EVALUATION OF PROTEIN VALUE OF DAIRY COW DIETS

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Introduction

The primary function of feed protein in the diet is to provide the ruminant with absorbed or metabolizable protein (**MP**) in the form of α-amino nitrogen. The MP requirement of ruminants is met from two sources, i.e. microbial protein synthesized in the rumen, and feed protein that escapes microbial degradation in the rumen. In addition, endogenous protein is included in computing the MP supply and requirements in some protein evaluation systems, e.g. NRC (2001).

Protein nutrition of ruminants is complex, because dietary supply of amino acids (**AA**) is modified both quantitatively and qualitatively in the fore-stomachs before digestion in the small intestine. Total CP and digestible CP are of little value in protein evaluation for ruminants, since dietary CP is only partly absorbed as AA. Often, especially when high CP diets are fed, a large proportion of apparent CP digestion is a result of ruminal ammonia production and absorption. In addition, microbial CP contains about 20% N in the form of non-amino N, mainly nucleic acids.

Protein is usually the most expensive component of dairy cow diets. During the last decades there has been increasing concerns of N emissions from dairy farms, both in the forms of evaporative losses, mainly as ammonia N, and leaching losses as nitrate to ground water. Feeding large amounts of supplemental protein is also associated with increased phosphorus (P) intakes and emissions, since protein supplements contain more P than forages or cereal grains

Accurate and precise evaluation of feed protein value is a prerequisite for optimizing production and minimizing environmental emissions from dairy operations. An ideal protein evaluation system should quantify the supply of MP from undegraded feed protein and microbial protein, the requirements of rumen degradable protein (**RDP**) by the rumen microbes, and MP requirements of the host animal. For prediction of environmental emissions an accurate estimation of the distribution of manure N between fecal and urinary N would be useful. Considerable improvements in feed protein evaluation were made when CP or

digestible CP systems were replaced with the modern systems based on MP, which differentiate the RDP requirements of rumen microbes and absorbed AA requirements of the host animal. In fact, meta-analysis of data from milk production trials (Huhtanen, 2005) indicated that metabolizable energy (**ME**) and even dry matter (**DM**) intake predicted global and within study milk protein yield responses better than CP or digestible CP, whereas MP intake was a better predictor of milk protein yield than ME intake.

The objective of this paper is to discuss the strengths and weaknesses of the methodologies used in estimating feed protein value for dairy cows. Validation of different approaches will be made using meta-analysis of data from milk production trials.

Microbial protein synthesis

Microbial protein synthesis (**MPS**) in the rumen provides the majority of AA flowing to the small intestine of dairy cows (Clark et al. 1992). Therefore, understanding the mechanisms regulating MPS is essential for optimizing the protein feeding of dairy cows. Microbial protein is of high quality, since its concentrations of lysine and methionine are similar or even greater than those in milk protein. These AA are often limiting or co-limiting milk production with typical U.S. dairy diets. Microbial protein synthesis is related to the amount of available substrates in the form of ATP. When the supply of RDP does not meet the requirements of rumen microbes, potential MPS from available energy is generally discounted, i.e. MPS is limited by RDP intake.

In feed protein evaluation systems, MPS is computed as a function of the intake of digestible organic matter (**DOM**), or from other corresponding parameters related to energy supply for rumen microbes. Usually DOM intake is discounted for dietary components, which provide either less or no fermentable energy for microbes. Such components include silage fermentation acids, fat, rumen undegraded protein (**RUP**), and starch and fiber digested post-ruminally

The effect of method of computing the microbial protein component of MP (**MicrMP**) on prediction accuracy of milk protein yield (**MPY**) was tested using a dataset from North European feeding experiments with dairy cows (988 diets, 204 trials). The diets in the dataset were based on grass silage, cereal grain-based energy supplements, and protein supplements. They covered a wide range in chemical composition, intake and milk production. The MP intake was estimated according to the Finnish version of Scandinavian protein evaluation system (MTT, 2006), in which microbial protein is derived from the intake of digestible

carbohydrates (**DCHO**) + RDP, i.e. RDP has a similar energy value for rumen microbes to DCHO. In addition, MicrMP was calculated excluding RDP and from intakes of digestible OM (**DOM**), ME, and total digestible nutrients (**TDN**). The TDN concentration of the diet was calculated from in vivo or in vitro digestibility of forages and from chemical composition and tabulated digestibility coefficients for concentrates taking into account the higher TDN coefficient for fat. Intakes of ME and TDN were calculated either at maintenance (m) or at production (p) levels of intake. The MP supply from RUP was kept equal to that estimated according to the feeding system (MTT, 2006), and the coefficients of MPS were adjusted so that the mean supply of microbial protein was similar for all methods of estimation MicrMP.

The method used to estimate MicrMP supply had only minor effects on the precision of **MPY** predictions (Table 1). Excluding RDP from the model had the strongest influence on the prediction error even though theoretically RDP provides less energy for rumen microbes than DCHO. This suggests that RDP may stimulate MPS by providing preformed AA and peptides for rumen microbes, which compensate for the lower energy supply from RDP compared with DCHO. The model was slightly improved when DOM, ME_m or TDN_m were used to calculate MPS. However, when ME_m and TDN_m intakes were discounted for the feeding level effects, the prediction errors increased compared with estimates at maintenance level. It is possible that discounting ME or TDN for level of intake includes an additional error. In the NRC (2001) system, discounted TDN is used to compute microbial MP.

Table 1. The effect of available substrate for microbial protein synthesis on the linear relationships between predicted supply of MP (kg/d) and milk protein yield (g/d; Y = A + BX) estimated by a mixed model regression analysis.

Substrate	A	SE[1]	B	SE[1]	RMSE[2]	AIC[3]
DCHO + RDP	117	16.7	407	10.1	19.5	9811.2
DCHO	93	18.3	421	11.3	20.6	9897.4
DOM	141	16.0	393	9.5	19.3	9791.7
ME_m	143	15.9	392	9.4	19.2	9777.5
ME_p	116	17.0	408	10.0	19.7	9826.0
TDN_m	146	16.1	390	9.5	19.4	9797.1
TDN_p	85	17.2	426	10.3	20.3	9854.3

[1]SE = Standard error
[2]RMSE = Residual mean square error (adjusted for random study effect)
[3]AIC = Akaike´s information criterion

The results of the meta-analysis indicate that the simple approaches to calculate MicrMP perform at least as well as more complex, and theoretically more correct, systems discounting for the substrates providing less or no energy for microbial growth. Although silage fermentation products (lactic acid and VFA) provide either no or very little energy for microbial growth, discounting these components from substrate supply increased prediction error of MPY (Rinne et al., 2008). This is in contrast with reduced MPS with increased extent of silage fermentation (Harrison et al., 2003) and may be related to increased propionate production from silage lactate. Increased gluconeogenesis from propionate can improve the utilization of absorbed AA for milk protein synthesis thereby compensating partially for the depressed MPS with extensively fermented silages. Similarly, increased starch digestion in the small intestine reduces the supply of fermentable energy for rumen microbes, but could improve the efficiency of AA utilization. Neither NDF digested post-ruminal provide any energy for microbial growth, but with typical dairy cow diets the contribution of the hind-gut to NDF digestibility is rather small (Huhtanen et al., 2006), and probably rather constant due to short retention time in post-ruminal fermentation compartments. Consequently, the effect of the variation in post-ruminal fiber digestion to the supply of fermentable energy for rumene microbes is likely to be small. It may be concluded that within factorial empirical models, very little can be gained by attempting to correct the intake of DOM (or ME/TDN) for components providing less energy for rumen microbes than digestible carbohydrates. This may be due to a small variation and/or errors in determination of these factors, and compensatory effects on AA utilization. When MPS was calculated as a function of DM intake, prediction error of MPY showed only a small increases from 19.2 – 20.6 to 21.9 g/d, which also supports the view that the accuracy of MP estimation is not markedly improved taking into account variable energy supply from different substrates.

Determination of microbial N synthesis

Microbial protein synthesis is traditionally determined using ruminally and duodenally cannulated animals and marker techniques. This approach involves several cannulated cows and measurements of the amount and composition of duodenal digesta flow. Problems have been related to inaccurate measurements of total digesta flow related to unrepresentative composition of digesta samples and to differentiation of microbial, feed, and endogenous protein flows. Difficulties related to marker methods have been at least partly overcome by using double marker techniques (Faichney, 1986) for digesta flow estimation and ^{15}N as microbial marker. However, a large proportion of published studies rely on a

single marker – usually Cr_2O_3 – in estimating digesta flow, which often results in large standard errors.

Less invasive methods have been developed to determine ruminal MPS. Hristov and Broderick (1996) reported satisfactory estimates of MPS in ruminally cannulated animals using rumen evacuation with dual-phase (solid and liquid) markers when microbial protein was labeled with ^{15}N. Recently Hristov (2007) described a method of determining microbial protein outflow from the rumen of dairy cows using reticular sampling. The flows of non-ammonia N and microbial N were similar when based on reticular or duodenal sampling.

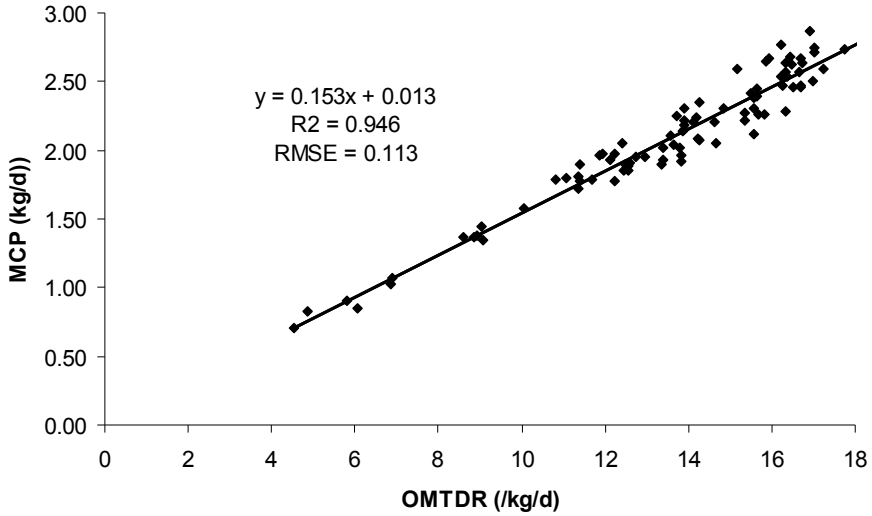

Figure 1. Relationship between OM truly digested in the rumen (OMTDR) and omasal flow of microbial CP. The values are adjusted for the random study effect. (Huhtanen et al., unpublished).

Huhtanen et al. (1997) developed an alternative system that estimates the flow of nutrients entering the omasal canal. Compared with sampling from the abomasum or duodenum, this technique offers several potential advantages. Surgical intervention is reduced, measurements obtained are less affected by endogenous secretions, and digestion of microbial cells is largely avoided. However, similarly to duodenal sampling, one major shortcoming of this approach arises from the collection of samples that are not representative of true digesta entering the omasal canal (Huhtanen et al. 1997; Ahvenjärvi et al. 2000). Accurate determination of nutrient flows requires marker systems (double- or triple-marker) differentially labeling specific digesta phases (Ahvenjärvi et al., 2003). Preliminary results from a meta-analysis suggest that the omasal technique estimates MPS accurately and relatively precisely (Figure 1). Microbial CP flow

increased 153 g/kg OM truly digested in the rumen (**OMTDR**). This value is higher than the default value of 130 g/kg TDN adopted by NRC (2001), even taking into account that the value of TDN intake is slightly greater than OMTDR. It should also be noted that either ^{15}N or purine bases were used as microbial markers in the studies included in the meta-analysis. Both enrichment of ^{15}N and purine to N ratio are higher in bacteria than in protozoa, which leads to underestimation of MPS, the extent depending on the contribution of protozoa to the total microbial N flow. Ahvenjärvi et al. (2002) separated protozoa from omasal digesta by centrifugation and estimated that protozoa comprised 10% of microbial N flow in cows fed a mixed forage-concentrate ration. With 50% lower ^{15}N enrichment of protozoa compared with bacteria, MPS is underestimated by 5% when the proportion of protozoa is 10% of the total microbial N flow.

Rumen undegraded protein

Although microbial protein is the major source of absorbed AA in ruminants, methodologies of determination and factors influencing ruminal protein degradability and the supply of rumen undegraded protein (**RUP**) to the small intestines have been studied more intensively than microbial protein synthesis. Many excellent reviews discussing techniques used to determine ruminal protein degradability have been published (e.g. Michalet-Doreau and Ould-Bah. 1992; Broderick, 1994; Hvelplund and Weisbjerg, 2000; NRC, 2001). Although increasing RUP supply by decreasing ruminal protein degradability should increase the total supply of MP and consequently MPY, the benefits of increased RUP supply in milk production have been small (Santos et al., 1998; Ipharraguerre and Clark, 2005). In the following section, possible reasons for the discrepancy between expected and observed MPY responses to increased RUP supply will be discussed.

In situ (nylon bag) method

In most feed protein evaluation systems, RUP is determined by incubation feed samples in nylon bags in the rumen for different periods of time. Kinetic parameters are estimated from the degradation data by the following widely accepted models:

$$\text{Degraded Protein} = A + B \times [k_d / (k_d + k_p)] \quad (1)$$

$$\text{Undegraded Protein} = B \times [k_p / (k_d + k_p)] + C \quad (2)$$

In these equations, CP is divided into three fractions, which sum to unity. Fraction A is the proportion of CP that has disappeared at 0-time. Fraction B is is non-soluble potentially degradable fraction of CP and C is completely indigestible CP. Fraction B is degraded at rate k_d and k_p is the fractional passage rate of feed particles.

Fraction A. The kinetic model (1) assumes that fraction A is degraded at infinite rate, i.e. there is no escape of this fraction. However, there is considerable evidence from studies using different experimental methods that this assumption is incorrect. Omasal measurements have shown a considerable outflow from the rumen of feed soluble non-ammonia N (**SNAN**), with peptides being quantitatively the most important component (Choi et al., 2002; Reynal et al., 2007). Volden et al. (2002) reported that approximately 10% of the silage soluble non-ammonia N given as a single dose to dairy cows escaped rumen degradation in the liquid phase. A similar value can be recalculated from the data of Choi et al. (2002). Hristov and Broderick (1996) estimated the flow of N fractions from rumen pool sizes and fractional passage rates of rumen solid and liquid phase. Outflow of alfalfa and corn silage SNAN in the liquid phase was approximately 24% of the dietary intake. Peltekova and Broderick (1996) using inhibitor in vitro technique estimated that 20% of silage SNAN escaped rumen fermentation. Hedqvist and Udén (2006) reported that proportionally 25% of soluble protein in ryegrass escaped ruminal degradation in vitro. Ahvenjärvi et al. (2007) labeled timothy grass with ^{15}N and reported that 19% of soluble SNAN in grass silage escaped ruminal fermentation. Consistent with the above-cited studies, the dietary proportion of SNAN had no effect on MPY when used in a model with MP, estimated using constant ruminal degradability and intestinal digestibility of RUP (Huhtanen et al., 2008). This suggests that the proportion of SNAN (A-fraction in situ) was not as strongly related to ruminal protein degradability and MP, as models estimating RUP from degradation kinetics would suggest.

Ruminal protein degradability of practical feed samples cannot be estimated by the in situ method, and therefore empirical equations have been developed to predict degradability from other feed parameters routinely analyzed. Yan and Agnew (2004) presented equations, which predicted in situ protein degradability from silage DM and concentrations of NDF, CP and soluble N ($R^2 \sim 0.80$). However, using constant ruminal CP degradability for silages, predicted MPY responses better than using degradability values estimated with the equation of Yan and Agnew (Rinne et al., 2008). One possible explanation for the failure of variable degradability values to improve MPY predictions is that the differences in the in situ degradability of forage CP reflect more differences in the extent of

microbial contamination of undegraded residues rather than true differences in feed CP degradability.

Kinetic model. The kinetic models used to compute rumen CP degradability consider the rumen a single compartment system with random passage of feed particles irrespective of their size or specific gravity. Marker kinetics data estimated from duodenal samples strongly indicates that the passage of feed particles cannot be described by assuming the rumen is a single first-order kinetic system (Ellis et al., 1994; Huhtanen et al., 2006). Both intrinsically (ADF-^{15}N) (Huhtanen and Hristov, 2001) and extrinsically (Lund et al., 2006) labeled forages showed an ascending phase of marker excretion curves. Using the passage rate estimated from the descending phase of marker excretion curves clearly underestimates the residence time in the rumen, during which the feed is subjected to degradation. The NRC (2001) model predicts a forage passage rate of about 0.05 per h (i.e. 20 h rumen retention time) for typical dairy cow diets. This value is markedly shorter than estimated from duodenal marker excretion curves (Huhtanen and Hristov, 2001; Lund et al., 2006), or from indigestible NDF passage rates estimated by rumen evacuation technique (Huhtanen et al., 2006). Broderick (1994) proposed a two-compartment kinetic model that had separate digestion and passage rates for soluble and insoluble proteins, but the model assumed one-compartment passage model for insoluble CP.

The assumptions of infinite digestion rate of protein fraction A and inappropriate passage model for fraction B would most likely increase the range in protein degradability determined by the in situ technique. Preliminary results from the meta-analysis of omasal sampling data support this suggestion (Huhtanen et al., unpublished). Dietary concentrations of RUP were predicted using NRC (2001) degradation parameters, which are based on large in situ database. Determined dietary RUP concentration was slightly higher than the predicted concentration (51.5 vs. 48.5 g/kg DM). The true difference is smaller or even negative, since endogenous N is included in the omasal "feed N" flow and microbial N is probably slightly underestimated. However, the slope between predicted and observed RUP concentration was only 0.58 (Figure 2). The slope was significantly ($P<0.001$) different from one, which suggests that NRC (2001) overestimated the differences in dietary RUP concentration. For RUP intake, the corresponding slope was 0.72, which was different ($P<0.001$) from one. It seems that the inherent problems related to the in situ method counterbalance each other resulting in a small mean bias, but considerable slope bias leading to errors in estimating relative RUP concentrations between the diets.

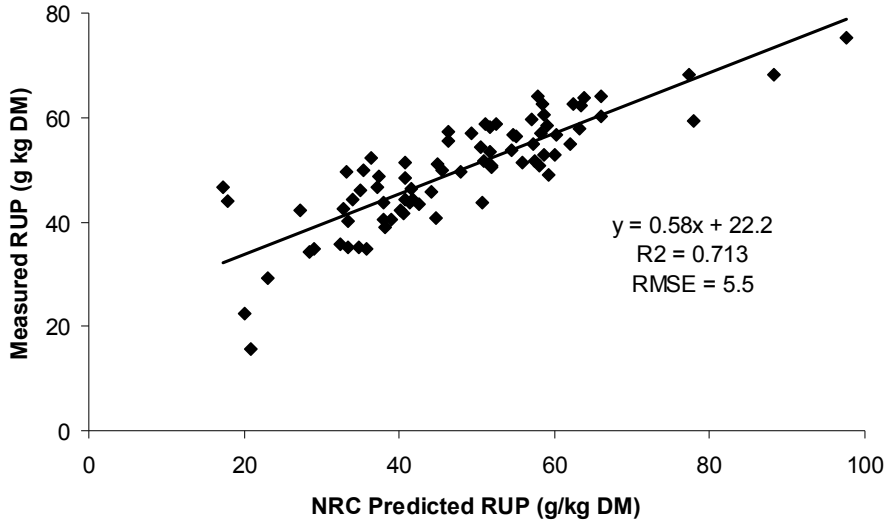

Figure 2. Relationship between predicted (NRC, 2001) and observed dietary RUP concentrations. The values are adjusted for the random study effect. (Huhtanen et al., unpublished).

Production data

Milk protein yield responses to MP supply is an ultimate test of feed protein value. Huhtanen and Hristov (2008) analyzed large North American (n = 739 diets) and North European datasets (n = 998 diets) to predict milk protein yield responses from various nutritive parameters using mixed model regression analysis with random study effect (intercept random).

In this meta-analysis, the effect of degradability on MPY was significant ($P<0.001$) when included in the CP- or TDN+CP-models. However, the MPY predictions were only slightly improved. This also indicates that the effects of dietary CP degradability (NRC, 2001) on MPY are overvalued. The residuals (observed – predicted) of MPY were positively ($P<0.001$) related to degradability of dietary CP and negatively related to dietary RUP concentration in both datasets. Thus, MPY was over-predicted for low degradability high RUP diets. Using CP degradability values estimated according to the Finnish protein evaluation system in the CP- and TDN+CP-models improved MPY predictions in the NE data compared to CP degradability estimated according NRC (2001). Noticeable differences between the systems were higher degradability (0.80 vs. 0.73), smaller variability (s.d. 0.023 vs. 0.044) and smaller range (0.73 – 0.88 vs. 0.54 – 0.83) in the Finnish compared with the NRC system.

Table 2. Residual mean square errors (RMSE) and Akaike´s information criteria (AIC) of models predicting milk protein yield (g/d) from dietary intake variables (from Huhtanen and Hristov, 2008)

Intake variable			RMSE		AIC	
X1	X2	X3	NA[1]	NE[2]	NA[1]	NE[2]
CP			56.3	29.8	8692	10639
CP	Degr		55.5	29.5	8667	10612
MP			52.6	27.3	8621	10438
MP	MP×MP		49.5	26.8	8550	10400
MPBact			50.9	27.4	8542	10371
TDN			47.6	26.6	8479	10289
TDN	CP		47.5	23.5	8465	10091
TDN	CP	Degr	46.9	23.2	8447	10059

[1]NA = North American data
[2]NE = North European data

Conclusions

Accurate estimation of feed protein value is a prerequisite for optimizing production and controlling N emissions in dairy farms. Ideally, the protein evaluation systems should describe the contribution of microbial and undegraded feed protein to meet the cow's MP requirements. The contribution of microbial protein to the MP supply is probably even more important than predicted by the current feed evaluation systems. Microbial protein synthesis in the rumen is a function of fermentable energy supply to rumen microbes. In practice, computing microbial protein from intake of digestible OM, or corresponding parameters is accurate enough. More complicated equations taking into account differences between dietary substrates in energy supply does not improve predictions of MPY, which is the ultimate goal in feed protein evaluation. However, mechanistic models may benefit from less aggregated equations in predicting MPS, when the interactions in nutrient metabolism (e.g. effect of glucose on AA metabolism) can better be taken into account. For example, differences in glucose supply can influence on the utilization of MP for milk protein production.

It appears that the role of dietary CP degradability as a determinant of MPY is overvalued in the current feeding models for dairy cows. This is partly due to inherent problems of the commonly used in situ technique in determining dietary

CP degradability. In addition, the kinetic models used to calculate degradability tend to increase the range of the values. Data from the meta-analysis of in vivo studies using omasal sampling technique are consistent with the hypothesis that the in situ technique overestimating differences in ruminal degradability of dietary CP. Ipharraguerre and Clark (2005) reported a 7% depression in the flow of microbial N in response to RUP supplementation. The true digestibility of incremental CP from heat-treated rapeseed meal (n = 24 diets) was markedly lower than that of the corresponding untreated meal (n=38; 0.82 vs. 0.92) in dairy cows (Huhtanen, 2005). Both depressions in MPS and intestinal digestibility in response to feeding more RUP at least partly offset the positive effects of the increased feed protein flow in response to decreasing ruminal degradability of dietary CP.

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