Estimating Microbial Protein Flow in Growing and Finishing Heifers

by

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University of Nebraska, 2003

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Growing, finishing, and metabolism trials were conducted with heifers to evaluate estimates of microbial CP (MCP) supply from allantoin in spot urine samples. Dietary treatments were expected to create different MCP flows in each trial. In the growing trial, three supplemental protein sources differing in degradability of protein were fed with and without steep liquor (SL). Inclusion of SL did not change protein efficiency with AminoPlus® and Soypass® being higher than soybean meal (SBM). Estimates of MCP and microbial efficiency were increased with inclusion of SL and increased with increasing level of supplemental protein. In the finishing and metabolism trial, corn bran inclusion and supplementation of SBM instead of urea in high-moisture corn diets were expected to increase MCP efficiency and flow. Corn bran and SBM only impacted performance early in the feeding period for finishing heifers. Estimates of MCP and microbial efficiency were unaffected by corn bran or SBM addition with high coefficients of variation for individual heifers. For heifers in the metabolism trial, microbial efficiency and MCP flow were lower for the control diet than diets containing corn bran or SBM. There was little diurnal variability in MCP estimates and day to day variability was due to changes in digestible intake. Microbial efficiency was well correlated with ruminal pH measurements, and MCP estimates followed estimates from the NRC model. Results of these trials indicate a high degree of individual animal variability for estimates of MCP; however, diurnal and day to day variability appears to be small. Additionally,

estimates for MCP flow and efficiency were feasible and related to performance and digestion parameters. More work is needed to obtain accurate and precise values, but spot sampling of urine shows promise as a non-invasive technique to estimate microbial flow and efficiency.

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Dedication

I dedicate this dissertation to my wife, Vanessa, and son, Hays.

Well baby, we finally made it! It has been a long road, but we trudged down it together. I want you to know how grateful I am for your love, sacrifice, patience, and understanding. We can now have the "real" life we have been looking forward to. The culmination of this part of our life is met by the birth of Hays. What better way to start this new chapter than as mommy and daddy. Thank you so much for our wonderful son. This degree belongs to you and Hays as much as it does to me, and I hope you are proud of what we have accomplished.

You are my love, my inspiration, and above all, my best friend.

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Introduction

The digestive system of ruminant animals is highly developed and specialized. Pregastric fermentation in the rumen makes protein nutrition more complex and allows more access to energy in the form of fibrous feeds than for nonruminants. The ruminant animal has the unique ability to survive and produce without a source of dietary true protein. This ability is a result of ruminal microbes that are capable of converting non-protein nitrogen (N) into microbial crude protein (MCP). Therefore, metabolizable protein (MP) available for absorption in the small intestine is the sum of MCP and protein that passes the rumen undegraded. As a result, protein nutrition in ruminants requires consideration of both animal and microbial requirements.

Ruminal microbes can contain between 20 and 60% of their dry matter as crude protein, and microbial N may represent up to 100% of the non-ammonia N entering the duodenum (Owens and Zinn, 1988). A summary of 152 dietary treatments in various research trials with dairy cows showed that microbial N represented 59% of non-ammonia N reaching the duodenum ranging from 34 to 89% (Clark et al., 1992). According to Smith (1979), 13 to 19% of total microbial N is in the form of nucleic acids with the remainder being in the form of protein. The biological value of the true protein component of MCP approaches 100. It resembles casein having a high level of lysine and threonine and a marginal level of methionine (Owens and Zinn, 1988).

Based on the above information, it is clear that understanding factors affecting MCP supply and being able to estimate supply to the duodenum are important for accurately assessing protein status of the ruminant animal. Several chemical, physiological, and nutritional components interact to influence the flow of microbes from

the rumen. The major chemical and physiological factors are rumen pH and passage rate. Both of these factors are affected by level of intake and nutritional characteristics of the diet including source and level of carbohydrate and degradable intake protein (DIP). All of the characteristics above either directly or indirectly affect microbial ecology, growth, and/or flow.

An estimate of MCP flow is a necessary component in any nutritional model. Several internal and external markers have been evaluated over time with the advantages and disadvantages of each reviewed in various publications (Broderick and Merchen, 1992; Stern et al., 1994). Based on the critical analyses of these publications, total purines have been suggested as one of the best procedures for estimating MCP flow to the duodenum. This procedure assumes that purines from the diet are totally degraded in the rumen and that the only purines passing to the duodenum are microbial in origin. Using this marker requires sampling duodenal digesta through a surgically inserted cannula (Harmon and Richards, 1997). This procedure is not only invasive to the animal but is also laborious and expensive. Therefore, only a small number of animals can be used in this type of research trial.

Topps and Elliot (1965) were the first to propose that purine derivative (PD) excretion in urine be used as a non-invasive marker of MCP flow. The PD are end products of purine catabolism in the animal. With this methodology, PD excretion is used to estimate total purines which are then used to estimate MCP flow. In addition to urine, PD are present in blood (Balcells et al., 1992) and milk (Tiemeyer et al., 1984). Using PD as a marker is non-invasive; however, it is still laborious and only few animals can be used because total urine or milk collection is required. The daily volume of milk

produced by a dairy cow would be readily available. However, researchers who wish to evaluate urinary PD excretion in spot urine samples need a marker of urine volume.

Creatine is a nitrogenous organic compound found primarily in muscle that can also be present in the form of phosphocreatine. The conversion of phosphocreatine to creatine provides energy during excitation and contraction of muscle tissue. Creatinine is derived on a purely physiochemical basis through the irreversible conversion of phosphocreatine or creatine (Heymsfield et al., 1983). Butcher and Harris (1957) suggested using urinary creatinine as an index metabolite. In this methodology, the ratio of the metabolite of interest to creatinine in spot urine samples is multiplied by a predicted daily output of creatinine to calculate daily excretion of the metabolite of interest.

There are several assumptions and predictions that must be made to use the above methodology to estimate MCP flow to the duodenum based on spot urine samples. The goal of this literature review is to examine: 1) determinants of MCP production and flow; 2) the use of purines and PD as markers of MCP supply at the duodenum; 3) the use of creatinine as a urine volume marker; and 4) the use of PD to creatinine ratios in spot urine sampling.

Literature Review

Determinants of Microbial Crude Protein Production and Flow

Level of Intake

Clark et al. (1992) summarized data from various experiments and showed that increasing organic matter intake (OMI) increased OM apparently digested in the rumen (OMAD) (r^2=0.53). However, as OMI increased OMAD as a percent of OMI decreased (r^2=0.39). The same trends held when the authors summarized experiments that calculated truly digested OM (OMTD). Passage of microbial nitrogen (N) was shown to be positively correlated (r^2=0.62) with OMI. Additionally, OMAD (r^2=0.25) and OMTD (r^2=0.39) explained a smaller percent of the variation in microbial N flow than OMI. These results indicate that increased substrate available for microbial fermentation in the rumen only partially explains increased microbial N flow.

Passage Rate

In addition to increasing available substrate, a higher level of OMI is positively correlated with rate of passage for liquid and particulate matter from the rumen (Russell et al., 1992). In a continuous culture system, Meng et al. (1999) evaluated the effect of passage rate on microbial production and efficiency with a corn-based substrate. They maintained fermenters at six passage rates: 2.5, 5.0, 7.5, 10, 15, and 20 %/h. Microbial N production (g/d) and microbial efficiency (g of microbial N/kg of OMTD) increased quadratically as passage rate increased.

An increase in passage rate leads to more microbes adhering to feed particles passing to the small intestine. This results in selection for faster growing microbes in the exponential growth phase reducing energy and N used for maintenance. Therefore,

microbial recycling is reduced leading to an increase in microbial growth and microbial efficiency (Febel and Fekete, 1996). In theory, maximal microbial yield would be achieved where passage rate and microbial division rate are equal (Orskov, 1992). Therefore, growth would be eliminated when passage rate exceeds division rate. This explains a quadratic response to increasing passage rate.

Ruminal pH

Normal rumen pH is usually between 5.5 and 7.0. Strobel and Russell (1986) conducted an in vitro study to examine the effects of pH on microbial protein synthesis by mixed cultures. Rumen fluid was collected 1.5 h post-feeding from a dairy cow fed a 50:50 concentrate:roughage diet. The fluid (pH=6.3) was separated and one part was adjusted to a pH of 6.7 and the other to a pH of 6.0. Several carbohydrate sources were tested as substrates for microbial growth. In all cases, those treatments that were initiated at a pH of 6.7 stayed at 6.7 and those initiated at 6.0 dropped below 6.0 after 10 h of incubation. Microbial protein synthesis and carbohydrate utilization were decreased by 34 to 69% and 14 to 53%, respectively, when initial pH was 6.0. Because the decrease in carbohydrate utilization did not completely explain the decrease in microbial protein synthesis, the authors concluded that low pH resulted in energy being used for nongrowth or energy spilling reactions to maintain H^+ balance. The mechanisms that various bacterial species use to spill energy (uncoupling of H^+ from ATP generation) in the rumen have been outlined by Russell (1998).

Carbohydrate Availability

Many of the empirical models of microbial growth have assumed that microbial growth is a constant function of DMI or OM digestion (Nocek and Russell, 1988).

Burroughs et al. (1974) were the first to propose that microbial crude protein (MCP) supply averaged 13.05% of total digestible nutrients (TDN). Level 1 of the NRC (1996) model uses 13% of TDN to estimate MCP flow. Fermentation of carbohydrates provides most of the energy to ruminal microbes. Therefore, using TDN to estimate MCP supply ignores the fact that protein, fat, and ash provide little energy for ruminal microbes. Additionally, lower efficiency would be expected for diets with both high and low TDN values.

Higher digestibility diets are based primarily on grain. The lower efficiencies for high TDN diets are a result of decreased ruminal pH (Russell et al., 1992). Spicer et al. (1986) fed six abomasally fistulated steers (448 kg) a sorghum grain, dry-rolled corn (DRC), or barley based diet (~82% concentrate) in a replicated 3 x 3 Latin-square design. They determined microbial efficiencies (g MCP/kg of digestible OMI) of 9.38, 10.06, and 12.93%, respectively. These values were not statistically different and averaged 10.8%. Cooper et al. (2002b) determined microbial efficiencies (g MCP/kg of digestible OMI) for six duodenally fistulated steers (523 kg) fed DRC, high-moisture corn (HMC), or steam-flaked corn (SFC) based diets (~90% concentrate) in a replicated 3 x 3 Latin-square design. The values for DRC (8.43%), HMC (9.26%), and SFC (8.35%) were not statistically different averaging 8.68%.

The lower efficiency for low TDN diets is a result of decreased passage rate and increased microbial turnover (Russell et al., 1992). Five studies that reported microbial efficiencies for cattle fed low-quality forages were summarized in the NRC (1996). Across all studies, MCP averaged 7.82% of digestible organic matter intake ranging from 5.0 to11.4%. Digestibilities ranged from 49.8 to 64.7%, but microbial efficiency was not

related to differences in digestibility. This may have been due to differences in intake and their disparate effect on passage rate.

The data reviewed above show that type and supply of ruminal carbohydrates have a significant impact on microbial protein flow from the rumen. Empirical models that represent MCP flow as a constant percentage of TDN provide statistical representation of direct observations, but they do not describe the underlying processes involved in digestion, absorption, and metabolism. In a paper by Russell et al. (1992), authors describe a mechanistic model for determining MCP flow from the rumen. It is a kinetic submodel of the Cornell Net Carbohydrate and Protein System (CNCPS). According to Dijkstra et al. (1998), a mechanistic model analyzes a system in terms of its key components. For example, a mechanistic model of rumen function would be based on amount and type of available OM, utilization of OM for microbial maintenance and other nongrowth functions, and microbial interaction.

The model described by Russell et al. (1992) will be briefly outlined here. Microbes in the rumen are divided into two groups: those that ferment structural carbohydrates (SC) and those that ferment nonstructural carbohydrates (NSC). This division reflects differences in growth efficiency, N utilization, and an almost exclusive partition of energy source utilization. The theoretical maximum growth yield (Y_G; g of bacteria/ g of carbohydrate) is the expected yield if bacteria had no maintenance requirements. Protozoal predation of bacteria is taken into account by decreasing Y_G. In addition, differences in maintenance requirements and types of N and fractions of carbohydrates supplied between SC and NSC fermenters are used to adjust Y_G. Ruminal

pH is estimated from neutral detergent fiber (NDF) concentration of the diets, and this is used to further adjust Y_G.

The adjustment for predicted ruminal pH described by Russell et al. (1992) has been incorporated into Level 1 of the NRC (1996) model. In diets with greater than 40% forage (~20% eNDF), 13% of TDN is used; however, in diets with less than 40% forage, MCP synthesis is decreased by 2.5% for every 1% decrease in eNDF below 20%.

Russell et al. (1992) compared published data of microbial flow with estimates from their model. The regression line comparing predicted and observed values had a slope of 0.94 with an r^2 value of 0.88. Kohn et al. (1998) state that regression of predicted versus observed values is ambiguous and lacks sensitivity. They contend that root mean square prediction error (RMSPE) should be used to compare predicted and observed values. They used twenty different treatment means from five lactating dairy cow studies to compare predictions made with level 1 of the NRC (1996) model and the CNCPS model. Results suggest that the CNCPS model was less accurate (RMSPE=0.77) than level 1 of the NRC (1996) model (RMSPE=0.54) for predicting MCP synthesis. Level 1 of the NRC (1996) model and the CNCPS model overpredicted MCP synthesis by 14% and 35%, respectively.

An estimate of TDN for various feedstuffs is the most readily available and easily determined descriptor of ruminal available energy. At this point in time, reliable estimates for rates of passage and degradation of carbohydrate fractions in a variety of feedstuffs do not exist (Klopfenstein, 1999). A model that is cumbersome and difficult to routinely run in the field has little value to producers. However, mechanistic models like

the CNCPS are needed to study and explain nutritional and physiological concepts to aid in the development of research ideas and hypotheses (Kohn et al., 1998).

Degradable Protein Availability

In level 1 of the NRC (1996) model, the requirement for degradable intake protein (DIP) is set equal to MCP synthesis. Therefore, the DIP requirement is directly proportional to the amount of energy supplied to the microbes. This concept is based on the assumption that there is no net recycling of ammonia back to the rumen. That is, the sum of ammonia passing to the duodenum and that leaving through absorption at the rumen wall is equal to the amount of ammonia returning to the rumen from the blood. This assumption of balance may be invalid as the fluxes are affected by various factors. When ammonia concentration in the rumen is low, recycling may be increased and vice versa (Russell et al., 1992). Complex modeling is required to account for and assess the quantity of recycled N (NRC, 1985; Russell et al., 1992).

Ruminal available energy limits MCP synthesis in most situations; however, particular diets, especially those based on consumption of low-quality forages, may be limited by DIP supply. A deficiency in DIP may lead to decreased MCP efficiency and reduced MCP supply from the rumen (Tedeschi et al., 2000). Even when adequate supply is present, some microbes respond differently to sources of N and N limitation (Russell et al., 1992). Level 1 of the NRC (1996) model and the CNCPS model both acknowledge the importance of adequate ruminal supply of N; however, neither of them have a direct method for adjusting MCP synthesis when supply is inadequate. Microbial efficiency can be adjusted up to increase the DIP requirement for particular diets in level

1 of the NRC (1996) model. Tedeschi et al (2000) devised a set of equations to adjust values in the CNCPS model.

The remainder of this section will be devoted to reviewing research concerning requirements of DIP for maximal MCP synthesis in forage and concentrate diets. Some of the studies reviewed contain information regarding levels of both DIP and carbohydrate. Additionally, the effects of degradable N source on MCP synthesis will be discussed.

DIP requirement. Koster et al. (1996) allowed five ruminally and duodenally fistulated cows (588 kg) ad libitum access to low-quality, tallgrass-prairie forage. They infused a DIP source (sodium caseinate; 90% CP) intraruminally twice daily at 0, 180, 360, 540, and 720 g/d. Forage OMI increased quadratically reaching a maximum (64.7 g/kg $BW^{0.75}$) at 540 g/d while OM digestibility, microbial N flow, and microbial efficiency increased linearly across all levels of DIP. The low and high values for microbial N flow and efficiency were 19.3 and 90.4 g/d and 12.2 and 20 g N/kg OMTD, respectively. Total duodenal flow of N was quadratic across levels of DIP reaching its maximum (111.7 g/d) at 540 g/d. The authors concluded that DIP supplementation increased forage utilization because OMI and OM digestibility were increased resulting in a greater flow of MCP from the rumen. This response was maximized when digestible OM contained 11% DIP (DM basis).

Stokes et al. (1991) evaluated responses in microbial efficiency and production to various levels of NSC and DIP in a continuous culture experiment. Diets were formulated for three levels (25, 37, and 54%) of NSC. Within each diet, peanut meal was used as a DIP source and was varied to give five levels of DIP (15 total diets). The DIP

levels as a percent of DM were not the same within each level of NSC. The lowest value for all 15 diets was 4.1% of DM and the highest value was 19.2% of DM. Passage rate of liquid (12%/h) and solids retention time (24 h) were held constant for all fermenters. Microbial synthesis increased linearly with increasing levels of DIP and NSC. There was a quadratic response in bacterial efficiency for DIP and NSC level. Efficiency was highest at the highest level of DIP and the middle level of NSC. The authors concluded that maximum fermentation was achieved when diets contained 37% or more NSC and 12.3% or more DIP. Ruminal ammonia concentration fell below 5 mg/dl when DIP decreased below 13%.

Satter and Slyter (1974) evaluated the effect of ammonia concentration on MCP production in continuous culture. Rumen fluid from steers fed a protein-free, 100% concentrate (DRC), or 23% forage and 77% concentrate diet were added to fermenters. A urea solution was continuously infused into the fermenter at increasing concentrations. Results suggest that when ammonia starts to accumulate, growth of ammonia-utilizing bacteria is not enhanced. A concentration of 5.0 mg/dl was enough to support maximal growth rates. Additionally, high levels of ammonia (up to 80.0 mg/dl) were not shown to inhibit microbial growth.

Kang-Meznarich and Broderick (1981) fed incremental levels of urea at 0, 0.4, 0.7, 1.1, 1.6 and 2.3% of the diet (air-dry basis) to two nonlactating, ruminally fistulated Holstein cows (583 kg). The basal diets (~79% TDN) were pelleted containing 75% ground corn, 20% cottonseed hulls, and 5% supplement. Ruminal ammonia concentration increased from 1.3 to 28.9 mg/dl at the lowest and highest levels of urea, respectively. Synthesis of MCP increased from 707 to 1227 g/d up to 1.1% urea and

declined thereafter. However, microbial efficiency (g MCP/kg digestible DM) reached a maximum of 15.5 at 0.7% urea. This level of urea led to a ruminal ammonia concentration of 8.5 mg/dl. There was a 4% increase in ruminal DM digestibility from 0.7 to 1.1% which explains the increase in MCP supply and decrease in efficiency between those two levels.

Shain et al. (1998) allowed four ruminally fistulated steers (380 kg) ad libitum access to a 90% concentrate DRC based diet. Levels of urea were fed at 0, 0.88, 1.34, or 1.96% of DM in a 4 x 4 Latin square design to determine the effects of increasing urea level on ruminal ammonia, volatile fatty acid (VFA) production, and pH. Increasing levels of urea resulted in dietary CP levels of 8.95, 11.2, 12.7, and 14.21% of DM. Ruminal ammonia concentration increased linearly from 1.65 to 7.89 mg/dl with increasing supplemental urea. There was no effect of urea level on ruminal pH or VFA concentrations. These authors also conducted two finishing trials with 306 crossbred yearling steers (359 kg) where the same diet and levels of urea were fed. Efficiency of gain increased for diets containing urea versus those that did not. However, efficiency did not differ between levels of urea. These results indicate that the DIP requirement was met with the first level of urea (0.88% urea; 3.89 mg/dl ammonia;6.4% DIP).

Milton et al. (1997a) fed four ruminally and duodenally fistulated steers (390 kg) in a 4 x 4 Latin square design to determine the effect of two DIP levels on ruminal fermentation and MCP production. Experimental diets were fed at 90% of ad libitum intake and were 92% concentrate DRC-based diets with urea added at either 0 or 0.91% of DM. The low and high levels of urea provided 8.9 and 11.5% dietary CP, respectively. Ruminal ammonia concentration increased from 7.65 to 12.24 mg/dl with

increasing supplemental urea. The flow MCP to the duodenum did not differ between levels of urea. However, microbial efficiency (g MCP/kg digestible OMI) was decreased by 15% (6.19 to 5.27%) when the higher level of urea was fed.

Milton et al. (1997b) fed four ruminally and duodenally fistulated steers (557 kg) a 90% concentrate DRC based diet with four levels of urea (0, 0.5, 1.0, and 1.5 % of DM) in a 4 x 4 Latin square design. Levels of urea provided 8.5, 9.9, 11.3, and 12.6% dietary CP. Steers were allowed ad libitum access to experimental diets. Ruminal ammonia concentration increased linearly with increasing urea levels from 3.74 to 15.47 mg/dl. With increasing levels of DIP, MCP flow to the duodenum and microbial efficiency were similar.

Cooper et al. (2002a) conducted three finishing trials to determine the effect of corn processing on DIP requirements. In trial 1, 252 yearling steers (379 kg) were used in a randomized complete block design (blocked by initial weight; n=3). Steers were fed 90% concentrate HMC based diets with 0, 0.4, 0.8, or 1.2% supplemental urea (DM basis). These levels of urea resulted in dietary DIP values of 7.0, 8.2, 9.3, and 10.5% (DM basis), respectively. Maximal feed efficiency was determined with nonlinear analysis and was equal to 10.2% dietary DIP. In trial 2, 264 yearling steers (355 kg) were used in a completely randomized design. A SFC based diet with 90% concentrate was fed with urea levels of 0, 0.4, 0.8, 1.2, or 1.6% of DM. These levels resulted in dietary DIP levels of 4.7, 5.8, 7.0, 8.2, 9.3, and 10.5% of DM. Nonlinear analysis of feed efficiency predicted a maximal DIP level of 7.1% of DM. In trial 3, 90 individually fed yearling steers (278 kg) were used in a completely randomized design with a 3 x 5 factorial arrangement of treatments. Diets were 90% concentrate, DRC, HMC, or SFC

based diets with 0, 0.5, 1.0, or 2.0% supplemental urea (DM basis) factored across treatments. Levels of urea provided dietary DIP values of 4.8, 6.3, 7.8, 9.2, and 10.7% for DRC, 6.7, 8.1, 9.6, 11.1, and 12.5% for HMC, and 4.7, 6.1, 7.6, 9.0, and 10.5% for SFC. Feed efficiency did not improve past the first level of dietary DIP for DRC diets. This implies that the DIP requirement was met at 6.3% of DM for DRC. Nonlinear analysis predicted maximal feed efficiency at dietary DIP levels of 10.0% of DM for HMC diets and 9.5% of DM for SFC diets.

Cooper et al. (2002b) fed six ruminally and duodenally fistulated yearling steers (523 kg) in a 3 x 3 Latin square design to determine the effects of corn processing on MCP production and efficiency. Diets were 90% concentrate, DRC, HMC, or SFC based diets. Each diet was fed with 2.0% supplemental urea (DM basis) resulting in dietary DIP levels of 10.7, 12.5, and 10.5% of DM for DRC, HMC, and SFC, respectively. The level of urea was chosen so that ruminal ammonia would not limit ruminal fermentation or MCP synthesis in any of the diets. Total flow of MCP to the duodenum was 29% higher for HMC (1125 g/d) than for DRC (863 g/d) or SFC (875 g/d) with DRC and SFC being similar. Microbial efficiency (g of microbial N/kg OMTD in rumen) did not differ statistically for diets; 18.5, 17.2, and 17.4% for DRC, HMC, and SFC, respectively. Ruminal OMTD was higher for HMC (10.49 kg/d) than for DRC (7.79 kg/d) or SFC (8.25 kg/d). When efficiency values are converted to g of MCP/kg digestible OMI, HMC (9.26%) becomes numerically higher than DRC (8.43%) or SFC (8.35%). Digestible OMI was 10.24, 12.14, and 10.48 kg/d for DRC, HMC, and SFC, respectively. Therefore, differences in MCP flow are due to differences in digestible OMI.