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Characterization of Proteins in Feeds*

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ABSTRACT

Several methods have been evaluated to partition feed crude protein (CP) into rumen-degradable protein (RDP) and rumen-undegradable protein (RUP) and to estimate the intestinal digestibility of RUP. These methods include in vivo, in situ, and a variety of in vitro methods. In situ-derived protein fractions were adopted for use to estimate RDP, RUP, and RUP digestibility in the most recent Dairy National Research Council model. In vitro, chemically determined protein fractions are used in the Cornell Net Carbohydrate and Protein System (CNCPS) that was subsequently adopted for use in level 2 of the Beef National Research Council model and in the Cornell-Penn-Miner (CPM) model (version 1). A comparison of the two methods for predicting RDP/RUP and RUP digestibility indicated remarkable similarity. Using data from 78 studies that reported measured flows of nonammonia nonmicrobial N (NANMN) to the small intestine of growing cattle and dairy cows fed 278 different diets, it was observed that the mean bias of prediction for NANMN was +1 g/d for NRC and –24 g/d for CNCPS. Whereas some disparity exists in predicted estimates of RUP for a few feeds, in most cases values are similar. To determine the impact of input uncertainty in the in situ protein system of NRC and the chemical partitioning method of the CNCPS on predicted metabolizable protein (MP) allowable milk and model-predicted RDP, a sensitivity analysis of the two models was conducted. For NRC, the highest variance in MP allowable milk was caused by variance in digestion rates of the B protein fraction, followed by the variance in the proportional sizes of the three CP fractions (i.e., A, B, and C), feed composition (e.g., CP), and RUP digestibility. For CNCPS, the highest variance in MP allowable milk was caused by the variance in feed composition, followed by the variance in the chemical components of feeds that affects the size of the five CP fractions in CNCPS (i.e., CP, soluble CP, neutral detergent insoluble CP [NDICP], acid detergent insoluble CP [ADICP], and nonprotein nitrogen [NPN]), RUP digestibility, and the digestion rates of the CP fractions. This analysis indicates that both models are sensitive to their respective inputs and that the size of the protein pools and the digestion rates of the potentially degradable protein fractions are strongly correlated to feed composition. Whenever possible, actual vs. model-default values for feed composition and pool sizes should be used.
(**Key words:** protein, feed)

Abbreviation key: ADICP = acid detergent insoluble CP, **AF** = adjustment factor, $\mathbf{K_d}$ = digestion rate, $\mathbf{K_p}$ = passage rate, **MP** = metabolizable protein, **MPMilk** = metabolizable protein allowable milk, **NANMN** = nonammonia nonmicrobial nitrogen, **NIRS** = near-infrared reflectance spectroscopy, **NDICP** = neutral detergent insoluble CP, **peNDF** = physically effective NDF.

INTRODUCTION

Feedstuffs contain a wide array of proteins and NPN compounds. Proteins are large molecules that differ in size, shape, solubility, and AA composition. Proteins are present in cell walls and cell contents of all plant and animal tissues, where they provide a variety of functions (e.g., structural, storage, catalytic, transport, and contractile). Nonprotein N compounds are smaller molecules that include peptides, free AA, nucleic acids, amides and amines, nitrate, and ammonia. Grasses and legume forages contain the highest and most variable concentrations of NPN with silages containing more NPN than hays (30 to 65 vs. 15 to 25%; NRC, 2001). The CP content of a feedstuff (percent N × 6.25) includes both true protein and NPN.

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Whereas numerous proteins and NPN compounds contribute to CP, it is understood that the nutritive value of CP in a feedstuff for ruminants is best described by its rate and extent of degradation in the rumen and the composition of the RDP and RUP fractions that result. Many factors affect the amount of CP that will be degraded in the rumen. These include the proportional content of proteins and NPN, the physical and chemical properties of proteins, the rumen retention time of the protein, microbial proteolytic activity, and ruminal pH (Broderick et al., 1991; NRC, 1985, 2001).

Much effort has been devoted to the development of feed analysis and computer models to characterize the nutritive value of CP in feedstuffs. This paper reviews the approaches that have been evaluated to estimate RDP/RUP in CP and RUP digestibility. Also presented are the results of a sensitivity analysis of the protein models as used in NRC (2001) and version 4.0 of CNCPS.

METHODS FOR ESTIMATING RDP AND RUP

The first goal in characterizing feed CP is to obtain reasonably accurate estimates of RDP and RUP. These two fractions of feed CP have separate and distinct functions in ruminant diets. Ruminally degraded CP is required for ruminal fermentation because it provides the mixture of peptides, free AA, and ammonia required for microbial growth, activity, and synthesis of microbial protein. The requirement for RDP in dietary DM to attain optimum ruminal digestion and synthesis of microbial protein is a function of the amount of carbohydrate digested in the rumen, the efficiency of microbial growth, and the degree of match between ruminal supply and microbial requirements for ammonia, AA, and peptides. In contrast, RUP provides a direct source of digestible AA to the animal. The animal's requirement for RUP in dietary DM in a given feeding situation is a function of need for metabolizable protein (**MP**) from RUP and the quality (i.e., digestibility and AA composition) of the RUP.

In Situ Method

The in situ approach is the most widely used research approach for measuring ruminal protein degradation. This method has been adopted for use in several countries and was the method adopted for use in the new Dairy NRC model (NRC, 2001).

In brief, the in situ procedure involves placing multiple samples of a feed into nylon or Dacron polyester bags with a 40- to 60-μm pore size and then placing the bags into the rumen of ruminally cannulated animals. The bags are removed at varying times of ruminal incubation and washed, and the quantity of undigested CP is determined. A minimum of three CP fractions (A, B, and C) and the digestion rate ($\mathbf{K_d}$) of fraction B can be determined. Fraction A is assumed to be completely degraded in the rumen (i.e., all RDP). This fraction is generally measured as the percentage of CP that escapes from the bag during an initial presoak period in 39°C tap water. It includes NPN, rapidly solubilized protein, and protein in particles of smaller size than the porosity of the nylon bags. Fraction B is that portion of the insoluble CP associated with particle sizes greater than the pore size of the bag that is "potentially degradable." It is that percentage of the original CP that disappears from the bag with unlimited exposure to fermentation. In most cases, unlimited fermentation refers to a defined endpoint of 48 to 72 h depending on the feed type. The NRC (2001) suggests that samples be incubated for 0, 2, 4, 8, 16, 24, and 48 h (72 h for forages). The amount of fraction B that is degraded in the rumen depends on competing K_d of fraction B and the passage rate ($\mathbf{K_p}$) of undigested feed. Fraction C is assumed to be undegradable in the rumen (i.e., all RUP). Ruminally undegradable protein is that percentage of the original CP remaining in the bag at the defined endpoint of degradation. The equations for computing RDP and RUP values (percentage of CP) are $RDP = A + B[K_d/(K_d + K_p)]$ and RUP = CP − RDP, or $B[K_p/(K_d + K_p)] + C$.

Two approaches have been used for arriving at estimates for the K_p of fraction B. One approach has been to assign fixed K_p values to feeds. In some cases, a fixed value is used for all feeds (e.g., 0.08 in the Scandinavian system and 0.06 in the French and Italian systems). In other cases, such as in the DVE/OEB system of the Netherlands and Belgium, different fixed values for forages and concentrates are used (i.e., 0.045 for forages and 0.06 for concentrates). The other approach has been to use variable K_p values for feedstuffs where the values are calculated from feed intake as well as feedstuff and diet characteristics (e.g., UK, Australian models, and 2001 Dairy NRC). Three equations are used in NRC (2001) for predicting K_p in the Dairy Model; one for wet forages ($K_p = 3.054 + 0.164 * [DMI, \% BW]$), one for dry forages ($K_p = 3.362 + 0.479 * [DMI, \% BW] - 0.007 * [\%$ concentrate in diet DM$] - 0.017 * [\%$ NDF in forage DM]), and one for concentrates ($K_p = 2.904 + 1.375 * [DMI, \% BW] - 0.020 * [\%$ concentrate in diet DM]). It is clear from these equations that both DMI and BW are important factors affecting K_p, which in turn affects the calculated RUP content of a feed. These equations do not consider some of the other factors that affect passage rate, such as particle size and particle density. However, data were too limited to make adjustments for those factors in the equations.

The greatest strength of the in situ method is that it allows exposure of feedstuffs to the digestive conditions thought to be similar to those existing in vivo. Coupled with the use of equations that predict rates of passage of undigested feed, a dynamic system is in place for predicting the RDP and RUP content of feedstuffs. Because several factors can affect an in situ-derived estimate of ruminal degradation (e.g., bag porosity, particle size, bag and sample size, bag location within the rumen, washing/rinsing procedures, microbial contamination, incubation times, mathematical models, animal diet, and frequency of feeding), adherence to proposed guidelines for standardizing these factors (e.g., Lind berg, 1985; Setälä, 1985; Nocek, 1988; Michalet-Doreau and Ould-Bah, 1992; Vanzant et al., 1998; Broderick and Cochran, 2000; NRC, 2001) increases the reproducibility of the results.

The primary shortcoming of the in situ method is that it is labor intensive and requires the use of cannulated animals, both of which makes it the most costly method for obtaining the RDP and RUP values for a feedstuff. A few commercial laboratories maintain ruminally cannulated animals and provide in situ-derived estimates of ruminal protein degradation. Another shortcoming of this method is the disappearance from the bag of soluble proteins. Soluble proteins vary in rate of ruminal degradation and therefore cannot be assumed to be degraded completely in the rumen. Thus, protein solubility is not synonymous with protein degradation (NRC, 2001).

In Vitro Enzymatic Methods

Several different in vitro enzymatic methods have been evaluated for their ability to yield accurate predictions of ruminal protein degradability. Understandably, a goal has been to identify a method that performs at least as well as in the in situ method. The methods can be categorized as "ruminal in vitro methods" that involve incubations with mixed ruminal microorganisms (i.e., ruminal digesta) and "nonruminal in vitro methods" that involve incubations with cell-free enzymes. In both cases, the rate of protein degradation is calculated from the rate of accumulation of AA and ammonia, the products of protein degradation.

Most "ruminal in vitro methods" are complicated by microbial uptake of the released AA and ammonia, causing degradability to be underestimated. They are also complicated by the release of AA and ammonia from the catabolism of the microbial and residual feed proteins present in the inoculum causing degradability to be overestimated. The release of AA and ammonia by microbial catabolism of inoculum protein is usually accounted for by using incubations that contain all components except the test protein, called "blanks." However, microbial uptake of AA and ammonia cannot be dealt with in the same way. To overcome this problem, Broderick (1987) developed an "inhibitor in vitro (IIV)" method in which hydrazine and chloramphenicol are added to the in vitro system. The result is a complete blocking of microbial uptake of AA and ammonia. Incubations are limited to 4 to 6 h because the system is subject to end-product inhibition.

The use of the IIV system, coupled with the use of blanks to correct for AA and ammonia production from the microbial catabolism of the inoculum proteins, has shown considerable promise for protein supplements and concentrate feeds. However, as reviewed by Broderick and Cochran (2000), the IIV system is less suitable for grass and legume forage silages as they contain high levels of AA and ammonia. The high "initial" concentrations of AA and ammonia constitute a high "background" and makes the determination of the rate of breakdown of the more slowly degraded residual proteins less accurate.

Many workers have reported results using "nonruminal in vitro systems" (e.g., Assoumani et al., 1992; Licitra et al., 1998, 1999). A goal has been to identify a protease or mixture of proteases that would yield estimates of degradation fractions and rates that are similar to those generated by mixed ruminal microorganisms. If that could be done, then such a system would have an advantage over the "ruminal in vitro systems" that must deal with the presence of the "interfering" microbes.

Most of the cell-free enzymes that have been evaluated have been commercial proteases rather than proteases extracted from mixed ruminal microbes. The two most studied enzymes have been *Streptomyces griseus* and ficin. Others that have been studied include Bromelain, papain, porcine pancreatin, *Aspergillus oryzae*, and *Bacillus subtilis*. The usual approach has been to correlate estimates of the "extent" of ruminal protein degradation obtained with the proteases to in situ estimates of the "extent" of degradation. Generally, the results have been well correlated. However, as pointed out by Broderick and Cochran (2000), cell-free proteases have not been shown to be very reliable in their ability to predict rates of ruminal protein. This was demonstrated by Luchini et al. (1996), who characterized the proteolytic activity of mixed ruminal microorganisms using 13 artificial substrates and then constituted blends of commercial proteases to try to match this proteolytic activity. Although three blends were identified that had hydrolytic activities similar to those of mixed ruminal microorganisms toward the artificial substrates, none of the blends degraded conventional feed proteins at the same rates as the use of mixed

Table 1. Degradation rate (% per hour) of feed proteins determined in incubations with strained rumen fluid or a blend of cell-free proteases (trypsin, carboxypeptidase B, chymotrypsin, and carboxypeptidase A).

Feeds	Rumen fluid	Cell-free proteases
Beet pulp	0.6	3.0
Corn meal	2.7	2.9
Distillers grain	3.2	1.3
Shell corn	5.4	4.2
Ear corn	7.5	4.8
Linseed	8.4	4.1
Barley	9.0	3.3
Canola meal	9.2	4.9
Solvent extracted soybean meal	10.0	4.2
Dried whey	10.1	7.0
Roasted soybeans	14.4	6.1
Oats	14.7	6.6
Wheat bran	15.6	4.6
Wheat	20.1	6.0
Casein	21.6	9.8

ruminal microorganisms. The degradation rates obtained for 15 feeds using one of the three protease blends compared with the rates obtained with the inhibitor in vitro system that uses strained rumen fluid are presented in Table 1.

The use of in vitro enzymatic methods to predict rates of protein degradation in the rumen offers laboratory utility and analytical precision. However, while considerable progress has been made (Licitra et al., 1999; Broderick and Cochran, 2000), no single method has emerged as being universally acceptable for all feedstuffs. Challenges associated with interfering compounds (e.g., starches and fiber), identification of the appropriate enzyme/substrate ratio, and so on still remain.

In Vitro Chemical Methods

A variety of different solvents have been evaluated for their ability, as individual solvents, to fractionate CP into RDP and RUP, or at the very least, have a high correlation between N solubility and protein degradability. However, as reviewed in NRC (2001), CP solubility (i.e., N solubility) in a single solvent is not synonymous with CP degradation in the rumen. Moreover, it is clear that soluble proteins are not equally susceptible to ruminal degradation, and that insoluble proteins are not equally resistant to degradation (NRC, 2001). Thus, it appears that a single solvent will not be identified that can fractionate feed CP into RDP and RUP fractions across diverse feed types. However, it is noted in the papers referenced by NRC (2001) that protein that goes into solution in a solvent generally has a higher probability of being degraded in the rumen than a protein that does not go into solution.

In Vitro Multi-Chemical Methods

The most widely used and sophisticated multi-chemical approach for quantifying N fractions in feedstuffs is the protein fractionation scheme used in the CNCPS (Sniffen et al., 1992; Fox et al., 2000) and adopted for use in the Beef NRC model (NRC, 1996). The CNCPS partitions CP into 5 fractions using 3 solvents and a protein-precipitating agent. The 5 fractions are: A (NPN; soluble in borate-phosphate buffer but not precipitated with tungstic acid), B_1 (rapidly degraded true protein; soluble in borate-phosphate buffer and precipitated with tungstic acid), B_2 (moderately degraded true protein and large peptides; calculated as the difference between total CP and the sum of the other 4 CP fractions), B_3 (slowly degraded true protein; calculated as the difference between neutral detergent insoluble CP [**NDICP**] and acid detergent insoluble CP [**ADICP**]), and C (undegraded true protein; measured as ADICP). The NDICP is derived by determining CP on the insoluble residue of an NDF extraction without the use of sodium sulfite. The ADICP is determined as the CP associated with the insoluble residue of an ADF extraction. Fraction A is assumed to be 100% degraded in the rumen and fraction C is assumed to be undegradable. Therefore, as with the A and C fractions in the in situ method, there is no need to assign rates of degradation (K_d) to these fractions. An exception to this are some specialty feeds whose K_d are assigned to the A fraction. The K_d that is assigned to each of the three B fractions vary (B_1 = 120 to 400%/h, B_2 = 3 to 16%/h, and B_3 = 0.06 to 0.55%/h). Published K_d and methodology can be found in Pichard and Van Soest (1977), Van Soest (1982), and Sniffen et al. (1992). While major classes of feeds had K_d determined in Sniffen et al. (1992), some values within feed classifications were radiated from similar species.

The authors of CNCPS also recognized that ruminal disappearance is a competitive function between K_d and K_p and that K_p varies with feed intake, feed, and diet characteristics. Two equations are used for predicting passage rate of undigested feed, one for forages (K_P = 0.388 + 22.0 * [DMI/$BW^{0.75}$] + 0.0002 * [% forage in diet DM]) and one for concentrates (K_P = -0.424 + [1.45 * K_P of forages]). The K_P are adjusted for individual feeds using a multiplicative adjustment factor (**AF**) for particle size using diet physically effective NDF (**peNDF**) (Russell et al., 1992; Mertens, 1997, 2002). There are two equations for calculating the adjustment factor, one for forages (AF = 100/[peNDF + 70]) and one for concentrates (AF = 100/[peNDF + 90]). In summary, the equations for calculating RDP and RUP values (as a percentage of CP) are RDP = A + B_1 (K_dB_1 / [K_dB_1 +

K_p]) + B_2 (K_dB_2 / [K_dB_2 + K_p]) + B_3 (K_dB_3 / [K_dB_3 + K_p]) and RUP = 1 − RDP.

The multi-chemical approach for characterizing feed CP was chosen for the CNCPS for 2 reasons. First, the procedures for determining the chemical fractions (NPN, soluble true protein, NDICP, and ADICP) that are required for determining the CP fractions (A, B_1, B_2, B_3, and C) can be performed under routine laboratory conditions. Other than separating NPN from true protein in soluble CP, the other chemical fractions are routinely analyzed for. This results in less reliance on model default values for pool sizes and, at least theoretically, offers a more robust method of estimating pool sizes. An ongoing goal of the CNCPS is for it to be a field-usable model with inputs that can be collected on-farm. Second, one of the original purposes of the CNCPS model was to be able to predict, as accurately as possible, microbial growth and ruminal fermentation (Russell et al., 1992). Two pools of microbes exist in the CNCPS, and microbial growth is a function of carbohydrate availability given availability of appropriate nitrogen sources (Russell et al., 1992). Nitrogen must be supplied as NPN or free AA and peptides. Structural carbohydrate fermenters can only utilize ammonia as their nitrogen source (CNCPS protein fraction A). Nonstructural carbohydrate fermenters also utilize ammonia, but their growth is enhanced via peptide availability. Peptide availability is the result of protein degradation in the rumen and is calculated by: B_1 (K_dB_1 / [K_dB_1 + K_p]) + B_2 (K_dB_2 / [K_dB_2 + K_p]) + B_3 (K_dB_3 / [K_dB_3 + K_p]) (Russell et al., 1992; Sniffen et al., 1992). Free AA and peptides can be fermented resulting in ammonia production by several microbial species, thus adding to the ammonia pool for structural carbohydrate and NSC microbial growth (Russell et al., 1992). These species are indirectly included in the rumen submodel.

A recognized deficiency of the equations for predicting RDP in both CNCPS and NRC (2001) is the lack of consideration of passage lag and digestion lag. This is of concern because meals are not equally sized and spaced and because feedstuffs exhibit and differ in passage lag (Murphy and Kennedy, 1993; Wylie et al., 2000) and digestion lag (Varga and Hoover, 1983; Nocek and Grant, 1987; Coblentz et al., 2000; Mustafa et al., 2000). Further research is needed to model particle size reduction via rumination and ruminal degradation and the impact of non-steady-state conditions (e.g., unequally spaced and sized meals) on these digestive processes.

METHODS FOR ESTIMATING RUP DIGESTIBILITY

Having accurate estimates of the intestinal digestibility coefficients of the RUP as provided by each feedstuff is fundamental to balancing diets for RUP. As a result, a growing number of feeding standards account for differences in RUP digestibility among feedstuffs.

Several methods have been used to obtain estimates of RUP digestibility. These include in vivo procedures, in vitro techniques, nonruminant animal bioassays, the in situ mobile nylon bag technique, and the use of ADICP. The most widely reported approach is the mobile bag technique. This approach consists of placing small amounts of washed ruminally undegraded feed residues in bags, preincubating them in a pepsin/HCL solution for 1 to 3 h, and then inserting them into the duodenum of cannulated ruminants. The bags are recovered from the feces, washed thoroughly to remove endogenous and other contaminating protein, and analyzed for protein content. Research has shown good correlation between estimates of RUP digestibility with this method and in vivo-derived estimates (Hvelplund, 1985; Todorov and Griginov, 1991).

An excellent alternative to the mobile bag technique, at least for protein supplements, is the 3-step in situ/in vitro procedure developed at the University of Minnesota (Calsamiglia and Stern, 1995). This procedure consists of incubating undegraded feed residues (after 16 h of in situ ruminal digestion) for 1 h in 0.1 *N* HCL solution containing 1 g/L of pepsin, neutralizing the mixture with 1 *N* NaOH and a pH 7.8 buffer containing pancreatin followed by a 24-h incubation, and precipitation of undigested protein with 100% TCA. Research is under way to extend the use of this procedure to other feedstuffs, such as forages and concentrates, and also microbial fractions (M. D. Stern, unpublished data).

The estimates of RUP digestibility assigned to each feedstuff in the NRC (2001) model are the approximate mean values as reported in the literature using the mobile bag technique and the 3-step procedure of Calsamiglia and Stern (1995). For feeds with limited or no data, the values used in the French protein system (Jarriage, 1989) were adopted. The values that were assigned range from a low of 50% for almond hulls, canola seeds, and cottonseed hulls to a high of 100% for molasses.

In the CNCPS, intestinal digestibility coefficients are assigned to each of the CP fractions (A = 1.00, B_1 = 1.00, B_2 = 1.00, B_3 = 0.80, and C = 0.00). Whereas digestibility coefficients are "assigned" to protein fractions in CNCPS, the coefficients are not assigned to the feedstuff, which means that model-predicted RUP digestibility values are not fixed as in NRC (2001) and vary with changes in distribution of CP among the five CP fractions. However, the extent to which this is an advantage for CNCPS is not known. It must be acknowledged that the intestinal digestibility coefficients for B_1, B_2, B_3, and C are not always as indicated. This is particularly true for the assumption that fraction C

(i.e., ADICP) always has a digestibility of 0.00. Several studies indicate that variable amounts of ADICP are digested in the small intestine (NRC, 2001; McNiven et al., 2002).

A COMPARISON OF THE IN SITU AND MULTI-CHEMICAL METHODS FOR PREDICTING FLOWS OF RUP

As noted previously, in situ-derived protein fractions are used in NRC (2001) and in vitro, chemically-determined protein fractions are used in CNCPS to estimate RDP/RUP in CP and RUP digestibility. Because the in situ and multi-chemical methods are two completely different approaches for determining pool sizes in feed CP, a reasonable question to ask is, how well do the two approaches compare for predicting RDP/RUP and RUP digestibility?

As with any method that is being evaluated for its accuracy in predicting the RUP and RDP content of a feed, no standard method of validation exists. However, the 2 models were compared for their ability to predict flows to the duodenum of nonammonia nonmicrobial nitrogen (**NANMN**) when a variety of diets were fed. The data used in this comparison consisted of 74 studies that reported flows of NANMN to the duodenum of growing cattle and cows fed 278 different diets. Of the 278 diets, 33 were fed to growing cattle and 245 were fed to dairy cows. The 278 diets are a subset of the 390 diets that were used in the initial evaluation of the protein model in NRC (2001). The diets selected for this model comparison were restricted to include only those diets that consisted of conventional ingredients found in the feed libraries of the two models.

The 278 diets were entered into the NRC (2001) and CNCPS (version 4.0) models. All of the required animal and diet data for the diets were entered into the models. The chemical composition of feedstuffs was entered if reported; otherwise, model default values were used. No data set was reported for in situ-determined CP fractions (A, B, and C) or the K_d of fraction B. In the 74 studies used in this analysis, a total of 138 forages and 245 grains were used. Out of the 138 total forages used, only 62 CP values, 12 soluble CP values, 0 NDICP values, 8 ADICP values, 51 NDF values, and 53 ADF values were reported. Out of the 245 total grains used, only 33 CP values, 7 soluble CP values, 0 NDICP values, 1 ADICP value, 18 NDF values, and 25 ADF values were reported. It is important to note that when using a computer model to evaluate diets, the outputs generated by the model are only as accurate as their inputs will allow. Therefore, relying on model default values is not an effective or reliable method for generating accurate outputs. However, for the purposes of this exercise, the authors had no other alternative than to rely on model default values for a large majority of the feedstuffs used. For NRC (2001), predicted NANMN values were calculated as the sum of predicted flows of RUP and endogenous protein multiplied by 0.16 to make the conversion from CP to N. For CNCPS, predicted NANMN values were calculated only from the predicted flow of RUP because the CNCPS does not consider a contribution of endogenous protein to NANMN.

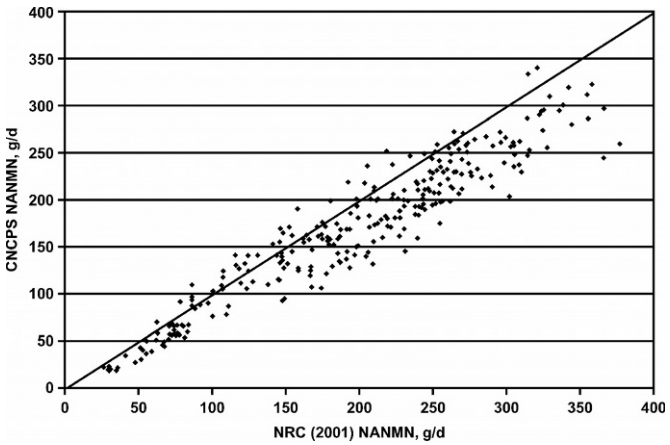

Figure 1. Plot of NRC (2001) vs. CNCPS (version 4.0) predicted flows of non-ammonia non-microbial N (NANMN) involving 278 diets fed to growing cattle (33 diets) and dairy cows (245 diets).

Figure 1 shows a plot of the NRC (2001) vs. CNCPS predicted flows of NANMN. The CNCPS model generally predicted lower flows, which was an expected observation because CNCPS does not predict a contribution of endogenous N. The NRC (2001) mean predicted flows of RUP-N and endogenous N were 160 g/d and 34 g/d, respectively. The mean predicted flow of RUP-N for CNCPS was 169 g/d. Therefore, the mean difference between the two models in predicting flow of RUP was only 9 g.

Figure 2 shows plots of the residuals for flows of NANMN (predicted values-measured values) as obtained from the above exercise regressed on DMI for the 2 models. The regression of residuals on DMI was done in recognition of the fact that feed intake affects passage rates of undigested feed that in turn also affects the RDP/RUP content of a feed. Although the number of observations for growing cattle is small (n = 33), the residual plots for both groups of animals are presented. On average, discrepancies are small between predicted and measured flows of NANMN. The mean biases for the combined data set for NRC and CNCPS were +1 g/d and –24 g/d, respectively. A similar amount of "scatter" of residuals about the "zero line" exists for the 2 models, indicating little difference in the use of in situ-

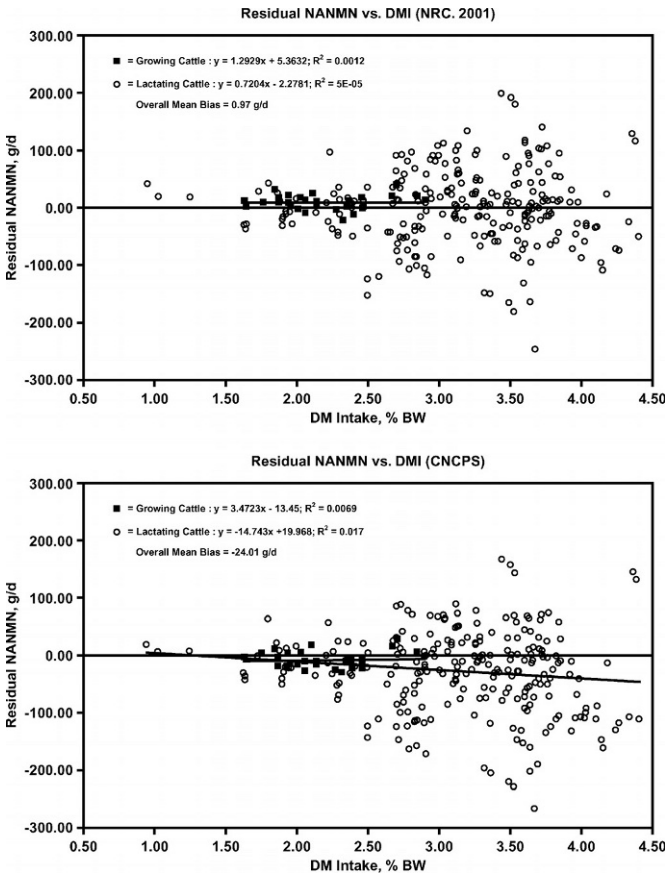

Figure 2. Plots of residual NANMN (predicted — measured) vs. DMI for NRC (2001) and CNCPS with best fit line for growing and lactating cattle datasets.

Table 2. Comparison of RUP digestibility values between NRC (2001) and CNCPS for selected feeds.

Forages	NRC	CNCPS
Alfalfa silage, mid-maturity	65	41
Alfalfa hay, immature	75	77
Bermudagrass hay, Tifton-85	65	64
Corn silage, normal	70	61
Grass silage, mid-maturity	60	53
Wheat silage, early head	70	65
Wheat straw	65	12
Energy and by-product feeds		
Almond hulls	50	24
Barley grain	85	83
Corn grain, ground, dry	90	86
Corn grain, steam-flaked	90	88
Brewers grains, dried	90	73
Beet pulp	80	66
Citrus pulp, dried	80	67
Corn distillers grains with solubles	80	59
Cottonseed hulls	50	73
Soybean hulls	70	63
Wheat midds	90	87
Cottonseed, whole w/ lint	80	77
Plant and animal protein		
Soybean meal, solvent extracted, 48%	93	93
Canola meal, mechanically extracted	75	79
Soybean meal, expellers	93	85
Soybean meal, nonenzymatically browned	93	78
Soybeans, heated	85	91
Blood meal	80	89
Corn gluten meal	92	94
Fish meal, Menhaden	90	92
Hydrolyzed feather meal	65	85
Meat meal	80	81

derived protein fractions as used in NRC (2001) vs. in vitro chemically derived protein fractions as used in the CNCPS to predict ruminal degradation of feed protein.

Of concern, however, are the negative slope bias and the negative mean bias that is evident for the CNCPS. As evidenced by the residual plots, both observations pertain only to dairy cows. As noted previously, the NRC (2001) and CNCPS models use different equations for predicting passage rate of undigested feed. It appears that the primary reason for the negative slope bias for the plot of residuals on DMI for the cow data set, and thus the under-prediction of NANMN, is the inability of the equation in CNCPS that predicts passage of undigested concentrate feeds to fully account for the effects of DMI as a function of cattle BW on protein degradability.

A final concern is the amount of "scatter" of residuals about the "zero line" for both models. Undoubtedly, part of the scatter is the result of errors in measuring passage of NANMN to the small intestine. Errors in measurement include errors in quantifying passage of digesta to the small intestine as well as errors in estimating the content of microbial N in NAN (NANMN = NAN – microbial N). The extent to which these "measurement errors" contribute to the "scatter" cannot be determined. However, it is the opinion of the authors that most of the scatter is the result of errors in predicting passage of NANMN to the small intestine. Errors in prediction would include model shortcomings (e.g., lack of or inadequate rumen submodels) as well as our need to rely so extensively on model default values for feed. Given the large variance in chemical compositions within feed types (Tylutki, 2002), the use of default composition values in a model evaluation is primarily an evaluation of the feed library. This introduces both type I and II errors into the evaluation. Therefore, this analysis can only be used as an index of model directionality.

A COMPARISON OF THE IN SITU AND MULTI-CHEMICAL METHODS FOR PREDICTING RUP DIGESTIBILITY

Twenty-nine common, but diverse, feedstuffs were selected to compare estimates of RUP digestibility as provided by NRC (2001) and CNCPS (Table 2). As noted

Table 3. Least squares means for NDF, CP, CP fractions (A, B, and C), and ruminal degradation rates (K_d) of fraction B for alfalfa silage, birdsfoot trefoil silage, and red clover silage from the NRC (2001) protein database.[1,2]

	NDF % DM	CP % DM	A % CP	B % CP	C % CP	K_d of B %/h
Alfalfa silage	36.0[a]	24.6[a]	58.9[a]	30.6[a]	10.5[a]	9.3[a]
Birdsfoot trefoil silage		22.3[a,b]	86.4[b]	11.2[b]	2.4[b]	17.6[b]
Red clover silage	28.3[b]	20.7[b]	48.2[a,c]	40.0[a,c]	11.8[a]	18.8[b]

[1]Provided by C. G. Schwab, NRC (2001) committee member.

[2]Means within columns with different superscripts differ at $P < 0.05$. Superscript c indicates a trend toward difference at $P < 0.10$.

previously, estimates, as derived from the mobile bag technique and the 3-step procedure of Calsamiglia and Stern (1995), are assigned to feeds in NRC (2001), whereas in CNCPS, the values are predicted from feed analysis (specifically soluble protein, NDICP, and ADICP) and the digestibility coefficients that are assigned to the five CP fractions. Again, whereas the methods for estimating RUP digestibility are different, values are similar for most feeds (Table 2). A diet formulated using all of the feeds in Table 2 and balanced to meet NRC (2001) requirements for 44 kg of milk resulted in an intestinal digestibility of diet RUP of 80.3 for NRC and 79.4% for CNCPS.

SENSITIVITY ANALYSIS OF IN SITU AND CHEMICAL APPROACHES

Modeling a biological system is a complex and daring task that must include rigorous testing to ensure that the model performs appropriately under a wide range of conditions. This testing procedure (i.e., sensitivity analysis) can be accomplished in several ways. The simplest method is to change all desired inputs to their most optimistic value followed by an analysis in which their most pessimistic values are inputted, basically testing model directionality. A second method is to change the desired inputs in combinations, acceptable during model development but not during full model evaluation because it is tedious and assumes that the user is aware of all potential correlations between inputs. For example, a model with 13 inputs, with each input changed to represent a minimum, mean, and maximum value, consists of 1,594,323 combinations that must be tested. The third method involves either Monte Carlo sampling or Latin hypercube sampling. These methods ensure sampling from the entire input distribution. The number of iterations required is much lower, while maintaining statistical relevance. Regardless of the method chosen, the objective of sensitivity analysis is to determine if the model "behaves" as expected and, if it does, it is assumed to be "robust." A robust model is one that generates the same general result pattern despite uncertainty in parameter values (Ford, 1999).

The objectives of this study were to determine the sensitivity of the in situ protein system utilized in NRC (2001) and the chemical partitioning method of the CNCPS to input uncertainty and to determine which components need to be analyzed to reduce risk of use under field conditions.

Methodology and Data Sources

A sensitivity analysis of the protein systems in the NRC (2001) and CNCPS version 4.0 was conducted using Monte Carlo sampling techniques. Both systems were reprogrammed in Microsoft Excel and a commercially available add-in. Monte Carlo sampling (@ Risk v. 4.5, Palisade Corporation, 2001) was utilized to conduct the simulations, allowing a distribution (mean and associated variance) to be assigned to any parameter (Conrow, 2000). As the simulation proceeds, samples are randomly drawn from the distribution, and a sample tally is recorded (Hardaker et al., 1997) forcing the

Table 4. Means and standard deviations (SD) for NDF, CP, CP fractions (A, B, and C), and ruminal degradation rate (K_d) of fraction B for alfalfa silage used in the sensitivity analysis of the two models.

	NDF % DM[1]	CP % DM[a]	A % CP	B % CP	C % CP	K_d of B %/h
Mean	36.0	24.6	58.9	30.6	10.5	9.3
SD	1.6	3.8	11.7	11.8	6.4	4.0

[a]NDF and CP means were used to develop the correlation matrix. The means and SD for the sensitivity analysis were from Tylutki (2002).

Table 5. Initial animal, environment, and ration inputs used in the sensitivity analysis of the models.

Animal factors	Input	Feeds in diet	Input, kg DM/d
BW kg	700	Corn silage	6.45
Mature weight, kg	750	Alfalfa silage	3.66
Milk production, kg/d	45.4	Grass silage	3.38
Milk fat, %	3.70	Whole cottonseed	2.29
Milk protein, %	3.10	Corn grain	7.13
		Soybean meal 48%	1.86
		Expellers soybean meal	1.63

cumulative distribution function to equal one at completion. An additional Monte Carlo constraint is that correlations among parameters must be included for model validity (for example, if the DM and NDF of a feed are positively correlated, as samples are drawn from DM, the corresponding NDF sample will be in the same direction, depending on the strength of the correlation) (Palisade Corporation, 2001). Results from such models include an estimate of the distribution, mean, and standard deviation for the parameters varied and any output. Sensitivity analysis can also be conducted utilizing either regression or correlation techniques (Palisade Corporation, 2001). While either regression or correlation values can be used, correlations are easily ranked and interpreted. These allow for the development of recommendations that will control these input parameters, thereby partially controlling output variance (Palisade Corporation, 2001).

The normal distribution (N[μ,σ]) was used for all feed composition inputs with the exception of protein intestinal digestibilities in the CNCPS, where the triangular distribution was used. Feed composition values were from 1 yr of a 2-yr intensive feed sampling project on the 600-cow dairy farm described by Tylutki and Fox (2000). Feeds from this study included corn silage, alfalfa silage, grass silage, corn meal, whole cottonseed, and solvent extracted soybean meal (47.5% CP). Analysis of feeds was conducted by a commercial laboratory (Dairy One, Ithaca, NY) via wet chemistry methods and represent the within-farm variance of home-raised and purchased feeds (Tylutki, 2002). Composition and variance values for expellers soybean meal were taken from the NRC (2001). The means, standard deviations, and correlations among components were calculated for all components using SAS (1999) with results entered into @Risk (expellers soy correlations were assumed to be the same as the solvent extracted soy).

Correlations for the in situ protein pools were calculated from the NRC (2001) protein development database. The NRC (2001) treats all legumes as one feed type, and our statistical analysis (least squares means and Bonforroni *t*-test) of the original data set indicates significant differences between legume types (Table 3). Based on this analysis, average protein fractions A, B, and C, and K_d for alfalfa silage, were used in the sensitivity analysis (Table 4). An example diet was formulated to support 45.4 kg of milk production. Initial inputs for animal, environment, and feed amounts can

Table 6. Feed composition values for the feeds used in the sensitivity analysis for both models.

Feed name component	Corn silage		Alfalfa silage		Grass silage		Corn grain		Whole cotton		Soy meal 48		Expellers soy	
	Mean	SD[1]	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Both models														
NDF, % DM	44.7	3.75	46.9	5.44	60.1	6.72	10.8	0.96	54.8	5.59	11.3	1.92	21.7	8.00
Lignin, % DM	3.8	1.04	8.3	1.73	6.5	2.01	1.5	0.81	15.6	2.14	1.4	0.95	1.5	0.80
CP, % DM	8.5	0.44	20.8	2.43	17.2	1.93	8.8	0.57	24.4	1.84	53.0	1.88	46.3	3.20
NDICP, % DM[2]	1.3	0.25	3.2	0.56	3.9	0.93	1.0	0.13	2.4	0.40	5.9	2.98	9.6	5.90
ADICP, % DM[3]	0.8	0.25	1.6	0.33	1.5	0.42	0.8	0.19	1.9	0.36	1.7	0.64	0.4	0.10
Fat, % DM	3.2	0.36	3.9	0.47	5.0	1.05	4.2	0.23	19.9	3.26	1.7	0.28	8.1	3.20
Ash, % DM	3.6	0.55	10.7	1.26	9.2	1.45	1.5	0.18	4.4	0.46	6.9	0.39	5.5	0.90
NRC (2001) only														
A, % CP	51.3	17.00	58.9	11.71	60.1	9.90	23.9	12.50	45.4	10.00	15.0	6.20	8.7	9.70
B, % CP	30.2	13.70	30.6	11.82	31.8	8.80	72.5	14.70	46.7	10.00	84.4	5.60	91.3	9.70
C, % CP	18.5	5.70	10.5	6.36	8.1	3.60	3.6	8.30	7.9	10.00	0.6	1.90	0.0	0.00
K_d of B, %/h	4.4	1.60	9.3	3.95	8.1	4.60	4.9	2.00	15.7	2.00	7.5	2.40	2.4	0.80
RUP digest, %	70.0	3.50	70.0	3.50	65.0	3.25	90.0	4.50	80.0	4.00	93.0	4.65	93.0	4.65
CNCPS only														
Soluble, % CP	57.2	1.29	61.3	7.28	54.4	9.12	15.8	3.40	23.9	6.39	21.8	5.73	8.0	2.00
NPN, % soluble	100.0	10.00	50.0	5.00	96.0	9.60	73.0	7.30	2.5	0.46	55.0	5.50	55.0	5.50
K_d of B_1, %/h	300.0		150.0		200.0		135.0		175.0		230.0		150.0	
K_d of B_2, %/h	15.0		11.0		9.0		7.0		8.0		11.0		5.0	
K_d of B_3, %/h	0.3		1.8		1.8		0.1		0.3		0.2		0.2	

[1]Standard deviation.

[2]CP fraction B_3 + C in CNCPS.

[3]CP fraction C in CNCPS.

Table 7. Evaluations conducted in the sensitivity analysis of NRC (2001) and the CNCPS (version. 4.0).

Components varied	NRC (2001)	CNCPS
Base	0	00
Feed composition	1	8
Protein fractions	2	9
Degradation rate	3	10
RUP digestibility	4	11
Feed composition and protein fractions	5	12
All protein inputs	6	13
Protein pools calculated from chemical analysis	7	
NRC (2001) degradation rates and RUP digestibilities		14
CNCPS pool calculations and intake equation	15	

Table 8. Mean and standard deviations (SD) for simulated metabolizable protein allowable milk (kg/d) and RDP (% DM) when input uncertainty is included.

	MP allowable milk, kg/d		Ration RDP, % DM	
Evaluation[1]	Mean	SD	Mean	SD
0 (base)	50.9		11.6	
00 (base)	49.0		11.2	
1	49.1	1.1	11.2	0.4
2	49.0	1.8	11.2	0.4
3	49.8	2.0	11.0	0.4
4	49.0	0.8	11.2	0.0
5	49.1	2.3	11.2	0.6
6	49.5	2.8	11.1	0.7
7	53.7	2.2	10.1	0.6
8	48.7	1.7	11.2	0.3
9	48.8	1.3	11.4	0.3
10	48.8	0.9	11.2	0.1
11	45.8	1.0	11.1	0.3
12	48.9	2.0	11.4	0.3
13	45.9	1.8	11.2	0.3
14	52.3	2.6	10.5	0.4
15	49.8	1.6	10.4	0.4

[1]See Table 7.

be found in Table 5. Table 6 lists the means and standard deviations for each feed and component used in the evaluation.

The diet was formulated using the average feed composition, ruminal degradation rates, and intestinal digestibilities. For all evaluations, DMI was forced to equal the respective model's predicted intake (27.8 and 26.4 kg/d for NRC [2001] and CNCPS, respectively). This was done because each system was developed to work with specific intake equations.

Using different intakes for each system (and forcing each iteration to equal predicted intake) does result in different model predicted passage rates; however, this evaluation was meant to determine the impact of feed composition and ruminal degradation rate variance on predicted RUP flow of the models as implemented. A model sensitivity analysis due to passage rate predictions, in addition to other predictive equations, needs to be conducted. For all evaluations, metabolizable protein allowable milk (**MPMilk**, kg/d) and diet RDP concentration were the outputs analyzed. Table 7 lists the evaluations conducted. Evaluations 1 (NRC, 2001) and 8 (CNCPS) varied all feed chemical composition inputs to determine the impact of chemical and in situ input uncertainty on desired outputs. Evaluations 2 (NRC, 2001) and 9 (CNCPS) varied only the inputs that impact protein fraction size (A, B, and C proportions for NRC [2001]; CP, soluble CP, NDICP, ADICP, and NPN for CNCPS). Evaluations 3 (NRC, 2001) and 10 (CNCPS) varied only the protein ruminal degradation rates. Evaluations 4 (NRC, 2001) and 11 (CNCPS) only varied the RUP digestibilities using either determined standard deviation (NRC, 2001) or assuming a 10% standard deviation (CNCPS). Evaluations 5 (NRC, 2001) and 12 (CNCPS) varied chemical composition, in situ protein pool proportions (NRC, 2001 only), and ruminal degradation rates. Evaluations 6 (NRC, 2001) and 13 (CNCPS) varied all the inputs related to protein utilization (feed composition, in situ proportions, K_d, and intestinal digestibilities). Evaluations 7 (NRC, 2001) using pools calculated from chemical composition) and 14 (CNCPS using NRC [2001] K_d and RUP digestibilities) required additional calculations. Protein pools for evaluation 7 were calculated using the method utilized by the CNCPS. The 3 CNCPS B pools were summed to represent an in situ B pool. These calculations result in assuming that protein pool A is NPN and protein pool C is ADICP. The purpose of evaluation 14 was to determine if in situ-derived K_d and NRC RUP digestibilities could be used in the CNCPS. Because CNCPS assumes intestinal flow of RUP from 5 sources (undegraded protein A, B_1, B_2, B_3, and C), evaluation 14 required correcting the NRC (2001) RUP digestibility for the CNCPS C pool. This was accomplished by determining what proportion pool C was of the total RUP and increasing the RUP digestibility for the other pools so that the total RUP digestibility was equal to the NRC (2001) RUP digestibility. The purpose of evaluation 15 was to determine if the MPMilk variance observed in evaluation 7 could be decreased by using the CNCPS equation for DMI. For all iterations in the NRC (2001) evaluations, protein fractions were forced to sum to 1 using a calculated proportion adjustment that was applied equally to each fraction. The resulting MPMilk and diet RDP distributions were tested for normality using the Anderson-Darling (A-D) method with normality assumed when A-D > 0.05.

Results

Predicted MPMilk by NRC (2001), without variance accounted for, was 50.9 kg/d (Table 8) with an RDP of

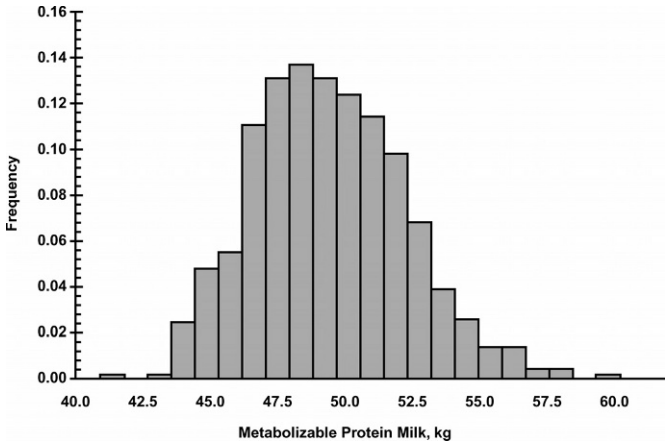

Figure 3. Histogram of metabolizable protein allowable milk distribution from evaluation 6.

11.6% DM. Predicted MPMilk decreased to 45.8 kg/d if the DMI equation from the CNCPS is used. This illustrates the sensitivity that models have relative to their development as a complete system. As shown in Table 8, evaluations 1 to 6 predicted lower mean MPMilk and ration RDP than the base evaluation (evaluation 0). In most evaluations of NRC (2001), MPMilk was nonnormally distributed and exhibited a large range with a long tail greater than the mean (i.e., positively skewed). If the distributions were normal (i.e., not skewed), the mean MPMilk differences would have been larger than the 1.0-kg observed difference from base MPMilk. Figure 3 portrays the simulated distribution of MPMilk from evaluation 6, highlighting the range and skewness with a mean MPMilk of 49.5 kg and a standard deviation of 2.8 kg. This behavior suggests that there are disconnects in the NRC (2001) model. A potential explanation is that the NRC (2001) assumes one microbial population and limits MP production if RDP is deficient using a fixed 85% efficiency of use for RDP that assumes RDP fractions are used equally by all rumen microbes. Additionally, it suggests that user risk may be increased if book values for feed composition and pool sizes are used. The positive skewness associated with MPMilk could result in a formulated ration containing excess protein (required to shift the mean MPMilk to a desired level to overcome the artificially inflated mean MPMilk). This is of particular concern given environmental concerns (ammonia emissions) and that excretion of excess N requires energy (NRC, 1985), areas that the NRC (2001) model does not address. An additional area to review with the NRC (2001) that may contribute to this behavior is ruminal pH and its impact on microbial growth at low pH.

The behavior exhibited by NRC (2001) was unexpected in that varying ruminal degradation rates resulted in the greatest MPMilk standard deviation (2.0 kg). It was hypothesized that the total protein pool size (i.e., CP content of each feed) would have the largest standard deviation in MPMilk, since dietary CP varied from 16.9 to 19.8% DM; however, total protein pool size resulted in the second smallest standard deviation (1.1 kg). Pool proportions resulted in intermediate standard deviations (1.8 kg) while, as anticipated, RUP digestibility variance represented the smallest standard deviation (0.8 kg). Standard deviations shown in Table 8 are not additive due to interactions between inputs and model parameters resulting in nonlinearity. Whereas the other evaluations allow sensitivity analysis within a variable, the greatest use of evaluation 6 is in the correlation ranking of varied inputs. Table 9 lists the top 3 inputs, and correlation coefficients, ranked by correlation coefficient. For evaluation 6, corn grain and soybean meal ruminal degradation rates were correlated the most with MPMilk followed by corn grain pool A. Corn grain pool A may have been ranked third because corn was the largest amount fed in the simulated ration. It is disconcerting that ruminal degradation rates ranked first and second because they are very difficult to analyze under field conditions. The highest ranking chemical component was ranked eighth (expellers soy CP, $r = 0.28$) with protein pools for corn silage, and expellers soy ranked fifth through seventh, all with correlation coefficients in the 0.28 to 0.30 range. Evaluation 7 attempted to use CNCPS pool calculations in the NRC (2001) protein system, resulting in a large shift in the mean MPMilk (53.7 kg) compared with evaluations 1 to 6 and a smaller standard deviation compared with evaluation 6 (2.2 vs. 2.8 kg for evaluations 7 and 6, respectively). This suggests that the NRC (2001) intake prediction was responsible. An additional evaluation was conducted using the CNCPS pool calculations and the CNCPS intake equation (evaluation 15). This resulted in a mean MPMilk (49.8 kg) similar to other NRC (2001) evaluations and a lower standard deviation (1.6 kg); MPMilk was normally distributed. This was expected because the CNCPS chemically derived protein fractions were developed to work with the CNCPS intake prediction equation. The shift to normality also suggests that correlations between feed chemical composition and in situ disappearance potentially introduce adequate levels of variance to invoke nonnormality. Whereas correlations were utilized, the composition of feeds from the NRC (2001) in situ development database contained primarily CP and, in several cases, NDF. Therefore, potential correlations between in situ fractions and other composition values (e.g., NDICP, ADICP, and lignin) could not be determined. Simulated ration RDP concentrations were all between 11.0 and 11.2% DM with variances in the 0.4 to 0.6 range. Varia-

Table 9. Three highest correlations with metabolizable protein allowable milk production for each evaluation.

Evaluation	Factor	Correlation coeffficient
1	Expellers soybean meal CP	0.68
	Corn grain CP	0.35
	Legume silage CP	0.32
2	Expellers soybean meal pool A	–0.45
	Corn silage pool A	–0.45
	Corn silage pool B	0.45
3	Soybean meal	–0.68
	Expellers soybean meal	–0.43
	Corn grain	–0.37
4	Expellers soybean meal	0.61
	Soybean meal	0.59
	Corn grain	0.53
5	Expellers soybean meal pool A	–0.62
	Expellers soybean meal CP	0.62
	Expellers soybean meal pool B	0.57
6	Corn grain K_d	–0.45
	Soybean meal K_d	–0.44
	Corn grain pool A	–0.41
7	Soybean meal K_d	–0.48
	Expellers soybean meal RUP digestibility	0.27
	Corn grain K_d	–0.26
8	Expellers soybean meal CP	0.31
	Cottonseed CP	0.28
	Cottonseed NDF	–0.27
9	Expellers soybean meal CP	0.44
	Soybean meal soluble CP	–0.38
	Grass silage soluble CP	–0.36
10	Corn grain carbohydrate B_1	0.64
	Soybean meal protein B_2	–0.33
	Corn silage carbohydrate B_2	0.32
11	Expellers soybean meal B_2 RUP digestibility	0.54
	Soybean meal B_2 RUP digestibility	0.47
	Expellers soybean meal B_3 RUP digestibility	0.36
12	Corn grain carbohydrate B_1	0.30
	Expellers soybean meal CP	0.27
	Corn silage lignin	–0.24
13	Expellers soybean meal protein B_2 RUP	0.30
	Expellers soybean meal NDICP	0.28
	Grass silage soluble CP	–0.28
14	Soybean meal NRC protein K_d	–0.35
	Corn grain NRC protein K_d	–0.32
	Expellers soybean meal CP	0.25
15	Expellers soybean meal CP	0.47
	Corn grain CP	0.39
	Expellers soybean meal RUP digestibility	0.34

tion in feed composition, protein pools, and degradation rates was equal (0.4 units).

Sensitivity analysis of the chemical composition approach used by the CNCPS highlighted the integration of energy and protein pools in the rumen submodel. Evaluation 12 (feed composition and degradation rates vary) resulted in a mean close to the formulated value (48.9 vs. 49.0 kg of MPMilk, for evaluation 12 and 00, respectively) and was normally distributed. The impact of energy supply can be observed in the correlations between inputs and MPMilk (Table 9), where 2 of the top 3 correlations related directly to energy supply (corn grain carbohydrate B1 K_d ($r = 0.30$) and corn silage lignin ($r = –0.24$)). Ration protein degradability was correlated with protein composition (Table 10) with grass silage soluble protein and NDICP ranking first and second, respectively. Attempting to use NRC (2001) RUP digestibilities and K_d (evaluation 14) increased the variance (2.6 kg) and predicted mean MPMilk (52.3 kg). An unlisted evaluation using just NRC K_d had a lower mean (49.8 kg of MPMilk), suggesting that RUP digestibility values were overestimated by the fraction C correction. Varying intestinal digestibility (evaluation 11) decreased MPMilk 3.2 kg compared with the base value. The CNCPS is much more sensitive to RUP digestibility than NRC (2001) (evaluation 4). This may be due to the CNCPS C fraction being assumed to be indigestible and proteins B1 and B2 being completely

Table 10. Three highest correlations with diet rumen degradable CP concentration for each evaluation.

Evaluation	Factor	Correlation coefficient
1	Legume silage CP	0.69
	Grass silage CP	0.50
	Legume silage NDF	–0.34
2	Corn silage pool A	0.51
	Corn silage pool B	–0.50
	Expellers soy pool A	0.40
3	Soybean meal	0.65
	Expellers soy	0.41
	Corn grain	0.37
4	NA	
	NA	
	NA	
5	Legume silage CP	0.53
	Legume silage pool C	–0.44
	Grass silage CP	0.37
6	Corn grain K_d	0.42
	Corn grain pool A	0.40
	Legume silage CP	0.38
7	Legume silage CP	0.47
	Legume silage K_d	0.41
	Soybean meal K_d	0.40
8	Grass silage soluble CP	0.43
	Alfalfa silage soluble CP	0.41
	Alfalfa silage NDF	–0.39
9	Grass silage soluble CP	0.44
	Alfalfa silage soluble CP	0.40
	Soybean meal NDICP	–0.39
10	Soybean meal protein B_2	0.58
	Expellers soybean meal protein B_2	0.49
	Corn grain protein B_2	0.41
11	NA	
	NA	
	NA	
12	Grass silage soluble CP	0.41
	Grass silage NDICP	–0.37
	Alfalfa silage NDF	–0.35
13	Grass silage soluble CP	0.44
	Alfalfa silage crude CP	0.38
	Expellers soy NDICP	–0.36
14	Soybean meal NRC protein K_d	0.43
	Corn grain NRC protein K_d	0.38
	Alfalfa silage NDF	–0.31
15	Legume silage CP	0.63
	Grass silage CP	0.43
	Legume silage NDF	–0.41

available in the lower tract, a debatable assumption. When only composition values that impact protein fractions were varied (evaluation 9), variance was reduced from 1.7 to 1.3 kg (evaluation 8 where all feed composition varied). There are several reasons why this may have occurred, with the primary one most likely being a change in maintenance protein requirements, namely metabolic fecal nitrogen. Metabolic fecal N in the CNCPS is calculated as 9% of indigestible DM, of which variance in lignin and NDF play a major role. Thus, by not varying the carbohydrate inputs, indigestible DM variance would decrease. When all inputs that impact protein pools, degradation, and RUP digestibility varied (evaluation 13), simulated variance was high (SD = 1.8 kg) and sensitivity analysis (Table 9) shows that expellers soy protein B2 RUP digestibility was correlated with MPMilk at 0.30, which was expected, given that expellers soy is the RUP protein source in this ration. The second highest correlation was expellers soy NDCIP (r = 0.28), indicating that the RUP pool size also impacted MPMilk. Diet RDP level (Table 10) was correlated with grass silage soluble protein (r = 0.44) and alfalfa silage CP (r = 0.38), again illustrating the impact of pool size on ruminal degradation.

Based on this sensitivity analysis and limited data, it appears that protein pools and feedstuff chemical composition are strongly correlated. The NRC (2001) protein development database was used to calculate

correlations between protein pools for feeds used in this evaluation, K_d, and limited chemical composition (CP and NDF). From the limited data set (CP and NDF, if available), it was found that alfalfa NDF was correlated with protein fraction B (r = –0.74), CP with fraction C (r = –0.62), and K_d with fraction A (r = –0.60). Similar results were observed with grass silage (NDF with fraction C with r = 0.62, and K_d with fraction A with r = –0.42), corn silage (NDF with fraction A with r = –.50, NDF with fraction B with r = 0.50), and corn grain (fraction B with K_d with r = –0.57). These correlation coefficients are moderate values; however, this is a relatively small data set with sample numbers ranging from 3 (expellers soy) to 57 (all grass species) and does not include other chemical composition data that may be more appropriate (NDICP, NDF of all feeds, ADICP, lignin, soluble protein).

CONCLUSIONS

Protein fractions based either on in situ analysis or chemical composition provide a framework for protein status evaluation and ration formulation. The NRC (2001) protein system represents a large step to the dairy industry in that it recognizes that RDP and RUP are not static values and that their concentrations in CP rely on the competitive function of ruminal degradation and passage of feed protein. It also recognizes, and is sensitive to, varying protein fractions and their potential site of fermentation. However, there are several areas that need refinement and further research including: an integration of protein and energy supply, limiting microbial growth using a variable discount depending on proportions of A and B protein fractions, and accounting for correlations between feed chemical composition and in situ disappearance. This analysis also illustrates the need for using actual feed analyses vs. default values for both models to reduce potential production variance due to composition variance. Overall, both methods are sensitive to their respective inputs; however, there is insufficient data to adequately determine whether analytical methods can be shared between systems. Research is required to develop a large feed database that includes all feedstuff chemical composition, in situ, RUP digestibility, ruminal degradation rates, and AA analysis. Coupled with this database is the need for a well-controlled study using several of the feeds in the database to obtain cattle (lactating, dry, and replacement heifer) performance data to further evaluate and refine each system. There needs to be a high priority placed on this research to aid nutritionists and dairy producers in meeting current and future environmental regulations and financial objectives.

NEAR-INFRARED REFLECTANCE SPECTROSCOPY (NIRS)

Since its development in the 1970s, major improvements have been made in NIRS hardware and calibration techniques to improve precision and accuracy of prediction of the nutrient composition of feeds. As a result, NIRS has become an increasingly routine laboratory procedure for determining the composition of feedstuffs. The NIRS technique, provided it is calibrated correctly to the primary reference method, is often preferable to traditional laboratory methods because of its accuracy, precision, speed, and unit cost of analysis. Somewhat surprising, however, is that NIRS has also proved relatively successful in predicting a multifaceted parameter, such as feed intake (Steen et al., 1995) and OM digestibility (Murray, 1993) of forages, both of which involve an interaction between the feed and the host animal.

Initial work by Halgerson et al. (1995) indicated that NIRS accurately predicted in situ and ficin CP degradability of forages on a DM basis. Hoffman et al. (1999a, 1999b, 1999c) and Dorshort and Hoffman (2000) showed that NIRS was effective in predicting the in situ RUP content of legume and grass silages and hays. In forages with similar CP content but different CP degradability, they reported that increasing wilting (proteolysis) time and increased plant maturity increased CP remaining in both forages (1999a). In addition, Hoffman et al. (1999b) demonstrated that NIRS accurately predicted in situ CP fractions (A, B, and C), and that in situ-derived protein fractions are better predicted by NIRS than by the chemical approach used in CNCPS. Although still within acceptable limits, NIRS accuracy in prediction of degradation rate was less than that for the specific fractions. In a final study, Dorshort and Hoffman (2000) developed an NIRS calibration equation to predict in situ RUP content of grass and legume hays.

Commercial NIRS equations to determine RUP in hays and haylages are available (NIRS Forage and Feed Testing Consortium, Marshfield, WI). Although these equations can be successfully transferred to other NIR instruments, the ability to monitor the accuracy of the transferred equations for prediction of concentration of fractions, such as RUP of unique new samples, may be challenging because of the necessity of conducting the reference analysis using cannulated animals. At present, we are reliant on software to warn of possible problems related to prediction of nutrient concentrations of new samples. The global H statistic (Infrasoft Software, Matilda, PA), where an H > 3.0, indicates the sample is significantly different than the population used to make the equation, and the value may or may not be

accurate. The ability to monitor these types of equations remains to be resolved. In addition, no commercial equations have been developed for prediction of protein fractions in concentrates.

SUMMARY AND CONCLUSIONS

The complexities of ruminal fermentation, coupled with the factors that affect rates of digestion and passage, are such that predicting the RDP and RUP content of feedstuffs and the intestinal digestibility of RUP are challenging tasks. Nevertheless, considerable progress has been made. In situ and multi-chemical derived protein fractions are used in NRC (2001) and CNCPS, respectively, to predict ruminal degradation of feed CP. By combining these data with rates of degradation and passage, both models appropriately recognize the fact that the proportional concentrations of RDP and RUP in feedstuffs are not static values and provide similar estimates of RUP and RUP digestibility. Moreover, both models are sensitive to protein inputs, and both provide a good framework for the industry. However, it is the opinion of the authors that a concerted effort be made to add substantially to the data sets from which these models have been developed. The relationships among pool sizes, digestion rates, and chemical composition of feeds must be established for the in situ method. Ultimately, a "gold standard" has to be identified against which simpler and less costly methods, such as NIRS, can be evaluated

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