses and *in vitro* starch and NDF degradability are recorded in Appendices A, B, and C.

3. Miner Institute Study:

Materials and Methods:

Protein and carbohydrate fractionation analysis of the feeds fed in the Miner Institute study were carried out as previously described for the MSU cannulated cow study feeds.

Feeds were also analyzed by the <u>in situ</u> technique previously described (concentrates: 2 h and 8 h, forages: 48 h) to estimate rate and extent of CP, starch, and available NDF degradation. Rates of B_2 degradation for each concentrate feed were determined according to the amount of 8 h <u>in situ</u> CP disappearance after correction for the A and B_1 fractions. The rates of degradation of B_2 for each concentrate feed were determined using the following equation: [[(ln 100 - ln(100-((initial B_2(%DM) - (initial B_2(%DM) - (total remaining CP at 8 h - (A + B_1))(%DM)) /initial B_2(%DM))*100)))/8]*100]. The measurements of the starch plus glucose fractions were based on 2 h and 8 h <u>in situ</u> starch disappearance. The amount of S_2 was calculated as the natural antilogarithm of: [(ln(starch remaining at 2h (%DM)) + [2 * [(ln(starch remaining at 2h (%DM)) - ln(starch remaining at 8h (%DM)))/6]]. The amount of S_1 was calculated as the difference between the total amount of starch in the feed and S_2. The rates of degradation of S_2 were determined using the following equation: [[(ln(starch remaining at 2h (%DM)) - ln(starch remaining at 8h (%DM)))/6]*100]. Rates of available NDF degradation in each

forage were determined based on 48 h *in situ* degradability. The rates of degradation of available NDF were determined using the following equation: [[(ln 100 - ln(100-((initial available NDF(%DM) - remaining available NDF(%DM) /initial available NDF(%DM))*100)))/48]*100]. A correction was made for the crude protein associated with NDF and for undegradable NDF which was estimated as lignin(%DM) multiplied by the factor 2.4 (Chandler et al., 1980).

Results:

The results of the protein and carbohydrate fractionation analyses are recorded in Appendices A, B, and C. The results of the *in situ* starch fractionation and the rate analyses are recorded in Appendix D.

Chapter V. Evaluation of Models for Prediction of Microbial Nitrogen Flow Measured *In Vivo*:

Introduction:

The 75 observation microbial nitrogen flow database described in Chapter IV was the basis for evaluation of the within-day model (Chapter III), NE_l consumed (Mcal/d), and other models currently in use for prediction of microbial nitrogen flow. In addition, regression analysis was used to evaluate those variables which greatly impact the prediction of measured microbial nitrogen flow.

Materials and Methods:

A. Evaluation of the Within-Day Model for Prediction of *In Vivo* Measured Microbial Nitrogen Flow:

The linear regression procedures of Quattro Pro (1992) were used to determine the linear relationship of microbial nitrogen flow predicted using the within-day model to *in vivo* measured microbial nitrogen flow in the 75 observation

database (Glenn et al., 1989, McCarthy et al., 1989, Klusmeyer et al., 1990, Robinson and Kennelly, 1990, Cameron et al., 1991, Clark, 1991, Cunningham et al., 1991, Klusmeyer et al., 1991a, Klusmeyer et al., 1991b, Stokes et al., 1991, Wonsil, 1991, Garrett and Polan, 1992, Glenn #8601, MSU cannulated cow study, Miner Institute study).

The General Linear Model procedure of SAS (SAS, 1982) was then used to relate microbial nitrogen flow predicted by the within-day model, within-day model estimated rumen fermented true protein, within-day model estimated rumen fermented NPN, within-day model estimated rumen fermented NSC, and within-day model estimated rumen fermented NDF, rumen available fat, and percent forage in the diet to <u>in vivo</u> measured microbial nitrogen flow. Laboratories also were put into the models as class variables in an attempt to separate out the individual variation associated with the different procedures conducted in the different laboratories. Quadratic effects of variables were also analyzed by putting each variable as well as the square of each into the model. Correlations between individual variables were determined.

The following models were used to determine the signifance of each variable, either alone or in combination with other variables, in the prediction of <u>in vivo</u> measured microbial nitrogen flow:

$$Y_{ijklmno} = u + Lab_i + TP_j + NPN_k + NSC_l + NDF_m + Fat_n + Forage_o + e_{ijklmno}$$

$$Y_i = u + Lab_i + e_i$$

$$Y_{ij} = u + Lab_i + WDME_j + e_{ij}$$

$$Y_{ijklmnop} = u + Lab_i + WDME_j + TP_k + NPN_l + NSC_m + NDF_n + Fat_o + Forage_p + e_{ijklmnop}$$

$$Y_{ijk} = u + Lab_i + WDME_j + TP_k + e_{ijk}$$

$$Y_{ijk} = u + Lab_i + WDME_j + NPN_k + e_{ijk}$$

$$Y_{ijkl} = u + Lab_i + WDME_j + TP_k + NPN_l + e_{ijkl}$$

$$Y_{ijk} = u + Lab_i + WDME_j + NSC_k + e_{ijk}$$

$$Y_{ijk} = u + Lab_i + WDME_j + NDF_k + e_{ijk}$$

$$Y_{ijkl} = u + Lab_i + WDME_j + NSC_k + NDF_l + e_{ijkl}$$

$$Y_{ijk} = u + Lab_i + WDME_j + Fat_k + e_{ijk}$$

$$Y_{ijk} = u + Lab_i + WDME_j + Forage_k + e_{ijk}$$

$$Y_{ijklmnopqrstuv} = u + Lab_i + WDME_j + TP_k + TP^2_l + NPN_m + NPN^2_n + NSC_o + NSC^2_p + NDF_q + NDF^2_r + Fat_s + Fat^2_t + Forage_u + Forage^2_v + e_{ijklmnopqrstuv}$$

$$Y_{ijklmnopqrstu} = u + Lab_i + TP_j + TP^2_k + NPN_l + NPN^2_m + NSC_n + NSC^2_o + NDF_p + NDF^2_q + Fat_r + Fat^2_s + Forage_t + Forage^2_u + e_{ijklmnopqrstu}$$

where: $Y_{i...}$ = <u>in vivo</u> measured microbial nitrogen flow, u = mean, $b_{i...}$ = the partial regression coefficient of the response variable on Lab=Laboratory, WDME=within-day model microbial nitrogen estimate, TP=kg fermented true protein, NPN=kg fermented non-protein nitrogen, NSC=kg fermented nonstructural carbohydrate, NDF=kg fermented NDF, Fat=kg rumen available fat, Forage=Percent forage in the diet, $e_{i...}$=random error

Although it was known that the correlation between the within-day model estimates and individual fermented nutrients might be high, both were put into some of the models. If the addition of a certain individual fermented nutrient

significantly affected the regression model beyond the within-day model estimate, it would indicate the extent to which its relationship to microbial nitrogen flow in the within-day model was inadequately accounted.

Type III R^2 describes the variance explained when a variable is included last in a model and is not order dependent. It was used as opposed to Type I R^2 in order to separate out effects of all other variables in the model prior to the variable of interest. This was especially important for determining the effects of different laboratory techniques and that of the within-day model. Standard partial regression coefficients were also calculated to evaluate the influence of each variable within a given model.

Comparisons of different laboratories were made by evaluating the y-intercept estimates generated for each laboratory in each model. The y-intercepts for all labs were relative to one lab (Hoover) which had a y-intercept of zero.

B. Evaluation of NE_l (Mcal/d) for Prediction of *In Vivo* Measured Microbial Nitrogen Flow:

The linear regression procedures of Quattro Pro (1992) were used to determine the linear relationship of NE_l (Mcal/d) to *in vivo* measured microbial nitrogen flow in the 75 observation database.

The General Linear Model procedure of SAS (SAS, 1982) was used to relate NE_l (Mcal/d) with and without the inclusion of the laboratory in which microbial nitrogen flow was measured as a class variable to <u>in vivo</u> measured microbial nitrogen flow using the models:

$$Y_{ij} = u + Lab_i + NE_{lj} + e_{ij}$$
$$Y_i = u + NE_{li} + e_i$$

where: Y_{ij} = <u>in vivo</u> measured microbial nitrogen flow, u = mean, Lab_i= the partial regression coefficient of the response variable on Laboratory, NE_{lj}= the partial regression coefficient of the response variable on NE_l, e_{ij}=random error

C. Evaluation of Other Models and Dietary Variables for Use in Prediction of <u>In Vivo</u> Measured Microbial Nitrogen Flow:

Dry matter intake (DMI), fat-corrected NE_l ($FCNE_l$), microbial nitrogen flow predicted by the Spartan Model (SpartanMN; Spartan Ration Evaluator, 1991), microbial nitrogen flow predicted by the Cornell Net Carbohydrate and Protein System (CNCPSMN), <u>in vivo</u> measured rumen-digested organic matter (KgOMD), <u>in vivo</u> measured rumen-digested starch (KgStarch), and <u>in vivo</u> measured rumen-digested NDF (KgNDF) were evaluated to determine their ability to predict <u>in vivo</u> measured microbial nitrogen flow at the duodenum. Using the General Linear Model procedure of SAS (SAS, 1982), the following models were evaluated:

$$Y_{ij} = u + Lab_i + DMI_j + e_{ij}$$
$$Y_i = u + DMI_i + e_i$$

$$Y_{ij} = u + Lab_i + FCNE_{lj} + e_{ij}$$
$$Y_i = u + FCNE_{li} + e_i$$

$$Y_{ij} = u + Lab_i + SpartanMN_j + e_{ij}$$
$$Y_i = u + SpartanMN_i + e_i$$

$$Y_{ij} = u + Lab_i + CNCPSMN_j + e_{ij}$$
$$Y_i = u + CNCPSMN_i + e_i$$

$$Y_{ij} = u + Lab_i + KgOMD_j + e_{ij}$$
$$Y_i = u + KgOMD_i + e_i$$

$$Y_{ij} = u + Lab_i + KgStarch_j + e_{ij}$$
$$Y_i = u + KgStarch_i + e_i$$

$$Y_{ij} = u + Lab_i + KgNDF_j + e_{ij}$$
$$Y_i = u + KgNDF_i + e_i$$

where: $Y_{i...}$ = <u>in vivo</u> measured microbial nitrogen flow, u = mean, $b_{i...}$ = the partial regression coefficient of the response variable on Lab=Laboratory, DMI=Dry Matter Intake, $FCNE_l$=Fat-corrected NE_l, SpartanMN=microbial N flow predicted by the Spartan Model, CNCPSMN=microbial N flow predicted by the Cornell Net Carbohydrate and Protein System, KgOMD=measured rumen-digested organic matter, KgStarch=measured rumen-digested starch, KgNDF=measured rumen-digested NDF, $e_{i...}$=random error

The linear regression procedures of Quattro Pro (1992) were used to determine the linear relationship of fat-corrected NE_l ($FCNE_l$), microbial nitrogen flow predicted by the Spartan Model (SpartanMN), microbial nitrogen flow predicted by the Cornell Net Carbohydrate and Protein System (CNCPSMN), and <u>in vivo</u> measured rumen-digested organic matter (KgOMD) to <u>in vivo</u> measured microbial nitrogen flow at the duodenum.

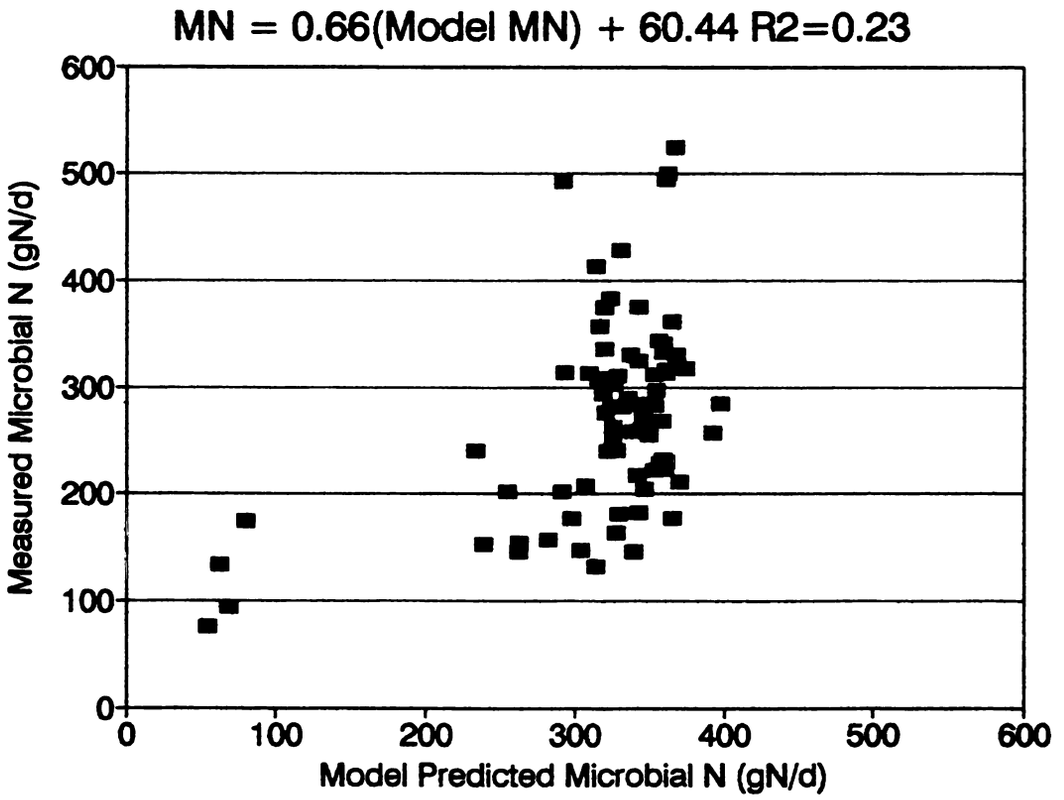
Results and Discussion:

A. Evaluation of the Within-Day Model for Prediction of *In Vivo* Measured Microbial Nitrogen Flow:

The accuracy with which the within-day model predicted measured microbial nitrogen flow for the 75 *in vivo* observation dataset is shown in Figure 5. The within-day prediction of microbial nitrogen flow accounted for only 23% of the variation in measured microbial nitrogen flow. The within-day model predicted microbial nitrogen flows for the majority of the diets to be within the range of 300 - 350 g N / day when measured flows were within the range of 150 - 525 g N / day for those diets (Appendix E).

One of the major problems associated with the prediction of daily microbial nitrogen flow is the large variation in methodology among laboratories. Table 7 shows the Type III R^2 associated with laboratory within the various regression models for prediction of microbial nitrogen flow. Unfortunately, effects such as cow size and stage of lactation would still be associated with the laboratory Type III R^2 but with the exception of the study of Glenn et al.(1989), these effects were expected to be minimal. Based on Run#13 and Run#14 (Table 7) which accounted for the most dietary variation, it can be concluded that the laboratory in which microbial protein flow was determined accounts for 31% of the

Figure 5. Microbial nitrogen flow (g N/d) predicted using the within-day model versus measured microbial nitrogen flow (g N/d) at the duodenum in the microbial nitrogen flow dataset (75 observations).



variation in this dataset.

Table 8 shows the relative Y-intercept estimates associated with each laboratory in the models generated for prediction of microbial nitrogen flow. Intercepts vary depending on dietary variables included in the models, especially those from the Glenn lab. However, general trends can be seen between those models which account for most of the dietary variation, such as, Run#1, Run#4, and Run#13. The lowest intercepts are associated with the MSU, Robinson, and VPI studies. The Miner, Clark, and Hoover studies tend to have intermediate intercepts while the studies of Glenn and Schwab had high intercepts.

Table 7. Variance associated with variables included in models for prediction of microbial nitrogen flow (75 obs.).

Run #1: Variable**	Type III R^2	(p<)	Estimate***	SE Est	SPRC*
Laboratory	0.39	0.0001	----(Table 8)---		-----
FermTP	0.04	0.0014	91.59	27.44	37.42
FermNPN	0.01	0.0472	57.29	28.29	21.08
FermNSC	0.03	0.0040	26.16	8.76	45.32
FermNDF	0.01	0.1100	36.80	22.69	18.34
AvailFat	0.04	0.0009	-93.86	26.91	30.67
%Forage	0.00	0.3717	-0.78	0.87	10.55
Total Type I R^2	0.80				

Run #2: Variable**	Type III R^2	(p<)	Estimate***	SE Est	SPRC*
Laboratory	0.57	0.0001	----(Table 8)---		-----
Total Type I R^2	0.57				

Run #3: Variable**	Type III R^2	(p<)	Estimate***	SE Est	SPRC*
Laboratory	0.45	0.0001	----(Table 8)---		-----
Model MN	0.12	0.0001	0.83	0.17	-----
Total Type I R^2	0.69				

Run #4: Variable**	Type III R^2	(p<)	Estimate***	SE Est	SPRC*
Laboratory	0.39	0.0001	----(Table 8)---		-----
Model MN	0.00	0.6453	0.21	0.44	13.94
FermTP	0.03	0.0076	85.22	30.86	34.82
FermNPN	0.01	0.0729	53.79	29.47	19.79
FermNSC	0.02	0.0244	23.72	10.27	41.09
FermNDF	0.00	0.3521	27.94	29.79	13.93
AvailFat	0.04	0.0009	-95.90	27.44	31.34
%Forage	0.00	0.7769	-0.36	1.27	4.85
Total Type I R^2	0.80				

Table 7.(cont'd). Variance associated with variables included in models for prediction of microbial nitrogen flow (75 obs.).

Run #5:

Variable**	Type III R^2	(p<)	Estimate***	SE Est	SPRC*
Laboratory	0.45	0.0001	----(Table 8)---		-----
Model MN	0.04	0.0038	0.54	0.18	36.52
FermTP	0.05	0.0013	93.43	27.80	38.18
Total Type I R^2	0.73				

Run #6:

Variable**	Type III R^2	(p<)	Estimate***	SE Est	SPRC*
Laboratory	0.47	0.0001	----(Table 8)---		-----
Model MN	0.08	0.0001	0.74	0.17	49.94
FermNPN	0.02	0.0708	49.00	26.69	18.04
Total Type I R^2	0.70				

Run #7:

Variable**	Type III R^2	(p<)	Estimate***	SE Est	SPRC*
Laboratory	0.45	0.0001	----(Table 8)---		-----
Model MN	0.03	0.0108	0.48	0.18	32.38
FermTP	0.04	0.0023	87.97	27.74	35.94
FermNPN	0.01	0.1289	38.79	25.21	14.27
Total Type I R^2	0.74				

Run #8:

Variable**	Type III R^2	(p<)	Estimate***	SE Est	SPRC*
Laboratory	0.45	0.0001	----(Table 8)---		-----
Model MN	0.00	0.3522	0.23	0.25	15.63
FermNSC	0.04	0.0021	28.73	8.98	49.76
Total Type I R^2	0.73				

Run #9:

Variable**	Type III R^2	(p<)	Estimate***	SE Est	SPRC*
Laboratory	0.42	0.0001	----(Table 8)---		-----
Model MN	0.14	0.0001	0.95	0.17	64.17
FermNDF	0.03	0.0128	-56.05	21.89	27.93
Total Type I R^2	0.72				

Table 7.(cont'd). Variance associated with variables included in models for prediction of microbial nitrogen flow (75 obs.).

Run #10:

Variable**	Type III R^2	(p<)	Estimate***	SE Est	SPRC*
Laboratory	0.43	0.0001	----(Table 8)---		-----
Model MN	0.01	0.1986	0.40	0.31	27.45
Ferm NSC	0.02	0.0455	22.72	11.14	39.36
FermNDF	0.00	0.3645	-24.18	26.48	12.05
Total Type I R^2	0.73				

Run #11:

Variable**	Type III R^2	(p<)	Estimate***	SE Est	SPRC*
Laboratory	0.46	0.0001	----(Table 8)---		-----
Model MN	0.13	0.0001	1.09	0.20	73.96
AvailFat	0.02	0.0315	-65.58	29.82	21.43
Total Type I R^2	0.71				

Run #12:

Variable**	Type III R^2	(p<)	Estimate***	SE Est	SPRC*
Laboratory	0.43	0.0001	----(Table 8)---		-----
Model MN	0.04	0.0046	0.92	0.31	62.42
%Forage	0.00	0.7484	0.45	1.38	6.01
Total Type I R^2	0.69				

Run #13:

Variable**	Type III R^2	(p<)	Estimate***	SE Est	SPRC*
Laboratory	0.31	0.0001	----(Table 8)---		-----
FermTP	0.00	0.3469	-79.91	84.23	32.65
FermTP^2	0.01	0.0542	56.88	28.91	58.85
FermNPN	0.00	0.9206	9.93	99.22	3.65
FermNPN^2	0.00	0.6854	16.24	39.88	13.37
FermNSC	0.02	0.0034	149.98	48.96	259.76
FermNSC^2	0.02	0.0095	-8.80	3.28	153.74
FermNDF	0.00	0.2206	-127.96	103.27	63.77
FermNDF^2	0.01	0.1063	41.26	25.13	77.21
AvailFat	0.02	0.0083	-319.23	116.66	104.32
AvailFat^2	0.01	0.0394	104.62	49.58	67.26
%Forage	0.00	0.3379	1.63	1.69	22.00
%Forage^2	0.01	0.1442	-0.01	0.009	15.08
Total Type I R^2	0.86				

Table 7.(cont'd). Variance associated with variables included in models for prediction of microbial nitrogen flow (75 obs.).

Run #14:

Variable**	Type III R^2	(p<)	Estimate***	SE Est	SPRC*
Laboratory	0.31	0.0001	----(Table 8)---		-----
Model MN	0.00	0.7316	0.15	0.42	9.89
FermTP	0.00	0.3319	-83.90	85.70	34.28
FermTP^2	0.01	0.0563	56.87	29.15	58.83
FermNPN	0.00	0.9799	2.58	102.27	0.95
FermNPN^2	0.00	0.6493	18.66	40.81	15.37
FermNSC	0.02	0.0080	144.00	52.31	249.42
FermNSC^2	0.02	0.0156	-8.51	3.41	148.66
FermNDF	0.00	0.2104	-133.54	105.36	66.54
FermNDF^2	0.01	0.1124	40.91	25.35	76.56
AvailFat	0.02	0.0093	-317.68	117.69	103.81
AvailFat^2	0.01	0.0430	103.74	50.04	66.69
%Forage	0.00	0.3144	1.79	1.76	24.11
%Forage^2	0.00	0.1399	-0.01	0.009	15.47
Total Type I R^2	0.86				

* Standard Partial Regression Coefficient

** Variables: Laboratory = lab effect as a class variable, Model MN = within-day model microbial N flow estimate, FermTP = fermented TP, FermNPN = fermented NPN, FermNSC = fermented NSC, FermNDF = fermented NDF, AvailFat = rumen available fat, %Forage = % forage in the diet

*** Estimate = Parameter Estimate

Table 8. Relative Y-intercept estimates associated with laboratory in models for prediction of microbial nitrogen flow (75 observations).

	Run#1	SE	Run#2	SE	Run#3	SE	Run#4	SE	Run#5	SE
Intercept	-63.7	117.7	284.0	37.1	-1.2	65.5	-97.9	139.6	-74.1	64.7
Robinson	-47.2	45.2	-117.0	58.6	-73.8	51.1	-46.6	45.5	-32.4	49.1
Glenn	162.4	64.9	-118.8	45.4	48.6	51.5	153.9	67.8	121.5	52.6
Clark	42.3	48.4	25.8	39.3	29.1	33.8	34.1	51.9	107.4	39.1
Miner	20.4	39.6	-5.0	41.5	34.3	36.5	18.4	40.1	45.4	34.1
Schwab	209.5	39.2	203.3	49.1	191.8	42.2	206.2	40.1	228.5	40.8
MSU	-45.9	42.8	-68.8	41.5	-76.0	35.7	-54.3	46.8	-7.3	39.0
VPI	-21.7	42.5	-48.5	41.5	-49.5	35.6	-26.7	44.1	10.7	37.7
Hoover	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

	Run#6	SE	Run#7	SE	Run#8	SE	Run#9	SE	Run#10	SE
Intercept	-8.5	64.5	-75.7	64.0	54.8	63.8	109.8	76.4	91.0	75.2
Robinson	-81.3	50.4	-40.8	48.9	-91.4	48.2	-72.1	49.1	-87.0	48.5
Glenn	35.1	51.1	106.5	52.9	14.2	49.4	-21.7	56.6	-8.9	55.6
Clark	14.2	34.2	91.1	40.2	-34.4	37.4	-12.6	36.3	-39.1	37.8
Miner	18.6	36.8	32.4	34.8	-27.3	39.2	-14.0	39.8	-35.3	40.2
Schwab	198.7	41.6	231.9	40.4	170.7	40.1	180.6	40.8	170.3	40.1
MSU	-66.9	35.4	-4.0	38.6	-109.5	35.0	-93.3	34.9	-110.0	35.0
VPI	-81.0	39.0	-17.7	41.6	-97.6	36.6	-76.3	35.8	-99.1	36.7
Hoover	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

	Run#11	SE	Run#12	SE	Run#13	SE	Run#14	SE
Intercept	-35.8	65.6	-47.8	159.3	-136.3	244.4	-140.3	246.6
Robinson	-65.0	49.9	-74.0	51.5	-73.8	49.1	-72.3	49.7
Glenn	79.8	52.0	47.0	52.1	112.5	70.8	105.0	74.6
Clark	15.0	33.5	26.5	34.9	-26.0	58.3	-29.8	59.8
Miner	45.8	35.9	32.9	37.0	19.0	42.2	19.7	42.6
Schwab	181.8	41.3	192.1	42.5	195.7	38.4	194.1	39.0
MSU	-99.3	36.2	-78.4	36.7	-95.9	53.6	-99.4	55.0
VPI	-31.9	35.5	-53.2	37.7	-50.2	47.3	-51.7	47.9
Hoover	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

In order to understand these laboratory differences, a closer look must be taken at the procedures of each. One reason for the lower estimates observed in the MSU cannulated cow study may be that the rumen fluid samples taken for bacterial analysis were frozen with no preservative such as 13.7% formaldehyde solution in physiological saline (McCarthy et al., 1989), prior to differential centrifugation for the pure bacterial pellet. It has been speculated that freezing rumen fluid samples with no preservative may result in cell lysis which could lead to inaccuracy in differential centrifugation (Robinson, personal communication) and possibly low nitrogen:purine ratios in the bacterial pellets obtained. Cecava et al.(1990b) found no differences in estimated bacterial nitrogen flows whether or not rumen samples were preserved with saline and frozen prior to centrifugation. Furthermore, in the MSU study ruminal and duodenal samples were not stored in the frozen state after being freeze-dried. The consequences of this action on purine stability are unknown but are expected to be minimal (F.N. Owens, personal communication). Robinson and Kennelly(1990) used DAPA as a bacterial marker. As previously discussed, DAPA probably underestimates bacterial nitrogen flow (Stern and Hoover, 1979, Broderick and Merchen, 1992). Cytosine was used as a bacterial marker in the VPI studies which yielded low y-intercept values. No particular evidence, however, has been found leading one to assume that cytosine as a marker would

result in lower microbial nitrogen flow estimates than those obtained using total purines.

It is interesting to note that the y-intercepts from the Miner Institute study were intermediate among all of the studies and similar to those of the Clark and Hoover studies. This does not necessarily imply that the results of these studies were more accurate but does indicate a strong association between the duodenally cannulated cow and total rumen evacuation methods for determination of microbial nitrogen flow from the rumen. As in the MSU study, ruminal samples were not stored in the frozen state after being freeze-dried. The consequences of this action on purine stability are unknown but are expected to be minimal (F.N. Owens, personal communication).

The studies conducted in the laboratories of Glenn and Schwab generally resulted in higher y-intercept values. Four out of six of the microbial nitrogen flow estimates obtained from Glenn were from heifers consuming all forage diets. Due to animal size and diet effects, rates of passage and microbial recycling taking place within these rumens may have been vastly different from that of cows on high concentrate diets, resulting in higher microbial efficiency. These effects may have been misrepresented as a laboratory effect in the models. The microbial nitrogen flow estimates from the laboratory of Schwab used in the models were calculated as the sum of the fluid-associated bacteria and the particle-

associated bacteria. Proper estimation of the percentage of each bacterial type flowing to the duodenum is necessary for accurate computation of total microbial nitrogen flow by this method (Lykos et al., 1991). Microbial nitrogen flow estimates calculated based on the nitrogen from the fluid-associated bacteria plus only those particle-associated bacteria which were removed by homogenization were also calculated for this study and found to be lower than the other estimates.

Once the variation associated with laboratory was accounted for, the performance of the within-day model and the value of additional individual dietary variables in models for prediction of microbial nitrogen flow were assessed (Table 7). Addition of kg fermented true protein, NPN, NSC, and NDF (as predicted using the within-day model, Appendix E), kg rumen available fat (Appendix F), and percent forage in the diet (Appendix D) to the model helped to account for 23% more of the variation in microbial nitrogen flow (Run#1) than did the model containing only the laboratory as a variable (Run#2). Type I R^2 increased from 0.57 in Run#2 with only laboratory as a class variable to 0.69 when the within-day model prediction was added in Run#3 indicating a significant improvement. However, total model performance was not improved when the within-day model estimate and all of the individual dietary variables were present in the model (Run#4) versus only inclusion of the individual dietary variables (Run#1) (Type I

R^2 = 0.80). Correlations between individual variables are recorded in Table 9.

Table 9. Correlations between variables included in regression models for the prediction of in vivo measured microbial nitrogen flow**.

	MeasMN	ModelMN	TP	NPN	NSC	NDF	Fat	%For
MeasMN	1.00	0.48	0.44	0.20	0.52	0.11	0.06	-0.50
ModelMN	0.48	1.00	0.50	0.29	0.85	0.63	0.35	-0.92
TP	0.44	0.50	1.00	0.17	0.43	0.25	0.39	-0.50
NPN	0.20	0.29	0.17	1.00	0.50	-0.16	0.56	-0.16
NSC	0.52	0.85	0.43	0.50	1.00	0.26	0.34	-0.75
NDF	0.11	0.63	0.25	-0.16	0.26	1.00	0.15	-0.60
Fat	0.06	0.35	0.39	0.56	0.34	0.15	1.00	-0.28
%For	-0.50	-0.92	-0.50	-0.16	-0.75	-0.60	-0.28	1.00

** Variables: MeasMN = in vivo measured microbial N, ModelMN = within-day model microbial N, TP = fermented TP, NPN = fermented NPN, NSC = fermented NSC, NDF = fermented NDF, Fat = rumen available fat, %For = % forage in the diet

It can be seen that in Run#1, which does not include the within-day model prediction, that fermented NSC ($p<0.0040$), fermented true protein ($p<0.0014$), and rumen available fat ($p<0.0009$) were the most important factors affecting prediction of measured microbial yield. Both fermented NSC and fermented true protein were positive effectors of microbial nitrogen yield as expected (Stern et al., 1978, Hoover et al., 1990, Stokes et al., 1991b). Available fat had a negative effect as expected (Palmquist and Jenkins, 1980, Maczulak et al., 1981, Boggs et al., 1987).

When the within-day model estimate of microbial nitrogen yield was added to the model in Run#4, fermented NSC ($p<0.0244$), fermented true protein ($p<0.0076$), and rumen available fat ($p<0.0009$) were still primary variables for describing the variation in the model. No attempt was made to account for rumen available fat in the within-day model while fermented NSC and true protein were included in that model. Fermented NSC was highly correlated with within-day model predicted microbial nitrogen flow (0.85), while the correlations of true protein and rumen available fat with within-day model predicted microbial nitrogen were lower (0.50 and 0.35, respectively).

In order to determine the extent to which individual dietary factors were not adequately accounted for in the within-day model, each was added separately in models with only laboratory and the within-day model estimates as the other variables. It appears from Run#5 and Run#8 that even though both fermented NSC and fermented true protein function to drive microbial nitrogen production in the within-day model, more work needs to be done to adequately describe their effect on microbial nitrogen flow. Since both are positive effectors in these models, one might surmise that Ymax should be higher in the within-day model and that the impact of degradable true protein is greater than the model currently predicts based on the degraded true protein:fermented NSC ratio. More <u>in vitro</u> studies need to be conducted with

cellulolytic and amylolytic bacteria grown with incremental degraded true protein:fermented NSC ratios in order to correctly define these relationships which may also be non-linear (Run#13).

The addition of fermented NDF ($p<0.0128$) and rumen available fat ($p<0.0315$) to the model were of intermediate importance in describing the variation in microbial nitrogen flow as compared to the microbial nitrogen predicted by the within-day model (Run#9 and Run#11). Both had negative effects. The correlations of fermented NDF and rumen available fat with within-day model predicted microbial nitrogen were 0.63 and 0.35, respectively.

The negative effect of fermented NDF is surprising due to the fact that one would expect diets higher in amount of forage to result in higher ruminal pH and increased microbial efficiency (Rode et al., 1985, Sniffen and Robinson, 1987). One might think that diets higher in fermentable NDF would often be lower in fermentable NSC resulting in fermented NDF being included in the model as a negative factor, however, the correlation between fermented NDF and fermented NSC was low (0.26). Fermented NDF and percent forage in the diet were negatively correlated (-0.60), leading one to believe that greater fermented NDF was not associated with higher ruminal pH and microbial efficiency within this dataset. More fermented NDF may have been associated with less effective fiber, thus compromising rumen productivity in this situation,

but this is highly speculative. Feng et al.(1993) found a decrease in efficiency of microbial nitrogen yield per kg of OM digested with higher NSC and higher degradable fiber diets and attributed it to increased microbial recycling within the rumen. It must also be recognized that in vitro rates of NDF degradation for the feeds in the current database may have been underestimated due to the long incubation intervals used.

It is apparent that rumen available fat negatively impacted microbial growth (Run#1 and Run#11). Based on the previous discussion, this result was expected (Palmquist and Jenkins, 1980). In vitro studies with different microbial types grown under different conditions need to be conducted in order to determine both the mechanism of inhibition and a functional relationship between amount and type of rumen available fat (saturated vs. unsaturated vs. type of unsaturation) and extent of inhibition according to bacterial type (gram negative vs. gram positive vs. type of gram positive) (Maczulak et al., 1981). The mechanism of inhibition would determine if Ymax or maintenance requirements would need adjustment. For example, if fat inhibits the microbes by reducing nutrient uptake and/or secretion of extracellular enzymes, Ymax would vary accordingly. However, if incorporation of fatty acids increases membrane permeability, this might impact maintenance requirements. These relationships may be non-linear, as indicated by Run#13 when squared variables were included in the model.

Fermented dietary NPN was not a driver in the within-day model and the results of these analyses would indicate that, if added, it would not greatly affect the performance of the within-day model for predicting measured microbial nitrogen flow. The correlation between fermented NPN and within-day model microbial nitrogen was 0.29. Total NPN present in the rumen might have a larger impact on microbial nitrogen flow but it is difficult to predict based on current knowledge (Kennedy and Milligan, 1980).

Addition of the percentage of the diet composed of forage as a dietary variable did little to help explain the variation in measured microbial nitrogen flow. Part of this effect might be explained by the fact that percent forage was correlated with fermented NSC (-0.75), fermented NDF (-0.60), and within-day model microbial nitrogen (-0.92). Particle size or effective NDF probably would have more of an impact on the model (Jaster and Murphy, 1983, Erdman, 1988). However, since most feed samples obtained from the investigators were shipped in dried, ground form, those variables were impossible to estimate for this study. More information is needed to describe saliva production, particle breakdown, microbial recycling, and rate of passage as a partial function of effective fiber in order to accurately predict the impact of effective fiber on microbial nitrogen flow.

Due to concern regarding the leverage of the four microbial nitrogen flow observations from heifers (Glenn et

al., 1989) on the dataset, the same regression analyses described above were carried out with these observations omitted from the dataset. The results are recorded in Appendix I. The same conclusions can be drawn from both the full 75 observation dataset and the 71 observation dataset.

One can conclude from these regression analyses that high variation between laboratories in the estimation of microbial nitrogen flow makes it difficult, if not impossible, to predict measured microbial nitrogen flow for all 75 observations using any one model. The relationships between fermented nutrients and microbial nitrogen flow need to be more adequately described in order to significantly improve microbial nitrogen flow prediction using the within-day model.

The imposed feeding behavior patterns in the model with the hourly prediction of available nutrients and ruminal pH appear to have little impact on model performance based on the similar Type I R^2 obtained for regression models including daily fermented nutrients and the within-day model predicted microbial nitrogen (0.80) (Run#4) or only including daily fermented nutrients (Run#1) (0.80). One cannot conclude from these regression analyses that within-day nutrient availability is not important since it may have been inadequately described in the model. Furthermore, variations in rates of passage of feed fractions through the rumen may significantly impact microbial nitrogen flow and may have been improperly described in the model.

B. Evaluation of NE_l (Mcal/d) for Prediction of <u>In Vivo</u> Measured Microbial Nitrogen Flow:

The accuracy with which NE_l (Mcal/d) predicted measured microbial nitrogen flow for the 75 <u>in vivo</u> observation dataset is shown in Figure 6. NE_l (Mcal/d) accounted for only 23% of the variation in measured microbial nitrogen flow.

Other relationships between <u>in vivo</u> measured microbial nitrogen flow and NE_l (Mcal/d) have been found, yielding the following equations:

Microbial N (g/d) = 11.45 NE_l (Mcal/d) - 30.93 R^2=0.77
(NRC, 1985)

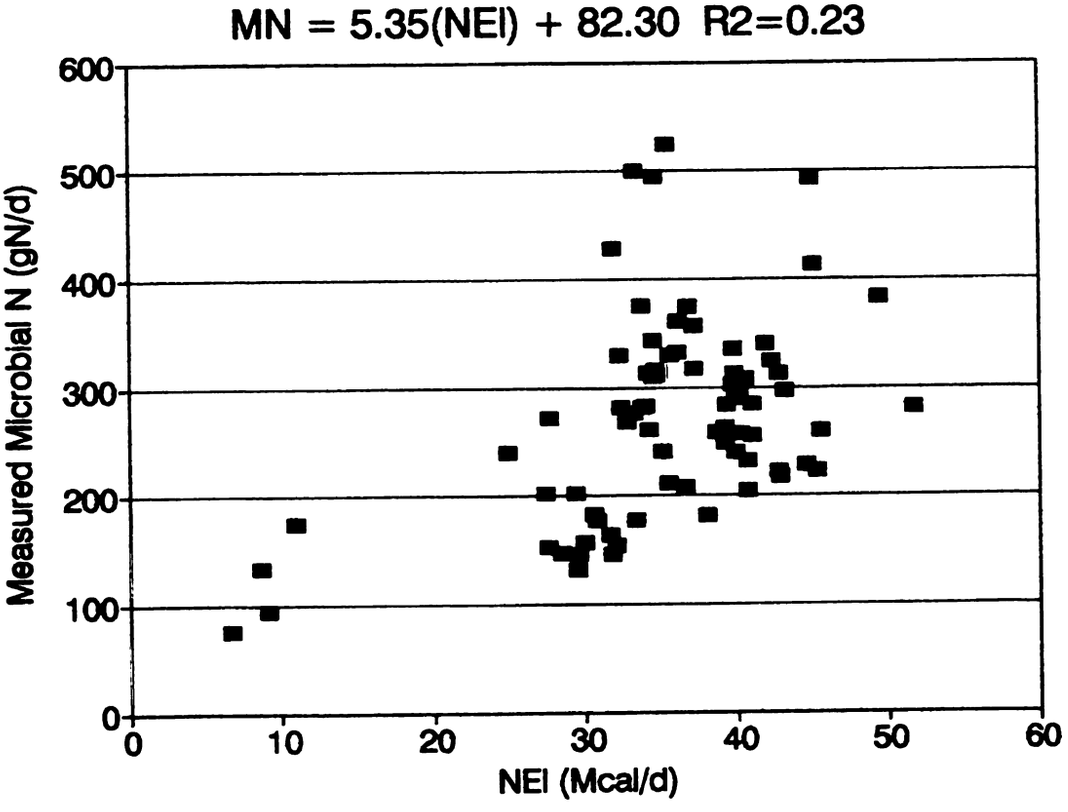
Microbial N (g/d) = 8.42 NE_l (Mcal/d) - 21.6 R^2=0.39
(Erdman and Komaragiri, 1991)

Microbial N (g/d) = 10.49 NE_l (Mcal/d) + 6.33 R^2=0.68

(Dataset compiled by Roe using: Cummins et al., 1983, Santos et al., 1984, Prange et al., 1984, Rooke et al., 1985, Stern et al., 1985, Chamberlain et al., 1986, Madsen, 1986, Madsen and Hvelplund, 1988, Glenn et al., 1989, Kirkpatrick and Kennelly, 1989, McCarthy et al., 1989, Robinson and Kennelly, 1990, Sadik et al., 1990)

Differences in the datasets result in vastly different predictive equations. The equations do not consider that proportion of energy which is fermented by the rumen microbes but only the total amount of energy utilized by the animal. Variations in rumen degradable fat, synchronization of protein and carbohydrate availability, source of degradable nitrogen and isoacid availability, ruminal pH, and rate of washout of the bacteria are all known to impact microbial nitrogen flow but are not considered in the equations based on NE_l (Mcal/d).

Figure 6. NE_l (mcal/d) versus measured microbial nitrogen flow (g N/d) at the duodenum (75 observations).



In Table 10, the amount of variation in measured microbial nitrogen flow described by NE_l (Mcal/d) and laboratory is recorded. The laboratory in which microbial nitrogen flow was measured accounted for 46% of the variation and only 12% more of the variation in measured microbial nitrogen flow could be accounted for when NE_l (Mcal/d) was added to the model.

Table 10. Variance associated with other variables without (variable wo/lab) and with a separate laboratory effect included in models for prediction of microbial nitrogen flow.

Variable	TypeIIIR^2	obs	(p<)	Estimate	SE	Intercept	SE
DMI(wo/Lab)	0.23	75	0.0001	9.54	2.0	69.7	43.9
DMI	0.11	75	0.0001	12.02	2.5	38.3	61.1
Lab	0.45	75	0.0001				
NEl(wo/Lab)	0.23	75	0.0001	5.35	1.2	82.3	41.9
NEl	0.12	75	0.0001	6.32	1.3	67.5	54.3
Lab	0.46	75	0.0001				
FCNEl(wo/Lab)	0.24	75	0.0001	5.52	1.2	77.9	41.7
FCNEl	0.12	75	0.0001	6.84	1.3	51.8	54.9
Lab	0.46	75	0.0001				
SpartMN(wo/L)	0.20	75	0.0001	0.44	0.1	113.5	38.2
SpartMN	0.10	75	0.0001	0.50	0.1	105.9	51.4
Lab	0.47	75	0.0001				
CNCPSMN(wo/L)	0.24	75	0.0001	0.62	0.1	78.3	41.3
CNCPSMN	0.15	75	0.0001	0.90	0.2	26.4	52.7
Lab	0.48	75	0.0001				
KgOMD(wo/Lab)	0.27	37	0.0009	20.13	5.6	117.0	52.2
OMD	0.00	37	0.7523	2.13	6.7	259.2	81.7
Lab	0.57	37	0.0001				
KgStarch(wo/L)	0.00	26	0.9488	0.67	10.3	295.8	47.8
Starch	0.08	26	0.0453	-15.68	7.4	382.7	35.3
Lab	0.58	26	0.0001				
KgNDF(wo/Lab)	0.02	35	0.3764	14.57	16.3	241.7	39.1
NDF	0.00	35	0.7282	-5.14	14.6	301.8	58.8
Lab	0.59	35	0.0001				

Lab=Laboratory, DMI=Dry Matter Intake, FCNE$_l$=Fat-corrected NE$_l$, SpartanMN=microbial N flow predicted by the Spartan Model, CNCPSMN=microbial N flow predicted by the Cornell Net Carbohydrate and Protein System, KgOMD=measured rumen-digested organic matter, KgStarch=measured rumen-digested starch, KgNDF=measured rumen-digested NDF, Estimate=Parameter Estimate

C. Evaluation of Other Models and Dietary Variables for Use in Prediction of <u>In Vivo</u> Measured Microbial Nitrogen Flow:

Table 10 shows the amount of variation in measured microbial nitrogen flow described by DMI (kg/d), fat-corrected NE_l (mcal/d), the Spartan model microbial nitrogen flow prediction (gN/d), the Cornell Net Carbohydrate and Protein System microbial nitrogen flow prediction (gN/d), measured amount of OM digested in the rumen (kg/d), measured amount of starch digested in the rumen (kg/d), and measured amount of NDF digested in the rumen (kg/d) (Appendix F). Once again, it can be seen that much of the variation in measured microbial nitrogen flow was associated with the laboratory in which the measurement was made and little progress was made toward improving the prediction with the addition of any of these variables.

The linear relationships between fat-corrected NE_l (mcal/d) (Figure 7), the Spartan model microbial nitrogen flow prediction (gN/d) (Figure 8), the Cornell Net Carbohydrate and Protein System microbial nitrogen flow prediction (gN/d) (Figure 9), measured amount of OM digested in the rumen (kg/d) (Figure 10) and measured microbial nitrogen flow to the duodenum were determined and plotted. The scatter associated with each of these plots is high.

Figure 7. Fat-corrected NE_l (mcal/d) versus measured microbial nitrogen flow (g N/d) at the duodenum (75 obs).

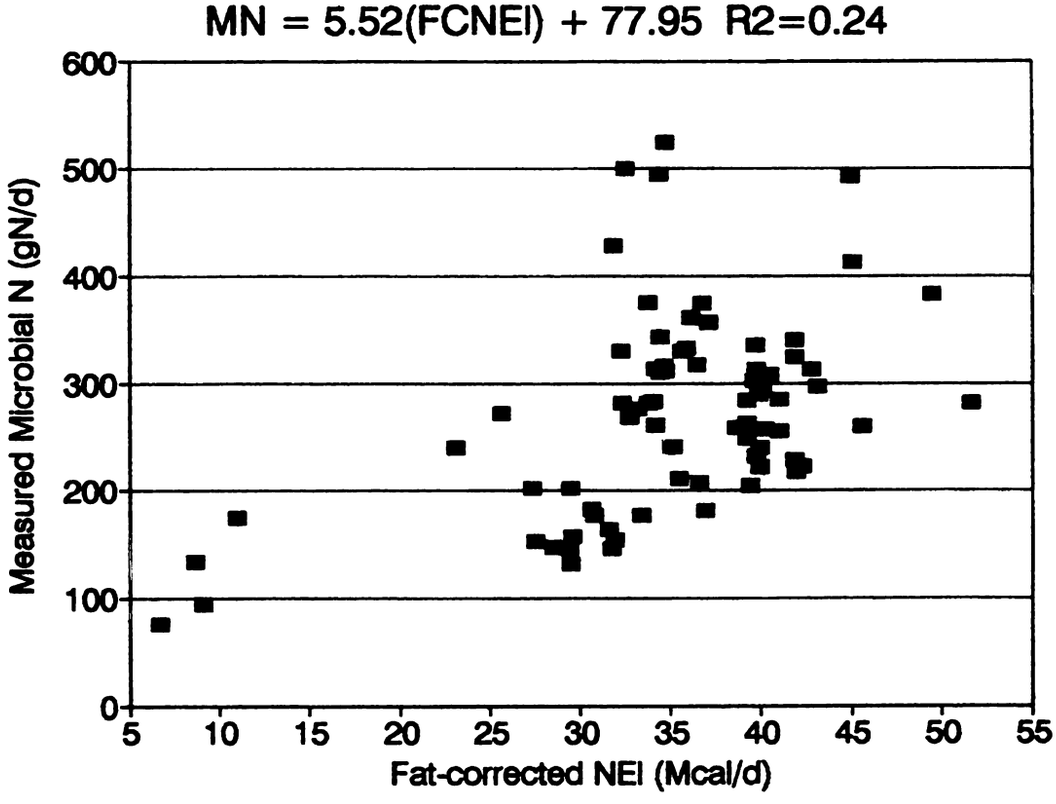


Figure 8. Spartan model predicted microbial nitrogen flow (g N/d) versus measured microbial nitrogen flow (g N/d) at the duodenum (75 observations).

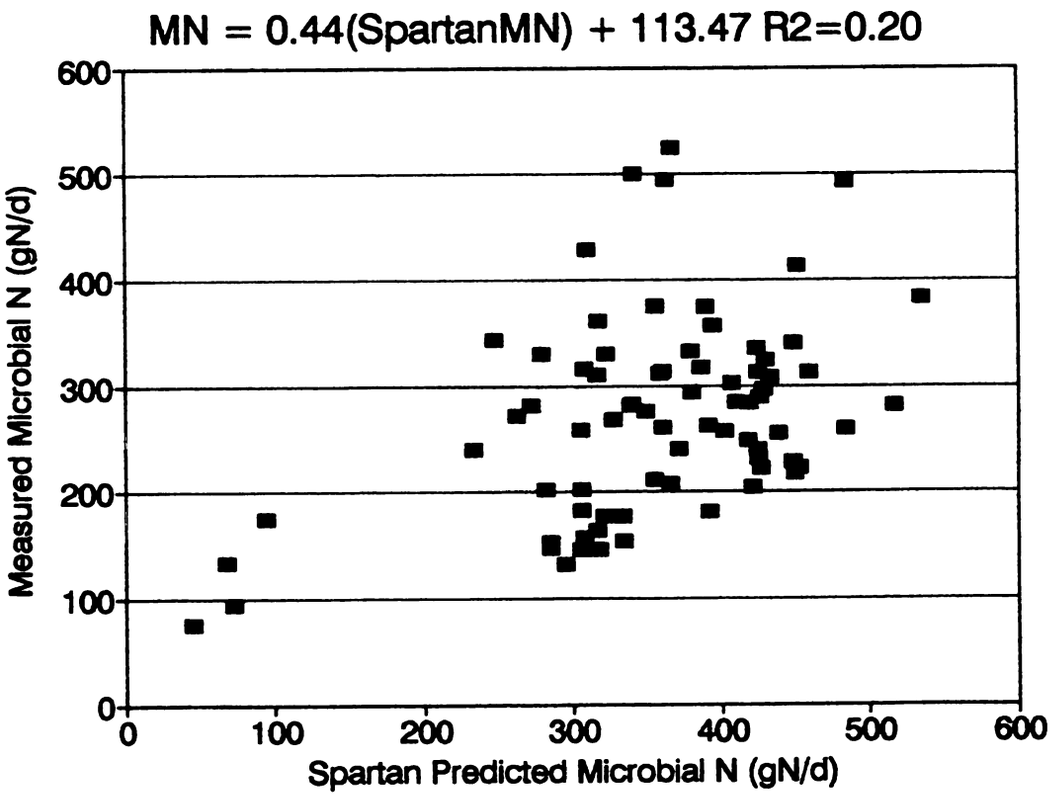


Figure 9. Cornell Net Carbohydrate and Protein System predicted microbial nitrogen flow (g N/d) versus measured microbial nitrogen flow (g N/d) at the duodenum (75 observations).

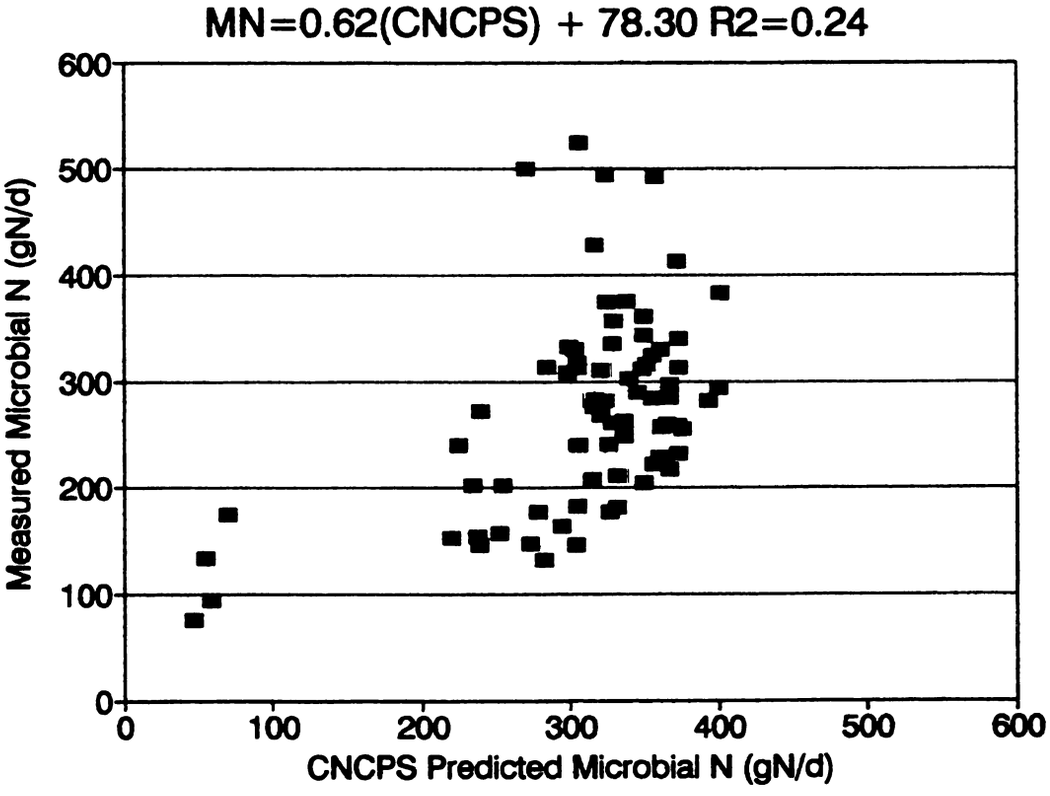
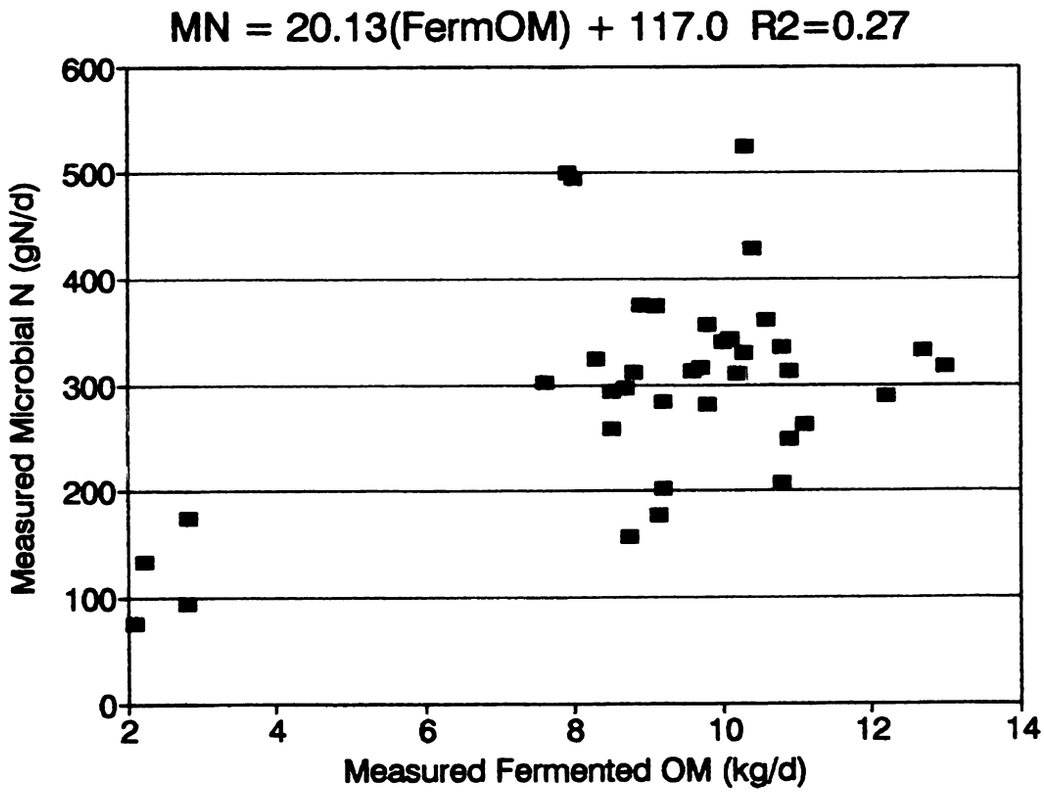


Figure 10. Measured rumen fermented organic matter versus measured microbial nitrogen flow (g N/d) at the duodenum (37 observations).



Chapter VI. Determination of Daily Microbial Nitrogen Flow from the Rumens of Cows Consuming Diets Varying in Carbohydrate and Protein Availability (Miner Institute Study):

Introduction:

In order to expand the microbial nitrogen flow database, a study was conducted at Miner Institute in which dietary treatments were designed to vary in carbohydrate and protein availability to the microbes. Since the rumen microbes require nitrogen, amino acids or isoacids, and carbohydrate for growth, variations in the ratios of these nutrients available to the microbes throughout the day should affect efficiency of growth. Microbial yield decreased curvilinearly from 34.2 to 10.3 g bacterial N per kg DM degraded in continuous culture as the nonstructural carbohydrate / rumen degradable crude protein ratio widened from 1.9 to 8.9 (Hoover, 1987, Stokes et al., 1991b). Russell and Sniffen (1984) added isovalerate, 2 methylbutyrate, valerate, and isobutyrate to batch cultures of rumen microbes not limited by ammonia and found an improvement in microbial protein

production of 18.7%. Newbold and Rust (1992) tested the effect of synchrony on batch culture microbial growth and concluded that the bacterial population was unaffected by synchronization of carbohydrate and protein availability. Unfortunately, bacterial nitrogen production per unit of OM degraded was not reported, making results very difficult to interpret since total OM availability was not equivalent among the diets. Furthermore, bacterial growth was minimal after approximately 10 h of incubation, indicating that end-product inhibition could have significantly affected the results of this experiment.

For the current study, it was necessary to develop a new technique in which microbial nitrogen flow was estimated as a function of the microbial concentration in the rumen liquids and solids and the pool sizes and rates of passage of each. The primary advantage of the method was that it required only rumen cannulation and no duodenal cannulation. Assumptions of this method were that: 1. the purine:nitrogen ratio was the same for the fluid- and particle-associated bacteria, 2. insoluble dietary purines were not degraded in the rumen while soluble dietary purines were totally degraded, 3. the liquid and solid microbial pools passed at the same rate as their phases and no selective retention of the microbes took place within either pool, 4. all particulate matter passed at the same rate as rumen undegradable NDF, and 5. the liquid rate of passage estimated by rumen dilution represented average daily

liquid flow. A study to determine the effect of diet on degradation of feeds in situ was also conducted concurrently with the microbial nitrogen flow study.

Materials and Methods:

A. Animals and Dietary Treatments:

Two early lactation Holstein cows (60 DIM) and two late lactation Holstein cows (142 DIM) were used. Diets expected to yield different amounts of microbial nitrogen at the small intestine but not necessarily different amounts of total nitrogen at the small intestine were fed. Diets varied in major source of rumen available carbohydrate and protein. Two separate 2x2 Latin squares were designed for the early lactation cows with treatments in the first square being high moisture ear corn (HMEC)/soybean meal (SBM) versus HMEC/corn gluten feed (CGF) and in the second square being ground shelled corn (GSC)/CGF versus GSC/SBM. Two separate 2x2 Latin squares were designed for the late lactation cows with treatments in the first square being HMEC/SBM versus GSC/SBM and in the second square being GSC/CGF and HMEC/CGF. The second square for the late lactation cows had to be discontinued due to problems with the acceptability of the forage source by the cows.

High-moisture ear corn (with very few intact kernels) and GSC were used to obtain differences in ruminally degraded

carbohydrate. CGF had a larger percentage of amino acids which were rapidly available (Fraction A) and less total ruminally degraded CP as compared to SBM which had a large percentage of more slowly available amino acids in Fraction B_2 (Appendix A). CGF was chosen over urea to eliminate an amino acid versus nitrogen effect and have only a carbohydrate and amino acid availability effect. The effect of reduced pH and carbohydrate and protein availability was expected to be confounded.

Protein and carbohydrate fractionation analysis of the feeds were carried out as previously described for the MSU cannulated cow study feeds. Feeds were also analyzed by the in situ technique previously described (concentrates: 2 h and 8 h, forages: 48 h) to estimate rate and extent of CP, starch, and available NDF degradation. Rates of B_2 degradation for each concentrate feed were determined according to the amount of 8 h in situ CP disappearance after correction for the A and B_1 fractions. The rates of degradation of B_2 for each concentrate feed were determined using the following equation: [[(ln 100-ln(100-((initial B_2(%DM) - (initial B_2(%DM) - (total remaining CP at 8 h - (A + B_1))(%DM))/initial B_2(%DM))*100)))/8]*100]. The measurements of the starch plus glucose fractions in each concentrate feed were based on 2 h and 8 h in situ starch disappearance. The amount of S_2 was calculated as the natural antilogarithm of: [(ln(starch remaining at 2h (%DM)) + [2 * [(ln(starch

remaining at 2h (%DM))- ln(starch remaining at 8h (%DM)))/6]]. The amount of S_1 was calculated as the difference between the total amount of starch in the feed and S_2. The rates of degradation of S_2 in each concentrate feed were determined using the following equation: [[(ln(starch remaining at 2h(%DM))-ln(starch remaining at 8h(%DM))) /6]*100]. Rates of available NDF degradation in each forage were determined based on 48 h <u>in situ</u> degradability. The rates of degradation of available NDF were determined using the following equation: [[(ln 100- ln(100-((initial available NDF(%DM)-remaining available NDF(%DM)/initial available NDF(%DM)) *100))) /48] *100]. A correction was made for the crude protein associated with NDF and for rumen undegradable NDF which was estimated as lignin(%DM) multiplied by the factor 2.4 (Chandler et al., 1980). All analyses were conducted by methods previously described. Two-hour, 8 h, and daily degraded CP and carbohydrate were predicted using the within-day model.

The cows remained on each diet for 16 day periods with the last 4 days being the collection period. Cows were body condition scored and weighed throughout the trial. Cows were housed in box stalls and were not moved for milking. Individual milk weights were recorded. Composite milk samples taken during the intensive analysis period were analyzed for fat, protein, and SCC (New York Dairy Herd Improvement Cooperative, Ithaca, NY). All cows were in their third lactation.

Diets were fed as a total mixed ration ad libitum at 12-hour intervals. Two samples of all forages and HMEC were taken each week and analyzed for dry matter, NDF, and CP. All concentrates besides HMEC were purchased at the beginning of the study and were assumed to be uniform throughout the study. Mixed diet offered and refused was weighed and sampled at each feeding and composite samples for each period were analyzed for DM, CP, NDF, and in vitro indigestible NDF(120 h). Total purines present in the insoluble portion of the diet were also determined. All analyses were conducted by methods previously described.

B. Microbial Nitrogen Flow:

Rumen contents were evacuated halfway between the AM and PM feedings on Day 12 and Day 14 of each period for each cow. Feed and water were taken away at the time of evacuation. A 5-mm pore-size screen was used as a lid on top of a 67.5 liter container to facilitate separation of particles and liquids. Each handful of rumen contents was hand squeezed over the screen and all fluids were collected in the container below the screen. Squeezed rumen particles were collected in a separate container. Weight of total rumen particles was determined. Total evacuation time was approximately 90 minutes.

During evacuation, every 10th handful of rumen particles was placed in a separate bucket. After evacuation, the

particles were mixed by hand, a 1 kg representative sample was taken, frozen immediately, and later freeze-dried and analyzed for DM, OM, *in vitro* undegradable NDF (120 h), N, and total purines by the methods previously described.

After evacuation, total rumen liquids were mixed and the volume and weight were determined. A representative 1.5-liter sample of rumen fluid was immediately cooled and stored prior to centrifugation as soon as possible after rumen evacuation was completed. Rumen fluid (500 ml) was saved for DM determination by toluene distillation (Knowlton, 1994). After all samples were taken, the remaining rumen liquids and particles were mixed and returned to the rumen.

The 400xg bacterial pellets and the 20,000xg bacterial pellets were obtained by differential centrifugation. Rumen fluid was blended for 2 minutes in a Waring blender and then strained through six layers of cheesecloth. Exactly 1.5 liters of strained rumen fluid was then centrifuged at 400xg for 20 minutes. The entire pellet was saved, frozen, and freeze-dried. The supernatant was centrifuged at 20,000xg for 30 minutes and the entire pellet was saved, frozen, and freeze-dried. Pellets were shipped from Miner Institute to Michigan in freeze-dried form and analyzed for total DM, OM, N, and purines by the methods previously described.

On Day 15 of each period halfway between the AM and PM feedings, rumen fluid was sampled for initial Co concentration. Then, 125 ml of Co-EDTA (Uden et al., 1980),

containing approximately 4.9 g of cobalt, was immediately dosed after initial sampling into each cow in five locations in the rumen. Samples of rumen fluid were collected at 1.5, 3, 6, 9, 12, 15, 18, 21, and 24 h after dosing. Rumen fluid samples were obtained by grabbing handfuls of rumen contents from 5 or 6 areas of the rumen and squeezing the fluid out of each handful. The pH of the rumen fluid samples was measured immediately. Samples of rumen fluid were analyzed for Co by first digesting samples according to the procedure of Hach et al.(1987) followed by flame emission analysis with atomic absorption spectrophotometry (Thermo Jarrell Ash Co., Model Smith/Hieffge 4000, 8E Forge Parkway, Franklin, MA).

Microbial Nitrogen Flow Calculations

Total Microbial N in Rumen Fluid (g MN / L fluid)

= N in 20,000*g pellet + N from bacteria in 400*g pellet

N in 20,000*g pellet = (%N in 20,000*g pellet) * (g of final pellet from 20,000*g spin/L)

N in 400xg pellet = [(%purine in 400xg pellet) * ((% N in 20,000xg pellet)/(% purine in 20,000xg pellet))] *(g of final pellet from 400xg spin /L)

Total Microbial N in Rumen Solids (g Microbial N / g of solids)
= ((% purine in rumen solid DM) - (% purine in insoluble TMR)) * ((% N in 20,000xg pellet)/(% purine in 20,000xg pellet))

Kp of particles (%/hour):

$$= \frac{((\text{Indig.NDF eaten (g/day)}) / \text{Rumen Indig.NDF(g)})}{24}$$

Indigestible NDF eaten = % INDF of feed consumed * DMI (g/d)
Rumen Indigestible NDF = % INDF of rumen DM * g Rumen DM

Kp of liquids (%/hour):

- regressed ln residual [Co] in the rumen versus time and determined % disappearance of Co per hour as the negative of the slope. Assumed instantaneous mixing of the Co in the rumen at dosing.

Microbial N Flow
= g Microbial N from rumen fluid + g Microbial N from solids

g Microbial N from rumen fluid = {(g Microbial N / l) * [(Total liters of rumen fluid) * Kp of liquids(%/hour)]} * 24

g Microbial N from rumen solids = {(g Microbial N / g) * [(Total g of rumen solids) * Kp of particles (%/hour)]} * 24

Comparisons of the treatment means within each 2x2 Latin square were made using the General Linear Model procedure of SAS (SAS, 1982). The model used was: $Y_{ijk} = u + cow_i + period_j + treatment_k + e_{ijk}$.

C. The Effect of Diet on Degradation of Feeds In Situ:

The three forages (alfalfa silage, corn silage, and grass silage) fed in the Miner Institute study, five of the concentrates fed in the Miner Institute study (ground shelled corn, high-moisture ear corn, corn gluten feed, corn gluten meal, and soybean meal), and five other concentrates (barley,

distillers' dried grains, canola, roasted soybeans, and wheat midds) were suspended in dacron bags in the rumen of a cow on each of the six treatments in the Miner Institute study. The in situ procedure previously described was followed. Forages were incubated for 30 h and the extent of DM and NDF degradation was determined. Concentrates were incubated for 8 h and the extent of DM and CP degradation was determined. Comparisons of the extent of degradation of each feed in the six dietary treatments were made using the Bonferroni t test of the General Linear Model procedure of SAS (SAS, 1982). The model used was: $Y_i = u + diet_i + e_i$.

Results and Discussion:

A. Dietary Treatments:

The ingredient and chemical composition of the diets is shown in Table 11. Nutrients degraded(%DM) were predicted using the within-day model. Total rumen degraded CP(%DM) was less for the diets containing CGF. Crude protein degraded within 2 h was similar among the diets. Nonstructural carbohydrate(%DM) degraded after 2 h was similar among the early lactation diets and but different between the late lactation diets. The HMEC early lactation diets contained less rumen degraded NSC than did the GSC early lactation diets.

Table 11. Ingredient and chemical composition of the diets fed in the Miner Institute study:

	Early Lactation Square #1:		Early Lactation Square #2:		Late Lactation Square #3:	
	A	B	C	D	E	H
CHO	HMEC	HMEC	GSC	GSC	HMEC	GSC
CP	SBM	CGF	CGF	SBM	SBM	SBM
% DM						
Grass Sil.	0.00	0.00	0.00	0.00	55.84	59.57
Alf Sil.	24.51	23.12	24.99	25.92	0.00	0.00
Corn Sil.	33.64	33.59	35.22	34.63	0.00	0.00
HMECorn	22.87	21.64	0.00	0.00	34.29	0.00
Cornmeal	0.00	0.00	19.87	21.30	0.00	30.69
SBM-48	11.79	0.00	0.00	13.29	8.27	8.16
Crnglutfd	0.00	10.92	9.06	0.00	0.00	0.00
Crnglutml	3.21	5.66	5.63	1.84	0.00	0.00
Bloodmeal	1.32	2.66	2.64	0.87	0.00	0.00
Megalac	1.02	0.81	1.01	0.60	0.00	0.00
Min&Vit.	1.64	1.60	1.58	1.55	1.60	1.58
Nutrients Degraded (%DM)						
True Prot.	8.58	6.04	5.40	7.86	6.34	6.32
NPN	4.04	4.49	4.90	4.45	4.42	4.51
NSC	27.80	26.72	29.70	30.52	22.00	22.57
NDF	6.09	7.67	5.92	4.41	10.95	9.67
2h CP	8.14	7.38	7.45	8.42	7.36	7.54
8h CP	10.79	9.27	9.19	10.86	9.42	9.53
2h NSC	15.22	15.39	15.35	15.28	12.95	10.51
8h NSC	24.24	23.66	26.32	27.01	19.01	19.45

B. Microbial Nitrogen Flow:

All cows maintained excellent milk production and intake throughout the study (Tables 12,13,14). There were no incidents of mastitis recognized.

Table 12. Least squares means for milk production, intake, rumen parameters, and microbial nitrogen estimates for the early lactation HMEC/SBM diet (A) vs. the early lactation HMEC/CGF diet (B)**.

Variable*	Mean A	Mean B	SEM	Signif.of Diff. (p<)
4% FCM(kg)	41.22	46.36	---	-----
DMI(kg)	21.74	24.88	---	-----
Solids(kgDM)	9.08	9.58	0.37	0.40
MN(g)/kgSolids	6.18	3.41	0.59	0.03
Liquids(kg)	33.07	36.19	2.02	0.34
MN(g)/l Liq.	1.44	1.15	0.15	0.25
Liquid Kp(%/h)	22.00	21.00	---	-----
Solids Kp(%/h)	4.80	5.30	0.001	0.06
Liquid MN(g/d)	244.81	207.16	28.17	0.40
Solids MN(g/d)	65.84	42.72	7.03	0.08
Total MN (g/d)	310.64	249.88	25.98	0.17
MN(g)/kg DMI	14.41	10.06	1.02	0.04
Ap Ferm OM (kg)	9.98	11.13	---	-----
MN(g)/AFOM(kg)	31.22	22.49	2.26	0.05
EstFerm CHO(kg)	7.23	8.53	---	-----
MN(g)/FCHO(kg)	43.01	29.43	2.93	0.03

* 4% FCM=4% Fat-corrected milk, DMI=Dry Matter Intake, Solids=Rumen Solids, MN=Microbial Nitrogen, Liquids= Rumen Liquids, Kp=Rate of passage, Ap Ferm OM= Apparently Fermented Organic Matter as estimated using the within-day model, EstFerm CHO= Rumen Fermented Carbohydrate as estimated using the within-day model

** Appendix G

Total rumen solids (kg DM) evacuated (Tables 12,13,14) were not affected by diet within squares ($p>0.10$) as expected because of similar days in milk and dry matter intakes. Although not compared statistically, amount of rumen solids

evacuated did tend to increase slightly from the first early lactation square to the second early lactation square (9.35, HMEC/SBM (A) and HMEC/CGF (B) early lactation diets vs. 10.6, GSC/CGF (C) and GSC/SBM (D) early lactation diets), possibly due to an increase in rumen volume (Remond, 1988) and a reduction in solids rate of passage with increased days in milk (Tables 12,13,14).

Table 13. Least squares means for milk production, intake, rumen parameters, and microbial nitrogen estimates for the early lactation GSC/CGF diet (C) vs. the early lactation GSC/SBM diet (D)**.

Variable*	Mean C	Mean D	SEM	Signif.of Diff. (p<)
4% FCM(kg)	39.35	39.50	---	-----
DMI(kg)	28.14	27.95	---	-----
Solids(kgDM)	10.96	10.16	0.45	0.28
MN(g)/kgSolids	5.93	5.74	1.19	0.92
Liquids(kg)	37.39	43.52	2.26	0.13
MN(g)/l Liq.	1.28	1.41	0.08	0.31
Liquid Kp(%/h)	25.00	26.00	---	-----
Solids Kp(%/h)	4.30	4.20	0.001	0.78
Liquid MN(g/d)	283.59	379.87	22.89	0.04
Solids MN(g/d)	64.09	58.07	13.57	0.77
Total MN (g/d)	347.68	437.97	25.22	0.06
MN(g)/kg DMI	12.57	15.80	0.91	0.07
Ap Ferm OM (kg)	12.70	12.88	---	-----
MN(g)/AFOM(kg)	27.59	34.34	1.96	0.07
EstFerm CHO(kg)	9.81	9.44	---	-----
MN(g)/FCHO(kg)	35.68	46.90	2.59	0.04

* 4% FCM=4% Fat-corrected milk, DMI=Dry Matter Intake, Solids=Rumen Solids, MN=Microbial Nitrogen, Liquids= Rumen Liquids, Kp=Rate of passage, Ap Ferm OM= Apparently Fermented Organic Matter as estimated using the within-day model, EstFerm CHO= Rumen Fermented Carbohydrate as estimated using the within-day model

** Appendix G

Microbial nitrogen (g) per kg of rumen solids was greater ($p<0.03$; Table 12) for the early lactation HMEC/SBM diet (A; 6.2 g/kg) than for the early lactation HMEC/CGF diet (B; 3.4 g/kg) . It was not different between the GSC/CGF (C) and GSC/SBM (D) early lactation diets (5.85 g/kg; $p>0.10$; Table 13). Microbial nitrogen (g) per kg of rumen solids was less ($p<0.10$; Table 14) for the late lactation HMEC/SBM diet (E; 2.1 g/kg) than for the late lactation GSC/SBM diet (H; 4.3 g/kg).

Table 14. Least squares means for milk production, intake, rumen parameters, and microbial nitrogen estimates for the late lactation HMEC/SBM diet (E) vs. the late lactation GSC/SBM diet (H)**.

Variable*	Mean E	Mean H	SEM	Signif.of Diff. (p<)
4% FCM(kg)	28.69	26.77	---	-----
DMI(kg)	19.41	20.32	---	-----
Solids(kgDM)	10.87	11.02	0.20	0.61
MN(g)/kgSolids	2.12	4.27	0.70	0.10
Liquids(kg)	45.03	45.34	0.88	0.81
MN(g)/l Liq.	0.76	0.78	0.08	0.88
Liquid Kp(%/h)	19.00	15.00	---	-----
Solids Kp(%/h)	3.60	3.00	0.001	0.02
Liquid MN(g/d)	155.99	120.20	18.37	0.24
Solids MN(g/d)	18.49	32.93	5.28	0.13
Total MN (g/d)	174.49	153.13	17.24	0.43
MN(g)/kg DMI	9.05	7.57	0.91	0.32
Ap Ferm OM (kg)	8.24	8.29	---	-----
MN(g)/AFOM(kg)	21.22	18.50	2.11	0.41
EstFerm CHO(kg)	6.16	6.10	---	-----
MN(g)/FCHO(kg)	28.41	25.16	2.84	0.46

* 4% FCM=4% Fat-corrected milk, DMI=Dry Matter Intake, Solids=Rumen Solids, MN=Microbial Nitrogen, Liquids= Rumen Liquids, Kp=Rate of passage, Ap Ferm OM= Apparently Fermented Organic Matter as estimated using the within-day model, EstFerm CHO= Rumen Fermented Carbohydrate as estimated using the within-day model

** Appendix G

A reduction in rumen pH would be expected to reduce the growth of the cellulolytic bacteria present within the rumen solids (Russell and Dombrowski, 1980), thus reducing the amount of microbial nitrogen per g of rumen solids. Since the late lactation GSC/SBM diet (H) was comprised of less rapidly available carbohydrate than the late lactation HMEC/SBM diet (E), pH was expected to be higher for the late lactation GSC/SBM diet (H), however, this was not seen (Tables 11 and 15). The percentage of total measurements of a pH less than 6.0 was 25% for both, with an average pH of 6.08 (Table 15).

Table 15. Rumen pH of cows on the Miner Institute Study

	Diet*									
Time	A		B		C		D		E**	H**
	Period									
	1	2	1	2	1	2	1	2	2	2
10AM	5.64	5.78	5.76	5.81	6.18	5.76	5.70	5.67	6.21	5.94
1PM	5.78	5.67	5.97	6.02	6.42	5.64	6.17	5.76	6.00	6.35
4PM	6.14	5.57	5.80	5.86	5.83	6.01	6.02	5.68	6.24	6.00
7PM	5.69	5.68	6.12	6.12	5.64	6.15	5.82	5.77	6.14	6.01
10PM	5.83	5.79	5.98	5.88	6.09	6.21	5.94	5.83	5.91	5.78
1AM	5.69	5.65	5.76	6.37	5.92	----	5.90	----	5.76	6.09
4AM	5.78	5.71	5.95	5.91	5.84	5.98	5.64	6.03	6.18	6.10
7AM	6.02	6.10	6.26	6.02	6.07	6.04	6.22	5.83	6.27	6.22
Mean	5.78		5.97		5.99		5.87		6.09	6.06
S.D.	0.17		0.18		0.22		0.18		0.18	0.17
Low	5.64		5.76		5.64		5.64		5.76	5.78
High	6.14		6.37		6.42		6.22		6.27	6.35
%<6.0	81.0		63.0		47.0		73.0		25.0	25.0

* A=HMEC/SBM, B=HMEC/CGF, C=GSC/CGF, D=GSC/SBM, E=HMEC/SBM, H=GSC/SBM

** pH values for Period #1 for diets E and H were not obtained

Increased availability of isoacids in the early lactation HMEC/SBM diet (A) may have had a stimulatory effect on the rumen cellulolytics causing the amount of microbial nitrogen per g of rumen solids to be greater than that found in the early lactation HMEC/CGF diet (B). However, this difference in isoacid availability should have also existed between the early lactation GSC/CGF diet (C) and the early lactation GSC/SBM diet (D) in which differences in microbial nitrogen per g of rumen solids were not found.

Total rumen liquids (kg) evacuated from the cows was not different between diets within squares ($p>0.10$; Tables 12,13,14). Microbial nitrogen (g) per liter of rumen fluid was not different between diets within squares ($p>0.10$; Tables 12,13,14). Although not statistically compared, the concentration of bacteria in the liquid did tend to be higher for the early lactation cows (1.32 g/l) than for the late lactation cows (0.77 g/l), as would be expected with the difference in energy concentration. More available energy should lead to a proportional increase in cell mass (Nocek and Russell, 1988).

Differences in liquid rate of passage (%/h) could not be statistically analyzed due to the fact that only one measurement was made for each period, however, means appeared to be similar for treatments within each square (Tables 12,13,14). These rates of passage are rather high (.24/h for the early lactation cows) relative to those observed by other

researchers. Cameron et al.(1991) observed an average liquid dilution rate of .13/h with cows consuming 22.2 kg DM/d at 146 d postpartum. Klusmeyer et al.(1991b) found the liquid dilution rate in cows consuming 24.4 kg DM/d at 113 d postpartum to be .14/h. Both researchers sampled from the duodenum. Dry matter intake for the early lactation cows on this study averaged 25.7 kg/d (Tables 12,13,14) and the cows were 92 d postpartum, on average. Thus, the higher liquid dilution rates observed under these conditions appear to be reasonable. It is difficult to compare those rates of passage estimated by rumen dilution techniques with those estimated by duodenal sampling. The extra length of tract and abomasal conditions prior to the duodenum could result in either a net decrease or increase in rate of passage estimates by duodenal sampling as compared to those obtained by rumen dilution techniques.

Rate of passage of solids from the rumen tended to be higher ($p<0.06$; Table 12) for the early lactation HMEC/CGF diet (B; 0.053/h) than for the early lactation HMEC/SBM diet (A; 0.048/h), similar (Table 13) for the early lactation GSC/CGF (C) and GSC/SBM (D) diets (0.043/h), and higher ($p<0.02$; Table 14) for the late lactation HMEC/SBM diet (E; 0.036/h) than for the late lactation GSC/SBM diet (H; 0.030/h). It is questionable as to whether these differences are biologically significant. Solids rate of passage did tend to decrease with increasing days in milk as would be expected

based on the increased amounts of total rumen solids evacuated (Table 12,13,14). Solids rates of passage averaging .056/h (Klusmeyer et al.,1991b) and .054/h (Klusmeyer et al, 1991a) have been measured with cows on similar types of diets by feeding a diet with ytterbium chloride sprayed on as an external marker followed by subsequent rumen sampling. Colucci et al.(1982) found rates of rumen particulate passage ranging from .023/h on a low forage (32%DM), low intake (12 g DM/kg BW) diet to .070/h on the same diet at high intake (42.1 g DM/kg BW). These measurements were obtained by feeding single-dose meals containing Cr-stained feed, sampling feces over time, and analyzing the descending slope of the fecal Cr concentration curve over time. It is difficult to compare the measurements obtained in the current trial using rumen undegradable NDF to those obtained using markers. Markers may not be totally recoverable and may not necessarily flow with the particulate phase depending on the extent of mordanting. Use of rumen undegradable NDF as a marker is dependent upon the assumption that all NDF remaining after a 120-h <u>in vitro</u> incubation is indeed undegradable.

Daily microbial nitrogen flows (g/d) from rumen liquids, rumen solids, and the sum total from the liquids and solids are recorded in Tables 12,13,14. Total microbial nitrogen flow (g/d) for the early lactation GSC/SBM (D) diet was greater ($p<0.06$) than that for the early lactation GSC/CGF (C) diet. Only slightly more OM (12.88 kg vs. 12.70 kg; Table 13)

was apparently fermented in the rumen with the GSC/SBM (D) diet than in the rumen with the GSC/CGF (C) diet; therefore, much of this difference in total microbial nitrogen flow might be attributed to differences in protein availability. The greatest amount of microbial nitrogen was derived from the liquid fraction. It must be recognized that this "liquid fraction" also included a large percentage of the small particle pool and, therefore, these high microbial nitrogen flow measurements derived from the "liquid fraction" may not necessarily contradict the conclusion of others that 25 - 50% of the rumen microbial mass is particle-associated (Minato et al., 1966). Measurements of total daily microbial nitrogen flow at the duodenum appear to be in the same range as those measurements obtained using duodenally cannulated cows on similar diets (Appendix E, McCarthy et al., 1989, Stokes et al., 1991a).

Daily microbial nitrogen flow (g) per kg DM intake, per kg estimated digested OM, and per kg estimated digested carbohydrate for each diet are recorded in Tables 12,13,14. Amounts of digested OM and carbohydrate were estimated using the within-day model and are recorded in Tables 12,13,14. Rumen pH is recorded in Table 15.

Differences in daily microbial nitrogen flow (g) per kg DM intake, per kg estimated digested OM, and per kg estimated digested carbohydrate for the dietary treatments were: early lactation GSC/SBM (D) greater than ($p<0.07$) early lactation

GSC/CGF (C) and early lactation HMEC/SBM (A) greater than ($p<0.05$) early lactation HMEC/CGF (B). The early lactation GSC/SBM diet (D) resulted in the highest microbial nitrogen flows per unit of OM or carbohydrate fermented possibly due to the greater availability of protein and better synchronization of protein and carbohydrate availability to the bacteria. The bacteria in the rumen containing the early lactation GSC/CGF diet (C) may have been limited by protein availability.

Unfortunately, the differences in 2 h nutrient degradability intended to exist between the dietary treatments were not shown to exist using the within-day model, with the exception that differences in carbohydrate availability between the late lactation diets were shown. It is recognized that perhaps the 2 h interval used as part of the diet comparison may not be adequate and differences in carbohydrate and protein availability did exist and did affect the rumen microbial population, but were not shown.

The extent of uniformity of each of the carbohydrate and protein fractions in the within-day model as well as the rates of passage imposed on each of the nutrient fractions may have also contributed to model error. For example, many of the fine particles present in both the HMEC and GSC may have immediately associated with the liquid pool in the rumen and therefore, passed at a faster rate than predicted. Rates of NDF degradation were probably underestimated when complete degradation of potentially fermentable NDF occurred before 48

hours.

Feng et al.(1993) attributed a reduction in microbial efficiency on a high NSC diet to lower ruminal liquid and solid dilution rates which resulted in increased microbial recycling. Although differences in rates of passage were not evident for the current study, within-day variations in rates of passage and recycling may have occurred and contributed to the variations in microbial flow estimates, especially with the early lactation HMEC/CGF (B) diet.

The microbial nitrogen flows (g) per kg DM intake, per kg estimated degraded OM, and per kg estimated degraded carbohydrate from the late lactation diets were not significantly different ($p>0.32$). It was expected that the late lactation GSC/SBM diet (H) would yield more microbial nitrogen due to the negative impact of reduced pH expected to result with the HMEC/SBM diet (E) which contained more rapidly available NSC (Table 11), however differences in rumen pH were not observed (Table 15). Microbial nitrogen flow per kg estimated OM and carbohydrate degraded was expected to be lower for the late lactation diets than for the early lactation diets due to the higher forage content of the diets, larger percentage of cellulolytic bacterial growth, and increased recycling.

C. The Effect of Diet on Degradation of Feeds <u>In Situ</u>:

Dry matter disappearances (%DM) following 30 h (forages) or 8 h (concentrates) <u>in situ</u> incubation of feeds in rumens

containing the six different diets fed in this study are recorded in Table 16. Consistent relationships between diet and DM degradability by feed type could not be found within this dataset.

Table 17 shows the effect of diet on crude protein disappearance (%CP) following 8 h in situ incubation of feeds. Crude protein degradability of distillers' grains was low (51.19% CP) and not affected by diet ($p>0.05$). Corn gluten meal protein was also degraded to only a limited extent (44.07% CP) and degradation was only increased ($p<0.05$) on the early lactation HMEC/SBM diet (A), the diet with maximum microbial nitrogen per l of rumen fluid. This result would be expected due to the high percentage of slowly degradable prolamine proteins contained in corn products (Blethen et al., 1990). Stern (personal communication) has found corn gluten meal tends to clump within dacron bags, thus lowering overall degradation and reducing repeatability of estimates. Feeds incubated in rumens containing the early lactation HMEC/SBM diet (A) consistently had the highest amount of protein degradation with feeds incubated in rumens containing less microbial nitrogen per l of rumen fluid tending to degrade less but with little consistency according to diet.

Rumen pH likely had a confounding effect on protein disappearance from the dacron bags. Bartle et al.(1986) found that soybean meal protein degradation responded to pH in a quadratic manner with degradation being highest at pH 6.0-6.5

and lower at pH 5.5 and pH 7.0. No degradability response to rumen pH was found in this study although the lack of any clear trend especially in canola, soybean meal, and roasted soy protein degradability, among the diets could lead one to conclude that pH had an influence.

Table 18 shows the effect of diet on NDF disappearance (%NDF) following 30 h in situ incubation of three forages. There was a trend toward higher NDF degradation in the dacron bags suspended in the rumens containing the most forage, the late lactation diets (E and H), perhaps indicating a higher percentage of cellulolytic bacteria.

It was shown that carbohydrate and protein availability to the microbes significantly impacts microbial nitrogen flow per kg of dry matter intake. Based on the preliminary comparisons, the rumen evacuation technique yields microbial nitrogen flow estimates which are in the same range as those estimated using duodenally cannulated cows. It can be concluded from the in situ study that extent of degradation of individual feeds within the rumen is dependent upon the total diet consumed by the animal.

Table 16. Dry matter disappearance (%DM) following 30 h (forages) or 8 h (concentrates) in situ incubation of feeds and microbial nitrogen (g) per l of rumen fluid in rumens containing six different diets

	Diet*					
	A	B	C	D	E	H
Alf Sil MSD=4.98**	83.26^{AB}	82.52^{B}	81.88^{B}	83.03^{AB}	87.52^{A}	83.34^{AB}
Corn Sil MSD=1.15	----	78.94^{A}	74.94^{B}	78.72^{A}	79.43^{A}	78.59^{A}
Grass Sil MSD=1.78	74.80^{AB}	75.03^{AB}	70.91^{C}	73.67^{B}	75.50^{A}	74.38^{AB}
Cornmeal MSD=5.39	69.81^{ABC}	69.25^{BC}	74.27^{AB}	72.48^{ABC}	67.68^{C}	74.79^{A}
HMEC MSD=4.56	77.86^{AB}	73.34^{B}	80.91^{A}	73.38^{B}	75.16^{B}	77.87^{AB}
Barley MSD=4.21	87.39^{A}	73.22^{C}	86.54^{A}	85.12^{AB}	85.80^{A}	81.57^{B}
CGF MSD=3.29	75.37^{A}	71.79^{B}	73.58^{AB}	73.20^{AB}	71.09^{B}	71.77^{B}
CGM MSD=1.86	57.42^{A}	54.95^{BC}	53.77^{C}	55.63^{AB}	54.33^{BC}	54.23^{BC}
Distillers MSD=3.37	66.32^{AB}	65.95^{AB}	62.34^{C}	64.41^{BC}	68.16^{A}	69.31^{A}
Canola MSD=3.57	74.21^{A}	68.20^{C}	69.38^{BC}	73.25^{A}	72.09^{AB}	69.40^{BC}
SBM MSD=4.36	77.53^{A}	71.81^{BC}	68.50^{C}	76.07^{AB}	71.77^{BC}	79.61^{A}
Roast Soy MSD=7.35	83.79^{A}	79.60^{AB}	77.28^{ABC}	79.11^{AB}	70.80^{C}	73.90^{BC}
Wht Midds MSD=2.17	82.70^{A}	82.44^{A}	82.27^{A}	82.86^{A}	80.00^{B}	82.00^{AB}
g MN per l	1.44	1.15	1.28	1.41	0.76	0.78

^{A,B,C} Means with unlike superscripts differ ($p<0.05$)

* A= Early HMEC/SBM, B= Early HMEC/CGF, C= Early GSC/CGF, D= Early GSC/SBM, E= Late HMEC/SBM, H= Late GSC/SBM

** MSD = Minimum Significant Difference

Table 17. Crude protein disappearance (%CP) following 8 h *in situ* incubation of feeds and microbial nitrogen (g) per l of rumen fluid in rumens containing six different diets

	Diet*					
	A	B	C	D	E	H
Cornmeal MSD=8.15**	58.85^{AB}	58.43^{AB}	63.25^{A}	60.99^{AB}	53.83^{B}	56.51^{AB}
HMEC MSD=6.26	70.79^{AB}	67.10^{B}	74.01^{A}	67.15^{B}	66.17^{B}	66.81^{B}
Barley MSD=4.78	89.22^{A}	65.22^{C}	85.01^{A}	85.12^{A}	84.88^{A}	79.19^{B}
CGF MSD=2.20	84.39^{A}	81.24^{B}	83.72^{A}	84.45^{A}	80.25^{B}	80.09^{B}
CGM MSD=2.31	46.13^{A}	44.25^{AB}	43.31^{B}	44.30^{AB}	43.26^{B}	43.14^{B}
Distil. MSD=4.79	52.49^{A}	51.70^{A}	48.62^{A}	49.24^{A}	51.86^{A}	53.25^{A}
Canola MSD=3.37	77.33^{A}	68.28^{D}	70.84^{DC}	74.93^{AB}	73.70^{BC}	71.40^{DC}
SBM MSD=6.43	66.14^{AB}	58.53^{CD}	55.01^{D}	63.64^{BC}	59.94^{BCD}	70.35^{A}
Roast Soy MSD=9.20	80.40^{A}	74.82^{AB}	71.49^{ABC}	73.54^{AB}	63.69^{C}	67.18^{BC}
Wht Midds MSD=0.90	94.53^{A}	93.28^{BC}	93.31^{BC}	93.79^{AB}	90.82^{D}	92.66^{C}
g MN per l	1.44	1.15	1.28	1.41	0.76	0.78

^{A,B,C,D} Means with unlike superscripts differ ($p<0.05$)

* A= Early HMEC/SBM, B= Early HMEC/CGF, C= Early GSC/CGF, D= Early GSC/SBM, E= Late HMEC/SBM, H= Late GSC/SBM

** = Minimum Significant Difference

Table 18. NDF disappearance (%NDF) following 30 h *in situ* incubation of feeds and microbial nitrogen (g) per l of rumen fluid in rumens containing six different diets

	Diet*					
	A	B	C	D	E	H
Alf Sil MSD=8.18**	72.65^{AB}	68.72^{B}	69.01^{B}	70.59^{B}	79.54^{A}	72.92^{AB}
Corn Sil MSD=2.02	---	63.25^{A}	56.31^{B}	62.36^{A}	63.77^{A}	62.72^{A}
Grass Sil MSD=2.30	68.02^{A}	65.48^{B}	62.13^{C}	64.66^{B}	69.39^{A}	68.79^{A}
g MN per l	1.44	1.15	1.28	1.41	0.76	0.78

^{A,B,C} Means with unlike superscripts differ ($p<0.05$)
* A= Early HMEC/SBM, B= Early HMEC/CGF, C= Early GSC/CGF, D= Early GSC/SBM, E= Late HMEC/SBM, H= Late GSC/SBM
** = Minimum Significant Difference

CONCLUSIONS

This research has made significant progress towards achieving the goal of developing an improved, practical model with which one can estimate microbial nitrogen flow at the duodenum of the dairy cow. The within-day model explained much of the variation in _in vivo_ measured microbial nitrogen flow after the laboratory effect was accounted for (R^2=0.69) and use of the within-day model to predict daily total amounts of individual rumen fermented nutrients was shown to further improve microbial nitrogen flow estimation (R^2=0.86). However, it is also apparent from this research that much more work must be done both _in vitro_ and _in vivo_ in order to refine the model further.

Conclusive results were not obtained regarding the ability of the within-day model to predict microbial nitrogen flow at the duodenum for a number of reasons. First, the assumption that the _in vivo_ estimates of microbial nitrogen flow are indeed accurate relies upon many more assumptions which have not been adequately tested. The "gold standard" to which a model based primarily on _in vitro_ data can be compared

to, simply may not exist. Secondly, high variation among laboratories hindered efforts to evaluate the within-day model since much of the variation in microbial nitrogen flow could be associated with the laboratory from which the estimate was obtained (R^2=0.31). Efforts must be made to standardize techniques among laboratories prior to the initiation of future collaborative work. Furthermore, it must be recognized that with the exception of one study (Glenn et al., 1989), the microbial nitrogen flow dataset used for testing of the model was primarily composed of diets designed for cows at high levels of production and did not contain much range in total fermented organic matter (8 - 11 kg/d). Microbial nitrogen flow data is needed from diets containing intermediate levels of fermentable organic matter (3 - 8 kg/d) in order increase confidence in microbial nitrogen flow models. Although not always recognized, other published datasets used for model development have had similar problems (Clark et al., 1992, Russell et al., 1992).

Based on the comparison of the within-day model estimates of microbial nitrogen flow to microbial nitrogen flow measurements made <u>in vivo</u>, it can be concluded that the within-day model explained much of the variation in yields after the laboratory effect was accounted for (R^2=0.69). For this dataset, the within-day model performance with correction for laboratory variation was similar to that of NEl (R^2=0.69), the Cornell Net Carbohydrate and Protein System (R^2=0.72), the

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Spartan model(R^2=0.67), and Fat-Corrected NE_l(R^2=0.69). It must be recognized, however, that to adequately compare the performance of the within-day model to that of steady-state models in existence, further experiments should be conducted under non-steady-state conditions, such as in commercial herd situations.

Use of the within-day model to predict daily total amounts of individual rumen fermented nutrients, especially fermented true protein, non-structural carbohydrate, and fat, was shown to improve microbial nitrogen flow estimation (R^2=0.86). It can therefore be concluded that rumen microbial growth is not a constant function of degraded organic matter, degraded carbohydrate, or NE_l (Mcal/d). Further research needs to be conducted to delineate the relationships of each of these nutrients to microbial nitrogen flow as well as to more accurately assess their rates and extents of fermentation.

The technique developed for estimation of microbial nitrogen flow as a function of the microbial concentration in the rumen liquids and solids and the rate of passage of each, offers promise for future studies. The question which arises when developing a new technique is: what does one compare to? Unfortunately, both the standard duodenally cannulated cow method and the new rumen evacuation method rely upon assumptions which need to be tested further. Many questions still remain for both methods, including: 1. What is the rate

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and extent of dietary purine degradation in the rumen? 2. How accurate are the current markers for estimating rates of passage? 3. Can steady-state kinetics be assumed? 4. How representative of the actual microbial population flowing from the rumen are the rumen bacterial pellets obtained by differential centrifugation? For the rumen evacuation method, the impact of the assumptions that the liquid and solid pools in the rumen are homogeneous and that selective retention of the microbes does not take place within either pool must be tested. The animal health problems leading to low dry matter intake and low milk production often inherent in studies using duodenally cannulated cows are avoided with the rumen evacuation technique.

Based on the preliminary comparisons, the rumen evacuation technique yields microbial nitrogen flow estimates which are in the same range as those estimated using duodenally cannulated cows fed similar diets. It was shown that carbohydrate and protein availability to the microbes impacts microbial nitrogen flow per kg of dry matter intake.

ADF = acid d
Ad libitum =
in
ADIN = acid
AOAC = Assoc
ATP = adenos
BW = body we
CHO = Carboh
CNCPS = The
CP = Crude p
CV = coeffic
df = degrees
DIM = Days
DIP = Rumen
DM = Dry ma
DMI = Dry ma
DNA = deoxy
EDTA = ethy
FCM = fat-c
JDS = Journa
MN = Rumen
n = number
N = Nitroge
NDF = Neutr
NDIN = Neut
NDFCP = Neu
NE = Net En
NPN = Non-p
NRC = Natio
NS = nonsig
NSC = Non-s
RNA = ribon
SAS = Stati
SCC = somat
SD = standa
SE = standa
SEM = stand
Synchrony =
TCA = trich
TDN = Total
TP = True p
UIP = Rumen
USDA = Unit
VFA = volat

GLOSSARY

ADF = acid detergent fiber
Ad libitum = Diet fed at 15% greater than expected dry matter intake
ADIN = acid detergent insoluble nitrogen
AOAC = Association of Official Analytical Chemists
ATP = adenosine triphosphate
BW = body weight
CHO = Carbohydrate
CNCPS = The Cornell Net Carbohydrate and Protein System
CP = Crude protein
CV = coefficient(s) of variation
df = degrees of freedom
DIM = Days in milk
DIP = Rumen degraded intake protein
DM = Dry matter
DMI = Dry matter intake
DNA = deoxyribonucleic acid
EDTA = ethylenediaminotetraacetate
FCM = fat-corrected milk
JDS = Journal of Dairy Science
MN = Rumen microbial nitrogen
n = number of samples
N = Nitrogen
NDF = Neutral detergent fiber
NDIN = Neutral detergent insoluble nitrogen
NDFCP = Neutral detergen insoluble crude protein
NE_l = Net Energy for Lactation
NPN = Non-protein nitrogen
NRC = National Research Council
NS = nonsignificant
NSC = Non-structural carbohydrate
RNA = ribonucleic acid
SAS = Statistical Analysis System
SCC = somatic cell count
SD = standard deviation
SE = standard error
SEM = standard error of the means
Synchrony = Ratio of available nutrients to each other
TCA = trichloroacetic acid
TDN = Total digestible nutrients
TP = True protein
UIP = Rumen undegraded intake protein
USDA = United States Department of Agriculture
VFA = volatile fatty acids

APPENDICES

Appendix A: Protein Fractions of Feeds Fed in Cow Studies:

Study	Feed	CP(%DM)	A(%CP)	B_1(%CP)	B_2(%CP)	B_3(%CP)	C(%CP)
Robinson&Kennelly	RDP Mix P1&2	17.30	7.46	10.52	68.44	8.38	5.20
Robinson&Kennelly	RDP Mix P3	19.40	13.09	14.18	60.05	8.20	4.48
Robinson&Kennelly	RDP Mix P4	21.60	13.66	12.64	60.65	8.94	4.12
Robinson&Kennelly	SDP Mix P1&2	19.70	13.15	6.14	40.61	32.99	7.11
Robinson&Kennelly	SDP Mix P3	21.30	16.76	5.31	36.20	34.60	7.14
Robinson&Kennelly	SDP Mix P4	22.80	14.21	5.13	41.89	34.34	4.43
Robinson&Kennelly	AlfSil. P1&2	19.30	63.37	3.26	23.78	2.07	7.51
Robinson&Kennelly	AlfSil. P3	19.60	63.27	2.76	24.80	2.24	6.94
Robinson&Kennelly	AlfSil. P4	20.60	63.69	3.83	23.11	2.52	6.84
Robinson&Kennelly	Oat Sil. P1	11.90	52.18	3.53	37.56	2.10	4.62
Robinson&Kennelly	Oat Sil. P2	10.10	43.86	3.07	45.84	3.07	4.16
Robinson&Kennelly	Oat Sil. P3	11.00	54.91	2.00	37.82	0.64	4.64
Robinson&Kennelly	Oat Sil. P4	10.20	58.53	2.35	30.29	5.29	3.53
Robinson&Kennelly	Alfalfa Hay	18.21	30.09	2.42	50.14	11.75	5.60
Stokes et al,1991	Diet #1 TMR	19.94	23.57	11.18	54.31	6.77	4.16
Stokes et al,1991	Diet #2 TMR	26.07	18.83	6.79	59.65	9.05	5.68
Stokes et al,1991	Diet #3 TMR	19.75	12.61	2.23	65.11	9.01	11.04
MSU Study	GSHCorn	10.19	4.71	7.85	84.49	1.08	1.86
MSU Study	Corn Gluten Ml	68.66	2.45	0.67	94.38	0.00	11.40
MSU Study	Soybean Meal	51.16	1.68	18.43	78.01	0.00	4.03
MSU Study	Corn Silage	7.99	54.07	1.25	33.42	6.13	5.13
Cunningham, 1991	Ration #1 Conc	17.82	9.43	6.51	72.90	1.80	9.37
Cunningham, 1991	Ration #2 Conc	20.96	10.16	11.35	75.10	2.00	1.38
Cunningham, 1991	Ration #3 Conc	15.38	16.32	5.46	65.08	9.82	3.32
Cunningham, 1991	Ration #4 Conc	18.10	13.04	8.23	65.14	9.83	3.76
Cunningham, 1991	Alfalfa Hay	24.10	27.18	1.78	56.10	11.58	3.36
Cunningham, 1991	Corn Silage	8.90	50.00	2.92	36.97	6.63	3.48
Cunningham, 1991	Haycrop Silage	11.80	27.63	3.56	37.88	16.69	14.24
McCarthy, 1989	TMR Barley/FM	14.60	26.85	1.71	23.22	36.64	11.58
McCarthy, 1989	TMR Corn/SBM	15.00	25.67	1.67	44.53	19.13	9.00
McCarthy, 1989	TMR Corn/FM	14.50	24.07	1.31	33.59	31.86	9.17
McCarthy, 1989	TMR Barley/SBM	14.90	30.13	2.15	31.07	23.36	13.29

Appendix A (cont'd): Protein Fractions of Feeds Fed in Cow Studies:

Study	Feed	CP(%DM)	A(%CP)	B_1(%CP)	B_2(%CP)	B_3(%CP)	C(%CP)
Klusmeyer, 1990	*TMR SBM 11%*	11.40	32.72	0.88	40.09	20.61	5.70
Klusmeyer, 1990	*TMR CGM 11%*	11.30	33.89	0.44	54.07	4.51	7.08
Klusmeyer, 1990	*TMR SBM 14.5%*	15.00	12.80	1.60	64.93	17.20	3.47

Appendix A (cont'd): Protein Fractions of Feeds Fed in Cow Studies:

Study	Feed	CP(%DM)	A(%CP)	B_1(%CP)	B_2(%CP)	B_3(%CP)	C(%CP)
Klusmeyer, 1990	TMR SBM 11%	11.40	32.72	0.88	40.09	20.61	5.70
Klusmeyer, 1990	TMR CGM 11%	11.30	33.89	0.44	54.07	4.51	7.08
Klusmeyer, 1990	TMR SBM 14.5%	15.00	12.80	1.60	64.93	17.20	3.47
Klusmeyer, 1990	TMR CGM 14.5%	14.50	23.10	0.97	55.52	13.79	6.62
Klusmeyer, 1991a	SBM Conc.	18.35	15.20	5.78	59.51	16.08	3.43
Klusmeyer, 1991a	SBM/FM Conc.	17.27	10.36	3.94	60.10	22.24	3.36
Klusmeyer, 1991a	SBM/Mega Conc	17.32	2.94	4.79	67.44	19.69	5.14
Klusmeyer, 1991a	SBM/FM/Megalac	17.53	10.55	5.08	54.48	26.41	3.48
Klusmeyer, 1991a	Corn Silage	9.80	39.29	4.08	43.06	4.69	8.88
Klusmeyer, 1991a	Alf.Haylage	23.80	51.60	1.85	22.35	16.89	7.31
Klusmeyer, 1991b	LF Conc.	19.82	5.20	6.31	66.60	18.26	3.63
Klusmeyer, 1991b	LF+Mega.Conc.	18.44	4.01	5.59	69.52	18.49	2.39
Klusmeyer, 1991b	HF Conc.	19.85	5.49	5.94	77.53	7.81	3.22
Klusmeyer, 1991b	HF+Mega.Conc.	23.89	10.59	5.65	78.82	1.63	3.31
Klusmeyer, 1991b	Corn Silage	9.60	36.98	1.15	48.65	6.35	6.88
Klusmeyer, 1991b	Alf.Haylage	20.70	48.50	1.88	31.11	10.82	7.68
Cameron, 1991	Control Conc.	16.54	42.56	1.45	43.41	9.25	3.33
Cameron, 1991	Starch Conc.	12.56	22.37	1.67	53.82	17.60	4.54
Cameron, 1991	Corn Silage	9.30	38.28	2.69	44.84	5.38	8.82
Cameron, 1991	Alf. Haylage	20.70	53.57	2.56	25.65	11.40	6.81
Illinois, 1991	CGM Conc.	28.40	20.00	1.20	69.89	5.32	3.59
Illinois, 1991	SBM Conc.	28.21	15.28	4.43	64.41	14.29	1.60
Illinois, 1991	BM Conc.	28.14	12.90	1.46	62.94	22.57	0.14
Illinois, 1991	BM/FM Conc.	27.88	16.36	1.11	57.21	23.85	1.47
Illinois, 1991	Corn Silage	7.81	56.21	1.79	28.43	6.40	7.17
Illinois, 1991	Alf. Haylage	18.53	50.67	2.37	23.53	12.79	10.63
Garrett, 1992	Corn Silage	7.65	46.41	1.18	45.36	4.44	2.61
Garrett, 1992	Alf. Haylage	22.92	57.02	1.57	24.08	9.86	7.46
Garrett, 1992	Corn	9.05	8.40	5.08	47.96	32.15	6.41
Garrett, 1992	Barley	11.94	9.55	7.12	59.05	20.69	3.60
Garrett, 1992	Soybean Meal	46.27	8.49	9.12	81.44	0.00	2.68
Garrett, 1992	Blood Meal	100.00	0.22	0.10	69.90	29.78	0.00

Appendix A (cont'd): Protein Fractions of Feeds Fed in Cow Studies:

Study	*Feed*	*CP(%DM)*	*A(%CP)*	B_1(%CP)	B_2(%CP)	B_3(%CP)	C(%CP)
Wonsil, 1991	*CornSil 1st*	*7.29*	*62.76*	*1.30*	*28.67*	*3.02*	*4.25*
Wonsil, 1991	*Alf Sil 1st*	*23.42*	*58.84*	*2.26*	*27.20*	*4.65*	*7.05*
Wonsil, 1991	*Tallow Conc*	*16.60*	*10.54*	*11.45*	*65.24*	*10.54*	*2.23*

Appendix A (cont'd): Protein Fractions of Feeds Fed in Cow Studies:

Study	Feed	CP(%DM)	A(%CP)	B_1(%CP)	B_2(%CP)	B_3(%CP)	C(%CP)
Wonsil, 1991	CornSil 1st	7.29	62.76	1.30	28.67	3.02	4.25
Wonsil, 1991	Alf Sil 1st	23.42	58.84	2.26	27.20	4.65	7.05
Wonsil, 1991	Tallow Conc.	16.60	10.54	11.45	65.24	10.54	2.23
Wonsil, 1991	Hyd Tal.Conc	16.24	10.22	10.53	65.70	10.96	2.59
Wonsil, 1991	CoatTal.Conc	16.77	7.16	13.48	62.43	14.91	2.03
Wonsil, 1991	Control Conc	16.47	6.92	9.35	63.02	18.70	2.00
Wonsil, 1991	CornSil 2nd	7.39	62.38	1.08	29.77	1.08	5.68
Wonsil, 1991	Alf Sil 2nd	23.86	61.94	1.55	27.33	2.60	6.58
Wonsil, 1991	Hyd FA Conc	15.21	10.12	3.88	78.50	4.21	3.29
Wonsil, 1991	FishOil Conc	15.39	8.25	6.89	66.28	15.14	3.44
Wonsil, 1991	SoyOil Conc	15.52	7.54	9.66	72.62	8.18	2.00
Glenn,#8601	TMR P#1(4/7)	17.11	44.65	2.92	45.76	0.00	8.07
Glenn,#8601	TMR P#1(4/21)	16.25	42.65	3.08	46.89	0.00	8.31
Glenn,#8601	TMR P#1(6/9)	17.02	40.36	2.94	50.59	2.17	3.94
Glenn,#8601	TMR P#2(5/12)	16.07	41.88	3.11	48.91	2.30	3.80
Glenn,#8601	TMR P#2(5/26)	16.78	38.38	2.98	52.92	0.00	8.05
Glenn,#8601	TMR P#2(7/7)	15.47	41.18	3.23	47.83	3.62	4.14
Glenn et al.1989	OG Sil P#1	19.24	41.16	2.08	26.51	25.73	4.52
Glenn et al.1989	OG Sil P#2	19.24	37.53	2.08	25.99	29.37	5.04
Glenn et al.1989	AlfSil P#1	24.05	53.85	2.08	30.73	8.94	4.41
Glenn et al.1989	AlfSil P#2	23.48	57.92	2.13	30.83	4.90	4.22
Glenn et al.1989	OG Sil P#3	19.47	46.69	2.05	27.02	18.85	5.39
Glenn et al.1989	OG Sil P#4	18.31	37.52	2.18	25.40	28.84	6.06
Glenn et al.1989	AlfSil P#3	23.15	55.77	2.16	30.41	6.22	5.44
Glenn et al.1989	AlfSil P#4	23.68	57.43	2.11	29.56	5.62	5.28
Miner Study	Alf.Silage	18.30	49.18	10.82	29.62	5.46	4.92
Miner Study	Corn Silage	8.50	35.29	12.71	36.71	8.24	7.06
Miner Study	Grass Silage	14.40	54.03	6.94	23.06	8.33	7.64
Miner Study	HMEC	8.70	11.49	14.48	64.83	8.05	1.15
Miner Study	GSHCorn	8.80	5.68	17.27	70.23	5.68	1.14
Miner Study	Soybean Meal	52.90	1.89	20.11	77.24	0.19	0.57
Miner Study	Corn Glut Fd	20.00	30.00	18.00	35.50	12.50	4.00
Miner Study	Blood Meal	91.40	0.00	2.00	76.66	18.38	2.95
Miner Study	Corn Glut Ml	60.10	4.99	1.21	89.13	0.00	4.66

Appendix B: Carbohydrate Fractions(%DM) of Feeds Fed in Cow Studies:

Study	Feed	NDF	ADF	Gluc&Starch	Lignin	K_dStarch	K_dAvNDF
Robinson&Kennelly	RDP Mix P1&2	24.73	12.84	37.63	4.84	15.31	------
Robinson&Kennelly	RDP Mix P3	23.40	11.84	32.86	3.60	14.25	------
Robinson&Kennelly	RDP Mix P4	25.57	12.94	32.31	4.28	20.50	------
Robinson&Kennelly	SDP Mix P1&2	28.94	11.65	33.18	3.99	13.61	------
Robinson&Kennelly	SDP Mix P3	25.90	9.95	37.50	3.92	11.47	------
Robinson&Kennelly	SDP Mix P4	36.38	11.61	25.50	3.03	-----	14.07
Robinson&Kennelly	AlfSil. P1&2	41.48	27.78	0.66	6.36	-----	5.26
Robinson&Kennelly	AlfSil. P3	42.02	28.53	0.00	6.86	-----	6.84
Robinson&Kennelly	AlfSil. P4	43.44	29.38	0.00	7.27	-----	6.31
Robinson&Kennelly	Oat Sil. P1	50.74	28.72	10.96	4.60	-----	1.97
Robinson&Kennelly	Oat Sil. P2	52.84	29.20	12.97	4.56	-----	2.30
Robinson&Kennelly	Oat Sil. P3	51.88	29.13	11.13	4.55	-----	2.24
Robinson&Kennelly	Oat Sil. P4	49.81	27.72	14.77	4.56	-----	1.89
Robinson&Kennelly	Alfalfa Hay	41.43	26.73	2.81	5.83	-----	5.66
Stokes et al,1991	Diet #1 TMR	28.99	15.66	30.21	3.60	6.30	5.52
Stokes et al,1991	Diet #2 TMR	30.09	14.11	22.16	3.67	-----	6.38
Stokes et al,1991	Diet #3 TMR	42.19	24.57	34.00	3.93	13.62	4.12
MSU Study	GSHCorn	11.28	3.74	69.68	1.08	10.48	------
MSU Study	Corn Gluten Ml	3.61	1.80	28.07	0.96	-----	------
MSU Study	Soybean Meal	14.33	8.80	11.15	1.14	-----	------
MSU Study	Corn Silage	43.87	24.93	30.13	2.82	12.55	3.01
Cunningham, 1991	Ration #1 Conc	17.71	6.57	40.09	2.16	15.70	------
Cunningham, 1991	Ration #2 Conc	17.98	10.39	33.39	1.90	16.96	------
Cunningham, 1991	Ration #3 Conc	19.44	9.60	41.47	1.16	7.29	------
Cunningham, 1991	Ration #4 Conc	23.75	12.82	30.07	2.38	18.92	------
Cunningham, 1991	Alfalfa Hay	32.23	19.87	0.00	4.87	-----	8.05
Cunningham, 1991	Corn Silage	45.48	26.69	27.47	3.31	-----	2.92
Cunningham, 1991	Haycrop Silage	69.10	43.00	0.61	7.53	-----	3.68
McCarthy, 1989	TMR Barley/FM	30.37	17.59	25.44	4.26	-----	4.51
McCarthy, 1989	TMR Corn/SBM	22.05	13.13	35.59	3.24	5.17	------
McCarthy, 1989	TMR Corn/FM	23.70	14.12	35.42	3.65	6.35	------
McCarthy, 1989	TMR Barley/SBM	31.94	18.78	17.63	4.60	-----	5.57

Appendix B (cont'd): Carbohydrate Fractions(%DM) of Feeds Fed in Cow Studies:

Study	Feed	NDF	ADF	Gluc&Starch	Lignin	K_dStarch	K_dAvNDF
Klusmeyer, 1990	TMR SBM 11%	29.12	14.54	36.16	2.28	2.18	------
Klusmeyer, 1990	TMR CGM 11%	29.14	15.49	37.86	2.23	6.22	------
Klusmeyer, 1990	TMR SBM 14.5%	28.66	14.98	28.78	2.18	-----	------
Klusmeyer, 1990	TMR CGM 14.5%	28.40	14.57	32.41	1.63	4.12	------
Klusmeyer, 1991a	SBM Conc.	11.61	3.39	46.64	0.76	-----	------
Klusmeyer, 1991a	SBM/FM Conc.	10.90	2.82	51.08	0.68	-----	------
Klusmeyer, 1991a	SBM/Mega Conc	10.46	3.35	40.97	0.75	-----	------
Klusmeyer, 1991a	SBM/FM/Megalac	10.52	2.67	44.22	0.67	-----	------
Klusmeyer, 1991a	Corn Silage	39.64	21.60	21.89	2.95	-----	1.65
Klusmeyer, 1991a	Alf.Haylage	41.88	27.82	0.00	7.49	-----	3.29
Klusmeyer, 1991b	LF Conc.	12.20	3.78	38.22	0.70	-----	------
Klusmeyer, 1991b	LF+Mega.Conc.	10.99	3.37	35.09	0.25	-----	------
Klusmeyer, 1991b	HF Conc.	10.85	3.83	36.83	0.48	-----	------
Klusmeyer, 1991b	HF+Mega.Conc.	8.93	3.16	31.33	0.34	-----	------
Klusmeyer, 1991b	Corn Silage	39.60	21.69	23.63	2.72	-----	2.18
Klusmeyer, 1991b	Alf.Haylage	40.19	27.11	0.00	8.15	-----	3.29
Cameron, 1991	Control Conc.	11.27	3.37	46.55	0.61	-----	------
Cameron, 1991	Starch Conc.	11.75	3.28	48.33	0.63	-----	------
Cameron, 1991	Corn Silage	41.38	22.26	22.38	2.67	-----	2.20
Cameron, 1991	Alf. Haylage	41.92	29.05	1.78	7.51	-----	2.33
Illinois, 1991	CGM Conc.	8.56	2.48	36.82	0.70	8.92	------
Illinois, 1991	SBM Conc.	9.91	3.72	26.90	0.48	-----	------
Illinois, 1991	BM Conc.	10.03	3.13	51.04	0.85	11.83	------
Illinois, 1991	BM/FM Conc.	9.04	2.73	49.97	0.71	-----	------
Illinois, 1991	Corn Silage	38.31	21.60	32.35	2.99	14.04	1.33
Illinois, 1991	Alf. Haylage	51.47	38.23	0.00	10.99	-----	3.46
Garrett, 1992	Corn Silage	38.96	19.20	29.56	2.22	-----	2.24
Garrett, 1992	Alf. Haylage	45.80	32.33	1.08	8.22	-----	4.02
Garrett, 1992	Corn	12.67	3.70	51.60	1.22	0.21	------
Garrett, 1992	Barley	24.22	8.04	51.01	1.68	13.62	------
Garrett, 1992	Soybean Meal	20.31	13.97	15.84	0.96	-----	------
Garrett, 1992	Blood Meal	0.93	0.21	0.00	0.40	-----	------

Appendix B (cont'd): Carbohydrate Fractions(%DM) of Feeds Fed in Cow Studies:

Study	Feed	NDF	ADF	Gluc&Starch	Lignin	K_dStarch	K_dAvNDF
Wonsil, 1991	CornSil 1st	36.88	19.91	31.38	1.98	-----	2.10
Wonsil, 1991	Alf Sil 1st	40.57	29.49	0.00	7.39	-----	5.01
Wonsil, 1991	Tallow Conc.	14.77	5.45	38.30	0.62	10.50	-----
Wonsil, 1991	Hyd Tal.Conc	15.28	5.53	38.33	0.89	10.92	-----
Wonsil, 1991	CoatTal.Conc	14.94	5.86	39.96	0.87	12.74	-----
Wonsil, 1991	Control Conc	15.28	5.63	44.87	0.91	8.03	-----
Wonsil, 1991	CornSil 2nd	37.63	19.85	30.77	2.26	27.96	1.94
Wonsil, 1991	Alf Sil 2nd	39.69	29.35	0.00	7.07	-----	4.65
Wonsil, 1991	Hyd FA Conc	13.07	4.53	40.51	0.74	13.81	-----
Wonsil, 1991	FishOil Conc	13.16	4.58	44.72	0.86	9.12	-----
Wonsil, 1991	SoyOil Conc	14.48	5.36	44.55	0.90	12.78	-----
Glenn,#8601	TMR P#1(4/7)	26.20	16.86	25.29	3.05	-----	2.90
Glenn,#8601	TMR P#1(4/21)	26.80	16.41	30.38	3.10	-----	2.83
Glenn,#8601	TMR P#1(6/9)	24.47	14.93	29.41	2.55	-----	2.91
Glenn,#8601	TMR P#2(5/12)	26.31	16.22	32.91	2.92	-----	2.38
Glenn,#8601	TMR P#2(5/26)	26.00	15.63	29.88	2.69	-----	3.16
Glenn,#8601	TMR P#2(7/7)	29.85	17.90	23.27	3.26	-----	2.62
Glenn et al.1989	OG Sil P#1	58.04	32.64	2.05	3.97	-----	3.87
Glenn et al.1989	OG Sil P#2	59.50	32.00	2.83	4.34	-----	4.54
Glenn et al.1989	AlfSil P#1	33.70	23.52	3.73	4.95	-----	5.51
Glenn et al.1989	AlfSil P#2	32.76	23.86	3.61	5.15	-----	5.02
Glenn et al.1989	OG Sil P#3	57.87	33.42	2.04	4.46	-----	5.37
Glenn et al.1989	OG Sil P#4	61.31	33.84	1.14	4.76	-----	5.47
Glenn et al.1989	AlfSil P#3	35.96	26.03	4.56	5.64	-----	8.43
Glenn et al.1989	AlfSil P#4	35.81	25.68	2.11	5.56	-----	7.01
Miner Study	Alf.Silage	41.63	36.20	1.73	(8.60)	5.06	2.91
Miner Study	Corn Silage	42.80	23.20	28.75	(3.13)	9.55	0.59
Miner Study	Grass Silage	65.40	39.20	(6.00)	(4.25)	-----	1.50
Miner Study	HMEC	16.80	4.27	44.70	(1.00)	12.51	-----
Miner Study	GSHCorn	10.20	2.90	31.74	(1.12)	12.48	-----
Miner Study	Soybean Meal	5.30	4.50	2.34	(0.16)	18.33	-----
Miner Study	Corn Glut Fd	34.50	10.50	18.47	(0.75)	20.06	-----
Miner Study	Blood Meal	41.70	4.00	(0.00)	(0.00)	-----	-----
Miner Study	Corn Glut Ml	5.80	4.70	10.52	(0.41)	20.20	-----

Appendix C: Other Fractions of Feeds Fed in Cow Studies:

Study	Feed	EE(%DM)	Ash(%DM)
Robinson&Kennelly	RDP Mix P1&2	2.81	6.67
Robinson&Kennelly	RDP Mix P3	2.63	6.12
Robinson&Kennelly	RDP Mix P4	3.11	7.44
Robinson&Kennelly	SDP Mix P1&2	2.91	6.10
Robinson&Kennelly	SDP Mix P3	6.14	7.14
Robinson&Kennelly	SDP Mix P4	3.22	8.45
Robinson&Kennelly	AlfSil. P1&2	5.37	9.53
Robinson&Kennelly	AlfSil. P3	5.62	9.18
Robinson&Kennelly	AlfSil. P4	5.87	8.44
Robinson&Kennelly	Oat Sil. P1	4.61	7.61
Robinson&Kennelly	Oat Sil. P2	4.71	7.51
Robinson&Kennelly	Oat Sil. P3	4.78	7.40
Robinson&Kennelly	Oat Sil. P4	4.89	7.41
Robinson&Kennelly	Alfalfa Hay	2.81	10.81
Stokes et al,1991	Diet #1 TMR	4.59	5.38
Stokes et al,1991	Diet #2 TMR	3.78	7.24
Stokes et al,1991	Diet #3 TMR	3.24	5.62
MSU Study	GSHCorn	1.64	1.38
MSU Study	Corn Gluten Ml	0.92	1.33
MSU Study	Soybean Meal	0.68	6.95
MSU Study	Corn Silage	3.04	4.29
Cunningham, 1991	Ration #1 Conc	4.00	7.57
Cunningham, 1991	Ration #2 Conc	4.92	7.01
Cunningham, 1991	Ration #3 Conc	6.38	5.44
Cunningham, 1991	Ration #4 Conc	5.79	5.47
Cunningham, 1991	Alfalfa Hay	2.54	13.02
Cunningham, 1991	Corn Silage	2.26	4.19
Cunningham, 1991	Haycrop Silage	3.52	6.95
McCarthy, 1989	TMR Barley/FM	2.19	9.02
McCarthy, 1989	TMR Corn/SBM	1.89	7.10
McCarthy, 1989	TMR Corn/FM	2.67	7.25
McCarthy, 1989	TMR Barley/SBM	2.28	9.32

Appendix C (cont'd): Other Fractions of Feeds Fed in Cow Studies:

Study	Feed	EE(%DM)	Ash(%DM)
Klusmeyer, 1990	TMR SBM 11%	2.42	7.84
Klusmeyer, 1990	TMR CGM 11%	2.39	8.06
Klusmeyer, 1990	TMR SBM 14.5%	2.61	8.73
Klusmeyer, 1990	TMR CGM 14.5%	2.47	7.25
Klusmeyer, 1991a	SBM Conc.	1.72	6.55
Klusmeyer, 1991a	SBM/FM Conc.	1.68	6.71
Klusmeyer, 1991a	SBM/Mega Conc	5.70	5.24
Klusmeyer, 1991a	SBM/FM/Megalac	3.10	6.62
Klusmeyer, 1991a	Corn Silage	2.67	4.96
Klusmeyer, 1991a	Alf.Haylage	2.43	13.64
Klusmeyer, 1991b	LF Conc.	1.95	7.90
Klusmeyer, 1991b	LF+Mega.Conc.	2.85	6.42
Klusmeyer, 1991b	HF Conc.	1.30	8.69
Klusmeyer, 1991b	HF+Mega.Conc.	2.71	9.39
Klusmeyer, 1991b	Corn Silage	3.69	5.45
Klusmeyer, 1991b	Alf.Haylage	4.22	12.63
Cameron, 1991	Control Conc.	1.66	12.98
Cameron, 1991	Starch Conc.	2.00	8.94
Cameron, 1991	Corn Silage	2.63	5.16
Cameron, 1991	Alf. Haylage	2.28	11.09
Illinois, 1991	CGM Conc.	1.39	9.65
Illinois, 1991	SBM Conc.	1.67	10.19
Illinois, 1991	BM Conc.	1.94	10.67
Illinois, 1991	BM/FM Conc.	1.87	11.04
Illinois, 1991	Corn Silage	3.57	3.31
Illinois, 1991	Alf. Haylage	2.93	8.02
Garrett, 1992	Corn Silage	3.72	0.87
Garrett, 1992	Alf. Haylage	2.90	8.07
Garrett, 1992	Corn	3.77	0.77
Garrett, 1992	Barley	1.16	0.82
Garrett, 1992	Soybean Meal	1.51	5.19
Garrett, 1992	Blood Meal	0.58	1.17

Appendix C (cont'd): Other Fractions of Feeds Fed in Cow Studies:

Study	Feed	EE(%DM)	Ash(%DM)
Wonsil, 1991	CornSil 1st	2.66	1.42
Wonsil, 1991	Alf Sil 1st	7.53	9.89
Wonsil, 1991	Tallow Conc.	8.56	2.10
Wonsil, 1991	Hyd Tal.Conc	9.18	2.59
Wonsil, 1991	CoatTal.Conc	8.40	1.84
Wonsil, 1991	Control Conc	3.09	2.65
Wonsil, 1991	CornSil 2nd	2.41	1.64
Wonsil, 1991	Alf Sil 2nd	3.45	10.13
Wonsil, 1991	Hyd FA Conc	7.88	2.20
Wonsil, 1991	FishOil Conc	7.70	1.94
Wonsil, 1991	SoyOil Conc	6.64	1.47
Glenn,#8601	TMR P#1(4/7)	8.99	5.51
Glenn,#8601	TMR P#1(4/21)	7.55	4.88
Glenn,#8601	TMR P#1(6/9)	6.78	5.31
Glenn,#8601	TMR P#2(5/12)	8.18	5.81
Glenn,#8601	TMR P#2(5/26)	8.59	5.37
Glenn,#8601	TMR P#2(7/7)	6.33	5.41
Glenn et al.1989	OG Sil P#1	9.65	5.64
Glenn et al.1989	OG Sil P#2	8.94	5.72
Glenn et al.1989	AlfSil P#1	12.57	6.39
Glenn et al.1989	AlfSil P#2	13.26	7.22
Glenn et al.1989	OG Sil P#3	11.09	5.39
Glenn et al.1989	OG Sil P#4	10.67	4.88
Glenn et al.1989	AlfSil P#3	14.19	7.41
Glenn et al.1989	AlfSil P#4	12.48	5.61
Miner Study	Alf.Silage	3.64	11.85
Miner Study	Corn Silage	2.90	3.76
Miner Study	Grass Silage	4.00	9.31
Miner Study	HMEC	4.15	1.17
Miner Study	GSHCorn	3.80	1.65
Miner Study	Soybean Meal	1.30	7.00
Miner Study	Corn Glut Fd	3.50	5.20
Miner Study	Blood Meal	0.50	3.15
Miner Study	Corn Glut Ml	4.50	2.88

Appendix D: Model Inputs for Cow Studies:

Study	Feed	%Forage	K_dB_1	K_dB_2	K_dB_3	S_1%DM	S_2%DM	K_dS_2	K_dNDF
Robinson&Kennelly	RDP Mix	0.0	285.0	12.0	0.30	16.41	17.86	5.0	14.00
Robinson&Kennelly	SDP Mix	0.0	251.5	9.1	0.35	15.35	16.71	5.0	14.00
Robinson&Kennelly	Alf Sil	100.0	150.0	12.0	1.80	0.22	0.00	5.0	6.14
Robinson&Kennelly	Oat Sil	100.0	300.0	12.0	0.20	6.23	6.23	5.0	2.10
Robinson&Kennelly	Alfalfa Hay	100.0	150.0	14.0	1.30	2.00	0.81	5.0	5.66
Stokes et al,1991	Diet #1 TMR	27.7	180.7	8.0	0.13	12.05	18.07	8.0	5.52
Stokes et al,1991	Diet #2 TMR	36.5	222.0	9.0	0.20	11.08	11.08	8.0	6.38
Stokes et al,1991	Diet #3 TMR	54.8	166.0	8.3	0.12	13.14	16.06	8.0	4.12
MSU Study	GSHCorn	0.0	150.0	2.9	0.09	21.27	48.41	8.0	14.00
MSU Study	Corn Gluten Ml	0.0	150.0	1.4	0.08	10.51	17.56	14.0	14.00
MSU Study	Soybean Meal	0.0	230.0	10.0	0.20	6.81	4.34	7.0	14.00
MSU Study	Corn Silage	70.0	300.0	10.1	0.20	15.07	15.07	5.0	3.01
Cunningham, 1991	Ration #1 Conc	0.0	186.9	7.0	0.17	12.24	27.85	8.0	14.00
Cunningham, 1991	Ration #2 Conc	0.0	163.5	7.0	0.14	10.19	23.20	8.0	14.00
Cunningham, 1991	Ration #3 Conc	0.0	147.6	5.9	0.09	12.66	28.81	8.0	14.00
Cunningham, 1991	Ration #4 Conc	0.0	184.3	7.4	0.24	9.18	20.89	8.0	14.00
Cunningham, 1991	Alfalfa Hay	100.0	150.0	14.0	1.30	0.00	0.00	5.0	8.05
Cunningham, 1991	Corn Silage	70.0	300.0	10.0	0.12	13.74	13.74	5.0	2.92
Cunningham, 1991	Haycrop Silage	100.0	175.0	12.0	1.50	0.61	0.00	5.0	3.68
McCarthy, 1989	TMR Barley/FM	45.0	255.0	13.0	0.75	12.18	13.26	5.0	5.57
McCarthy, 1989	TMR Corn/SBM	45.0	255.0	13.0	0.75	10.87	24.72	8.0	5.57
McCarthy, 1989	TMR Corn/FM	45.0	189.8	9.9	0.64	10.81	24.61	8.0	5.57
McCarthy, 1989	TMR Barley/SBM	45.0	257.0	13.0	0.74	8.44	9.19	5.0	5.57
Klusmeyer, 1990	TMR SBM 11%	42.0	248.0	8.4	0.16	11.04	25.12	8.0	5.00
Klusmeyer, 1990	TMR CGM 11%	42.0	242.8	8.1	0.15	11.56	26.30	8.0	5.00
Klusmeyer, 1990	TMR SBM 14.5%	42.0	254.0	8.9	0.17	8.79	19.99	8.0	5.00
Klusmeyer, 1990	TMR CGM 14.5%	42.0	243.7	8.0	0.16	9.89	22.52	8.0	5.00
Klusmeyer, 1991a	SBM Conc.	0.0	166.2	6.2	0.11	14.24	32.40	8.0	14.00
Klusmeyer, 1991a	SBM/FM Conc.	0.0	152.5	5.2	0.17	15.59	35.49	8.0	14.00
Klusmeyer, 1991a	SBM/Mega Conc	0.0	168.2	6.4	0.12	12.51	28.46	8.0	14.00
Klusmeyer, 1991a	SBM/FM/Megalac	0.0	153.0	5.2	0.17	13.50	30.72	8.0	14.00
Klusmeyer, 1991a	Corn Silage	70.0	300.0	10.0	0.20	10.95	10.95	5.0	2.00
Klusmeyer, 1991a	Alf.Haylage	100.0	150.0	12.0	1.80	0.00	0.00	5.0	3.00

Appendix D (cont'd): Model Inputs for Cow Studies:

Study	Feed	%Forage	K_dB_1	K_dB_2	K_dB_3	S_1%DM	S_2%DM	K_dS_2	K_dNDF
Klusmeyer, 1991b	LF Conc.	0.0	167.6	6.3	0.11	11.67	26.55	8.0	14.00
Klusmeyer, 1991b	LF+Mega.Conc.	0.0	170.7	6.6	0.12	10.71	24.38	8.0	14.00
Klusmeyer, 1991b	HF Conc.	0.0	174.7	6.9	0.12	11.24	25.59	8.0	14.00
Klusmeyer, 1991b	HF+Mega.Conc.	0.0	179.5	7.2	0.13	9.57	21.76	8.0	14.00
Klusmeyer, 1991b	Corn Silage	70.0	300.0	10.0	0.20	11.82	11.82	5.0	2.00
Klusmeyer, 1991b	Alf.Haylage	100.0	150.0	12.0	1.80	0.00	0.00	5.0	3.00
Cameron, 1991	Control Conc.	0.0	146.3	4.7	0.14	14.21	32.34	8.0	14.00
Cameron, 1991	Starch Conc.	0.0	143.8	4.5	0.18	24.17	24.17	5.0	14.00
Cameron, 1991	Corn Silage	70.0	300.0	10.0	0.20	11.19	11.19	5.0	2.00
Cameron, 1991	Alf. Haylage	100.0	150.0	12.0	1.80	1.00	0.78	5.0	3.00
Illinois, 1991	CGM Conc.	0.0	150.0	4.7	0.09	11.24	25.58	8.0	14.00
Illinois, 1991	SBM Conc.	0.0	184.3	7.6	0.14	8.21	18.69	8.0	14.00
Illinois, 1991	BM Conc.	0.0	142.3	4.4	0.24	15.58	35.46	8.0	14.00
Illinois, 1991	BM/FM Conc.	0.0	137.6	4.0	0.27	15.26	34.71	8.0	14.00
Illinois, 1991	Corn Silage	70.0	300.0	10.0	0.20	16.18	16.18	5.0	1.33
Illinois, 1991	Alf. Haylage	100.0	150.0	12.0	1.80	0.00	0.00	5.0	3.46
Garrett, 1992	Corn Silage	70.0	300.0	10.0	0.20	14.78	14.78	5.0	2.24
Garrett, 1992	Alf. Haylage	100.0	150.0	12.0	1.80	1.00	0.08	5.0	4.02
Garrett, 1992	Corn	0.0	150.0	5.0	0.09	15.75	35.85	8.0	14.00
Garrett, 1992	Barley	0.0	300.0	12.0	0.35	24.42	26.59	5.0	14.00
Garrett, 1992	Soybean Meal	0.0	230.0	11.0	0.20	9.68	6.16	7.0	14.00
Garrett, 1992	Blood Meal	0.0	100.0	1.0	0.80	0.00	0.00	5.0	14.00

Appendix D (cont'd): Model Inputs for Cow Studies:

Study	Feed	%Forage	K_dB_1	K_dB_2	K_dB_3	S_1%DM	S_2%DM	K_dS_2	K_dNDF
Wonsil, 1991	CornSil 1st	70.0	300.0	10.0	0.20	15.69	15.69	5.0	2.10
Wonsil, 1991	Alf Sil 1st	100.0	150.0	12.0	1.80	0.00	0.00	5.0	5.01
Wonsil, 1991	Tallow Conc.	0.0	164.4	6.2	0.16	11.69	26.61	8.0	14.00
Wonsil, 1991	Hyd Tal.Conc	0.0	164.4	6.2	0.16	11.70	26.63	8.0	14.00
Wonsil, 1991	CoatTal.Conc	0.0	164.4	6.2	0.16	12.20	27.76	8.0	14.00
Wonsil, 1991	Control Conc	0.0	164.4	6.2	0.16	13.70	31.17	8.0	14.00
Wonsil, 1991	CornSil 2nd	70.0	300.0	10.0	0.20	15.39	15.39	5.0	2.10
Wonsil, 1991	Alf Sil 2nd	100.0	150.0	12.0	1.80	0.00	0.00	5.0	5.01
Wonsil, 1991	Hyd FA Conc	0.0	164.4	6.2	0.16	12.37	28.14	8.0	14.00
Wonsil, 1991	FishOil Conc	0.0	164.4	6.2	0.16	13.65	31.07	8.0	14.00
Wonsil, 1991	SoyOil Conc	0.0	164.4	6.2	0.16	13.60	30.95	8.0	14.00
Glenn,#8601	TMR P#1	44.0	186.4	8.7	0.66	8.66	19.70	8.0	2.88
Glenn,#8601	TMR P#2	44.0	186.4	8.7	0.66	8.76	19.93	8.0	2.72
Glenn et al.1989	Alf Sil LOW	100.0	150.0	12.0	1.80	3.00	0.50	5.0	6.49
Glenn et al.1989	Alf Sil HIGH	100.0	150.0	12.0	1.80	3.00	0.50	5.0	6.49
Glenn et al.1989	OG LOW	100.0	135.0	11.0	0.09	2.00	0.02	5.0	4.81
Glenn et al.1989	OG HIGH	100.0	135.0	11.0	0.09	2.00	0.02	5.0	4.81
Miner Study	Alf.Silage	100.0	150.0	12.0	1.80	0.85	0.85	5.0	2.91
Miner Study	Corn Silage	70.0	300.0	10.0	0.20	15.00	15.00	5.0	0.59
Miner Study	Grass Silage	100.0	200.0	12.0	2.00	3.50	3.50	5.0	1.50
Miner Study	HMEC	0.0	135.0	6.7	0.15	29.92	32.58	4.4	14.00
Miner Study	GSHCorn	0.0	150.0	6.3	0.09	9.69	22.05	7.9	14.00
Miner Study	Soybean Meal	0.0	230.0	9.0	0.20	1.43	0.91	6.6	14.00
Miner Study	Corn Glut Fd	0.0	150.0	22.9	0.08	13.04	5.43	4.8	14.00
Miner Study	Blood Meal	0.0	100.0	1.0	0.80	1.25	1.25	5.0	14.00
Miner Study	Corn Glut Ml	0.0	150.0	5.9	0.08	3.94	6.58	14.3	14.00

Appendix E: Outputs of the Within-Day Model for Prediction of Microbial Nitrogen Flow:

Diet	MeasuredMN (gN/d)	Model MN (gN/d)	FermTP (kg)	FermNPN (kg)	FermNSC (kg)	Ferm NDF (kg)
McCarthy: Corn/SBM	294.0	319.2	1.165	0.932	8.861	1.777
McCarthy: Barley/FM	282.0	331.3	0.638	0.804	6.429	2.267
McCarthy: Barley/SBM	310.0	328.2	0.793	0.938	6.303	2.380
McCarthy: Corn/FM	259.0	338.9	0.800	0.813	8.257	1.818
Klusmeyer: SBM 14.5%	361.0	364.9	1.294	0.419	6.866	2.609
Klusmeyer: SBM 11%	344.0	356.1	0.581	0.780	7.188	2.568
Klusmeyer: CGM 14.5%	316.0	360.7	0.985	0.700	6.963	2.660
Klusmeyer: CGM 11%	330.0	367.8	0.747	0.827	7.320	2.635
Klusmeyer: SBM	340.0	358.9	1.248	1.468	8.016	1.826
Klusmeyer: SBM/LCFA	325.0	342.9	1.235	1.121	7.718	1.669
Klusmeyer: FM	284.0	342.0	1.073	1.252	7.705	1.705
Klusmeyer: FM/LCFA	303.0	326.2	1.028	1.200	7.393	1.626
Klusmeyer: HighForage	336.0	320.0	1.343	1.321	7.018	1.726
Klusmeyer: LoFor/LCFA	297.0	354.2	1.362	0.982	7.890	1.801
Klusmeyer: LowForage	313.0	360.5	1.447	1.080	7.928	1.881
Klusmeyer: HiFor/LCFA	313.0	309.2	1.453	1.357	6.509	1.581
Cameron: Control	357.0	316.6	0.810	1.794	7.060	1.809
Cameron: Urea	374.0	320.0	0.802	1.943	6.968	1.791
Cameron: Starch	312.0	353.1	0.771	1.245	7.778	1.722
Cameron: Starch/Urea	376.0	342.4	0.754	1.366	7.535	1.678
Stokes: Diet #1	317.0	374.2	1.820	1.029	6.552	2.445
Stokes: Diet #2	333.0	359.5	2.314	1.036	5.214	2.607
Stokes: Diet #3	202.0	291.2	1.464	0.456	3.958	2.912
Glenn: Alf Low	134.0	62.9	0.273	0.616	0.928	0.557
Glenn: Alf High	175.0	80.0	0.343	0.800	1.180	0.697
Glenn: OG Low	76.0	54.6	0.165	0.331	0.425	1.014
Glenn: OG High	94.0	69.5	0.208	0.429	0.541	1.262
Robinson: SDP	157.0	281.9	1.043	0.905	4.319	2.792
Robinson: RDP	177.0	297.8	1.474	0.874	5.212	2.371

Appendix E (cont'd): Outputs of the Within-Day Model for Prediction of Microbial Nitrogen Flow:

Diet	MeasuredMN (gN/d)	Model MN (gN/d)	FermTP (kg)	FermNPN (kg)	FermNSC (kg)	Ferm NDF (kg)
Cunningham: #1	429.0	330.6	1.115	0.543	5.877	2.523
Cunningham: #2	495.0	361.2	1.694	0.710	6.495	2.359
Cunningham: #3	525.0	367.0	1.598	0.934	6.514	2.444
Cunningham: #4	500.0	362.7	1.658	0.718	6.164	2.602
Wonsil: Coat Tallow	228.9	357.1	1.292	1.557	7.201	2.290
Wonsil: Tallow	222.7	353.2	1.255	1.596	7.121	2.300
Wonsil: Hydrg Tallow	223.7	359.8	1.263	1.654	7.255	2.358
Wonsil: 1st Control	232.7	359.1	1.216	1.548	7.558	2.323
Wonsil: 2nd Control	255.8	349.9	1.186	1.746	7.707	2.280
Wonsil: Hydrg FA	204.8	346.4	1.109	1.667	7.272	2.040
Wonsil: Fish Oil	181.8	328.9	1.030	1.532	6.889	1.942
Wonsil: Soy Oil	217.4	342.2	1.206	1.714	7.537	2.187
Garrett: #1	240.5	327.7	1.378	1.224	6.541	1.924
Garrett: #4	275.9	320.6	1.128	1.134	6.165	2.028
Garrett: #6	281.3	324.4	1.242	1.246	6.090	2.068
Garrett: #9	260.7	345.3	1.395	1.206	5.950	2.414
Glenn: Week#6	240.0	233.3	0.816	1.037	4.964	1.184
Glenn: Week#11	272.0	246.1	0.889	1.048	5.262	1.316
Miner: Diet E P#1	202.8	254.9	1.170	0.890	4.270	2.040
Miner: Diet E P#2	146.2	261.9	1.290	0.820	4.260	2.210
Miner: Diet H P#1	152.6	239.0	1.180	0.920	4.430	1.830
Miner: Diet H P#2	153.7	262.8	1.390	0.900	4.710	2.100
Miner: Diet A P#1	313.5	292.8	1.700	0.730	5.990	1.130
Miner: Diet A P#2	307.8	315.7	2.030	1.040	6.010	1.530
Miner: Diet B P#1	259.8	325.5	1.510	1.100	7.460	1.830
Miner: Diet B P#2	239.9	321.9	1.480	1.120	5.880	1.960
Miner: Diet C P#1	413.4	313.9	1.460	1.280	8.130	1.570
Miner: Diet C P#2	281.9	331.0	1.570	1.480	8.530	1.760
Miner: Diet D P#1	383.3	323.4	2.280	1.310	9.090	1.230
Miner: Diet D P#2	492.6	291.9	2.110	1.180	7.980	1.230

Appendix E (cont'd): Outputs of the Within-Day Model for Prediction of Microbial Nitrogen Flow:

Diet	MeasuredMN (gN/d)	Model MN (gN/d)	FermTP (kg)	FermNPN (kg)	FermNSC (kg)	Ferm NDF (kg)
MSU: Lysine P#1	330.3	337.0	1.110	0.645	6.335	2.263
MSU: Lysine P#2	183.0	342.8	1.131	0.616	6.311	2.358
MSU: Lysine P#3	147.2	303.9	1.006	0.568	5.694	2.069
MSU: Lysine P#4	210.7	370.6	1.212	0.713	6.935	2.462
MSU: Control P#1	268.6	358.6	1.183	0.653	6.654	2.454
MSU: Control P#2	163.6	327.7	1.079	0.633	6.170	2.191
MSU: Control P#3	285.5	397.8	1.333	0.821	7.740	2.657
MSU: Control P#4	145.7	339.6	1.117	0.636	6.328	2.292
MSU: Casein P#1	257.0	392.2	1.316	0.807	7.624	2.629
MSU: Casein P#2	282.8	354.1	1.158	0.687	6.631	2.348
MSU: Casein P#3	177.0	365.9	1.210	0.675	6.789	2.504
MSU: Casein P#4	131.5	313.9	1.038	0.595	5.884	2.134
Illinois: SBM	290.0	335.3	1.243	1.239	7.476	1.623
Illinois: CGM	249.0	325.5	1.019	1.338	7.432	1.489
Illinois: BM	263.0	325.3	0.949	1.173	7.334	1.570
Illinois: FM&BM	208.0	307.5	0.822	1.172	6.911	1.473

Appendix F: Other Dietary Variables Used for Model Development for Prediction of Microbial Nitrogen Flow:

Diet	DMI (kg/d)	AvailFat (kg/d)	NE_l (mcal)	$FCNE_l$ (mcal)	SpartanMN (gN/d)	CNCPS (gN/d)	DigOM (kg)	DigStr (kg)	DigNDF (kg)
McCarthy: Corn/SBM	24.2	0.46	40.2	40.2	380.38	369.24	8.5	5.1	2.1
McCarthy: Barley/FM	20.5	0.45	33.8	33.8	272.30	296.46	9.8	6.3	1.8
McCarthy: Barley/SBM	20.9	0.48	34.5	34.5	316.53	299.25	10.2	6.7	0.8
McCarthy: Corn/FM	23.3	0.62	38.7	38.7	305.25	343.73	8.5	5.3	2.7
Klusmeyer: SBM 14.5%	21.8	0.57	36.2	36.2	317.30	325.76	10.6	5.3	3.1
Klusmeyer: SBM 11%	20.9	0.51	34.5	34.5	247.45	323.33	10.1	5.2	2.7
Klusmeyer: CGM 14.5%	20.9	0.52	34.7	34.7	308.10	326.11	9.7	5.1	3.0
Klusmeyer: CGM 11%	21.6	0.52	35.6	35.6	279.38	332.37	10.3	5.8	2.5
Klusmeyer: SBM	25.1	0.53	41.9	41.9	449.02	346.54	10.0	5.2	2.9
Klusmeyer: SBM/LCFA	23.8	0.54	42.4	41.9	430.77	331.64	8.3	3.5	2.4
Klusmeyer: FM	23.4	0.49	39.3	39.3	419.19	329.21	9.2	4.4	2.5
Klusmeyer: FM/LCFA	22.3	0.51	39.7	39.7	407.53	316.33	7.6	3.7	2.1
Klusmeyer: HighForage	24.7	0.77	39.8	39.8	424.40	309.07	10.8	3.5	2.4
Klusmeyer: LoFor/LCFA	24.0	0.72	43.2	43.2	428.77	345.08	8.7	3.6	1.7
Klusmeyer: LowForage	25.5	0.76	42.8	42.8	459.59	349.35	10.9	4.6	2.4
Klusmeyer: HiFor/LCFA	23.3	0.78	39.8	39.8	424.63	287.91	9.6	3.1	2.1
Cameron: Control	23.1	0.48	37.2	37.2	395.09	306.03	9.8	3.4	2.4
Cameron: Urea	23.0	0.47	36.8	36.8	390.43	302.28	9.1	2.6	2.4
Cameron: Starch	21.6	0.46	34.8	34.8	359.35	327.55	8.8	4.1	1.0
Cameron: Starch/Urea	21.0	0.45	33.8	33.8	356.38	317.75	8.9	4.2	1.3
Stokes: Diet #1	21.9	1.01	37.2	36.5	387.15	284.47	13.0	---	2.9
Stokes: Diet #2	21.1	0.80	36.1	35.9	380.17	284.38	12.7	---	3.9
Stokes: Diet #3	18.3	0.59	29.5	29.5	306.42	229.64	9.2	---	3.6
Glenn: Alf Low	4.6	0.14	8.7	8.7	68.42	53.53	2.2	---	0.4
Glenn: Alf High	6.0	0.18	11.0	11.0	94.73	67.77	2.8	---	0.6
Glenn: OG Low	4.3	0.13	6.7	6.7	45.47	45.95	2.1	---	1.9
Glenn: OG High	5.5	0.17	9.1	9.1	73.17	57.63	2.8	---	2.1
Robinson: SDP	18.3	0.77	30.0	29.6	308.24	245.53	8.8	2.9	3.5
Robinson: RDP	19.4	0.70	30.8	30.8	321.29	270.39	9.1	3.2	2.9

Appendix F (cont'd): Other Dietary Variables Used for Model Development for Prediction of Microbial Nitrogen Flow:

Diet	DMI (kg/d)	AvailFat (kg/d)	NE_l (mcal)	$FCNE_l$ (mcal)	SpartanMN (gN/d)	CNCPS (gN/d)	DigOM (kg)	DigStr (kg)	DigNDF (kg)
Cunningham: #1	19.8	0.68	31.9	31.9	310.60	293.77	10.4	---	---
Cunningham: #2	21.0	0.70	34.7	34.4	363.29	307.04	8.0	---	---
Cunningham: #3	21.5	0.72	35.5	34.7	366.84	299.95	10.3	---	---
Cunningham: #4	20.2	0.71	33.3	32.6	341.89	275.90	7.9	---	---
Wonsil: Coat Tallow	24.1	1.75	44.6	41.9	448.96	337.43	---	---	---
Wonsil: Tallow	23.8	1.75	42.8	40.0	427.42	334.85	---	---	---
Wonsil: Hydrg Tallow	24.8	1.89	45.4	42.3	453.34	341.30	---	---	---
Wonsil: 1st Control	24.1	1.14	40.7	39.9	425.32	349.60	---	---	---
Wonsil: 2nd Control	24.6	0.77	41.1	41.1	439.46	354.44	---	---	---
Wonsil: Hydrg FA	22.9	1.22	40.8	39.5	421.56	330.66	---	---	---
Wonsil: Fish Oil	21.4	1.12	38.1	37.0	392.51	312.98	---	---	---
Wonsil: Soy Oil	24.1	1.15	42.9	42.0	449.90	345.72	---	---	---
Garrett: #1	20.5	0.64	35.2	35.2	371.81	304.47	---	---	---
Garrett: #4	19.7	0.52	33.2	33.2	349.50	292.61	---	---	---
Garrett: #6	19.2	0.51	32.4	32.4	339.83	293.26	---	---	---
Garrett: #9	20.3	0.44	34.2	34.2	361.11	302.33	---	---	---
Glenn: Week#6	14.5	1.13	24.9	23.1	233.37	208.62	---	---	2.3
Glenn: Week#11	16.1	1.24	27.7	25.7	262.92	221.90	---	---	1.1
Miner: Diet E P#1	18.7	0.70	27.5	27.3	282.15	213.99	---	---	---
Miner: Diet E P#2	20.1	0.76	29.6	29.4	305.91	218.01	---	---	---
Miner: Diet H P#1	18.8	0.69	27.7	27.6	284.87	200.50	---	---	---
Miner: Diet H P#2	21.8	0.80	32.1	32.0	335.12	216.00	---	---	---
Miner: Diet A P#1	19.9	0.62	34.2	34.2	360.98	267.73	---	---	---
Miner: Diet A P#2	23.6	0.74	40.6	40.6	433.45	280.34	---	---	---
Miner: Diet B P#1	26.5	0.89	45.6	45.6	484.46	347.14	---	---	---
Miner: Diet B P#2	23.3	0.78	40.0	40.0	424.88	290.32	---	---	---
Miner: Diet C P#1	26.2	0.86	45.1	45.1	451.59	353.02	---	---	---
Miner: Diet C P#2	30.1	0.99	51.7	51.7	517.73	372.49	---	---	---
Miner: Diet D P#1	29.3	0.89	49.5	49.5	535.65	376.78	---	---	---
Miner: Diet D P#2	26.6	0.80	45.0	45.0	483.99	334.65	---	---	---

Appendix F (cont'd): Other Dietary Variables Used for Model Development for Prediction of Microbial Nitrogen Flow:

Diet	DMI (kg/d)	AvailFat (kg/d)	NE_l (mcal)	$FCNE_l$ (mcal)	SpartanMN (gN/d)	CNCPS (gN/d)	DigOM (kg)	DigStr (kg)	DigNDF (kg)
MSU: Lysine P#1	19.8	0.46	32.3	32.3	322.73	297.82	---	---	---
MSU: Lysine P#2	18.8	0.44	30.6	30.6	306.43	300.67	---	---	---
MSU: Lysine P#3	17.5	0.41	28.5	28.5	285.24	268.89	---	---	---
MSU: Lysine P#4	21.8	0.51	35.5	35.5	355.33	325.42	---	---	---
MSU: Control P#1	20.1	0.47	32.8	32.8	327.62	315.82	---	---	---
MSU: Control P#2	19.4	0.45	31.6	31.6	316.21	289.70	---	---	---
MSU: Control P#3	25.2	0.59	41.1	41.1	410.74	359.64	---	---	---
MSU: Control P#4	19.5	0.46	31.8	31.8	317.84	298.73	---	---	---
MSU: Casein P#1	24.7	0.58	40.3	40.3	402.59	354.79	---	---	---
MSU: Casein P#2	20.9	0.49	34.1	34.1	340.66	310.86	---	---	---
MSU: Casein P#3	20.5	0.48	33.4	33.4	334.14	322.25	---	---	---
MSU: Casein P#4	18.1	0.42	29.5	29.5	295.02	277.91	---	---	---
Illinois: SBM	24.2	0.67	39.9	39.9	426.27	320.33	12.2	4.7	2.4
Illinois: CGM	23.8	0.64	39.3	39.3	418.71	309.98	10.9	5.6	1.9
Illinois: BM	24.1	0.69	39.3	39.3	391.74	306.92	11.1	5.2	2.9
Illinois: FM&BM	22.5	0.64	36.7	36.7	366.27	287.67	10.8	5.4	2.7

Appendix G: Mean milk production, intake, diet analyses, rumen parameter, and microbial nitrogen estimates for cows on the Miner Institute Study.

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1. 4% fat-corrected milk production (kg) of cows on the Miner Institute Study

Early Lactation:

	Period		Mean	S.D.
Diet*	1	2		
A	38.97	43.47	41.22	3.18
B	53.75	38.97	46.36	10.45

Early Lactation:

	Period		Mean	S.D.
Diet*	1	2		
C	38.54	40.15	39.35	1.14
D	41.14	37.85	39.50	2.33

Late Lactation:

	Period		Mean	S.D.
Diet*	1	2		
E	29.65	27.72	28.69	1.36
H	28.82	24.72	26.77	2.90

* A=Early HMEC/SBM, B= Early HMEC/CGF, C= Early GSC/CGF, D= Early GSC/SBM, E= Late HMEC/SBM, H= Late GSC/SBM

2. Milk production (kg) of cows on the Miner Institute Study

Early Lactation:

	Period		Mean	S.D.
Diet*	1	2		
A	38.56	43.84	41.20	3.73
B	49.24	40.05	44.65	6.50

Early Lactation:

	Period		Mean	S.D.
Diet*	1	2		
C	39.80	38.59	39.20	0.86
D	41.89	39.57	40.73	1.64

Late Lactation:

	Period		Mean	S.D.
Diet*	1	2		
E	32.37	28.54	30.46	2.71
H	30.13	28.43	29.28	1.20

* A=Early HMEC/SBM, B= Early HMEC/CGF, C= Early GSC/CGF, D= Early GSC/SBM, E= Late HMEC/SBM, H= Late GSC/SBM

Appendix G (cont'd): Mean milk production, intake, diet analyses, rumen parameter, and microbial nitrogen estimates for cows on the Miner Institute Study.

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3. Percent fat of milk from cows on the Miner Institute Study

Early Lactation:

	Period		Mean	S.D.
Diet*	1	2		
A	4.07	4.02	4.05	0.035
B	4.36	3.82	4.09	0.382

Early Lactation:

	Period		Mean	S.D.
Diet*	1	2		
C	3.79	4.27	4.03	0.339
D	3.88	3.71	3.80	0.120

Late Lactation:

	Period		Mean	S.D.
Diet*	1	2		
E	3.44	3.81	3.63	0.262
H	3.71	3.13	3.42	0.410

* A=Early HMEC/SBM, B= Early HMEC/CGF, C= Early GSC/CGF, D= Early GSC/SBM, E= Late HMEC/SBM, H= Late GSC/SBM

4. Percent crude protein of milk from cows on the Miner Institute Study

Early Lactation:

	Period		Mean	S.D.
Diet*	1	2		
A	3.15	3.18	3.17	0.02
B	3.36	3.27	3.32	0.06

Early Lactation:

	Period		Mean	S.D.
Diet*	1	2		
C	----	3.56	3.56	-----
D	----	3.33	3.33	-----

Late Lactation:

	Period		Mean	S.D.
Diet*	1	2		
E	3.25	3.11	3.18	0.10
H	2.95	3.29	3.12	0.24

* A=Early HMEC/SBM, B= Early HMEC/CGF, C= Early GSC/CGF, D= Early GSC/SBM, E= Late HMEC/SBM, H= Late GSC/SBM

Appendix G (cont'd): Mean milk production, intake, diet analyses, rumen parameter, and microbial nitrogen estimates for cows on the Miner Institute Study.

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5. Percent true protein of milk from cows on the Miner Institute Study

Early Lactation:

	Period		Mean	S.D.
Diet*	1	2		
A	2.98	2.97	2.98	0.01
B	3.06	3.05	3.06	0.01

Early Lactation:

	Period		Mean	S.D.
Diet*	1	2		
C	3.34	3.30	3.32	0.03
D	3.60	3.09	3.35	0.36

Late Lactation:

	Period		Mean	S.D.
Diet*	1	2		
E	3.03	2.94	2.99	0.06
H	2.76	3.09	2.93	0.23

* A=Early HMEC/SBM, B= Early HMEC/CGF, C= Early GSC/CGF, D= Early GSC/SBM, E= Late HMEC/SBM, H= Late GSC/SBM

6. Dry matter intake (kg) of cows on the Miner Institute Study

Early Lactation:

	Period		Mean	S.D.
Diet*	1	2		
A	19.90	23.58	21.74	2.60
B	26.51	23.25	24.88	2.31

Early Lactation:

	Period		Mean	S.D.
Diet*	1	2		
C	26.22	30.06	28.14	2.72
D	29.28	26.61	27.95	1.89

Late Lactation:

	Period		Mean	S.D.
Diet*	1	2		
E	18.70	20.12	19.41	1.00
H	18.82	21.82	20.32	2.12

* A=Early HMEC/SBM, B= Early HMEC/CGF, C= Early GSC/CGF, D= Early GSC/SBM, E= Late HMEC/SBM, H= Late GSC/SBM

Appendix G (cont'd): Mean milk production, intake, diet analyses, rumen parameter, and microbial nitrogen estimates for cows on the Miner Institute Study.

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7. Total rumen solids (kg DM) evacuated from cows on the Miner Institute Study

Early Lactation:

Diet*	Period 1	Period 2	Mean	S.D.	Signif.of Diff. (p<)
A	8.7	9.4	9.1	0.9	0.40
B	8.8	10.4	9.6	1.0	

Early Lactation:

Diet*	Period 1	Period 2	Mean	S.D.	Signif.of Diff. (p<)
C	11.4	10.5	11.0	1.1	0.28
D	9.1	11.2	10.2	1.2	

Late Lactation:

Diet*	Period 1	Period 2	Mean	S.D.	Signif.of Diff. (p<)
E	10.0	11.7	10.9	1.1	0.61
H	9.9	12.1	11.0	1.3	

* A=Early HMEC/SBM, B= Early HMEC/CGF, C= Early GSC/CGF, D= Early GSC/SBM, E= Late HMEC/SBM, H= Late GSC/SBM

8. Microbial nitrogen (g) per kg rumen solids from cows on the Miner Institute Study

Early Lactation:

Diet*	Period 1	Period 2	Mean	S.D.	Signif.of Diff. (p<)
A	5.5	6.9	6.2	0.9	0.03
B	4.7	2.3	3.4	1.9	

Early Lactation:

Diet*	Period 1	Period 2	Mean	S.D.	Signif.of Diff. (p<)
C	7.7	4.2	5.9	3.2	0.92
D	5.8	5.8	5.8	1.2	

Late Lactation:

Diet*	Period 1	Period 2	Mean	S.D.	Signif.of Diff. (p<)
E	3.1	1.2	2.1	1.2	0.10
H	5.1	3.6	4.3	1.8	

* A=Early HMEC/SBM, B= Early HMEC/CGF, C= Early GSC/CGF, D= Early GSC/SBM, E= Late HMEC/SBM, H= Late GSC/SBM

Appendix G (cont'd): Mean milk production, intake, diet analyses, rumen parameter, and microbial nitrogen estimates for cows on the Miner Institute Study.

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9. Total rumen liquids (kg) evacuated from cows on the Miner Institute Study

Early Lactation:

	Period		Mean	S.D.	Signif.of Diff.
Diet*	1	2			(p<)
A	27.8	38.3	33.1	7.5	
					0.34
B	34.8	37.6	36.2	2.1	

Early Lactation:

	Period		Mean	S.D.	Signif.of Diff.
Diet*	1	2			(p<)
C	37.2	37.6	37.4	5.0	
					0.13
D	41.5	45.6	43.5	2.8	

Late Lactation:

	Period		Mean	S.D.	Signif.of Diff.
Diet*	1	2			(p<)
E	43.5	46.6	45.0	2.4	
					0.81
H	38.8	51.9	45.3	7.7	

* A=Early HMEC/SBM, B= Early HMEC/CGF, C= Early GSC/CGF, D= Early GSC/SBM, E= Late HMEC/SBM, H= Late GSC/SBM

10. Microbial nitrogen (g) per liter of rumen fluid from cows on the Miner Institute Study

Early Lactation:

	Period		Mean	S.D.	Signif.of Diff.
Diet*	1	2			(p<)
A	1.75	1.13	1.44	0.38	
					0.25
B	1.11	1.19	1.15	0.32	

Early Lactation:

	Period		Mean	S.D.	Signif.of Diff.
Diet*	1	2			(p<)
C	1.61	0.95	1.28	0.38	
					0.31
D	1.42	1.39	1.41	0.18	

Late Lactation:

	Period		Mean	S.D.	Signif.of Diff.
Diet*	1	2			(p<)
E	0.82	0.71	0.76	0.19	
					0.88
H	0.75	0.81	0.78	0.07	

* A=Early HMEC/SBM, B= Early HMEC/CGF, C= Early GSC/CGF, D= Early GSC/SBM, E= Late HMEC/SBM, H= Late GSC/SBM

Appendix G (cont'd): Mean milk production, intake, diet analyses, rumen parameter, and microbial nitrogen estimates for cows on the Miner Institute Study.

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11. Liquid rate of passage (h^{-1}) of cows on the Miner Institute Study

Early Lactation:

	Period		Mean	S.D.
Diet*	1	2		
A	0.23	0.22	0.22	0.00
B	0.21	0.20	0.21	0.01

Early Lactation:

	Period		Mean	S.D.
Diet*	1	2		
C	0.23	0.27	0.25	0.03
D	0.23	0.29	0.26	0.04

Late Lactation:

	Period		Mean	S.D.
Diet*	1	2		
E	0.21	0.17	0.19	0.03
H	0.17	0.12	0.15	0.04

* A=Early HMEC/SBM, B= Early HMEC/CGF, C= Early GSC/CGF, D= Early GSC/SBM, E= Late HMEC/SBM, H= Late GSC/SBM

12. Solids rate of passage (h^{-1}) of cows on the Miner Institute Study

Early Lactation:

	Period		Mean	S.D.	Signif.of Diff. (p<)
Diet*	1	2			
A	0.046	0.051	0.048	0.004	
					0.06
B	0.063	0.043	0.053	0.012	

Early Lactation:

	Period		Mean	S.D.	Signif.of Diff. (p<)
Diet*	1	2			
C	0.037	0.049	0.043	0.007	
					0.78
D	0.047	0.037	0.042	0.006	

Late Lactation:

	Period		Mean	S.D.	Signif.of Diff. (p<)
Diet*	1	2			
E	0.032	0.040	0.036	0.004	
					0.02
H	0.026	0.033	0.030	0.005	

* A=Early HMEC/SBM, B= Early HMEC/CGF, C= Early GSC/CGF, D= Early GSC/SBM, E= Late HMEC/SBM, H= Late GSC/SBM

Appendix G (cont'd): Mean milk production, intake, diet analyses, rumen parameter, and microbial nitrogen estimates for cows on the Miner Institute Study.

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13. Daily microbial nitrogen flow (g) from rumen fluids of cows on the Miner Institute Study

Early Lactation:

	Period		Mean	S.D.	Signif.of Diff.
Diet*	1	2			(p<)
A	261.51	228.10	244.81	24.66	
					0.40
B	198.27	216.06	207.16	64.04	

Early Lactation:

	Period		Mean	S.D.	Signif.of Diff.
Diet*	1	2			(p<)
C	335.48	231.70	283.59	68.94	
					0.04
D	323.87	435.88	379.87	76.24	

Late Lactation:

	Period		Mean	S.D.	Signif.of Diff.
Diet*	1	2			(p<)
E	179.28	132.72	155.99	49.81	
					0.24
H	120.28	120.13	120.20	6.48	

* A=Early HMEC/SBM, B= Early HMEC/CGF, C= Early GSC/CGF, D= Early GSC/SBM, E= Late HMEC/SBM, H= Late GSC/SBM

14. Daily microbial nitrogen flow (g) from rumen solids of cows on the Miner Institute Study

Early Lactation:

	Period		Mean	S.D.	Signif.of Diff.
Diet*	1	2			(p<)
A	51.98	76.70	65.84	17.12	
					0.08
B	61.57	23.87	42.72	26.46	

Early Lactation:

	Period		Mean	S.D.	Signif.of Diff.
Diet*	1	2			(p<)
C	77.95	50.23	64.09	33.21	
					0.77
D	59.38	56.77	58.07	11.74	

Late Lactation:

	Period		Mean	S.D.	Signif.of Diff.
Diet*	1	2			(p<)
E	23.54	13.45	18.49	7.29	
					0.13
H	32.27	33.59	32.93	11.39	

* A=Early HMEC/SBM, B= Early HMEC/CGF, C= Early GSC/CGF, D= Early GSC/SBM, E= Late HMEC/SBM, H= Late GSC/SBM

Appendix G (cont'd): Mean milk production, intake, diet analyses, rumen parameter, and microbial nitrogen estimates for cows on the Miner Institute Study.

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15. Total daily microbial nitrogen flow (g) of cows on the Miner Institute Study

Early Lactation:

	Period		Mean	S.D.	Signif.of Diff.
Diet*	1	2			(p<)
A	313.50	307.80	310.64	17.30	
					0.17
B	259.84	239.93	249.88	58.68	

Early Lactation:

	Period		Mean	S.D.	Signif.of Diff.
Diet*	1	2			(p<)
C	413.42	281.93	347.68	86.37	
					0.06
D	383.25	492.64	437.94	75.39	

Late Lactation:

	Period		Mean	S.D.	Signif.of Diff.
Diet*	1	2			(p<)
E	202.81	146.17	174.49	50.12	
					0.43
H	152.55	153.71	153.13	11.93	

* A=Early HMEC/SBM, B= Early HMEC/CGF, C= Early GSC/CGF, D= Early GSC/SBM, E= Late HMEC/SBM, H= Late GSC/SBM

16. Microbial nitrogen flow (g) per kg dry matter intake of cows on the Miner Institute Study

Early Lactation:

	Period		Mean	S.D.	Signif.of Diff.
Diet*	1	2			(p<)
A	15.76	13.06	14.41	1.78	
					0.04
B	9.80	10.32	10.06	2.22	

Early Lactation:

	Period		Mean	S.D.	Signif.of Diff.
Diet*	1	2			(p<)
C	15.77	9.38	12.57	4.00	
					0.07
D	13.09	18.51	15.80	3.43	

Late Lactation:

	Period		Mean	S.D.	Signif.of Diff.
Diet*	1	2			(p<)
E	10.85	7.26	9.05	2.89	
					0.32
H	8.11	7.05	7.57	0.87	

* A=Early HMEC/SBM, B= Early HMEC/CGF, C= Early GSC/CGF, D= Early GSC/SBM, E= Late HMEC/SBM, H= Late GSC/SBM

Appendix G (cont'd): Mean milk production, intake, diet analyses, rumen parameter, and microbial nitrogen estimates for cows on the Miner Institute Study.

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17. Apparent organic matter digested (kg/d) by cows on the Miner Institute Study

Early Lactation:

	Period		Mean	S.D.
Diet*	1	2		
A	9.47	10.49	9.98	0.72
B	11.86	10.40	11.13	1.03

Early Lactation:

	Period		Mean	S.D.
Diet*	1	2		
C	12.27	13.13	12.70	0.61
D	13.56	12.19	12.88	0.97

Late Lactation:

	Period		Mean	S.D.
Diet*	1	2		
E	8.14	8.34	8.24	0.14
H	7.95	8.63	8.29	0.48

* A=Early HMEC/SBM, B= Early HMEC/CGF, C= Early GSC/CGF, D= Early GSC/SBM, E= Late HMEC/SBM, H= Late GSC/SBM

18. Microbial nitrogen flow (g) per kg apparent OM digested by cows on the Miner Institute Study

Early Lactation:

	Period		Mean	S.D.	Signif.of Diff.
Diet*	1	2			(p<)
A	33.09	29.35	31.22	2.80	
					0.05
B	21.91	23.07	22.49	4.96	

Early Lactation:

	Period		Mean	S.D.	Signif.of Diff.
Diet*	1	2			(p<)
C	33.70	21.48	27.59	7.81	
					0.07
D	28.27	40.42	34.34	7.64	

Late Lactation:

	Period		Mean	S.D.	Signif.of Diff.
Diet*	1	2			(p<)
E	24.92	17.53	21.22	6.31	
					0.41
H	19.19	17.82	18.50	1.68	

* A=Early HMEC/SBM, B= Early HMEC/CGF, C= Early GSC/CGF, D= Early GSC/SBM, E= Late HMEC/SBM, H= Late GSC/SBM

Appendix G (cont'd): Mean milk production, intake, diet analyses, rumen parameter, and microbial nitrogen estimates for cows on the Miner Institute Study.

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19. Estimated total carbohydrate fermented (kg) in the rumens of cows on the Miner Institute Study

Early Lactation:

	Period		Mean	S.D.
Diet*	1	2		
A	7.04	7.42	7.23	0.27
B	9.25	7.80	8.53	1.03

Early Lactation:

	Period		Mean	S.D.
Diet*	1	2		
C	9.53	10.08	9.81	0.39
D	9.97	8.90	9.44	0.76

Late Lactation:

	Period		Mean	S.D.
Diet*	1	2		
E	6.08	6.23	6.16	0.11
H	5.85	6.34	6.10	0.35

* A=Early HMEC/SBM, B= Early HMEC/CGF, C= Early GSC/CGF, D= Early GSC/SBM, E= Late HMEC/SBM, H= Late GSC/SBM

20. Microbial nitrogen flow (g) per kg estimated digested carbohydrate by cows on the Miner Institute Study

Early Lactation:

	Period		Mean	S.D.	Signif.of Diff.
Diet*	1	2			(p<)
A	44.53	41.48	43.01	2.98	
					0.03
B	28.09	30.76	29.43	6.51	

Early Lactation:

	Period		Mean	S.D.	Signif.of Diff.
Diet*	1	2			(p<)
C	43.39	27.97	35.68	9.89	
					0.04
D	38.44	55.36	46.90	10.60	

Late Lactation:

	Period		Mean	S.D.	Signif.of Diff.
Diet*	1	2			(p<)
E	33.36	23.47	28.41	8.45	
					0.46
H	26.08	24.25	25.16	2.27	

* A=Early HMEC/SBM, B= Early HMEC/CGF, C= Early GSC/CGF, D= Early GSC/SBM, E= Late HMEC/SBM, H= Late GSC/SBM

Appendix G (cont'd): Mean milk production, intake, diet analyses, rumen parameter, and microbial nitrogen estimates for cows on the Miner Institute Study.

21. Chemical Analyses of Diets Throughout the Miner Institute Study*

		Offered (Predicted)			Offered (TMR)			Refused (TMR)		
Diet	Period	CP(%DM)	NDF(%DM)	DM	CP(%DM)	NDF(%DM)	DM	CP(%DM)	NDF(%DM)	DM
A	1	19.0	35.1	42.1	20.2	35.5	43.0	20.8	36.0	37.5
A	2	19.8	34.4	41.3	19.7	36.2	45.2	19.1	36.9	47.9
B	1	17.3	37.4	42.7	19.9	45.1	43.7	18.0	39.3	44.9
B	2	18.0	36.7	42.7	17.9	42.0	45.5	18.0	43.9	45.1
C	1	17.3	31.3	46.4	19.1	31.6	48.4	18.3	35.6	46.5
C	2	17.2	31.5	45.3	18.0	34.0	48.5	16.9	35.4	46.6
D	1	18.9	29.0	45.5	19.0	33.2	49.4	18.3	35.9	47.7
D	2	18.8	29.1	44.6	19.4	30.4	48.7	19.2	33.1	46.4
E	1	15.5	46.3	34.1	17.1	41.8	36.0	16.2	45.5	32.6
E	2	15.0	47.7	34.9	15.5	50.6	38.2	15.5	50.9	34.2
H	1	15.6	44.1	34.4	17.2	41.8	35.8	16.9	47.4	33.5
H	2	15.1	45.6	35.6	15.6	49.2	39.2	15.7	53.2	35.4

* Offered (Predicted) = Diet Analyses Based on Individual Ingredient Analyses
Offered (TMR) = Analyses of Daily TMR Sampled
Refused (TMR) = Analyses of Refused TMR Sampled
A=Early HMEC/SBM, B= Early HMEC/CGF, C= Early GSC/CGF,D= Early GSC/SBM,
E= Late HMEC/SBM, H= Late GSC/SBM

Appendix H: Diet composition, nutrient degradation, intake, body weight, rumen parameter, and microbial nitrogen estimates for cows on the MSU Cannulated Cow Study.

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1. Ingredient and chemical composition of the diet fed in the MSU cannulated cow study

Ingredients (%DM):		Chemical composition:	
Corn silage	62.00	NEl (mcal/kg)	1.63
Ground shell corn	22.37	CP (%DM)	14.70
Corn gluten meal	1.54	Sol CP (%CP)	35.20
Soybean meal, 44%	10.52	ADF (%DM)	17.20
Urea	0.37	NDF (%DM)	31.30
Mineral & Vitamin	3.20		

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2. *In situ* DM degradation(%DM) of individual dietary ingredients fed in the MSU cannulated cow study.

	Feed							
	GSHCorn	SD	SBM	SD	CGM	SD	CornSil	SD
Time (h)								
0	17.37	(5.7)	30.40	(0.5)	9.80	(1.4)	26.27	(4.3)
1	25.01	(5.1)	35.23	(2.4)	16.55	(1.5)	33.71	(3.5)
4	24.90	(7.3)	51.11	(2.6)	23.33	(0.8)	36.87	(5.9)
8	49.44	(3.6)	71.09	(2.5)	32.50	(1.4)	46.69	(2.9)
12	62.44	(3.8)	79.36	(3.5)	35.73	(2.3)	55.41	(3.0)
24	88.84	(4.1)	95.10	(1.4)	46.05	(2.7)	64.76	(1.7)
48	96.35	(0.5)	98.09	(0.2)	80.47	(2.6)	72.35	(1.0)
72	-----		-----		-----		78.68	(0.9)

Appendix H (cont'd): Diet composition, nutrient degradation, intake, body weight, rumen parameter, and microbial nitrogen estimates for cows on the MSU Cannulated Cow Study.

3. In situ CP degradation(%CP) of individual dietary ingredients fed in the MSU cannulated cow study.

	Feed							
	GSHCorn	SD	SBM	SD	CGM	SD	CornSil	SD
Time (h)								
0	12.75	(6.0)	15.83	(0.6)	3.22	(1.5)	64.86	(2.0)
1	17.91	(5.6)	21.93	(2.9)	9.54	(1.6)	62.21	(2.0)
4	13.75	(8.4)	40.93	(3.1)	8.43	(1.0)	66.68	(3.1)
8	30.13	(5.0)	62.96	(3.2)	13.37	(1.8)	71.21	(1.6)
12	43.21	(5.8)	75.46	(4.1)	16.17	(3.0)	70.62	(1.9)
24	80.07	(7.3)	96.85	(0.9)	30.80	(3.5)	74.74	(1.2)
48	94.60	(0.7)	99.49	(0.1)	75.38	(3.2)	77.97	(0.8)
72	-----		-----		-----		83.03	(0.7)

4. Dry matter intake of cows in MSU cannulated cow study.

	Period				Mean	SEM
Treatment	1	2	3	4		
Control	20.1	19.4	25.2	19.5	21.0[a]	0.19
Lysine	19.8	18.8	17.5	21.8	19.5[b]	
Casein	24.7	20.9	20.5	18.1	21.1[a]	

[a,b] Means with unlike superscripts differ ($p<0.05$)

Appendix H (cont'd): Diet composition, nutrient degradation, intake, body weight, rumen parameter,and microbial nitrogen estimates for cows on the MSU Cannulated Cow Study.

5. Body weight of cows in MSU cannulated cow study.

	Period				Mean	SEM
Treatment	1	2	3	4		
Control	692	512	593	585	596	3.38
Lysine	546	694	516	598	589	
Casein	593	556	710	535	599	

* Differences between treatment means were not significant (p>0.10)

6. Rumen ammonia nitrogen concentrations (mg NH_3N/dl) of cows in MSU cannulated cow study.

	Period				Mean	SEM
Treatment	1	2	3	4		
Control	11.8	11.0	12.6	13.2	12.2	1.28
Lysine	8.7	12.4	12.7	14.3	12.0	
Casein	6.5	12.4	9.5	15.8	11.1	

* Differences between treatment means were not significant (p>0.10)

7. Average rumen pH of cows in MSU cannulated cow study.

	Period				Mean	SEM
Treatment	1	2	3	4		
Control	6.22	5.72	5.92	5.73	5.90	0.05
Lysine	5.88	5.87	6.03	6.05	5.96	
Casein	6.05	5.68	5.97	5.81	5.88	

* Differences between treatment means were not significant (p>0.10)

Appendix H (cont'd): Diet composition, nutrient degradation, intake, body weight, rumen parameter,and microbial nitrogen estimates for cows on the MSU Cannulated Cow Study.

8. DM Flow at the duodenum (kg/d) of cows in MSU cannulated cow study.

	Period				Mean	SEM
Treatment	1	2	3	4		
Control	10.31	9.62	13.44	5.56	9.73	0.91
Lysine	11.77	8.02	6.91	9.34	9.01	
Casein	12.60	7.61	9.98	5.52	8.93	

* Differences between treatment means were not significant (p>0.10)

9. Microbial nitrogen (g/d) at the duodenum of cows in MSU cannulated cow study.

	Period				Mean	SEM
Treatment	1	2	3	4		
Control	268.63	163.57	285.53	145.69	215.9	27.6
Lysine	330.26	183.01	147.16	210.74	217.8	
Casein	257.03	282.84	176.95	131.48	212.1	

* Differences between treatment means were not significant (p>0.10)

10. Microbial nitrogen (g/d) at the duodenum per kg of dry matter intake in cows on the MSU cannulated cow study.

	Period				Mean	SEM
Treatment	1	2	3	4		
Control	13.36	8.43	11.33	7.47	10.15	1.31
Lysine	16.68	9.73	8.41	9.67	11.12	
Casein	10.41	13.53	8.63	7.26	9.96	

* Differences between treatment means were not significant (p>0.10)

Appendix H (cont'd): Diet composition, nutrient degradation, intake, body weight, rumen parameter, and microbial nitrogen estimates for cows on the MSU Cannulated Cow Study.

11. Microbial nitrogen (g/d) per kg of estimated organic matter apparently fermented in cows on the MSU cannulated cow study.

	Period				Mean	SEM
Treatment	1	2	3	4		
Control	27.42	17.14	24.55	10.89	20.0	3.47
Lysine	38.95	16.78	14.43	17.20	21.8	
Casein	21.33	22.10	16.03	10.97	17.6	

* Differences between treatment means were not significant (p>0.10)

12. Microbial nitrogen (g/d) per kg of estimated organic matter truely fermented in cows on the MSU cannulated cow study.**

	Period				Mean	SEM
Treatment	1	2	3	4		
Control	20.73	14.26	19.05	9.65	15.9	2.11
Lysine	26.71	14.02	12.34	14.30	16.8	
Casein	17.05	17.54	13.49	9.72	14.5	

* Differences between treatment means were not significant (p>0.10)
** Assumed bacterial OM contained 8.5% N

Appendix H (cont'd): Diet composition, nutrient degradation, intake, body weight, rumen parameter, and microbial nitrogen estimates for cows on the MSU Cannulated Cow Study.

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13. Microbial nitrogen (g/d) per kg of estimated carbohydrate fermented in cows on the MSU cannulated cow study.

	Period				Mean	SEM
Treatment	1	2	3	4		
Control	30.05	19.97	28.10	17.24	23.8	3.12
Lysine	39.22	21.48	19.34	22.91	25.7	
Casein	25.65	32.18	19.40	16.73	23.5	

* Differences between treatment means were not significant ($p>0.10$)

Appendix I. Variance associated with variables included in models for prediction of microbial nitrogen flow with observations from Glenn et al.(1989) omitted (71 obs.).

Run #1:

Variable**	Type III R^2	(p<)	Estimate***	SE Est	SPRC*
Laboratory	0.38	0.0001	----	---	-----
FermTP	0.05	0.0012	95.38	28.00	33.45
FermNPN	0.02	0.0394	62.54	29.67	22.28
FermNSC	0.03	0.0113	23.82	9.10	26.69
FermNDF	0.01	0.0779	42.26	23.53	18.09
AvailFat	0.03	0.0059	-82.25	28.74	25.44
%Forage	0.00	0.1408	-2.22	1.49	13.06
Total Type I R^2	0.77				

Run #2:

Variable**	Type III R^2	(p<)	Estimate***	SE Est	SPRC*
Laboratory	0.54	0.0001	----	---	-----
Total Type I R^2	0.54				

Run #3:

Variable**	Type III R^2	(p<)	Estimate***	SE Est	SPRC*
Laboratory	0.55	0.0001	----	---	-----
Model MN	0.11	0.0001	1.17	0.27	-----
Total Type I R^2	0.65				

Run #4:

Variable**	Type III R^2	(p<)	Estimate***	SE Est	SPRC*
Laboratory	0.38	0.0001	----	---	-----
Model MN	0.00	0.6932	0.18	0.46	5.90
FermTP	0.03	0.0060	89.84	31.48	31.50
FermNPN	0.02	0.0563	59.79	30.68	21.30
FermNSC	0.02	0.0489	21.61	10.74	24.21
FermNDF	0.01	0.2734	34.32	31.03	14.70
AvailFat	0.03	0.0058	-83.82	29.22	25.92
%Forage	0.00	0.2787	-1.89	1.72	11.08
Total Type I R^2	0.77				

Appendix I.(cont'd). Variance associated with variables included in models for prediction of microbial nitrogen flow with observations from Glenn et al.(1989) omitted (71 obs.).

Run #5:

Variable**	Type III R^2	(p<)	Estimate***	SE Est	SPRC*
Laboratory	0.51	0.0001	----	---	-----
Model MN	0.04	0.0039	0.83	0.28	26.99
FermTP	0.05	0.0027	87.93	28.16	30.84
Total Type I R^2	0.70				

Run #6:

Variable**	Type III R^2	(p<)	Estimate***	SE Est	SPRC*
Laboratory	0.56	0.0001	----	---	-----
Model MN	0.09	0.0001	1.09	0.27	35.70
FermNPN	0.02	0.0844	47.58	27.12	16.95
Total Type I R^2	0.67				

Run #7:

Variable**	Type III R^2	(p<)	Estimate***	SE Est	SPRC*
Laboratory	0.52	0.0001	----	---	-----
Model MN	0.04	0.0055	0.79	0.27	25.73
FermTP	0.04	0.0047	82.64	28.13	28.98
FermNPN	0.01	0.1470	37.88	25.78	13.49
Total Type I R^2	0.71				

Run #8:

Variable**	Type III R^2	(p<)	Estimate***	SE Est	SPRC*
Laboratory	0.53	0.0001	----	---	-----
Model MN	0.01	0.1105	0.54	0.33	17.59
FermNSC	0.04	0.0046	26.83	9.13	30.07
Total Type I R^2	0.69				

Run #9:

Variable**	Type III R^2	(p<)	Estimate***	SE Est	SPRC*
Laboratory	0.50	0.0001	----	---	-----
Model MN	0.13	0.0001	1.31	0.27	42.80
FermNDF	0.03	0.0154	-56.19	22.54	24.06
Total Type I R^2	0.68				

Appendix I.(cont'd). Variance associated with variables included in models for prediction of microbial nitrogen flow with observations from Glenn et al.(1989) omitted (71 obs.).

Run #10:

Variable**	Type III R^2	(p<)	Estimate***	SE Est	SPRC*
Laboratory	0.51	0.0001	----	---	-----
Model MN	0.02	0.0655	0.76	0.41	24.87
Ferm NSC	0.02	0.0826	20.21	11.45	22.65
FermNDF	0.00	0.3413	-26.64	27.77	11.41
Total Type I R^2	0.70				

Run #11:

Variable**	Type III R^2	(p<)	Estimate***	SE Est	SPRC*
Laboratory	0.55	0.0001	----	---	-----
Model MN	0.12	0.0001	1.33	0.28	43.33
AvailFat	0.02	0.0602	-58.35	30.48	18.05
Total Type I R^2	0.67				

Run #12:

Variable**	Type III R^2	(p<)	Estimate***	SE Est	SPRC*
Laboratory	0.49	0.0001	----	---	-----
Model MN	0.06	0.0025	1.01	0.32	32.83
%Forage	0.01	0.3396	1.70	1.76	9.96
Total Type I R^2	0.66				

Run #13:

Variable**	Type III R^2	(p<)	Estimate***	SE Est	SPRC*
Laboratory	0.35	0.0001	----	---	-----
FermTP	0.00	0.7017	-79.91	89.30	12.06
FermTP^2	0.00	0.2687	56.88	31.48	35.10
FermNPN	0.00	0.5152	9.93	114.29	26.68
FermNPN^2	0.00	0.8821	16.24	45.15	5.51
FermNSC	0.03	0.0054	149.98	66.20	215.50
FermNSC^2	0.02	0.0116	-8.80	4.63	175.62
FermNDF	0.01	0.0543	-127.96	157.64	132.98
FermNDF^2	0.02	0.0280	41.26	37.94	148.63
AvailFat	0.02	0.0134	-319.23	119.29	94.49
AvailFat^2	0.01	0.0566	104.62	50.66	63.93
%Forage	0.00	0.4315	1.63	1.78	8.27
%Forage^2	0.01	0.1321	-0.01	0.01	17.15
Total Type I R^2	0.85				

Appendix I.(cont'd). Variance associated with variables included in models for prediction of microbial nitrogen flow with observations from Glenn et al.(1989) omitted (71 obs.).

Run #14:

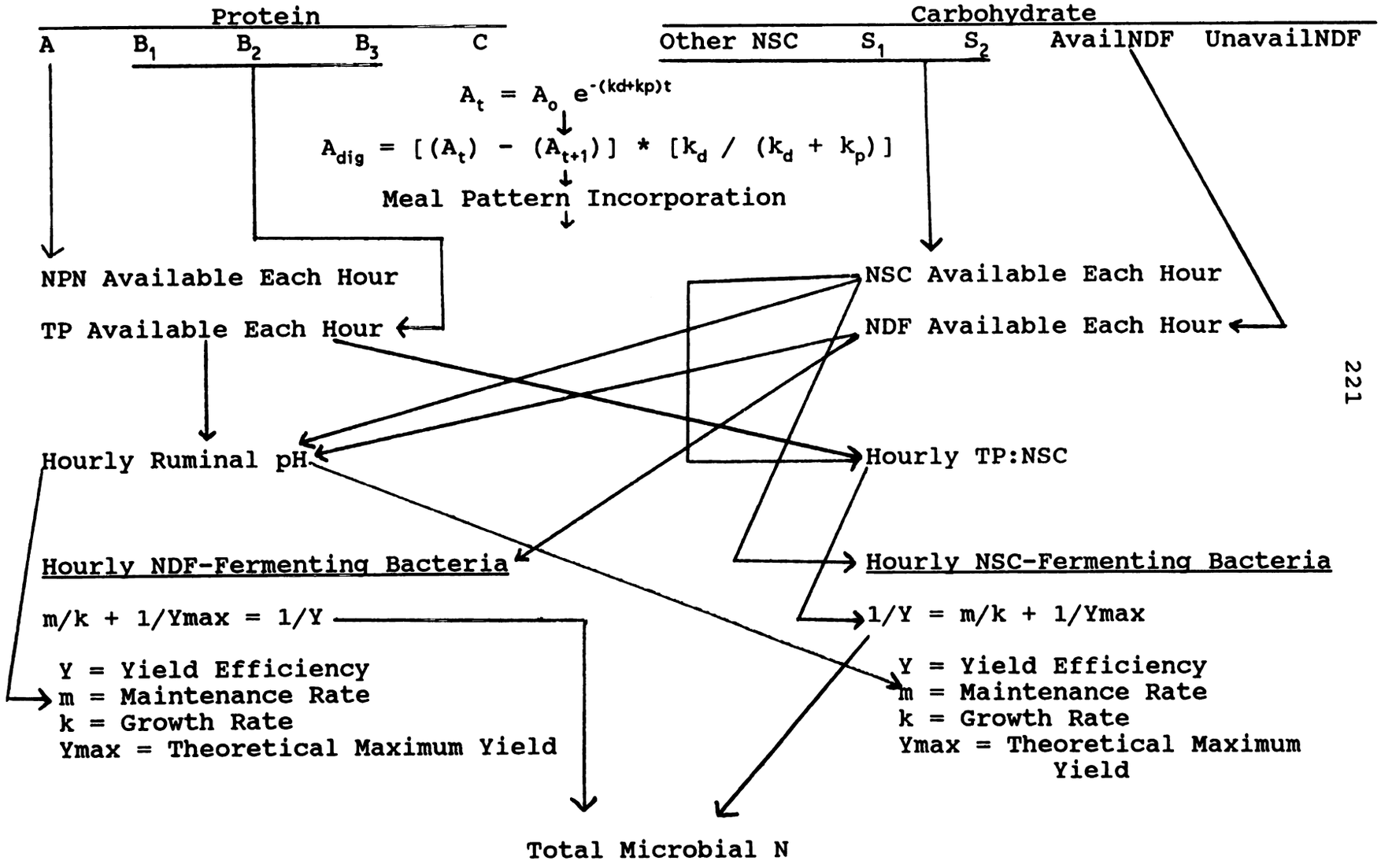
Variable**	Type III R^2	(p<)	Estimate***	SE Est	SPRC*
Laboratory	0.35	0.0001	----	---	-----
Model MN	0.00	0.9799	-0.01	0.46	0.38
FermTP	0.00	0.7184	-33.80	93.20	11.85
FermTP^2	0.00	0.2783	35.10	32.03	35.00
FermNPN	0.00	0.5244	75.49	117.76	26.89
FermNPN^2	0.00	0.8815	-6.92	46.23	5.66
FermNSC	0.02	0.0149	193.27	76.61	216.56
FermNSC^2	0.02	0.0233	-12.17	5.20	176.46
FermNDF	0.01	0.0591	-310.03	160.54	132.76
FermNDF^2	0.02	0.0296	85.84	38.33	148.67
AvailFat	0.02	0.0144	-305.53	120.48	94.49
AvailFat^2	0.01	0.0591	98.85	51.17	63.94
%Forage	0.00	0.4643	1.39	1.89	8.18
%Forage^2	0.01	0.1469	-0.02	0.01	17.09
Total Type I R^2	0.85				

* Standard Partial Regression Coefficient

** Variables: Laboratory = lab effect as a class variable, Model MN = within-day model microbial N flow estimate, FermTP = fermented TP, FermNPN = fermented NPN, FermNSC = fermented NSC, FermNDF = fermented NDF, AvailFat = rumen available fat, %Forage = % forage in the diet

*** Estimate = Parameter Estimate

Appendix J. Within-Day Model Flow Chart



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