Effects of silage protein degradability and fermentation acids on metabolizable protein concentration: A meta-analysis of dairy cow production experiments

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ABSTRACT

A meta-analysis was conducted using data from dairy cow production studies to evaluate silage metabolizable protein (MP) concentrations. The data consisted of 397 treatment means in 130 comparisons, in which the effects of silage factors (e.g., date of harvest, wilting, silage additives) were investigated. Within a comparison, a fixed amount of the same concentrate was fed. A prerequisite of data to be included in the analysis was that silage dry matter (DM), crude protein (CP), ammonia N, lactic acid (LA), and total acid (TA) concentrations and digestibility were determined. A smaller data set ($n = 248$) comprised studies in which silage water-soluble N concentration was also analyzed. The supply of MP was estimated as amino acids absorbed from the small intestine using a model with constant values for ruminal effective protein degradability (EPD) and intestinal digestibility of rumen undegraded protein. Microbial protein was calculated on the basis of digestible carbohydrates and rumen degradable protein (RDP). Alternative models were used to estimate microbial protein formation, assuming the energy values of RDP and TA to be equivalent to 1.00, 0.75, 0.50, 0.25, and 0 times that of digestible carbohydrates. Because EPD values are seldom determined in production trials, they were derived using empirical models that estimate them from other feed components. The goodness of fit of models was compared on the basis of root mean squared error (RMSE) of milk protein yield (MPY) predicted from MP supply (adjusted for random study effect) and Akaike's information criterion. Metabolizable protein supply calculated from basal assumptions predicted MPY precisely within a study (RMSE = 16.2 g/d). Variable contribution of RDP to the energy supply for microbial synthesis influenced the precision of MPY

prediction very little, but RMSE for MPY increased markedly when the energy supply of rumen microbes was corrected for TA concentration. Using predicted rather than constant EPD values also increased RMSE of MPY prediction. These observations do not mean that the supply of MP from undegraded feed protein is constant. However, it suggests that our current methods overestimate the range in EPD values and that the techniques have so many inherent technical problems that they can mask the true differences between the feeds. Including new elements in feed protein evaluation models may not improve the precision of production response predictions unless the consequent effects on the supply of other nutrients are taken into account.

Key words: in situ protein degradation, microbial protein synthesis, metabolizable protein, milk protein yield

INTRODUCTION

Microbial protein synthesized in the rumen and feed protein escaping ruminal degradation are the 2 main sources of MP for ruminant animals. In addition, endogenous protein is included in computing the MP supply in some protein evaluation systems; for example, NRC (2001). In the modern feed protein evaluation systems, a wide range of equations is used to predict microbial protein synthesis (MPS) from digestible OM or other comparable feed components. In most systems, variable corrections are made to account for the differences between substrates in the energy supply to rumen microbes.

Some silages may contain up to 200 g/kg of DM of fermentation end-products, mainly lactic acid (LA) and VFA, which provide no or very little energy to rumen microbes (Chamberlain, 1987). Silage fermentation acids are excluded completely from the substrate providing energy for microbial growth in the French (Vérité and Peyraud, 1989) and United Kingdom (AFRC, 1993; Givens et al., 2004) systems. In the Dutch system (Tamminga et al., 1994), silage fermentation acids are

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assumed to provide, proportionally, 0.50 of the energy of digestible carbohydrates for microbial growth. The NRC (2001) and the Scandinavian systems (Madsen et al., 1995) do not differentiate between carbohydrates and fermentation acids as an energy source for rumen microbes.

At present, the in situ technique is the most widely used method to estimate effective protein degradability (EPD) in the rumen and subsequent RUP concentration of ruminant feeds. However, silage EPD values are seldom determined in milk production trials, which makes the validation of the MP values based on the in situ data difficult. Another problem in the validation is the poor reproducibility between research sites of the in situ EPD estimates (Madsen and Hvelplund, 1994). On the other hand, a good fit in regression equations between EPD and routinely analyzed silage parameters within research sites has been demonstrated (Yan and Agnew, 2004). Using EPD values of silages estimated with these equations may be a more reliable approach because the large variation between research sites can be avoided. Thus, EPD values calculated from silage chemical composition were used in this study instead of in situ estimates.

The objectives of this study were to evaluate how different methods in estimating MPS and variation in estimated EPD values influence the predictions of milk protein yield (MPY) from estimated supply of MP. A mixed model regression analysis was used to investigate the relationships within a study.

MATERIALS AND METHODS

Data and Calculations

Mean treatment values were collected from dairy cow studies (list of studies is available as an online data supplement: <http://jds.fass.org/content/vol92/issue4/>) using ad libitum feeding of grass silage varying in quality, or grass silage partly (below 0.50) replaced with legume or whole-crop cereal (barley, wheat) silages. The prerequisite for an experiment to be included in the data set was that the concentrations of DM, CP, digestible OM (g/kg of DM), fermentation acids (LA and VFA) and ammonia N in silage were determined. Silage digestible OM was estimated either in vivo using sheep or by in vitro procedures based on either rumen fluid or pepsin-cellulase incubations. Silage total acid (TA) concentration was calculated as LA + VFA. The diets were supplemented with concentrate feeds differing both in amount and composition between the experiments, but within comparisons all silages were supplemented with a fixed amount of the same concentrate. The total data included 397 treatment means from 130 compari-

sons. A smaller data set (248 silages in 77 comparisons) comprised experiments in which silage water-soluble N concentration also was determined.

Metabolizable protein concentrations expressed as amino acids absorbed from the small intestine were calculated using a modification of the Scandinavian system (Tuori et al., 1998; MTT, 2006). This system uses constant EPD for grass silages (0.85) and legume and whole-crop silages (0.80). Also, the intestinal digestibility of RUP (RUPD) is assumed to be constant (0.82). Metabolizable protein from microbial protein (g/kg of DM) is calculated as $0.179 \times [\text{digestible carbohydrates (g/kg of DM)} + \text{EPD} \times \text{CP (g/kg of DM)}] \times 0.70 \times 0.85$, where the coefficients are the efficiency of MPS (0.179), proportion of amino acid N in microbial N (0.70), and digestibility of microbial protein (0.85). Digestible carbohydrates are calculated as digestible crude fiber + digestible nitrogen-free extract, and equal fermentable energy for microbial growth from digestible carbohydrate and RDP is assumed. For concentrates, the MP concentrations were calculated using analyzed chemical composition and published digestibility and degradability coefficients for each ingredient (MTT, 2006).

Because silage ammonia N provides neither energy for rumen microbes nor RUP for intestinal absorption, silage CP concentrations were corrected for ammonia N when computing microbial protein and RUP. This discount slightly improved the precision of MPY prediction from MP supply (Huhtanen et al., 2008). The effects of energy providing substrates on microbial growth were investigated by comparing alternative models by assuming that ATP yield from RDP or TA is proportionally 0, 0.25, 0.50, 0.75, or 1.00 of that of digestible carbohydrates.

Effective protein degradability is seldom analyzed in production trials, and therefore published empirical relationships between silage characteristics and EPD were used. Yan and Agnew (2004) published the following multiple regression equations from a comprehensive data set of 136 silages that were used to predict EPD:

$$\begin{aligned} \text{EPD}_{1a} \text{ (g/g)} &= 0.758 + 0.701 \times \text{CP} - 0.167 \\ &\times \text{DM} - 0.191 \times \text{NDF} + 0.251 \times \text{SN/N} \\ &+ 0.002 \times \text{LA/VFA}; \end{aligned} \quad [1]$$

$$\begin{aligned} \text{EPD}_{1b} \text{ (g/g)} &= 0.866 + 0.863 \times \text{CP} - 0.139 \\ &\times \text{DM} - 0.180 \times \text{NDF} + 0.003 \times \text{LA/VFA}; \end{aligned} \quad [2]$$

$$\begin{aligned} \text{EPD}_{2a} \text{ (g/g)} &= 0.696 + 0.287 \times \text{CPD} - 0.191 \\ &\times \text{NDF} + 0.140 \times \text{SN/N}; \end{aligned} \quad [3]$$

Table 1. Description of the experimental silages in the large and small data sets

Item	Large data set					Small data set				
	n	Mean	SD	Minimum	Maximum	n	Mean	SD	Minimum	Maximum
DM (g/kg)	397	260	60.1	163	695	248	267	63.9	183	695
In DM (g/kg)										
CP	397	156	26.2	107	234	248	158	24.2	108	234
NDF	397	522	48.8	312	647	248	517	48.5	404	645
Indigestible NDF	397	80	27.0	21	199	248	82	27.5	32	199
Digestible OM	397	669	40.8	495	756	248	666	43.4	495	738
Lactic acid	397	65	33.1	1	176	248	57	31.9	1	138
VFA	397	20	11.4	1	80	248	19	11.1	3	70
Total acids	397	85	37.7	7	218	248	76	35.3	7	161
CP digestibility	397	0.720	0.047	0.566	0.816	248	0.727	0.0450	0.581	0.816
In total N (g/kg)										
Ammonia N	397	63	29.1	12	179	248	55	23.6	12	150
Soluble N	248	575	86.7	274	782	248	575	86.7	274	782

$$\text{EPD}_{2b} \text{ (g/g)} = 0.729 + 0.341 \times \text{CPD} - 0.170 \times \text{NDF}. \quad [4]$$

In the equations, CP, DM, and NDF are the concentrations of CP, DM, NDF as g/g; SN/N is soluble N as a proportion of total N (g/g); LA/VFA is the ratio between LA and VFA; and CPD is the CP digestibility. Digestibility of CP was computed as digestible CP/CP, where digestible CP was estimated from the data described by Huhtanen et al. (2006) using the Lucas test separately for primary growth and regrowth silages. The coefficients of determination for equations [1]–[4] were 0.82, 0.71, 0.82, and 0.79, respectively (Yan and Agnew, 2004). Equations [1] and [3] were used when soluble N was determined and equations [2] and [4] when soluble N was not determined. Equations [1]–[4] predict EPD at a passage rate of 0.02/h, and these values were converted to equivalent values at passage rate of 0.05/h using the equation described by Yan and Agnew (2004).

Statistical Analysis

The relationships between estimated MP supply and MPY were analyzed using the mixed model procedure of SAS (Littell et al., 1996). The model was $Y = B_0 + B_1X_{ij} + b_0 + B_2X_{ij} + e_{ij}$, where $B_0 + B_1X_{ij} + B_2X_{ij}$ is the fixed part of the model, b_0 is the random study effect (intercept), $i = 1 \dots 78$ or 130 are the studies, and $j = 1 \dots n_i$ the values. Milk protein yield was the dependent Y variable. In multivariate models only the first independent variable was a random factor. The models estimating the effects of dependent variables on MPY did not converge and therefore only random intercept was used in these models. Variation in experimental protocols, animal types, and laboratory assays would contribute to study effects in these regressions.

Simple regression analysis was conducted to estimate the relationships between silage parameters and residuals (observed minus predicted) of MPY from the mixed model analysis. In the tables, root mean squared error (RMSE) values after adjusting for the random study effect are presented. The goodness of fit of models was also compared using Akaike's information criterion (AIC), which indicates the model likely to be the most correct with the smallest number of parameters. Rationale and further details of using mixed model analysis to integrate quantitative findings from multiple studies are described by St-Pierre (2001).

RESULTS AND DISCUSSION

The experimental silages displayed a large variation in DM concentration and in parameters describing both nutritive value (e.g., CP, NDF, and digestible OM concentrations,) and extent and type of fermentation in the silo (TA, VFA, and ammonia N concentrations; Table 1). The mean silage parameter values and their variation were similar in the small data set, in which the silage soluble N concentration was also analyzed.

Total DM intake varied from 10.9 to 25.2 kg/d and milk yield from 13.3 to 38.2 kg/d (Table 2). Concentration of MUN and efficiency of N utilization varied widely, indicating that the data covered a large part of the practical range in protein feeding and production levels of dairy cows. In the small data set, total DM intake (19.2 vs. 18.2 kg/d) and milk yield (26.6 vs. 25.3 kg/d) were higher than in the large data set, whereas coefficients of variation for intake and production parameters were slightly smaller than in the large data set.

The overall RMSE of MPY predicted as a linear function of ME supply was small (16.4 g/d in the whole data set, Table 3). This value is well within the 95%

Table 2. Description of feed intake and milk production of dairy cows in the large and small data sets

Item	Large data set					Small data set				
	n	Mean	SD	Minimum	Maximum	n	Mean	SD	Minimum	Maximum
DM intake (kg/d)										
Silage	397	11.1	1.94	5.3	17.4	248	11.5	1.65	7.8	17.4
Concentrate	397	7.1	1.99	0.0	13.5	248	7.6	1.66	4.1	13.5
Total	397	18.2	2.89	10.9	25.2	248	19.2	2.49	13.7	25.2
ME intake (MJ/d)	397	208	32.8	149	297	248	105.1	12.28	79.0	139.0
DIM	397	103	20.1	45	194	248	26.6	4.57	16.5	38.2
Milk yield (kg/d)	397	25.3	4.73	13.3	38.2	248	28.1	4.54	18.4	40.2
ECM yield (kg/d)	397	26.4	4.99	14.3	40.2	248	32.3	1.34	28.7	35.9
Milk protein content (g/kg)	397	32.1	1.67	25.9	35.9	248	856	154.1	546	1,265
Milk protein yield (g/d)	397	809	162.4	429	1,265	248	275	81.5	83	557
MUN (mg/100 mL)	206	12.7	3.74	3.8	25.6	248	285	29.4	208	364
Milk N/N intake (g/kg)	397	278	34.2	177	364	248	11.5	1.65	7.8	17.4

confidence interval of MPY of the whole data set (22.6 g/d), assuming a 5% coefficient of variation in MPY and 12 observations per treatment, and only slightly higher than that of MP using the basal model (16.2 g/d). Comparison of these values suggests that the potential to improve the precision of MPY predictions by a more accurate estimation of MP is rather limited, and that silage MP value is mainly dictated by the digestible OM concentration. Therefore, accurate determination of digestibility is much more important than that of EPD, RUPD, or the substrates for MPS in estimation of MP concentration of grass silage.

Contribution of RDP to MPS

Changing the value of RDP relative to digestible carbohydrates as an energy source for rumen microbes had only minor effects on RMSE of MPY (Table 3). Based on both RMSE and AIC, a value of 0.50 seems the most appropriate. With this value, the residuals (observed minus predicted) of MPY were not significantly related to any of the silage parameters, whereas with relative values of 0.25 and 0.00, the residuals were significantly correlated with silage CP concentration and estimated

CP digestibility. Thus, a relative value of 0.50 for RDP as an energy source for rumen microbes was chosen for further analyses. To maintain the total MP supply and proportion of MP derived from forage at a similar level as in the original model, the EPD values were decreased by 0.05 units.

Ruminal degradation of CP produces less ATP for microbial growth than degradation of carbohydrates (Russell, 2002). However, in the present evaluation using variable proportions for the contribution of RDP to ATP (fermentable OM) supply relative to digestible carbohydrates had only a minor effect on the precision of MPY prediction (Table 3). The small effects may be related to the 2 partly contradictory roles of RDP in MPS. Ruminal degradation of CP provides amino acids and peptides, which can be directly incorporated into microbial protein, thereby sparing ATP required for biosynthesis of amino acids (Walker et al., 2005), but complete degradation of protein to ammonia produces less ATP than carbohydrates per unit of substrate fermented.

Most feed protein evaluation systems; for example, INRA (Vérité and Peyraud, 1989), that of Tamminga et al. (1994), and NRC (2001), assume the same value

Table 3. The effects of ME intake and the proportional value of silage RDP to digestible carbohydrates (dCHO) as an energy source for rumen microbes on prediction of milk protein yield from estimated MP supply ($Y = A + BX$; $n = 397$)

Item	A	SE	B	SE	RMSE ¹	AIC ²
ME intake (MJ/d)	130	20.6	3.29	0.096	16.40	3,958.4
RDP/dCHO						
0.00	58	20.2	0.491	0.0128	16.36	3,918.1
0.25	67	19.8	0.474	0.0122	16.22	3,913.2
0.50	78	19.6	0.457	0.0118	16.17	3,913.3
0.75	91	19.4	0.439	0.0114	16.17	3,917.4
1.00	106	19.4	0.420	0.0111	16.19	3,924.4

¹Root mean squared error.

²Akaike's information criterion.

Table 4. The effects of discounting for silage total acids (TA) from the substrate available for rumen microbial growth [0.00 = same relative value as digestible carbohydrates (dCHO); 1.00 = no energy from TA to microbial growth] on prediction of milk protein yield from estimated MP supply ($Y = A + BX$; $n = 397$)

TA discount	A	SE	B	SE	RMSE ¹	AIC ²
0.00	92	19.4	0.437	0.0114	16.2	3,917.6
0.25	110	19.1	0.433	0.0114	16.4	3,924.9
0.50	134	19.0	0.424	0.0115	17.0	3,946.0
0.75	161	19.1	0.413	0.0117	17.9	3,976.9
1.00	192	19.2	0.400	0.0119	19.0	4,013.6

¹Root mean squared error.

²Akaike's information criterion.

for RDP as for other fermentable substrates. The Scandinavian system (Madsen et al., 1995) assumes that RDP provides no energy for rumen microbes. In the British feed evaluation system (Feed into Milk), the ATP supply from rumen degradable DM is a function of CP concentration (Givens et al., 2004). In that equation, the ATP supply per kilogram of rumen degradable DM decreases with increasing CP concentration of the diet.

Contribution of Fermentation Acids to MPS

Including the discount for TA concentration increased RMSE of MPY markedly (Table 4) although silage fermentation products provide no (VFA) or very little (LA) energy for rumen microbes (Chamberlain, 1987). Also, AIC and standard error of the slope increased when the TA concentration was subtracted

from fermentable energy substrates. When no discount was made for TA concentration, the residuals of MPY were not significantly correlated with any of the silage parameters. In contrast, the residuals were significantly ($P < 0.001$) related to silage TA and LA concentrations when the proportional discount of TA concentration was 0.50 or greater. The residuals for discount factor 1.00 are plotted against TA intake in Figure 1.

The greater water-soluble carbohydrate contents of restrictively fermented silages increase MPS in the rumen (Jaakkola et al., 2006). Increased duodenal microbial N flow (Jaakkola et al., 2006) and increased plasma essential amino acid concentrations (Nagel and Broderick, 1992; Miettinen and Huhtanen, 1997), when silage fermentation was restricted by formic acid application, are also consistent with the theoretical considerations.

Benefits of increased MPS, and consequently MP supply when using restrictively fermented silages, may

Figure 1. Plot of observed minus predicted milk protein yield (residual) vs. total acid intake.

partly be offset by increased utilization of amino acids for gluconeogenesis in the liver. Propionic acid, the major glucose precursor in ruminants, appears to be the main end-product from ruminal lactic acid fermentation, whereas residual sugars in restrictively fermented silages are fermented to acetate or butyrate (Harrison et al., 2003). This has been demonstrated by an intraruminal lactate infusion (Jaakkola and Huhtanen, 1992) and by analysis of rumen fermentation data of cattle fed grass silage-based diets (Sveinbjörnsson et al., 2006).

Reduced plasma glucose concentration in cows fed restrictively fermented silages compared with those fed high-lactate silages (Miettinen and Huhtanen, 1997; Shingfield et al., 2002) supports the hypothesis that glucose supply may be more limiting with restrictively fermented silages, and therefore less amino acids are available for milk protein synthesis than predicted from MP supply corrected to account for fermentation acids. Data compiled by Huhtanen (1998) from intraruminal propionate and abomasal glucose infusion studies, and by Rigout et al. (2002) from duodenal glucose infusion studies indicated, on average, a small positive effect of increased supply of glucogenic substrates on MPY, but the responses have not been consistent.

Extents of silage fermentation may affect the amino acid profile of MP as residual sugars promote MPS (Jaakkola et al., 2006). Histidine is the first-limiting amino acid in dairy cow diets based on grass silage and cereal grain supplements (Vanhatalo et al., 1999; Kim et al., 2001). Because the histidine concentration is markedly lower in microbial protein than in milk protein (20 to 21 vs. 26 to 27 g/kg of amino acids), utilization of additional MP derived from microbial protein may be compromised by a suboptimal amino acid profile.

A third factor explaining why correcting energy supply to rumen microbes to account for fermentation acids increased prediction error of MPY may be related to differences in diet digestibility. In the present data,

diet digestibility was determined either by total fecal collection ($n = 71$) or by using acid insoluble ash as an internal marker ($n = 151$) for a total of 222 diets. Diet OM digestibility (kg/kg) increased 0.00013 units ($P < 0.001$) per 1 g/kg of DM increase in silage TA concentration when silage digestible OM concentration was used as a second independent variable in the model. A difference of 100 g/kg of TA concentration between extensively and restrictively fermented silages was equal to a difference of 0.24 kg/d in digestible OM intake.

Effect of Variable Ruminal Protein Degradability

Using variable EPD values estimated from other silage parameters increased RMSE of MPY compared with the basal model using constant EPD values (Table 5). The AIC followed the same pattern. The differences between the models were similar for the large and small (soluble N determined) data sets. The residuals of MPY predictions with the basal model were not related to any of the silage parameters ($P > 0.20$). However, estimated EPD values were positively ($P < 0.001$) related to the residual of MPY prediction; that is, the model underestimated MPY potential of the high EPD silages (Figure 2). When the models with variable EPD values were used in estimating MP, the residuals of MPY were related to silage CP concentration (slope 0.10 g of milk protein per g/kg CP; $P < 0.01$). The residuals were positively [$P = 0.001$ ($n = 248$); $P = 0.03$ ($n = 397$)] related to silage LA concentration when the equation including LA/VFA [1] was used to estimate EPD.

When both variable EPD values and TA discount for the fermentable substrate were used in the model, the increases in RMSE were more than additive (Table 6). For example, incorporating the TA correction to the basal model increased RMSE of MPY by 2.8 g/d and variable EPD value (equation [1]) by 1.4 g/d, but when both variables were used simultaneously, the increase in RMSE was 5.6 g/d.

Table 5. The effects of different equations of effective protein degradability (EPD) estimation on prediction of milk protein yield from estimated MP supply ($Y = A + BX$)

Data set	n	A	SE	B	SE	RSME ¹	AIC ²
All data							
Basal model	397	92	19.4	0.437	0.011	16.16	3,917.6
Yan and Agnew, 2004 (1) ³	397	76	20.7	0.456	0.012	17.55	3,957.0
Yan and Agnew, 2004 (2) ⁴	397	60	20.6	0.471	0.013	16.96	3,937.4
Soluble N data							
Basal model	248	103	22.1	0.442	0.012	14.53	2,381.9
Yan and Agnew, 2004 (1) ³	248	95	24.2	0.454	0.014	16.40	2,422.2
Yan and Agnew, 2004 (2) ⁴	248	72	23.4	0.474	0.014	15.31	2,394.2

¹Root mean squared error.

²Akaike's information criterion.

³Effective protein degradability (EPD)₁ estimated using equations [1] and [2].

⁴EPD₂ estimated using equations [3] and [4].

Figure 2. Plot of observed minus predicted milk protein yield (residual) vs. estimated silage effective protein degradability (EPD) calculated using equations [1] and [2] (Yan and Agnew, 2004).

It is widely accepted that silage characteristics such as herbage maturity at harvest affect the CP degradation pattern in the rumen and consequently the digestibility of RUP (Rinne et al., 1997; Harrison et al., 2003). Most current feed protein evaluation systems use the in situ technique to determine silage EPD. The present analysis was based on EPD values estimated from other silage parameters using empirical relationships with high correlation coefficients (Yan and Agnew, 2004). Therefore, it is likely that the EPD values estimated using these equations based on the parameters of routine silage analysis such as CP, NDF, fermentation acids, and soluble N as a proportion of total N concentrations reflect the true differences in the in situ EPD values better than those estimated at different research sites using a wide range of modifications of the in situ technique (Madsen and Hvelplund, 1994). Furthermore, silage EPD values are seldom determined in production trials.

When variable EPD values were estimated using the equations of Yan and Agnew (2004), the RMSE of MPY increased compared with the model using constant values both for EPD and RUPD. The increase in RMSE and AIC was greatest for the EPD₁ model, which resulted in the largest range and highest coefficient of variation of the EPD values. The highly significant positive relationship between the estimated EPD values and the residuals of predicted MPY indicates that MPY responses were under-predicted for the high EPD silages and over-predicted for the low EPD silages. The significant relationships of the residuals to silage CP (positive) and NDF (negative) concentrations suggest that the differences in estimated EPD values may be related to inherent technical problems of the in situ technique rather than reflecting real differences in ruminal CP degradability between the silages.

The models predicted higher EPD values for high CP and low NDF silages compared with low CP and

Table 6. The effects of different equations of estimation on prediction of milk protein yield from estimated MP supply when the substrate for microbial synthesis was discounted for total acids ($Y = A + BX$; $n = 397$)

Model	A	SE	B	SE	RSME ¹	AIC ²
Basal model	92	19.4	0.437	0.011	16.16	3,917.6
Total acids discount	192	19.2	0.400	0.012	19.00	4,013.6
Yan and Agnew, 2004 (1) ³	207	20.6	0.398	0.013	21.84	4,091.5
Yan and Agnew, 2004 (2) ⁴	184	20.4	0.418	0.013	20.92	4,061.0

¹Root mean squared error.

²Akaike's information criterion.

³Effective protein degradability (EPD)₁ estimated using equations [1] and [2].

⁴EPD₂ estimated using equations [3] and [4].

high NDF silages. These differences may result from relative differences in the contribution of microbial contamination of undegraded feed residues. Incubation of intrinsically ^{15}N -labeled feeds demonstrated that a large proportion of residual N is of microbial origin (Varvikko and Lindberg, 1985), especially for feeds of low CP and high NDF concentration. Because forages typically have relatively low CP and high NDF concentration, microbial contamination can severely influence forage EPD estimation. Michalet-Doreau and Ould-Bah (1989) developed 2 equations to correct the EPD values for microbial contamination; one was related to CP and the other to CP and NDF. Interestingly, their coefficient for correcting EPD values for the differences in NDF concentration was similar to those in the equations of Yan and Agnew (2004) for estimating EPD values from the laboratory measurements. This suggests that the variation in silage EPD values resulting from NDF concentration can reflect differences in the extent of microbial contamination.

Another problem of the in situ method in estimating silage EPD values is that the kinetic model (Ørskov and McDonald, 1979) assumes that the *a* fraction is immediately degraded. However, there is increasing evidence that the assumption of infinite degradation rate of soluble N fraction does not hold true. Omasal flow measurements have shown a considerable flow of feed soluble nonammonia N (SNAN) with peptides being quantitatively the most important component (Choi et al., 2002; Reynal et al., 2007). Volden et al. (2002) fed a single dose of silage soluble N to dairy cows and estimated that proportionally 0.08 to 0.10 of SNAN escaped rumen degradation in the liquid phase. 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 feed SNAN for lucerne and maize silage in the liquid phase was approximately 24% of the dietary intake of this N fraction. Peltekova and Broderick (1996) estimated using inhibitor in vitro technique that 20% of SNAN in alfalfa silage escaped rumen fermentation.

The Dutch feed protein evaluation system assumes, on the basis of discrepancies between estimated MP value and production responses, that proportionally 0.05 of the *a* fraction escapes ruminal degradation (Tamminga et al., 1994). More recently, in the British Feed into Milk system the degradation rate of the *a* fraction was assumed to be 0.90 per h (Givens et al., 2004), which corresponds to a proportional escape of 0.14 for this fraction, if the passage rate is 0.15 per h.

It is possible that the degradation rate and escape are not constant for the *a* fraction, because the composition of silage SNAN can be highly variable. Nsereko and Rooke (2000) showed that the proportion of peptides in

silage soluble N was much higher for formic acid-treated silages compared with untreated silages. Degradation of peptides has been demonstrated to be the rate-limiting step (Chen et al., 1987; Choi et al., 2002; Reynal et al., 2007) of ruminal proteolysis.

In a recent study, proportionally 0.19 of SNAN of ^{15}N -labeled formic acid treated silage escaped ruminal degradation when estimated from ruminal ^{15}N kinetics (Ahvenjärvi et al., 2007). This and other studies cited earlier suggest that the differences in ruminal degradability of soluble and insoluble silage N fractions are much smaller than previously thought, and that our current experimental methods are not sensitive enough to detect the possible differences. Even though the degradation rates of silage soluble N fractions are greater than those of insoluble N fractions, a markedly faster liquid passage rate compensates for the difference in degradation rates. Our previous study (Huhtanen et al., 2008) demonstrated that the proportion of SNAN in silage N had no influence on MPY responses predicted from MP estimated using a constant value for EPD, and that the adverse effect of silage soluble N fraction could entirely be related to ammonia N concentration.

If the EPD of early-harvested silages is higher than that of late-harvested silages as most in situ data suggest, it is possible that the differences in EPD are at least partly offset by corresponding differences in RUPD. Proportion of ADIN in grass N tends to increase with advancing maturity (e.g., Rinne et al., 1997; Elizalde et al., 1999) leading to reduced digestibility. However, EPD and digestibility were positively correlated, indicating that higher EPD of early harvested silages is compensated for by a lower proportion of indigestible N. Many feed protein evaluation systems use the mobile bag technique in estimating intestinal digestibility, but that method has many technical problems including extensive disappearance of ADIN (Vanhatalo, 1994).

Our observations that using constant values for EPD and digestibility of RUP to compute MP supply predicted MPY responses better than models incorporating variable parameter values agree with those of Tuori et al. (1998). In their evaluation, a model with a constant EPD for all feeds resulted in a smaller variation in the efficiency of MP utilization within study than using EPD values determined in situ. The EPD values were determined in 2 laboratories using the same procedures, and the values were corrected for microbial contamination and particle loss.

These observations do not mean that the supply of MP from undegraded feed protein is constant. However, it tends to suggest that our current methods overestimate the range in EPD values and that the techniques have so many inherent technical problems that they can mask the true differences between the feeds.

CONCLUSIONS

The present meta-analysis showed that correcting the supply of fermentable energy for microbial protein synthesis in the rumen to account for silage fermentation acids increased prediction error of milk protein yield despite being a biologically correct concept. This demonstrates that including new and theoretically correct elements to feed protein evaluation systems may not improve the models unless the consequent effects (e.g., efficiency of MP utilization, diet digestibility, amino acid balance) are simultaneously taken into account. The inclusion of empirical models estimating the EPD values of grass silage did not improve the predictions of milk protein yield compared with using constant EPD values. This can, at least partly, be because the differences in estimated EPD may reflect the inherent problems of the in situ technique rather than true differences in EPD. In addition, high EPD is associated with higher intestinal digestibility of RUP; that is, the differences in EPD are partly compensated for by differences in the digestibility of RUP. Also, the differences in EPD of soluble and insoluble N in silage seem to be smaller than previously estimated. It may be concluded that in relation to the most likely small true differences in EPD of grass silages and methodological difficulties, the value of in situ EPD determination of grass silages is questionable. Whether this conclusion can be applied to other forage types and concentrate feeds needs further evaluation.

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