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Improved Feed Protein Fractionation Schemes for Formulating Rations with the Cornell Net Carbohydrate and Protein System

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ABSTRACT

Adequate predictions of rumen-degradable protein (RDP) and rumen-undegradable protein (RUP) supplies are necessary to optimize performance while minimizing losses of excess nitrogen (N). The objectives of this study were to evaluate the original Cornell Net Carbohydrate Protein System (CNCPS) protein fractionation scheme and to develop and evaluate alternatives designed to improve its adequacy in predicting RDP and RUP. The CNCPS version 5 fractionates CP into 5 fractions based on solubility in protein precipitant agents, buffers, and detergent solutions: A represents the soluble nonprotein N, B1 is the soluble true protein, B2 represents protein with intermediate rates of degradation, B3 is the CP insoluble in neutral detergent solution but soluble in acid detergent solution, and C is the unavailable N. Model predictions were evaluated with studies that measured N flow data at the omasum. The N fractionation scheme in version 5 of the CNCPS explained 78% of the variation in RDP with a root mean square prediction error (RMSPE) of 275 g/d, and 51% of the RUP variation with RMSPE of 248 g/d. Neutral detergent insoluble CP flows were overpredicted with a mean bias of 128 g/d (40% of the observed mean). The greatest improvements in the accuracy of RDP and RUP predictions were obtained with the following 2 alternative schemes. Alternative 1 used the inhibitory in vitro system to measure the fractional rate of degradation for the insoluble protein fraction in which A = nonprotein N, B1 = true soluble protein, B2 = insoluble protein, C = unavailable protein (RDP: R^2 = 0.84 and RMSPE = 167 g/d; RUP: R^2 = 0.61 and RMSPE = 209 g/d), whereas alternative 2 redefined A and B1 fractions as the non-amino-N and amino-N in the soluble fraction respectively (RDP: R^2 = 0.79 with RMSPE = 195 g/d and RUP: R^2 = 0.54 with RMSPE = 225 g/d). We concluded that implementing alternative 1 or 2 will improve the accuracy of predicting RDP and RUP within the CNCPS framework.
Key words: feed protein fractionation, protein supply, nutritional model

INTRODUCTION

Excess feeding of N can contribute to air and water pollution (NRC, 1993). To mitigate negative environmental N pollution of farming, it is important that diets are formulated to meet, but not exceed, N requirements of rumen microbes and AA requirements of the ruminant animal (Schwab et al., 2005). Nutritional models help in the process of farm decision making by predicting animal performance and nutrient excretion and assessing diet adequacy under a range of management and feeding situations (Fox et al., 2004). At present, to formulate diets that minimize N excretion, some aspects of current nutritional models require further improvements, in particular predictions of dietary supply of RDP and RUP, N requirements of rumen microorganisms, and microbial protein supply (Schwab et al., 2005; Lanzas et al., 2007b).

The Cornell Net Carbohydrate and Protein System (**CNCPS**) accounts for effects of variation in feed protein fractions in predicting feed MP supply, rumen N, and AA balances when developing diets to meet cattle nutrient requirements (Fox et al., 2004). The CNCPS fractionates CP into 5 fractions based on solubility in protein precipitant agents, buffers, and detergent solutions. This system of protein fractionation was first described 25 yr ago (Van Soest et al., 1981). Some limitations of the system have been identified because of recent research and its implementation by nutritionists. There are several limitations of this fractionation system: 1) the assumption that the N insoluble in neutral detergent and in acid detergent represents slowly degradable and unavailable protein fractions, respectively, is not valid for all feeds (Waters et al., 1992; Nakamura et al., 1994; Coblentz et al., 1999); 2) the assumption that all of the NPN fraction enters the ammonia pool completely and does not provide amino N that can stimulate microbial

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Table 1. List of alternative protein fractionation schemes

Scheme	Modifications[1]
1	Original scheme
2	Original scheme with adjusted B3 rates
3	A fraction as NAAN
4	A fraction as NAAN and adjusted B1 rates
5	A fraction as NAAN and adjusted B3 rates
6	A fraction as NAAN and adjusted B1 and B3 rates
7	Aggregated insoluble fraction, A fraction as NPN
8	Aggregated insoluble fraction, A fraction as NAAN
9	Aggregated insoluble fraction, A fraction as NPN, adjusted B1 rates
10	Aggregated insoluble fraction, A fraction as NAAN, adjusted B1 rates

[1]NAAN = nonamino N, A fraction computed as indicated in Eq. 6 (A´ fraction); NPN: A fraction computed as indicated in Eq. 1 (A fraction); B2 fraction computed as indicated in Eq. 8 (B2´ fraction). The fractional rates for the B2´ were obtained by the inhibitory in vitro method.

growth has caused underprediction of microbial protein production (Aquino et al., 2003); 3) the assumption that fraction A is completely degraded does not account for the contributions of free amino acids and peptides to the RUP flows (Choi et al., 2002; Volden et al., 2002; Reynal et al., 2007); and 4) despite the RUP flow being very sensitive to degradation rates for the B2 fraction, no method is currently recommended for determining B2 rates (Lanzas et al., 2007b).

The objectives of this study were to evaluate the original CNCPS protein fractionation system and to develop and evaluate alternatives to improve its ability to accurately predict RDP and RUP using existing literature and currently available methodology. Our approach consisted of developing several modifications that addressed some of the limitations of the original scheme. These modifications were combined to create new fractionation schemes. The original and proposed alternative schemes were evaluated against in vivo RDP supply and RUP flow data and were ranked based on their accuracy.

MATERIALS AND METHODS

Feed Protein Fractionation Schemes

Original CNCPS Scheme. The original CNCPS protein fractionation divides feed protein into 5 fractions (Sniffen et al., 1992). The A fraction is the N soluble in buffer and not precipitated by protein precipitating agents such as TCA. It contains peptides, free AA, ammonia, amides, amines, ureides, nucleotides, and nitrates (Reid, 1994). It is determined as

$$PA_j = NPN_j \times (SolCP_j/1{,}000) \times (CP_j/1{,}000) \quad \text{(g/kg of DM)}, \qquad [1]$$

where CP_j is the CP content of the jth feed, g/kg of DM; NPN_j is the nonprotein content of the jth feed, g/kg of SolCP; PA_j is the protein A fraction content of the jth feed, g/kg of DM; and $SolCP_j$ is the buffer-soluble CP content, g/kg of CP.

Fraction B1 is measured as the buffer-soluble protein that is not precipitated by protein precipitating agents and is assumed to be very rapidly degraded in the rumen with degradation rates greater than 1.0/h;

$$PB1_j = (SolCP_j\ /1{,}000) \times (CP_j/1{,}000) - PA_j \quad \text{(g/kg of DM)}, \qquad [2]$$

where CP_j is the CP content of the jth feed, g/kg of DM; PA_j is the protein A fraction content of the jth feed, g/kg of DM; $PB1_j$ is the protein B1 fraction content of the jth feed, g/kg of DM; and $SolCP_j$ is the buffer-soluble CP content, g/kg of CP.

Fraction C is the N insoluble in acid detergent solution that, when multiplied by 6.25, is assumed to be the protein associated with lignin, tannin-protein complexes, and Maillard products. Ruminal degradation rates and intestinal digestibility for the C fraction are 0:

$$PC_j = ADICP_j \times (CP_j/1{,}000) \text{ (g/kg of DM)}, \qquad [3]$$

where $ADICP_j$ is the acid detergent insoluble CP (**ADICP**) content of the jth feed, g/kg CP; CP_j is the CP content of the jth feed, g/kg DM; and PC_j is the protein C fraction content of the jth feed, g/kg DM.

The B3 fraction is the CP insoluble in neutral detergent solution, but soluble in acid detergent:

$$PB3_j = (NDICP_j - ADICP_j) \times (CP_j/1{,}000) \quad \text{(g/kg of DM)}, \qquad [4]$$

where $ADICP_j$ is the CP insoluble in acid detergent solution content of the jth feed, g/kg of CP; CP_j is the CP content of the jth feed, g/kg of DM; **NDICP** is the insoluble CP in neutral detergent solution content

of the jth feed, g/kg of CP; and $PB3_j$ is the protein B3 fraction content of the jth feed, g/kg of DM. It is assumed that the CP insoluble in neutral detergent solution is protein associated with the cell wall and it is very slowly degraded (<0.02/h); thus, a high percentage escapes degradation in the rumen.

The B2 fraction represents the intermediate degradable protein with rates of degradation within the range 0.03 to 0.16/h, and it is calculated by difference:

$$PB2_j = CP_j - PA_j - PB1_j - PB3_j - PC_j \text{ (g/kg of DM)}. \quad [5]$$

Modifications to the Original CNCPS Scheme. The following modifications were investigated: 1) fractions A and B1 were redefined as nonamino N and buffer-soluble amino N fractions, respectively; 2) degradation rates for the B1 were adjusted as shown below; 3) the original insoluble B2 and B3 were combined into one fraction; and 4) degradation rates of B3 were adjusted as shown below.

First, the A and B1 fractions were redefined as non-AA N (**NAAN**; PA´) and AA N (**AAN**; PB1´) in the buffer-soluble fraction:

$$PA'_j = (1{,}000 - AAN_j) \times (SolCP_j/1{,}000) \times (CP_j/1{,}000) \text{ (g/kg of DM)} \quad [6]$$

$$\text{and } PB1'_j = (SolCP_j/1{,}000) \times (CP_j/1{,}000) - PA'j \text{ (g/kg of DM)}, \quad [7]$$

where CP_j is the CP content of the jth feed, g/kg DM; AAN_j is the AA N content of the jth feed, g/kg of SolCP; PA'_j is the protein A fraction content of the jth feed, g/kg of DM; $PB1'_j$ is the protein B1 fraction content of the jth feed, g/kg of DM; and $SolCP_j$ is the buffer-soluble CP content, g/kg of CP. In addition, the B1´ fraction was assumed to pass at the same rate as liquids leaving the rumen.

Second, degradation rates of the B1 fraction in the CNCPS feed library exceed most of the published values for in vitro soluble proteins (Mahadevan et al., 1980; Broderick et al., 1989; Peltekova and Broderick, 1996; Hedqvist and Udén, 2006). The effects of adjusting the B1 rates to reflect observed in vitro rates were investigated (Table 1). The adjustment of B1 rates was assumed independent of the definition of A and B1 fractions because the ranges of reported fractional degradation rates of soluble protein and peptides degradation are similar (Volden et al., 2002).

Third, recent studies in which the kinetics of NDICP disappearance has been determined indicated that digestion rates of NDICP are considerably greater than the rates found in the CNCPS feed library for the B3 fraction (Rossi et al., 1997; Juarez, 1998; Coblentz et al., 1999; McBeth et al., 2003). The rates for the B3 fraction were increased.

Fourth, the aggregation of the original B2 and B3 fractions into one fraction (PB2´) was performed because previous sensitivity analyses indicated that unless the rates for fractions within the true insoluble protein (B2 and B3) differed by several magnitudes (> ×10), the model predictions were insensitive to the presence of different fractions (Lanzas et al., 2007b). The new fraction was renamed PB2´:

$$PB2'_j = CP_j - PA_j - PB1_j - PC_j \text{ (g/kg of DM)}. \quad [8]$$

Degradation rates based on the inhibitory in vitro (**IIV**) method were used for the PB2´ fraction (Broderick, 1987; Broderick and Clayton, 1992; Broderick et al., 2004a,b).

Alternatives schemes were developed by incorporating various combinations of the modifications described above into the CNCPS protein fractionation scheme. Both the original and alternative schemes tested in this study are listed in Table 1.

Evaluation of the Feed Protein Fractionation Schemes

The ability of the original and alternative schemes to predict RDP supply and RUP flows determined using omasal sampling technique was assessed. There are some advantages of using omasal data for estimating N fractions (Ahvenjarvi et al., 2000): 1) there is substantially less endogenous N secreted into the rumen than into the duodenum, and 2) rumen microbes are measured before they reach the abomasum where they are digested, allowing the digesta N to be separated into particle- and liquid-associated bacteria, protozoa, and soluble and insoluble dietary N fractions.

Database Description. Five studies designed to test the effect of dietary protein content and supplementation on N metabolism and animal performance in lactating dairy cows in which omasal flows were determined were used to evaluate the ability of the protein fractionation schemes to predict RDP supply and RUP flows (Reynal and Broderick, 2003, 2005; Reynal et al., 2003, 2005; Olmos Colmenero and Broderick, 2006a,b; Brito and Broderick, 2006, 2007; Brito et al., 2006, 2007; Table 2).

Table 2. Descriptive statistics for the studies used to evaluate the ability of the protein fractionation schemes to predict RDP supply and flow of RUP

Item	Descriptive statistics				
	n	Mean	SD	Minimum	Maximum
Diet composition and intake					
DM intake, kg/d	22	23.9	1.55	21.4	26.8
NDF, g/kg DM	22	250	2.4	22.4	30
N, g/kg DM	22	27.8	2.63	21.6	32.5
NE_L, MJ/kg DM	22	6.28	0.251	5.94	6.90
Production and N excretion					
BW, kg	22	602	27.5	561	634
DIM, d	22	91	19	72	120
Milk, kg/d	22	39	2.9	32.9	42.8
Fat yield, kg/d	22	1.3	0.12	1	1.6
True protein yield, kg/d	22	1.2	0.11	0.9	1.3
Urine N, g/d	17	154	48.7	63	240
Fecal N, g/d	17	211	28.7	154	275
Omasal N flows					
Total N, g/d	22	562	163.7	233	709
Free AA N, g/d	18	42.1	19	16	70
Total NAN, g/d	22	551	161.8	226	695
Dietary NAN, g/d	22	236	80.8	74	403
Bacterial NAN, g/d	22	397	87.2	238	480
NDIN, g/d	17	25	7.7	14	45
ADIN, g/d	18	20	23.6	3	66

Simulations and Evaluation. Simulations were made using a spreadsheet version of the rumen submodel of the CNCPS as described by Fox et al. (2004) with new passage rate equations developed by Seo et al. (2006) and a revised feed carbohydrate fractionation scheme (Lanzas et al., 2007a). The following predicted outputs were evaluated against the in vivo data:

1. RUP flows (g/d):

$$\text{Observed RUP flows} = (\text{NAN} - \text{Microbial NAN}) \times 6.25; \quad [9]$$

$$\text{Predicted RUP flows} = \sum_{j=1}^{j} REPB1_j + REPB2_j + REPB3_j + REPC_j; \quad [10]$$

2. RDP supply (CP intake − RUP flows) (g/d):

$$\text{Observed RDP supply} = \text{Total CP intake} - \text{RUP flow}; \quad [11]$$

$$\text{Predicted RDP flows} = \sum_{j=1}^{j} RDPA_J + RDPB1_J + RDPB2_j + RDPB3_j; \quad [12]$$

3. NDICP flows (g/d):

$$\text{Observed NDICP flow} = \text{NDIN flow} \times 6.25; \quad [13]$$

$$\text{Predicted NDICP flow} = \sum_{j=1}^{j} REPB3_j + REPC_j; \quad [14]$$

where NDIN is the neutral detergent insoluble nitrogen, $RDPA_j$ is ruminally degraded protein A fraction of the jth feedstuff, $RDPB_{ij}$ is the ruminally degraded protein B_i fraction of the jth feedstuff, $REPB_{ij}$ is the ruminally escaped protein B_i fraction of the jth feedstuff, and $REPC_j$ is the ruminally escaped protein C fraction of the jth feedstuff.

The following statistical tests were used to assess the adequacy of the model predictions (Tedeschi, 2006). Accuracy was determined using the concordance correlation coefficient (**CCC**) and accuracy index (**Cb**; Lin, 1989). The concordance correlation coefficient evaluates the agreement between observed and predicted by measuring the variation from the 45° line through the origin (Lin, 1989). Mean square error (**MSE**), mean square prediction error (**MSPE**) and its partition into 3 independent and additive components (Theil, 1961; mean bias, slope bias, and random unexplained errors), and linear regression were also performed.

RESULTS

Tables 3 and 4 present the fractions and degradation rates for the feeds used in the evaluation. Table 3 has the average values for the protein fractions in the feeds

Table 3. Feed protein fractions in the feeds included in the evaluation

Feed	Protein fractions[1]							
	CP	Soluble CP	NPN	True protein	NAAN	AA N	NDICP	ADICP
	(g/kg of DM)	(g/kg of CP)	(g/kg of sol CP)				(g/kg of CP)	
Alfalfa silage	224.8	496.2	829.5	170.5	136.4	863.6	92.2	28.9
Blood meal	1,000.0	50.0	60.0	940.0	10.0	990.0	64.0	12.0
Canola meal	427.0	323.2	652.2	347.8	170.0	830.0	71.7	40.3
Corn gluten meal	651.9	41.4	740.7	259.3	10.0	990.0	81.0	64.0
Corn silage	72.7	565.3	889.4	110.6	199.2	800.8	74.3	13.5
Cottonseed meal	484.0	200.4	402.1	597.9	180.0	820.0	27.3	19.5
Expeller soybean meal (SBM)	489.4	61.3	533.3	466.7	10.0	990.0	107.0	23.0
Lignosulfonate SBM	496.6	48.3	500.0	500.0	20.0	980.0	323.6	74.6
Roasted soybeans	400.0	57.5	1,000.0	0.0	20.0	980.0	82.5	34.4
Rolled HMSC[2]	86.4	321.9	935.4	64.6	103.5	896.5	34.7	6.1
Solvent SBM	530.8	199.7	537.8	462.2	20.0	980.0	15.2	5.2

[1]NAAN = non-AA N; NDICP = neutral detergent insoluble CP; ADICP = acid detergent insoluble CP. The NPN fraction was assayed with TCA; NPN is the A fraction of the original scheme (Eq. 1); true protein is the B1 fraction of the original scheme (Eq. 2); NPN values were corrected by subtracting total amino nitrogen from total nitrogen; NAAN is the modified A fraction (Eq. 6) (= A´ fraction); AAN is the modified B1 fraction (Eq. 7) (= B1´ fraction).

[2]HMSC = high-moisture shelled corn.

included in the evaluation. For the protein concentrates, the A fraction of the original scheme represented approximately 500 g/kg of the soluble CP. When the soluble protein was corrected for its amino N content, the average amino N content was greater than 800 g/kg of soluble CP. Table 4 lists the current feed library rates, the adjusted rates for the B1 and B3 fractions, and the rates for the B2´ fractions.

The original scheme overpredicted the RDP supply with a mean bias of 149 g/d (5% of the predicted and observed mean; Table 5 and Figure 1). The regressed residuals (observed − predicted) against predicted RDP had significant intercept and slope [$Y = -148.7 - 0.28(X - 3{,}050.8)$], indicating the presence of significant slope and mean bias. The original scheme explained more variation in the RDP supply ($R^2 = 0.78$) than for the RUP flows ($R^2 = 0.51$; Table 5). The original scheme underpredicted RUP flow with a mean bias of 152 g/d (12% of the predicted mean and 14% of the observed mean). Similar to RDP, the regressed residuals against predicted RUP flow had significant intercept and slope [$Y = 151.8 - 0.39(X - 1{,}086.7)$]. Four studies that also measured omasal NDICP flows were used to evaluate the predictions of the flow of NDICP out of the rumen (Reynal and Broderick, 2005; Brito et al., 2006, 2007; Olmos Colmenero and Broderick, 2006b). The original

Table 4. Degradation rates (/h) for the protein fractions of the feeds used in the evaluation.

Feed	Cornell Net Carbohydrate and Protein System				
	B1 rate	AdjB1 rate[1]	B3 rate	AdjB3 rate[2]	IIV rate[3]
Alfalfa silage	1.5	0.28	0.0180	0.14	0.04
Blood meal	1.35	0.2	0.0009	0.01	0.01
Canola meal	2.3	0.46	0.0002	0.05	0.12
Corn gluten meal	1.5	0.2	0.0050	0.02	0.02
Corn silage	1.5	0.28	0.0180	0.03	0.04
Cottonseed meal	1.75	0.46	0.0175	0.04	0.10
Expeller soybean meal (SBM)	2.3	0.46	0.0020	0.05	0.04
Lignosulfonate SBM	2.3	0.46	0.0020	0.04	0.04
Roasted soybeans	2.3	0.46	0.0020	0.04	0.05
Rolled HMSC	1.5	0.5	0.0200	0.02	0.02
Solvent SBM	2.3	0.46	0.0100	0.06	0.17

[1]AdjB1 rates are the adjusted rates for the fraction B1 and were based on several published sources (Broderick et al., 1989; Peltekova and Broderick, 1996; Hedqvist and Udén, 2006).

[2]AdjB3 rates are the adjusted rates for the fraction B3 and were based on several published sources (Rossi et al., 1997; Juarez, 1998; Coblentz et al., 1999; McBeth et al., 2003; Pichard et al., 2005; Ogden et al., 2006).

[3]IIV = inhibitory in vitro. Corn silage, rolled high-moisture shelled corn (HMSC) and canola meal rates were assigned based on relative ranking compared with the other feeds.

scheme overpredicted the omasal flow of NDICP (Table 6), with a mean bias of 62.3 g/d, which represented 28.5% of the predicted mean and 40% of the observed mean. For the study with the greatest proportion of protein as B3 and C fraction (Reynal and Broderick, 2005), the averaged mean bias for the study was as great as 204 g/d, representing 40% of the predicted mean and 97% of the observed mean. Adjusting the B3 rates to reflect available data (scheme 2; Table 1) resulted in a decrease in the RMSPE and lower mean bias (21 g/d; Table 6). However, the NDICP flows were still overpredicted when the adjusted rates were used. Overall, the predicted contribution of the NDICP to the RUP flows was greater than observed because the NDICP fraction was more extensively degraded in the rumen (Table 2).

Statistical measures for the evaluation of the alternative protein fractionation schemes listed in Table 1 are summarized in Table 5. Table 7 ranks the schemes by their accuracy in predicting RDP and RUP. The original scheme ranked seventh and fifth in predicting RDP and RUP, respectively, whereas scheme 7 (in which the insoluble fraction was combined into 1 fraction, and fraction A = NPN) was the best. The CCC ranked the schemes similarly. As a general trend, adjusting for the AAN in the soluble protein (A´ and B1´ fractions, Eq. 6; schemes 3 and 5) decreased RDP compared with the original scheme. Aggregating B2 and B3 pools (B2´ fraction, Eq. 8; schemes 7 to 10) resulted in an increase in the RUP flows. Schemes 3 (A´ and B1´ fractions), 7 (aggregated insoluble B2´ fraction), and 9 (aggregated insoluble B2´ fraction, A fraction as NPN, and adjusted B1 rates) were the schemes that resulted in an overall improvement in the accuracy of both RDP supply and RUP flow predictions. The scheme that performed the worst was scheme 10, in which A fraction and B1 rates were adjusted and the insoluble fraction was aggregated. Scheme 10 overpredicted the amount of escaping soluble and insoluble protein fractions.

DISCUSSION

The original scheme overpredicted the RDP supply and underpredicted RUP flows when compared with omasal flow data. Evaluations using previous versions of the CNCPS model reported the same directionality for biases (Kohn et al., 1998; Bateman et al., 2001b), but the RMSPE in this study are considerably lower than previously reported (Kohn et al., 1998; Bateman et al., 2001a). The greater accuracy observed in this study is probably because of a more homogeneous database and the use of actual feed analyses rather than feed library values. Likely, contributing factors to the overprediction of RDP supply in the original scheme are the predicted high degradability of the B2 fraction and the almost complete degradation of the soluble protein (B1 + A). For most feeds, the B2 fraction represents the largest protein pool size (Sniffen et al., 1992) and the feed library degradation rates for the B2 fraction are greater than most of the in situ and in vitro estimates (NRC, 2001). In addition, for most feeds, the B1 fraction represents a small percentage of the total soluble protein (Table 3), and most of the soluble protein is allocated into the A fraction, which is assumed to be immediately converted to ammonia. As a result, and similar to results with the in situ method, almost no soluble protein is predicted to be in the RUP. On average, the predicted RUP contained mostly B2 protein (~75%), B3 + C fractions (~20%), and small amounts of B1 (~5%). However, for the studies included in the evaluation, the free AA-N was escaping in a proportion similar to NDICP flows (Table 2). In other studies, the peptide-N was identified to be the most important amino N flowing out from the rumen in the liquid phase (Choi et al., 2002). Within the insoluble fraction, contributions of the B3 and C fractions were also overestimated. The original scheme overpredicted the NDICP flow out of the rumen (Table 6). The CNCPS feed library values for the degradation rates of the B3 fraction are virtually zero; therefore, it is predicted to almost completely escape microbial degradation in the ruminal. When values for the degradation rates for the B3 fraction were reassessed and adjusted (Table 4), the predictions of NDICP were improved (Table 6). However, adjusting rates for the B3 fraction (which are greater than the default feed library rates) with no other changes in the fractionation (scheme 2; Table 1) increased the bias in predicting RDP and RUP. The CNCPS model was only sensitive to NDICP measurements for feeds that contain high proportions of protein as NDICP (Lanzas et al., 2007b), but it is for those feeds (i.e., tropical forages) that rates have been reported (Juarez, 1998; Coblentz et al., 1999; Ogden et al., 2006) to be consistently greater than CNCPS B3 feed library values. Reported values for NDICP degradation rates might be similar or slightly greater than NDF degradation rates (Pichard, 1977). This fact has lead to the question of the appropriateness of using the N isolated in detergent solution as an indicator of the slowly protein degradation fraction.

Changes in the fractionation scheme were proposed to address some of the issues indicated previously. The contribution of the soluble N fractions to the RUP flows was improved by accounting for all the AAN pool in the soluble protein and adjusting B1 rates. Adjusting the B1 fraction to represent all of the AAN pool (scheme 3; Table 1) resulted in the lowest bias in RDP and RUP of all the schemes. From a nutritional point of view, the AAN fraction represents a more homogeneous

Table 5. Evaluation of the ability of alternative protein fractionation schemes to predict RDP supply and RUP flow (n = 22)

Item[1]	Scheme[2]									
	1	2	3	4	5	6	7	8	9	10
RDP										
Intercept	702.1	723.0	771.4	957.7	1,061.0	742.0	447.5	488.3	422.0	675.5
Slope	0.72	0.70	0.73	0.72	0.63	0.78	0.87	0.90	0.89	0.89
R^2	0.78	0.79	0.79	0.78	0.70	0.85	0.84	0.88	0.86	0.77
RMSE	167.7	163.7	164.7	170.6	197.7	139.9	144.8	123.7	134.4	174.2
Mean bias (MB)	−148.7	−198.7	−59.6	210.1	−28.4	123.0	80.4	210.0	125.0	411.7
MB as % of predicted mean	5	7	1	8	1	5	3	8	4	17
MB as % of observed mean	5	6	1	8	1	5	3	7	4	14
MSPE	61,653	80,769	38,103	44,142	65,536	41,209	27,761	59,363	33,599	198,292
Partition of MSPE										
Mean bias (U^M), %	35.8	48.9	1.1	52.3	1.2	37.1	23.3	74.2	46.7	85.4
Slope not equal to 1 (U^R), %	22.6	21.0	34.1	16.2	44.6	20.0	8.0	2.0	4.4	0.1
Lack of correlation (U^D), %	41.6	30.1	64.8	31.5	54.2	42.9	68.7	23.8	48.9	14.5
RMSPE	248.3	284.2	195.2	210.1	256.0	203.0	166.6	243.6	183.3	445.3
CCC	0.81	0.77	0.87	0.76	0.80	0.87	0.89	0.80	0.88	0.52
Cb	0.91	0.87	0.98	0.86	0.96	0.94	0.98	0.86	0.94	0.59
RUP										
Intercept	572.1	563.9	483.4	396.9	527.3	401.2	237.8	206.3	257.5	112.0
Slope	0.61	0.63	0.62	0.58	0.59	0.60	0.75	0.72	0.72	0.68
R^2	0.51	0.53	0.54	0.53	0.47	0.53	0.61	0.56	0.54	0.54
RMSE	201.8	197.8	196.1	198.9	209.4	197.3	181.2	191.8	195.1	196.4
Mean bias (MB)	151.8	194.1	20.5	−209.5	43.0	−163.0	−94.6	−201.5	−127.2	−416.9
MB as % of predicted mean	12	16	2	17	13	3	7	14	9	25
MB as % of observed mean	14	19	2	14	12	4	8	16	10	34
MSPE	75,625	85,264	50,850	100,679	58,516	80,486	43,890	80,698	571,667	217,902
Partition of MSPE										
Mean bias (U^M), %	30.5	44.1	0.8	43.6	3.2	33.1	20.4	50.3	28.3	79.7
Slope not equal to 1 (U^R), %	20.6	14.3	5.1	20.7	28.7	23.0	11.6	8.3	11.2	4.2
Lack of correlation (U^D), %	48.9	41.6	94.1	35.7	68.1	43.9	68.0	41.4	60.5	16.1
RMSPE	275.0	292.2	225.5	317.3	241.9	283.7	209.5	284.1	239.1	466.8
CCC	0.63	0.60	0.72	0.58	0.68	0.63	0.74	0.60	0.67	0.36
Cb	0.88	0.82	0.98	0.80	0.98	0.86	0.95	0.80	0.91	0.50

[1]RMSE = root mean square error; MSPE = mean square prediction error; RMSPE = root mean square prediction error; Cb = bias correction factor; CCC = concordance correlation coefficient. Mean bias = observed − predicted.

[2]Scheme descriptions: 1 = original; 2 = original with adjusted B3 rates; 3 = A fraction as non-AA N (NAAN; A′ fraction); 4 = A fraction as NAAN and adjusted B1 rates; 5 = A fraction as NAAN and adjusted B3 rates; 6 = A fraction as NAAN and adjusted B1 and B3 rates; 7 = aggregated insoluble fraction [inhibitory in vitro (IIV) rates], A as NPN; 8 = aggregated insoluble fraction (IIV rates), A as NAAN; 9 = aggregated insoluble fraction (IIV rates), A fraction as NPN, and adjusted B1 rates; and 10 = aggregated insoluble fraction (IIV rates), A fraction as NAAN, and adjusted B1 rates.

Table 6. Evaluation of the Cornell Net Carbohydrate and Protein System (CNCPS) predictions of the escape of the neutral detergent CP using the original protein fractionation scheme with either the default feed library B3 rates or adjusted B3 rates based on published data (n = 17)

Item[1]	Default B3 rates	Adjusted B3 rates
Intercept	96.4 ($P < 0.0001$)	101 ($P < 0.0001$)
Slope	0.27 ($P < 0.0001$)	0.31 ($P < 0.0001$)
R^2	0.77	0.78
RMSE	24.0	24.0
Mean bias (MB)	−62.31	−21
MB as % of predicted mean	28.5	11.8
MB as % of observed mean	39.8	13.4
MSPE[2]	16,281.8	9,604.0
Partition of MSPE		
Mean bias (U^M), %	23.8	4.5
Slope not equal to 1 (U^R), %	73	90.3
Lack of correlation (U^D), %	3.2	5.2
RMSPE	127.6	98
CCC	0.43	0.54
Cb	0.50	0.61

[1]RMSE = root mean square error; MSPE = mean square prediction error; RMSPE = root mean square prediction error; Cb = bias correction factor; CCC = concordance correlation coefficient. Mean bias = observed − predicted.

fraction than the NPN fraction. The NPN fraction (fraction A of the original scheme) contains both AAN (i.e., peptides and free AA) and NAAN (i.e., ammonia, amides, amines, ureides, nucleotides and nitrates). However, AAN and NAAN represent 2 distinct nutritional fractions. Peptides and free AA may stimulate microbial growth more than ammonia (Van Kessel and Russell, 1996). In addition, although the original A fraction is assumed to be completely degraded in the rumen, recent studies showed that peptides and free AA contributed to the RUP flows (Choi et al., 2002; Volden et al., 2002; Reynal et al., 2007). For corn- and alfalfa-silage based diets, the amount of N flowing as free AA out of the rumen exceeds the outflow of N insoluble in neutral detergent (Olmos Colmenero and Broderick, 2006b). Therefore, the distinction between the fraction containing NAAN and AAN is important in predicting both RUP and microbial protein flows. In addition, having the B1 fraction include all AAN may be less variable than the current B1 fraction for most feeds, and therefore, it may be more robust for use as default feed library values. Silages are the feeds with the greatest variation in the composition of the soluble protein fraction (McDonald et al., 1991). But in well-fermented silages, with predominantly lactic acid fermentation, free AAN is still the main fraction within the NPN because lactic acid bacteria have limited ability to ferment AA, with the exception of serine and arginine (Givens and Rulquin, 2004).

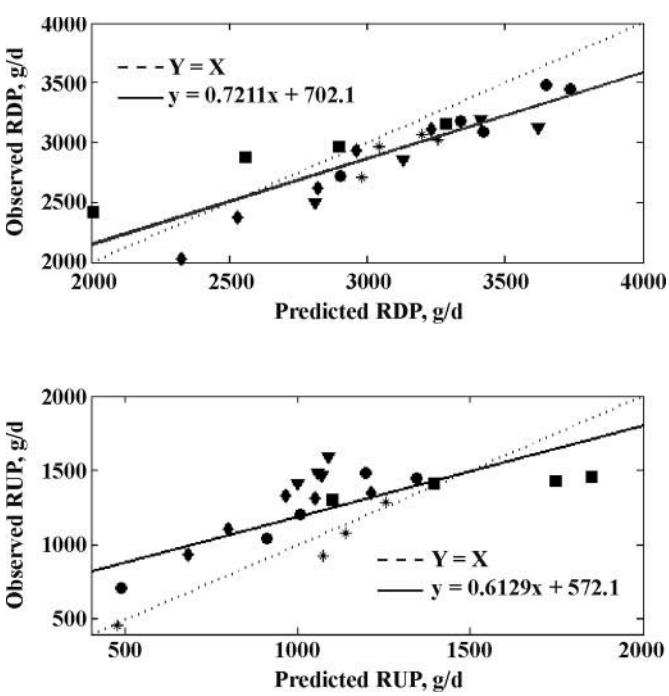

Figure 1. Predictions of the RDP supply and RUP flow using the original Cornell Net Carbohydrate and Protein System scheme for the following studies: Reynal et al. (2003) (●), Reynal and Broderick (2005) (■), Olmos Colmenero and Broderick (2006b) (◆), Brito and Broderick (2007) (*), and Brito et al. (2006) (▲).

Aggregating the insoluble fractions and using the IIV rates for the combined fraction (scheme 7; Table 1) resulted in the scheme with the greatest accuracy for both RDP and RUP (Table 7). It also resulted in a change in the sign of the bias (overpredicting RUP and underpredicting RDP), but did not address the underrepresentation of the soluble N fractions in the RUP flows. Predicted RDP, RUP, and AA flows were very sensitive to protein B2 degradation rates (Lanzas et al.,

Table 7. Ranking of the protein fractionation schemes based on their ability to predict RDP supply and RUP flow as assessed by their root mean square prediction error (RMSPE)

	RDP		RUP	
Scheme[1]	RMSPE	Ranking	RMSPE	Ranking
1	248.3	7	275	5
2	284.2	9	292.2	8
3	185.2	3	225.5	2
4	210.1	5	317.3	9
5	256	8	241.9	4
6	203	4	283.7	6
7	166.6	1	209.5	1
8	243.6	6	284.1	7
9	183.3	2	239.1	3
10	445.3	10	466.8	10

[1]Scheme descriptions: 1 = original; 2 = original with adjusted B3 rates; 3 = A fraction as non-AA N (NAAN; A´ fraction); 4 = A fraction as NAAN and adjusted B1 rates; 5 = A fraction as NAAN and adjusted B3 rates; 6 = A fraction as NAAN and adjusted B1 and B3 rates; 7 = aggregated insoluble fraction [inhibitory in vitro (IIV) rates], A as NPN; 8 = aggregated insoluble fraction (IIV rates), A as NAAN; 9 = aggregated insoluble fraction (IIV rates), A fraction as NPN, and adjusted B1 rates; and 10 = aggregated insoluble fraction (IIV rates), A fraction as NAAN, and adjusted B1 rates.

2007b). Combining both insoluble fractions (B2 + B3) makes the currently infeasible task of measuring degradation rates of these 2 fractions much easier. An implicit assumption in using the IIV rates for the insoluble fraction is that the rate for the insoluble fraction is directly proportional to the overall rate. The incubation time for the IIV method is short; therefore, the overall fractional rate may be bias toward the fastest degradable protein. For most feeds, the true soluble protein B1 represents a small percentage of the total protein. An approach not tested but that would likely increase the contribution of the soluble protein and reduce the overprediction of the RUP flow is defining the A fraction as NAAN, and the using of the Michaelis-Menten variant of the IIV method (Broderick and Clayton, 1992) to obtain rates for the combined insoluble fraction.

Proposed Modifications

To implement the best-ranked scheme (scheme 7 = aggregated insoluble fraction, A as NPN), the following aspects should be considered: 1) to implement the scheme within the current feed library, the new insoluble rate should be applied to both the B2 and B3 fractions, which would in practice collapse the 2 fractions into 1 fraction in the current versions of CNCPS versions 5 and 6; 2) the IIV method can be simplified by determining total N of the TCA-supernatants with either the combustion assay or Kjeldahl (Broderick et al., 2004b); and 3) for some groups of feeds the method may be less accurate, and modifications or alternative methods should be considered for these feeds. Degradation rates for feeds containing high levels of ammonia and free AA (e.g., grass and legume silages) are less accurate (Broderick, 1994). For those feeds, to reduce the background levels of the ammonia and free AA background levels, only the residue insoluble in borate phosphate buffer could be used for the IIV method. The method may also not be accurate for tannin-containing forages; for those forages, the Michaelis-Menten variant of the IIV method may be a more feasible approach (Broderick, 1994).

In conclusion, although further testing of more diverse dairy rations will be necessary, improvements in the accuracy of RDP and RUP predictions by the CNCPS protein fractionation scheme can be obtained when the insoluble fractions B2 and B3 are combined, resulting in a single pool and degradation rate, which can be determined by the IIV method. Evaluations of the NDICP flows indicated the ruminal escape of the NDICP was overpredicted, and thus the concept that the N insoluble in neutral detergent represents the slow degradable protein might not be appropriate and needs revision. Improvements in the accuracy of the predictions were also achieved when AAN was accounted for in the soluble fraction.

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