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# Evaluation of the National Research Council (2001) dairy model and derivation of new prediction equations. 2. Rumen degradable and undegradable protein[1]

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## ABSTRACT

This work evaluated the National Research Council (NRC) dairy model (2001) predictions of rumen undegradable (RUP) and degradable (RDP) protein compared with measured postruminal non-ammonia, nonmicrobial (NANMN) and microbial N flows. Models were evaluated using the root mean squared prediction error (RMSPE) as a percent of the observed mean, mean and slope biases as percentages of mean squared prediction error (MSPE), and concordance correlation coefficient (CCC). The NRC (2001) over-estimated NANMN by 18% and under-estimated microbial N by 14%. Both responses had large mean biases (19% and 20% of MSPE, respectively), and NANMN had a slope bias (22% of MSPE). The NRC NANMN estimate had high RMSPE (46% of observed mean) and low CCC (0.37); updating feed library A, B, and C protein fractions and degradation rate ($K_d$) estimates with newer literature only marginally improved fit. The re-fit NRC models for NANMN and microbial N had CCC of 0.89 and 0.94, respectively. When compared with a prediction of NANMN as a static mean fraction of N intake, the re-derived NRC approach did not have improved fit. A protein system of intermediate complexity was derived in an attempt to estimate NANMN with improved fit compared with the static mean NANMN model. In this system, postruminal appearance of A, B, and C protein fractions were predicted in a feed-type specific manner rather than from estimated passage and degradation rates. In a comparison to independent data achieved through cross-validation, the new protein system improved RMSPE (34 vs. 36% of observed mean) and CCC (0.42 vs. 0.30) compared with the static mean NANMN model. When the NRC microbial N equation was re-derived, the RDP term dropped from the model. Consequently, 2 new microbial protein equations were formulated, both used a saturating (increasing at a decreasing rate) form: one saturated with respect to TDN and the other saturated over increasing intakes of rumen degraded starch and NDF. Both equations expressed maximal microbial N production as a linear function of RDP intake. The function relating microbial N to intake of rumen degradable carbohydrate improved RMSPE (24 vs. 28% of the observed mean) and CCC (0.63 vs 0.30) compared with the re-derived NRC model. The newly derived equations showed modest improvements in model fit and improved capacity to account for known biological effects; however, substantial variability in NANMN and microbial N estimates remained unexplained.

**Key words:** National Research Council (2001) dairy model, duodenal flow, model evaluation

## INTRODUCTION

Predicting microbial protein and flow of RUP from the rumen is of key importance in designing dairy cattle diets because these flows make up the majority of MP

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supply and affect the composition of AA absorbed from the digestive tract. Accurate and precise estimates of these variables should allow more precise matching of MP (or AA) supply and requirements, thus improving animal efficiency and reducing N excretion. Several systems to predict microbial protein and RUP flow have been derived (NRC, 2001; Fox et al., 2004; Huhtanen and Hristov, 2009). Although a series of evaluations of predicted postruminal microbial N flows (Bateman et al., 2001; Yu et al., 2003; Tedeschi et al., 2015) and RUP percentages of CP (Seo et al., 2006; Broderick et al., 2010) have been undertaken, few have explicitly addressed errors in predicting equation inputs and how those errors contribute to estimates of protein flow.

One challenge in constructing and evaluating nutrient requirement models is the source data. There are often multiple methods of measuring fluxes in the animal, and even application of a common method can vary across laboratories. This variation in method and application of method may affect measurement accuracy and precision (Nocek, 1988; Broderick and Merchen, 1992; Owens and Hanson, 1992). Incomplete nutrient input data are often reported in the literature (Angel et al., 2015; McNamara et al., 2016), necessitating use of tabular values to represent the missing dietary nutrients (White et al., 2017). Substituting missing data with mean book values does not account for the source of variation and may also result in mean bias in inputs. Systematic deviations in both input and output data will result in biased model equations, and failure to consider input variation may inhibit opportunities to evaluate underlying system behavior. The potential for data-related errors is greater for measurements that are more complicated to make using methods that are not standardized. Examples are microbial N and non-ammonia, non-microbial N (**NANMN**) flows from the rumen because they compound errors from sampling, different flow marker approaches, and different microbial markers. The risk is even greater for NANMN because it is calculated by difference from total N flow and microbial N flow. As such, a re-evaluation of the intermediate steps in the calculation of MP supply is warranted to better understand the source of errors within the model. In a companion paper (White et al., 2017), the digestibility predictions within the NRC (2001) lactating dairy cow model were evaluated, and new equations were derived to estimate digestibility with minimal mean and slope bias. At present, the degree to which imprecise and inaccurate estimates of nutrient digestibility resulted in misrepresented estimates of microbial N and NANMN is unknown.

The objectives of this work were to evaluate predicted ruminal outflows of microbial N and NANMN provided by the NRC (2001) dairy model against a literature data set and, when necessary, to derive and test new equation forms. We hypothesized that (1) ruminal outflow estimates would have poor accuracy when compared with measured data, and (2) accuracy would be improved by re-deriving coefficients used in the current equation forms.

## MATERIALS AND METHODS

This study used a 5-part methodology that will be described in order. First, data were collected from the literature and any missing input data were simulated. The NRC (2001) model predictions were evaluated against literature NANMN and microbial N measurements. The NRC (2001) model was then evaluated using a library of revised and updated A, B, and C protein fractions for feeds. The NRC (2001) models were then re-derived, and new equation forms were fitted to the data. Re-derived models were fit using both old and new A, B, and C protein fractions. The re-derived NRC (2001) model and the new equation forms were compared using Monte Carlo cross-validation (Lendasse et al., 2003). Details of each step are provided in the subsequent sections.

### *Data Collection*

Data were collected as described in White et al. (2017). Briefly, the collection of papers used in deriving the NRC (2001) was updated with more recent work published between the early 2000s and mid-2015. The complete data set contained usable data from 550 treatment means from 147 studies conducted on lactating or dry dairy cows. In total, 125 of those studies reported duodenal or omasal N flow measurements, leaving 525 treatments for use in estimating microbial N and 507 treatments for use in estimating NANMN. The summary statistics for major production variables are included in White et al. (2017), and a copy of the data can be downloaded from the National Animal Nutrition Program (2015) website. Summary statistics of the key variables evaluated in this study are included in Table 1.

### *Evaluating and Correcting Ingredient Biases*

All studies reported the inclusion rates of the ingredients used in diets; however, few studies reported the complete nutrient composition of all ingredients. When ingredient-specific data were not available, data were populated from the NRC (2001) feed tables. Library feed nutrient compositions were adjusted as described in White et al. (2017) and by Hanigan et al. (2013). For variables where dietary composition was not re-

ported (neutral detergent insoluble protein, **NDFIP**; acid detergent insoluble protein, **ADFIP**), feed library values were used without adjustment. In the case of NDFIP, the NRC (2001) feed values were determined with sodium sulfite so the NDFIP values used herein will best reflect that methodology.

The NRC (2001) estimates of RDP and RUP are heavily dependent on the feed library estimates of A, B, and C protein fractions and rumen degradation rate ($\mathbf{K_d}$). Because the literature contains many additional observations of those values that have been published since the NRC (2001) release, the feed library was updated to include these data. Available data were collected from published studies (published at any time) that reported A, B, and C fractions and $K_d$ from CP disappearance in situ for individual feeds in dairy cattle. Studies were excluded from the analysis if the last time point was <48 h of incubation because this may have compromised estimation of the C fraction. This was done because these kinetics are highly correlated (Woods et al., 2003) such that a poor estimate of C fraction can also compromise the B and $K_d$ estimates, particularly for proteins with high RUP such as animal-based protein sources. The mean estimate of each protein fraction and $K_d$ was calculated for each feedstuff and used in model evaluation or derivation in place of the NRC (2001) feed library. The mean, SD, minimum, and maximum of each nutrient for each feedstuff evaluated are presented in Supplemental Table S1 (https://doi.org/10.3168/jds.2015-10801).

### Calculating NRC Predictions

The model equations used in the NRC (2001) dairy model, which are available as a text file on the compact disk distributed with the publication, and the equations listed in the NRC (2001) publication were used to reconstruct the model in R (version 3.1.0; R Core Team, 2014) as described in White et al. (2017). Many studies did not report all animal descriptor data required as inputs to the NRC model. When treatment-specific data were not available for an input, reference input data (averages from the unadjusted data set or NRC software default values) were used, as described in White et al. (2017).

### Evaluating Prediction Errors

In the NRC (2001) nutrient supply model, feed RUP and RDP, along with estimates of digestibility, were used to calculate digestible RUP and microbial protein, respectively. Together with endogenous protein, digestible RUP and microbial protein were used to estimate MP supply. Although numerous assessments of the NRC (2001) protein system have been conducted (Seo et al., 2006; Lanzas et al., 2007; Krizsan et al., 2010), few have addressed the potential for compounding errors in this calculation method. We evaluated the modeled microbial protein, RUP, and RDP estimates using either the original NRC (2001) TDN equation or the updated digestibility equations presented in White et al. (2017). Because the NRC (2001) protein equations were found to have notable prediction error, the parameter estimates in the NRC (2001) equation form were re-fit and new equations were also derived. Prediction errors in the NRC (2001) calculations were assessed using root mean squared error of prediction (**RMSPE**) as a percentage of observed mean and SD, mean and slope biases as a percentage of the mean squared error (**MSE**; Bibby and Toutenburg, 1978), and concordance

**Table 1**. Summary statistics for data used to evaluate the NRC (2001) estimates of RUP and RDP

| Variable[1] | N[2] | Mean | SD | Minimum | Maximum |
|---|---|---|---|---|---|
| Dietary CP, % of DM | 525 | 17 | 1.9 | 10 | 25 |
| Dietary NDFIP, % of DM | 525 | 2.2 | 0.9 | 0.8 | 6.2 |
| Dietary ADFIP, % of DM | 525 | 1.0 | 0.3 | 0.5 | 2.1 |
| NRC[3] A, % of CP | 525 | 36 | 10 | 13 | 66 |
| NRC B, % of CP | 525 | 55 | 10 | 30 | 76 |
| NRC C, % of CP | 525 | 9 | 3 | 4 | 35 |
| New[4] A, % of CP | 525 | 39 | 9 | 19 | 68 |
| New B, % of CP | 525 | 52 | 9 | 26 | 70 |
| New C, % of CP | 525 | 9 | 4 | 4 | 34 |
| NANMN, g/d | 507 | 212 | 82 | 26 | 576 |
| Microbial N, g/d | 525 | 287 | 94 | 74 | 642 |

[1]NDFIP = neutral detergent insoluble protein; ADFIP = acid detergent insoluble protein; NANMN = nonammonia, nonmicrobial N.

[2]Number of treatment means used in calculating input data summaries.

[3]The variables labeled NRC A, B, and C represent mean dietary A, B, and C protein as calculated by the NRC (2001) feed library.

[4]The variables labeled New A, B, and C represent dietary A, B, and C protein fractions reported in the updated feed library included in Supplemental Table S1 (https://doi.org/10.3168/jds.2015-10801).

correlation coefficients (**CCC**; Lin, 1989). Because the models herein were evaluated against the same data used for derivation, they were assessed using the root mean squared error (**RMSE**), mean and slope bias as a percentage of MSE, and CCC. Although calculated in the same way, RMSPE and RMSE should be interpreted differently as the former reflects evaluation of a prediction against independent data, whereas the latter reflects evaluation of a prediction against data used for derivation. As new models were derived, the corrected Akaike information criterion (**AICc**; Hurvich and Tsai, 1993) was also reported to identify tradeoffs between model complexity and goodness of fit. Ideal models were selected by choosing those with RMSE or RMSPE closest to 0, CCC closest to 1, mean and slope biases closest to 0, and smallest AICc. Optimal models were also evaluated for biological adequacy. Variance inflation parameters (VIF) were used to evaluate covariance of all models. In all models tested, all non-intercept parameters had VIF less than the cutoff of 10.

### Model-Fitting Procedure

Two model-fitting approaches were used. First, nonlinear mixed-effects regression (**NLME**) was performed using the "nlme" function of R statistical software (version 3.1.0; R Core Team, 2014). Fixed effects varied by equation, and a random study effect was included in all NLME models. Nonlinear least squares regression (**NLS**) was used to solve for parameters without consideration of random intercepts associated within each study. The NLS regression was performed using the "nls" function of R. For each model, multiple fitting algorithms were evaluated, and a series of initial values were tested to ensure parameter estimates were robust with respect to initial conditions and fitting method. The optimal model was identified by a low RMSPE, minimal mean and slope biases, and high CCC from cross-validation and small AICc from derivation.

Although inclusion of random effects in models is recommended for analyses deriving equations from literature summaries (St-Pierre, 2001; Sauvant et al., 2008), the comparison in White et al. (2017) identified some tradeoffs between inclusion of random study effects and usefulness of models for field application. To further evaluate these tradeoffs, models derived herein were fit using TDN predicted by NLME models and NLS models. For clarity, these results are presented in separate but analogous tables. These TDN values reflect those derived in White et al. (2017). Additionally, the fit of NLME and NLS models were evaluated by estimating the RMSE and CCC from fitted values of the NLME models without accounting for the study effect. Although statistically an inaccurate representation of NLME model predictions, this approach allows for some more analogous comparison of NLME and NLS model approaches.

### Cross-Validation Procedure

As an additional assessment of the effects of small groups of studies on the model responses, a Monte Carlo cross-validation was performed. In the cross-validation procedure, the data were randomly divided into 2 groups; 60% of the data were used for model derivation, and 40% were used for independent model evaluation. This data splitting, model derivation, and model evaluation was repeated 500 times. The means and SD of each parameter estimate were collected for all 500 replicates and were compared with the parameter estimates derived from the full data set. The RMSPE and CCC calculated from the independent evaluation data in each run were also collected to compare how the newly derived models and the re-fit NRC model would be expected to perform against independent data.

### Addressing Prediction Biases

***Postruminal NANMN.*** The fractionation scheme used to estimate postruminal N flows was evaluated compared with measured flow data. Because variables throughout the analysis were specific to a certain level of aggregation (feed, *f*; dietary treatment, *t*; study, *s*), subscripts were used throughout the paper to denote the appropriate level for which a variable held unique values. Variables with subscript *f* were sourced from the NRC (2001) feed table or from Supplemental Table S1 (https://doi.org/10.3168/jds.2015-10801) because they held a specific value for each feed that did not vary with treatment or study.

In the NRC (2001) protein model, passage rate (**Kp**; %/h) for each feed was calculated based on an equation aggregated for all concentrates or for forage type classification:

$$Kp_{f,t,s} = \begin{cases} if\ ForageType_f = "Concentrate" \\ \quad 2.904 + 1.375 \times \frac{DMI_{t,s}}{BW_{t,s}} - 0.020 \times ConcPct_{t,s} \\ else,\ if\ ForageType_f = "Dry" \\ \quad 3.362 + 0.479 \times \frac{DMI_{t,s}}{BW_{t,s}} - 0.017 \times NDF_{f,t,s} \\ \quad + 0.0070 \times ConcPct_{t,s} \\ else,\ if\ ForageType_f = "Wet" \\ \quad 3.054 + 0.614 \times \frac{DMI_{t,s}}{BW_{t,s}} \end{cases} \quad [1]$$

where $DMI$ was in kilograms per day, $BW$ was in kilograms, $ConcPct$ was concentrate percentage in the diet, and $NDF_{f,t,s}$ was feed NDF (% of DM). In practice, Kp is often calculated based on flow markers assuming a single compartment first-order elimination. In this work, Kp was only calculated using the NRC (2001) equation. Although some studies did report $K_d$ estimated in vitro and Kp calculated from postruminal flow and rumen volume, these values were not used as inputs to the evaluation because the objective was to evaluate the system of equations.

Flow of RDP (kg/d) was subsequently predicted for each feedstuff in the diet as a function of Kp, rumen degradation rate ($K_d$, %/h), and the A and B protein fractions ($PrA$ and $PrB$, both % of CP) from the NRC (2001) feed library or using the newly updated feed library provided in Supplemental Table S1 (https://doi.org/10.3168/jds.2015-10801):

$$RDP_{f,t,s} = \begin{cases} if\ Kp_{f,t,s} + Kd_f > 0 \\ \quad \left[\left(\frac{Kd_f}{Kd_f + Kp_{f,t,s}} \times \frac{PrB_f}{100} \times \frac{CP_{f,t,s}}{100}\right) + \frac{PrA_f}{100} \right. \\ \quad \left. \times \frac{CP_{f,t,s}}{100}\right] \times DMI_{f,t,s} \\ else \\ \quad 0 \end{cases} \quad [2]$$

The Kp equations used in this analysis were those corrected by Seo et al. (2006), which fixed a typographical error reported in the NRC (2001). Rumen undegraded protein (kg/d) was then calculated by difference:

$$RUP_{f,t,s} = CP_{f,t,s} - RDP_{f,t,s}. \quad [3]$$

Measured postruminal NANMN contains both RUP and endogenous protein. The latter was assumed to be 96.1 g + 7.54 g/kg of DMI (Lapierre et al., 2016), and digesta protein was assumed to be composed of 16% N. Thus, the predicted NANMN flow (g/d) was calculated as

$$NANMN_{t,s} = \left[\sum_{f=1}^{k}(RUP_{f,t,s} \times 1{,}000) + (96.1 + 7.54 \times DMI_{t,s})\right] \times 0.16. \quad [4]$$

Initial evaluations of the model identified mean and slope biases in the ruminal outflow protein predictions. To assess what proportion of these biases were due to poorly specified feed A, B, or C fractions or $K_d$ in the NRC (2001) feed library, the data were updated with more recent studies (Supplemental Table S1; https://doi.org/10.3168/jds.2015-10801). The NRC (2001) model was re-evaluated using these new feed descriptions (Table 2; NRC+Feed), and minimal improvement in estimation accuracy or precision was observed. Because updated estimates of A, B, C, and $K_d$ did not improve model fit, potential errors in estimating Kp were evaluated. The original estimates of Kp were derived from marker-based data that have been shown to overestimate true passage of particles (Firkins et al., 1998); however, the NRC (2001) Kp equations have previously evaluated favorably against literature data (Seo et al., 2006). Even so, markers were applied to and evaluated against data for intact feeds and therefore could have misrepresented passage of feed B protein fraction. Thus, despite the previous and favorable evaluation, the coefficients in the current Kp calculation were rederived using stepwise backward elimination (starting with the terms in the NRC equation) to determine if predictions of NANMN could be improved by adjusting the Kp prediction. The model was fit using the NLME approach as previously described, and the result of the backward elimination yielded

$$Kp_{f,t,s} = \begin{cases} if\ ForageType_f = "Concentrate" \\ \quad a + b \times \frac{DMI_{t,s}}{BW_{t,s}} + c \times ConcPct_{t,s} \\ else,\ if\ ForageType_f = "Dry" \\ \quad d \\ else,\ if\ ForageType_f = "Wet" \\ \quad e \end{cases} \quad [5]$$

where $a$ through $e$ were derived parameters, and DMI/BW is DMI per unit of BW (kg/kg). The additional terms included in the NRC equation (Eq. [1]) were dropped due to nonsignificance ($P > 0.10$).

Although not presented here, attempts were also made to derive (1) new Kp equations, (2) equations to adjust $K_d$, and (3) new equations for Kp and $K_d$, concurrently. Among other approaches, these attempts included (1) linear and nonlinear adjustments to NRC (2001) feed library $K_d$ values based on nutrient (NDF, CP, FA, starch) concentration or protein fractions (A, B, C); (2) linear and nonlinear adjustments to previously predicted Kp values; (3) new feed groupings for Kp es-

**Table 2**. Parameter estimates and overall model fit of NRC (2001) and selected new equations for predicting non-ammonia, non-microbial N (n = 507)

| Item[1] | NRC | NRC+Feed | Eq. [5] | Eq. [6] | Eq. [7] to [12] | Eq. [9] to [14] |
|---|---|---|---|---|---|---|
| Parameter[2] | | | | | | |
| *a* | | | 2.65 (<0.01) | 203 (<0.01) | 0.499 (0.10) | 1.27 (<0.01) |
| *b* | | | −1.09 (<0.01) | 35.8 (0.01) | 0.0194 (<0.01) | |
| *c* | | | −3.08 (<0.01) | | −0.0734 (<0.01) | −0.0693 (<0.01) |
| *d* | | | 7.61 (<0.01) | | −0.723 (<0.01) | 0.459 (<0.01) |
| *e* | | | 34.2 (0.059) | | 0.831 (<0.01) | 1.28 (<0.01) |
| *f* | | | | | −0.0994 (<0.01) | −0.156 (<0.01) |
| *g* | | | | | 0.00728 (0.02) | 0.00913 (<0.01) |
| *h* | | | | | −3.65 (<0.01) | −4.40 (<0.01) |
| *i* | | | | | 0.715 (<0.01) | 0.381 (<0.01) |
| *j* | | | | | −0.183 (<0.01) | |
| *k* | | | | | 0.174 (0.02) | 0.311 (<0.01) |
| *l* | | | | | 0.400 (<0.01) | 0.325 (<0.01) |
| *m* | | | | | 0.336 (<0.01) | |
| Fitting method[3] | | | NLME | NLME | NLME | NLS |
| Mean random effect | | | 33.6 | 51.0 | 29.6 | |
| Observed mean, g/d | 215 | 215 | 215 | 215 | 215 | 215 |
| Predicted mean, g/d | 253 | 243 | 210 | 210 | 213 | 210 |
| RMSE or RMSPE, % of observed mean | 46 | 43 | 18 | 17 | 15 | 29 |
| Mean bias, % of MSE or MSPE | 19 | 13 | <1 | <1 | <1 | <1 |
| Slope bias, % of MSE or MSPE | 22 | 20 | <1 | <1 | <1 | <1 |
| RMSE/SD | 1.2 | 1.1 | 0.45 | 0.43 | 0.43 | 0.82 |
| CCC | 0.37 | 0.37 | 0.89 | 0.90 | 0.90 | 0.51 |
| AICc | | | 5,781 | 5,741 | 5,701 | 5,915 |
| $\sigma_s$ | | | 92 | 97 | 80 | |
| $\sigma_e$ | | | 43 | 41 | 38 | |
| Unadjusted RMSE | | | 39 | 38 | 32 | |
| Unadjusted CCC | | | 0.20 | 0.23 | 0.34 | |
| Monte Carlo cross-validation[4] | | | | | | |
| RMSPE, % of observed mean | | | 37 ± 1.7 | 36 ± 1.6 | 38 ± 1.6 | 34 ± 0.5 |
| Mean bias, % of MSPE | | | <1 ± 1.9 | 1.7 ± 1.9 | 1.9 ± 1.4 | 1.2 ± 1.8 |
| Slope bias, % of MSPE | | | 4.4 ± 2.2 | 2.1 ± 1.7 | 2.7 ± 1.9 | 2.2 ± 0.9 |
| CCC | | | 0.29 ± 0.08 | 0.30 ± 0.05 | 0.15 ± 0.05 | 0.42 ± 0.03 |

[1]Model evaluation criteria included root mean squared prediction error as a percent of observed mean (RMSPE), mean and slope bias as a percent of mean squared prediction error (MSPE), RMSPE as a proportion of observed standard deviation (RMSPE/SD), and concordance correlation coefficient (CCC) for the NRC (2001). Evaluation criteria for derived equations included root mean squared prediction error (RMSE), mean and slope bias as a percent of mean squared error (MSE), RMSE as a fraction of observed standard deviation (RMSE/SD), CCC, corrected Akaike information criterion (AICc), variance from study ($\sigma_s$) and residual error ($\sigma_e$), and RMSE and CCC unadjusted for study effects.

[2]Parameter names are as referenced in each equation, and parameter estimates are presented with significance values in parentheses.

[3]Specifies models fit using nonlinear mixed effect model derivation (NLME) or nonlinear least squares (NLS).

[4]Cross-validation (±SD of the variables) was performed using 500 iterations of a repeated random sampling approach, in which 60% of the data was used for derivation and 40% used as an independent evaluation.

timation; (4) no feed groupings for Kp estimation; and (5) combinations of the above $K_d$ and Kp adjustments. All approaches returned estimates of NANMN that had notable slope biases or poor RMSE and CCC, or combinations thereof. As such, a series of new NANMN calculation approaches was tested. To better evaluate potential errors associated with grouping omasal and duodenal sampling, a binary variable ($Omasal_{t,s}$; 1 if omasal sampling was used, 0 if duodenal) was added to all new equations. The first equation tested was a simple model representing NANMN (g/d) as a static fraction of nitrogen intake (*NI,* kg/d):

$$NANMN_{t,s} = (a \times NI_{t,s} + b \times Omasal_{t,s}) \times 1{,}000, \quad [6]$$

where *NI* was calculated from N intake (kg/d) multiplied by 16%. These parameters were estimated using NLME.

Although parsimonious, Eq. [6] fails to account for factors known to affect postruminal protein flow and, thus, from a biological perspective may be considered a step backward in protein modeling efforts, given that the NRC (2001) accounted for these factors. Although all efforts to improve accuracy of NANMN predictions by deriving new estimates of Kp and $K_d$ failed, the concept of the Kp, $K_d$ system has merit biologically. To more completely evaluate the concept of a 3-pool protein fractionation scheme, a protein system of intermediate complexity was derived. Protein was viewed as a 3-pool system from which pool A is mostly ruminally

degradable, pool B is partially ruminally degradable, and pool C is ruminally undegradable. Rather than predicting passage from $K_d$ and Kp, we estimated a simple daily percentage of CP intake appearing postruminally (%/d; i.e., a daily turnover rate for each pool). This simpler approach uses one parameter estimate for each pool and thus is more parsimonious, but it retains the conceptual structure of the protein system. Moreover, this approach would be preferable for field application over Eq. [6] because it should allow predicted NANMN to reflect known differences in postruminal appearance of different feeds, whereas Eq. [6] would predict no differences among feeds.

This new protein system was derived using NLME regression and is described by Eq. [7] to [12]. The proportion of B protein exiting the rumen for any given feed was considered to be feed-type specific; it depended on its ADFIP concentration and DMI per unit of metabolic BW (**DMIMBW**), where metabolic BW was $BW^{0.75}$ (in $kg^{0.75}$):

$$kRUPB_{f,t,s} = \begin{cases} if\ TypeForage_{f,t,s} = "Forage", \\ \quad a + b \times ADF_{f,t,s} + c \times DietCP_{t,s} \\ else,\ if\ Category_{f,t,s} = "AnimalProtein", \\ \quad d \\ else,\ if\ Category_{f,t,s} = "PlantProtein", \\ \quad e + f \times ADFIP_f + g \times NDFIP_f + h \\ \quad \times DMIMBW_{t,s} \\ else,\ if\ Category_{f,t,s} = "Byproduct\ /\ Other", \\ \quad i + j \times ADFIP_f \\ else, \\ \quad k \end{cases} \quad [7]$$

In this function, $a$ through $k$ were parameter estimates to be derived, $Category_{f,t,s}$ was the feed category as defined in the NRC feed library and $kRUPB_{f,t,s}$ was the proportion of B protein (g/g) escaping the rumen for any given feed $f$. Equation [7] was derived using a stepwise backward elimination approach, from which initial variables within feed category included ADF, NDF, CP, ADFIP, NDFIP, and DMIMBW. The approach was repeated several times using different starting feed categories. When all variables for a feed category were eliminated as nonsignificant, that category-specific $kRUPB$ was removed from the model.

Previous meta-analyses have suggested that some A protein may pass as RUP (Huhtanen and Hristov, 2009). To account for this passage, an appearance rate of A protein was also derived:

$$RUPA_{f,t,s} = \begin{cases} if\ Category = "Forage" \\ \quad l \times \frac{PrA_{f,t,s}}{100} \times \frac{CP_{f,t,s}}{100} \times DMI_{f,t,s} \times 1{,}000 \\ else, \\ \quad m \times \frac{PrA_{f,t,s}}{100} \times \frac{CP_{f,t,s}}{100} \times DMI_{f,t,s} \times 1{,}000 \end{cases} \quad [8]$$

where $RUPA_{f,t,s}$ is appearance of A protein postruminally (g/d) and $l$ and $m$ are derived parameter estimates. The $kRUPB$ was used to estimate RUP from B protein ($RUPB_{f,t,s}$):

$$RUPB_{f,t,s} = kRUPB_{f,t,s} \times \frac{PrB_{f,t,s}}{100} \times \frac{CP_{f,t,s}}{100} \times DMI_{f,t,s} \times 1{,}000, \quad [9]$$

where $RUPB_{f,t,s}$ is appearance of B protein postruminally (g/d). The RUP from C protein ($RUPC_{f,t,s}$; g/d) was predicted as a function of feed CP and the percent of feed CP that was C fraction protein ($PrC_{f,t,s}$), assuming 100% of C protein appeared postruminally:

$$RUPC_{f,t,s} = \frac{PrC_{f,t,s}}{100} \times \frac{CP_{f,t,s}}{100} \times DMI_{f,t,s} \times 1{,}000. \quad [10]$$

Rumen undegradable protein was calculated by summing the $RUPA_{f,t,s}$, $RUPB_{f,t,s}$, and $RUPC_{f,t,s}$ estimates:

$$RUP_{t,s} = \sum_{f,i=0}^{j} RUPA_{f,t,s} + RUPB_{f,t,s} + RUPC_{f,t,s}. \quad [11]$$

Endogenous protein flow was added to dietary RUP and converted to N to predict NANMN (g/d):

$$NANMN = \left[(96.1 + 7.54 \times DMI_{t,s}) + RUP_{t,s}\right] \times 0.16. \quad [12]$$

A binary variable for omasal versus duodenal sampling was originally included in the model, but this variable was nonsignificant ($P = 0.68$). This system of equations was first derived using the NLME approach.

Fixed-effects NLS regression was also used to estimate parameters for $RUPA_{t,s}$ and $RUPB_{t,s}$ prediction. The parameters in Eq. [7] and [8] were used in the initial model, and the backward elimination regression was used to remove parameters that were not significant under the fixed-effects fitting approach. The resulting equations (Eq. [13] and [14]) retained the same

parameter estimate names ($a$ through $l$) for more direct comparison to Eq. [7] and [8]:

$$kRUPB_{f,t,s} = \begin{cases} if\ TypeForage_{f,t,s} = "Forage", \\ \quad a + c \times DietCP_{t,s} \\ else,\ if\ Category_{f,t,s} = "AnimalProtein", \\ \quad d \\ else,\ if\ Category_{f,t,s} = "PlantProtein", \\ \quad e + f \times ADFIP_f + g \times NDFIP_f + h \\ \quad \times DMIMBW_{t,s} \\ else,\ if\ Category_{f,t,s} = "Byproduct\ /\ Other", \\ \quad i \\ else, \\ \quad k \end{cases} \quad [13]$$

$$RUPA_{f,t,s} = \begin{cases} if\ Category = "Forage" \\ \quad l \times \frac{PrA_{f,t,s}}{100} \times \frac{CP_{f,t,s}}{100} \times DMI_{f,t,s} \times 1{,}000 \\ else, \\ \quad 0 \times \frac{PrA_{f,t,s}}{100} \times \frac{CP_{f,t,s}}{100} \times DMI_{f,t,s} \times 1{,}000 \end{cases} \quad [14]$$

Although a proportion of nonforage A protein escaping the rumen was greater than zero in the NLME derivation, this parameter was not statistically different from zero in the NLS derivation (Eq. [14]); therefore, parameter $m$ was replaced with a value of zero. The binary effect of sampling location was also included in this model at the beginning of backward elimination; however, this parameter was dropped due to nonsignificance ($P = 0.78$).

***Microbial Nitrogen.*** In the NRC (2001) model, ruminal outflow of microbial N (*MicrN*; g/d) was calculated from discounted TDN ($DiscTDN_{t,s}$, kg/d) and capped at 85% of the RDP available. Here we converted this equation to an N basis (g/d):

$$MicrN_{t,s} = 0.16 \times \begin{cases} if\ 0.13 \times DiscTDN_{t,s} > 0.85 \times RDPI_{t,s} \\ \quad 0.85 \times RDPI_{t,s} \\ else, \\ \quad 0.13 \times DiscTDN_{t,s} \end{cases} \quad [15]$$

where *RDPI* was RDP intake (g/d) and *DiscTDN* was TDN intake (g/d). This calculation scheme was used to predict microbial N (g/d) with the NRC (2001) estimates of RUP and RDP and with the newly derived estimates of RUP and RDP (Eq. [7] to [12] or Eq. [9] to [14]). In both systems, RDP (% of CP) was assumed to be 100 − RUP (% of CP). Additionally, microbial N was predicted using the NRC (2001) estimate of TDN and using TDN predicted from NLS and NLME models calculated in White et al. (2017). For clarity, the results from the NLS and NLME models are presented in separate tables. After the NRC (2001) equation was evaluated using the NRC (2001) RUP/RDP and TDN estimates and the RUP/RDP estimates derived herein and the TDN estimates from White et al. (2017), new equation forms were tested. Equation [16] was fit using both NLME and NLS regressions:

$$MicrN_{t,s} = 0.16 \times \begin{cases} if\ a \times DiscTDN_{t,s} > RDP_{t,s} \\ \quad RDP_{t,s} + b \times Omasal \\ else, \\ \quad a \times DiscTDN_{t,s} + b \times Omasal \end{cases} \quad [16]$$

where *Omasal* is a binary indicator variable with a value of 0 if duodenal sampling was used and a value of 1 if omasal sampling was used.

Equation [16] suggests that there is a single limiting nutrient for microbial N production within the rumen because microbial N flows are modeled as the minimum of protein-predicted microbial N (0.85 g of microbial protein/g of RUP) or energy-predicted microbial N (130 g of protein/kg of TDN). However, evidence suggests that a single limiting nutrient model fails to reproduce biological responses at the tissue level (Arriola Apelo et al., 2014a,b) and fails to account for the fact that both energy and protein must be metabolized simultaneously to optimize efficiency of microbial growth (Hackmann and Firkins, 2015). To evaluate whether a continuous response of microbial N production to $DiscTDN_{t,s}$ (kg/d) and RDP supplies (% of dietary DM) was an improved representation of the observed responses, Eq. [17] was derived:

$$MicrN_{t,s} = 0.16 \times \left( \frac{a + b \times RDP_{t,s}}{1 + \frac{c}{TDN_{t,s}}} \right) + d \times Omasal, \quad [17]$$

where $a$ through $d$ were derived parameters. Originally, Eq. [17] was fit using NLME regression; however, this approach resulted in a nonsignificant variable $c$, so stepwise regression would result in a base linear model. Thus, parameter estimates from NLS regression only are presented for Eq. [17]. The TDN in Eq. [17] was

predicted from both the NLS and NLME models calculated in White et al. (2017). For clarity, the results from the NLS and NLME TDN estimates are presented in separate tables.

A more mechanistic approach to predicting rumen microbial protein synthesis is achieved through the use of ruminally degradable carbohydrate, rather than TDN, as a driving variable. The estimates of apparent ruminal digestibilities of starch and NDF derived in White et al. (2016) were used to predict rumen-degraded NDF (Eq. [18]; kg/d) and starch (Eq. [19]; kg/d):

$$RDNDF_{t,s} = NDF_{t,s} \times \left( \begin{array}{l} -31.9 + 0.721 \times NDF_{t,s} - 0.247 \times Starch_{t,s} + 6.63 \times CP_{t,s} \\ -\ 0.211 \times CP_{t,s}^2 - 0.387 \times \dfrac{ADF_{t,s}}{NDF_{t,s}} - 0.121 \times WetFor_{t,s} \\ +\ 1.51 \times DMI_{t,s} \end{array} \over 100 \right), \quad [18]$$

where $ADF_{t,s}$, $NDF_{t,s}$, $Starch_{t,s}$, and $CP_{t,s}$ were dietary percentages and $WetFor_{t,s}$ was dietary wet forage percentage. Ruminal starch degradation was also fit by dietary nutrient percentages and forage NDF percentage ($ForNDF_{t,s}$):

$$RDSt = Starch \times \left( \begin{array}{l} 67.5 + 18.4 \times Omasal - 1.45 \times DMI_{t,s} + 0.424 \times ForNDF_{t,s} \\ +\ 1.39 \times Starch_{t,s} - 0.0219 \times Starch_{t,s}^2 - 0.154 \times WetFor_{t,s} \end{array} \over 100 \right). \quad [19]$$

These ruminal NDF and starch degradation estimates were used as inputs to a final method of predicting microbial N:

$$MicrN_{t,s} = \frac{a + b \times RDPIn_{t,s}}{\left(1 + \dfrac{c}{RDNDF_{t,s}} + \dfrac{d}{RDSt_{t,s}}\right)}, \quad [20]$$

where $RDPIn_{t,s}$ was RDP intake (kg/d), and $RDNDF_{t,s}$ and $RDSt_{t,s}$ were predicted in Eq. [18] and [19]. The equation is a multi-substrate Michaelis-Menten form, which allows for a response variable (microbial N) to saturate against 2 or more driving variables ($RDNDF$ and $RDSt$). In this case, we follow the assumption that maximal microbial N production can be limited by RDP intake and therefore express the horizontal asymptote of this equation as a linear function of RDP intake. This function was derived using both NLME and NLS. In the initial fitting steps, a term for ruminally degradable OM (calculated as specified in White et al., 2016) was also included but it was not significant and thus was omitted from the final model. This term was likely nonsignificant because ruminally degradable OM, NDF, and starch will be highly correlated and some variation in OM or residual OM will be encompassed by changes in NDF and starch. It is likely that on some diet types, residual OM will play an important role in driving microbial N, independent of degradable NDF or starch. Data in this analysis were inadequate to identify such a role but future work should focus on defining ruminal degradability of residual OM fractions to better test whether or how these chemical compounds affect microbial N.

## RESULTS AND DISCUSSION

### NANMN

***Evaluation of the NRC (2001) Model.*** On average, the NRC (2001) model over-estimated NANMN flows by 38 g/d (Table 2; NRC), and this mean bias was responsible for 19% of the prediction error. A substantial slope bias (22% of MSPE) was also identified. Concordance (CCC = 0.37) indicated poor agreement between the modeled and the observed values when the NRC (2001) model was used to estimate NANMN (Table 2; NRC).

In the NRC (2001) model, RDP is estimated from feed protein fractions, Kp, and $K_d$ (Ørskov and McDonald, 1979), and RUP is calculated by difference. Comparison of the modeled and observed data indicated that the over-prediction in NANMN increased as the predicted amount of NANMN increased (Table 2; slope bias = 22% of MSE). This work is not the first to identify bias in the NRC (2001). Bateman et al. (2005) also identified bias in NANMN predictions (when compared with duodenal sampling) and derived linear and nonlinear adjustments to tabular RUP values to improve NANMN prediction accuracy. Over-prediction of RUP supply was also noted when the NRC (2001) estimates were compared with data collected from omasal sampling approaches (Broderick et al., 2010).

Overestimation of RUP supply might be due to mis-specified A, B, and C protein fractions and $K_d$ estimates. When values from the new feed library (Supplemental Table S1; https://doi.org/10.3168/jds.2015-10801) were used, fit of the NRC (2001) model

was only marginally improved (Table 2; NRC+Feed). Alternatively, the errors in estimating RUP may be due to estimates of Kp or to changes in animal characteristics over time. The data used in this analysis were from more recent studies than those used to parameterize the NRC (2001). The older data used in the NRC (2001) likely had lower intake and thus lower passage rates. As such, it is possible that the NRC (2001) equations were more accurately calibrated to this data range. The Kp equations used by the NRC (2001) were biased compared with Kp measurements from studies using indigestible NDF as a marker (Krizsan et al., 2010). A recent study of Kp on forage-based diets also supports errors in prediction of particulate Kp (Gregorini et al., 2015). Markers should be inherent to the fraction being studied and not affect passage of that fraction, but no markers that specifically mark protein have been used. Poor agreement between marked particles and particles of interest may be an underlying cause for some bias in Kp estimates within the NRC (2001) model, and such bias will cause errors in both RUP and RDP because both depend on Kp (NRC, 2001).

Deriving new parameters for the NRC (2001) protein system (Table 2; Eq. [5]) resulted in negligible mean and slope bias (Table 2; <1% of MSE). Equation [5] (fitted using NLME) resulted in an RMSE of 18% of the observed mean and had high CCC (0.89; Table 2). Although not statistically appropriate, estimating fit statistics for Eq. [5] by adjusting to remove the random study effects demonstrated that the model fit explained a similar proportion of the variance in the observed data as the NRC (2001; unadjusted RMSE = 39%; Table 2).

Because the NRC (2001) model and derivations thereof continued to return somewhat poor fit statistics, a linear model was derived predicting NANMN with an intercept (Table 2, Eq. [6], *a*) and slope of N intake (Table 2, Eq. [6], *b*). This model (Table 2, Eq. [6]; fitted with NLME) had marginally improved RMSE and CCC compared with the re-derived NRC (2001) model, indicating that the added complexity of the NRC (2001) approach provided minimal benefit in terms of fit. Although congruent with other work based on large literature database evaluations, the improved statistical agreement of Eq. [6] contrasts with some experimental work evaluating NANMN responses to differing feedstuffs (Cunningham et al., 1993; Erasmus et al., 1994), forage types (Abreu et al., 2004), energy densities (Cecava et al., 1988), or feed additive inclusion (Erasmus et al., 1992). If applied in the field, Eq. [6] would estimate a constant percentage of CP intake appearing postruminally, irrespective of dietary protein source used. Although Eq. [6] represents limited knowledge about RUP responses to diet, further development of an RUP/RDP model should be compared with this simple function to ensure that work performs better than a simple average. Obviously, if the more complicated system does not perform better than, or at least as well as, this simple representation, then it also has limited utility.

***Derivation of a New System.*** To test the idea that a 3-pool protein system with pool-specific Kp or $K_d$ could be used to model rumen protein dynamics, 2 new protein systems (Eq. [7] to [12] or Eq. [9] to [14]) were derived. Both equation sets estimated postruminal appearance of A and B protein fractions as a function of feed intake and chemical composition. The NLME system (Eq. [7] to [12]; Table 2) returned the lowest RMSE (15% of observed mean) and lowest AICc (5,701) compared with other estimated NLME models (Table 2); however, the improvement in fit was minimal given the number of additional parameters used. The system fit with NLS (Eq. [9] to [14]) had larger RMSE, lower CCC, and higher AICc compared with the NLME approaches but the lack of random study effects in this system makes directly comparing the fit of Eq. [9] to [14] to the fit of the other equations inappropriate. A more robust comparison of the equations was achieved through cross-validation.

In contrast with a simpler model, which included an intercept shift for sampling type (Roman-Garcia et al., 2016), the current term included to differentiate between omasal and duodenal sampling methods became nonsignificant when the more complex system of equations was used to define NANMN flows. This change in significance potentially suggests that studies that used omasal sampling also had similar dietary characteristics and these dietary characteristics drove shifts in measured NANMN more so than any measurement difference between these methods. However, other studies also identified consistent differences associated with omasal sampling; therefore, differences in the mean measurement between these methods likely do exist.

***Cross-Validation and Model Selection.*** The results of cross validating the NANMN equations derived herein against independent literature data are presented in Table 2. In general, the new protein system derived with NLS (Eq. [9] to [14]) performed the best out of the evaluated equations, returning the lowest RMSPE (34 ± 0.5; Table 2) and highest CCC (0.42 ± 0.03; Table 2). In fact, the approach using Eq. [9] to [14] was the only NANMN prediction that performed better than the simple average model (Eq. [6]). Although the RMSPE of these 2 modeling approaches are similar (36 ± 1.6 vs. 34 ± 0.5), the improvement in CCC (0.30 ± 0.05 vs. 0.42 ± 0.03) was fairly substantial. Despite returning the most promising prediction of NANMN, the approach in Eq. [9] to [14] still had large errors

of prediction, likely attributable to the aggregation of experimental errors inherent in measuring NANMN.

To further investigate the potential implications of selecting a model derived with NLS rather than NLME, the NLME (Eq. [9] to [14]) and NLS (Eq. [7] to [12]) equations were used to predict postruminal NANMN for different feed types (Table 3). The RUP estimates predicted by Eq. [7] to [12] returned some improbable values that appear incongruent with biology. For example, the postruminal appearance rate of B protein from forages and animal proteins was estimated at −14% and −72%, respectively, and the RUP of animal protein (% of CP) was estimated to be negative (−13%; Table 3) before the mean study effect was added in. Even after the mean study effect was added, a 17% RUP for animal protein appears to conflict with previous literature highlighting animal protein as a good source of RUP (Santos et al., 1998).

Compared with the predictions from the NLME system (Eq. [7] to [12]), the NLS system (Eq. [9] to [14]) returned estimates of RUP that are in accord with expectations (Santos et al., 1998; Ipharraguerre and Clark, 2014). Animal protein was predicted to have the highest RUP (51% of CP; Table 3), followed by plant proteins (36% of CP), and byproduct feeds (34% of CP). When evaluating these estimates of RUP, it is important to recall that the RUP values estimated here are expected to be lower, on average, than those predicted by NRC (2001) because the NRC (2001) model over-predicted postruminal NANMN appearance (Table 2). The current model rectifies passage of A, but the NRC (2001) model was never verified against measured NANMN data and was biased (Bateman et al., 2005) When the improved biological interpretability of Eq. [9] to [14] is coupled with the more accurate fit in cross-validation (Table 2), the case for using the NLS models in this analysis becomes more compelling. For these reasons, Eq. [9] to [14] were used for subsequent model derivation efforts.

### *Microbial Protein*

***Evaluation of the NRC (2001) Approach.*** The NRC (2001) model (using original TDN equations) estimated microbial N with poor precision and accuracy (RMSPE = 37% of observed mean; CCC = 0.39; Table 4). Although slope bias was negligible (<1% MSE; Table 4), 20% of the prediction error was explained by under-predicting microbial N (by 40 g/d). Huhtanen and Hristov (2009) evaluated equations predicting microbial N against data from a European and a North American data set. They compared predicting microbial N from CP, TDN, RUP, or RDP where TDN, RUP, and RDP were predicted by the NRC (2001) model. In that

**Table 3.** Average feed composition and predicted postruminal appearance of A, B, and C protein fractions and feed CP for different feed types as predicted by Eq. [7] to [12] or Eq. [9] to [14] (Table 2) using A, B, and C protein fraction data from Supplemental Table S1 (https://doi.org/10.3168/jds.2015-10801)

| Item | Forages | | Plant protein | | Animal protein | | Energy source | | By-product feed | |
|---|---|---|---|---|---|---|---|---|---|---|
| Nutrients[1] reported or estimated from feed library | | | | | | | | | | |
| NDF, % of DM | 48 | | 21 | | 0 | | 11 | | 53 | |
| ADFIP, % of DM | 1.6 | | 1.2 | | 0 | | 0.3 | | 1.6 | |
| NDFIP, % of DM | 2.2 | | 4.3 | | 0 | | 0.9 | | 4.8 | |
| CP, % of DM | 14 | | 47 | | 84 | | 9.3 | | 17 | |
| A, % of CP | 50 | | 19 | | 17 | | 35 | | 26 | |
| B, % of CP | 40 | | 78 | | 59 | | 58 | | 64 | |
| C, % of CP | 10 | | 3 | | 24 | | 7 | | 10 | |
| Nutrients[2] estimated with Eq. [7]–[12] or Eq. [9]–[14] | | | | | | | | | | |
| Fitting method[2] | NLME | NLS | NLME | NLS | NLME | NLS | NLME | NLS | NLME | NLS |
| Postruminal A, % of A | 40 | 32 | 34 | 0 | 34 | 0 | 34 | 0 | 34 | 0 |
| Postruminal B, % of B | −14 | 16 | 16 | 43 | −72 | 46 | 17 | 31 | 42 | 38 |
| Postruminal CP, % of CP | 24 | 33 | 22 | 36 | −13 | 51 | 29 | 25 | 46 | 34 |
| Postruminal CP + mean study effect, % of CP | 54 | | 51 | | 17 | | 58 | | 75 | |
| Postruminal CP, g/g of DM | 7.6 | 4.6 | 24 | 17 | 14 | 43 | 5.4 | 2.3 | 13 | 5.8 |

[1]Nutrients included acid detergent insoluble protein (ADFIP), neutral detergent insoluble protein (NDFIP), and protein A, B, and C fractions (A, B, C).
[2]Estimated postruminal CP fractions are presented as predicted by the nonlinear mixed effect model (NLME; Eq. [7] to [12]) and the nonlinear least squares model (NLS; Eq. [9] to [14]).

**Table 4**. Parameter estimates and overall model fit of NRC (2001) and selected new equations for predicting for microbial N using TDN equations derived in White et al. (2017) using nonlinear mixed-effect regression (n = 525)

| Item[1] | NRC (2001) | +TDN | +TDN +RUP | Eq. [16] | Eq. [16] | Eq. [17] |
|---|---|---|---|---|---|---|
| Fitting method[2] | | | | NLME | NLS | NLS |
| Parameter[3] | | | | | | |
| *a* | | | | — | | 2.03 (<0.01) |
| *b* | | | | 0.0992 (<0.01) | 0.129 (<0.01) | 0.643 (<0.01) |
| *c* | | | | 23.4 (0.08) | 66.8 (<0.01) | 13.9 (0.02) |
| *d* | | | | | | 61.2 (<0.01) |
| Mean random effect | | | | 66 | | |
| Observed mean, g/d | 286 | 286 | 286 | 286 | 286 | 286 |
| Predicted mean, g/d | 246 | 253 | 259 | 285 | 286 | 286 |
| RMSE or RMSPE, % of observed mean | 37 | 31 | 30 | 11 | 27 | 25 |
| Mean bias, % of MSE or MSPE | 20 | 14 | 10 | <1 | <1 | <1 |
| Slope bias, % of MSE or MSPE | <1 | <1 | <1 | <1 | <1 | <1 |
| RMSE/SD | | 0.95 | 0.91 | 0.35 | 0.82 | 0.78 |
| CCC | 0.39 | 0.34 | 0.37 | 0.94 | 0.49 | 0.56 |
| AICc | | | | 5,816 | 6,060 | 6,013 |
| $\sigma_s$ | | | | 115 | | |
| $\sigma_e$ | | | | 38 | | |
| Unadjusted RMSE | | | | 28 | | |
| Unadjusted CCC | | | | 0.36 | | |
| Monte Carlo cross-validation[4] | | | | | | |
| RMSPE, % of observed mean | | | | 28 ± 1.3 | 27 ± 1.0 | 26 ± 0.9 |
| Mean bias, % of MSPE | | | | <1 ± 0.8 | <1 ± 0.3 | <1 ± 0.5 |
| Slope bias, % of MSPE | | | | 1.60 ± 0.8 | <1 ± 0.2 | <1 ± 0.8 |
| CCC | | | | 0.38 ± 0.04 | 0.50 ± 0.03 | 0.56 ± 0.02 |

[1]Model evaluation criteria included root mean squared prediction error as a percent of observed mean (RMSPE), mean and slope bias as a percent of mean squared prediction error (MSPE), RMSPE as a proportion of observed standard deviation (RMSPE/SD), and concordance correlation coefficient (CCC) for the NRC (2001). Evaluation criteria for derived equations included root mean squared prediction error (RMSE), mean and slope bias as a percent of mean squared error (MSE), RMSE as a proportion of observed standard deviation (RMSE/SD), CCC, corrected Akaike information criterion (AICc), variance from study ($\sigma_s$) and residual error ($\sigma_e$), and RMSE and CCC unadjusted for study effects.

[2]Fit method refers to the model derivation approach with NLME indicating that mixed-effect regression was used (with random study effect), and NLS indicating that nonlinear least squares regression was used (no study effect).

[3]Parameter names are as referenced in each equation, and parameter estimates are presented with significance values in parentheses.

[4]Cross-validation (±SD of the variables) was performed using 500 iterations of a repeated random sampling approach, in which 60% of the data was used for derivation and 40% used as an independent evaluation.

analysis, dietary CP was identified as a better predictor of measured microbial N flows than RDP. However, the authors used tabular RUP, RDP, and TDN values from the NRC (2001); thus, they assumed these values were accurate representations of feed chemical composition. In contrast to this assumption, White et al. (2017) identified notable biases in estimates of nutrient digestibility by the NRC (2001).

To better account for errors associated with predicting nutrient digestibility, the adjusted estimates of TDN derived in White et al. (2017) were applied and the modeled microbial N flows were evaluated. Table 4 shows the evaluation and subsequent equation fitting using the NLME nutrient digestibility models presented in White et al. (2017). Table 5 presents the equivalent evaluation using the NLS nutrient digestibility models presented in White et al. (2017). Applying the adjusted nutrient digestibility equations reduced mean bias from 20 to 14% of MSE for the NLME models (Table 4; +TDN) and to 10% for the NLS models (Table 5; +TDN), suggesting that part of the microbial N under-prediction was due to improperly characterized nutrient digestibilities. Applying the RUP prediction derived using NLS (Eq. [9] to [14]) in addition to the nutrient digestibility equations (using either NLME- or NLS-based estimates) further reduced mean bias (to 10 and 5.5% of MSE; Table 4 and Table 5; +TDN+RUP). Although the NRC (2001) RMSPE was higher than that of the +TDN+RUP model (37 vs. 30% of observed mean; Table 4), the CCC was also higher (0.39 vs. 0.37; Table 4), making it difficult to objectively identify whether the new TDN and RUP equations improved the NRC (2001) prediction of microbial N.

When the microbial N flow equation coefficients were re-derived, the cap to limit microbial N prediction based on availability of RDP dropped from the model because the system was singular, irrespective of fitting method. At the point that it dropped from the NLS model, the coefficient had a value of 1.45, suggesting that microbial N needed to exceed 145% of RDP before RDP became limiting, which contrasts with the NRC (2001) prediction of 85%. Although this disparity might

suggest that RDP is less important for microbial synthesis than previously estimated, it could also reflect limitations with the data because few studies actually tested RDP limiting diets and the equation form does not account for N recycling. The coefficient describing the RDP restraint on microbial N production was nonsignificant; however, the RDP restraint was retained assuming that RDP became limiting when microbial N predicted by TDN was equal to RDP. Parameter estimates for this equation form (Eq. [16]) were derived 4 times using either NLS or NLME nutrient digestibility equations (Table 4 or Table 5, respectively) and using either NLS or NLME model fitting.

Equation [16] derived using NLME suggested either 9.9 or 10.5 g of microbial protein per kg of TDN intake (parameter *b*; Table 4 and Table 5). An effect for omasal versus duodenal sampling (parameter *c*; Eq. [16]) was significant in the model using NLME TDN measurements (Table 4) but was dropped due to nonsignificance in the model fit with NLS TDN measurements (Table 5). A recent meta-analysis identified that sampling location had a significant effect on estimates of rumen starch digestibility (White et al., 2016). The relationship between sampling location and microbial N estimation might be partially related to differing estimates of starch digestibilities when samples are taken from the duodenum or omasum. The NLS total-tract starch digestibility model presented in White et al. (2017) was an accurate prediction of apparent total-tract starch digestibility and thus may have partially explained some of the variability in starch digestibility that co-varied with sampling location.

Equation [16] derived using NLS suggested either 12.9 or 12.3 g of microbial protein per kg of TDN intake (parameter *b*; Table 4 and Table 5). Unlike the NLME model, fitting Eq. [16] using NLS or NLME TDN estimates (Table 4 and Table 5, respectively) both resulted in a significant omasal sampling effect. Both the TDN and sampling location coefficients (*b* and *c*, respectively) were higher in Eq. [16] derived using NLS than Eq. [16] derived using NLME, likely because of the large mean random effect that was identified in the NLME models. The study effects estimated using NLME were not accounted for in estimating model fit to allow for better comparison to NLS regression (see unadjusted RMSE and CCC). Although this is not strictly statistically appropriate because study effects are an implicit component of mixed-effect regression, the comparison suggested minimal shifts in RMSE (28 vs. 27% of observed mean, Table 4; 30 vs. 27% of observed mean, Table 5) across fitting methods, although CCC was lower in the NLME models (0.36 vs. 0.49, Table 4; 0.27 vs 0.45, Table 5).

***Derivation of a New Model.*** The lack of significant RDP effect contrasts with experimental work demonstrating that microbial N can be limited by reduced dietary protein concentration (Hume et al., 1970; Dewhurst et al., 2000; Boucher et al., 2007). The failure to identify an effect of RDP on microbial N could suggest that few studies actually fed diets low enough in RDP to detect an effect; however, this is somewhat paradoxical because the RDP effect dropped from the re-derived NRC (2001) models with parameter estimates of >1 (conversion of RDP into microbial protein with over 100% efficiency). Biologically, the only way to achieve CP conversion efficiency above 100% is for dietary CP to be very low. Although urea recycling can help offset rumen N restriction (Reynolds and Kristensen, 2008), it is unlikely to be the cause of the failure to identify an RDP cap in Eq. [16] because few of the source studies restricted CP. Most likely, failure to identify a RDP cap was a mathematical issue, rather than a biological or computational artifact. The discontinuous relationships fit in the NRC (2001) can be challenging to characterize mathematically, particularly when the data are nearly linear with respect to one driving variable. Microbial growth responds to energy and protein supplies in a continuous manner (Hackmann and Firkins, 2015). Thus, a new equation form was tested that predicted microbial N as a saturating function (multi-substrate Michaelis-Menten form) of TDN intake and RDP supply. Because the NLME version of this function was nonsignificant, only results from NLS derivation are presented. Allowing RDP to have a continuous effect on microbial N (Eq. [17]), rather than a discontinuous effect (Eq. [16]) resulted in a significant linear RDP effect ($P < 0.001$; Table 4, Table 5), reduced RMSE (25 vs. 27%; Table 4, Table 5) and improved CCC (0.56 vs. 0.49, Table 4; 0.55 vs. 0.45, Table 5), although the magnitude of the reduction was small. Nonlinear responses to RDP were also tested but did not have improved fit compared with the linear effect presented here. The fact that a different equation form is able to detect a significant effect of RDP suggests that the inability to detect this effect in Eq. [16] was due to equation functional form, rather than any real support in the database.

Deviations from the NRC (2001) calculation approach are not a new tactic in modeling microbial N. Both Broderick et al. (2010) and Ipharraguerre and Clark (2014) predicted microbial N as a continuous function of N supply (either by CP or RDP intake) and ruminally degradable OM. Although simple linear models, these papers moved toward accounting for the site of carbohydrate digestion in microbial N models. Indeed, TDN is a somewhat paradoxical variable to use for predicting microbial N because it contains many components

**Table 5**. Parameter estimates and overall model fit of NRC (2001) and selected new equations for predicting for microbial N using TDN equations derived in White et al. (2017) using nonlinear least-squares regression (n = 525)

| Item[1] | NRC (2001) | +TDN | +TDN +RUP | Eq. [16] | Eq. [16] | Eq. [17] | Eq. [20] |
|---|---|---|---|---|---|---|---|
| Fitting method[2] | | | | NLME | NLS | NLS | NLS |
| Parameter[3] | | | | | | | |
| *A* | | | | — | | 1.79 (<0.01) | 7.47 (<0.01) |
| *B* | | | | 0.105 (<0.01) | 0.123 (<0.01) | 0.666 (<0.01) | 0.574 (<0.01) |
| *C* | | | | | 73.5 (<0.01) | 13.0 (0.02) | 3.60 (<0.01) |
| *D* | | | | | | 63.5 (<0.01) | 12.3 (<0.01) |
| Mean random effect | | | | 50 | — | | |
| Observed mean, g/d | 286 | 286 | 286 | 286 | 286 | 286 | 286 |
| Predicted mean, g/d | 246 | 251 | 266 | 285 | 286 | 286 | 286 |
| RMSE or RMSPE, % of observed mean | 37 | 31 | 30 | 11 | 27 | 25 | 25 |
| Mean bias, % of MSE or MSPE | 20 | 10 | 5.5 | <1 | <1 | <1 | <1 |
| Slope bias, % of MSE or MSPE | <1 | <1 | <1 | <1 | <1 | <1 | <1 |
| RMSE/SD | | 0.95 | 0.91 | 0.35 | 0.84 | 0.78 | 0.76 |
| CCC | 0.39 | 0.33 | 0.36 | 0.94 | 0.45 | 0.55 | 0.60 |
| AICc | | | | 5,785 | 6,078 | 6,017 | 5,982 |
| $\sigma_s$ | | | | 105 | | | |
| $\sigma_e$ | | | | 38 | | | |
| Unadjusted RMSE | | | | 30 | | | |
| Unadjusted CCC | | | | 0.27 | | | |
| Monte Carlo cross-validation[4] | | | | | | | |
| RMSPE, % of observed mean | | | | 28 ± 1.6 | 27 ± 1.7 | 26 ± 1.1 | 24 ± 1.1 |
| Mean bias, % of MSPE | | | | <1 ± 0.5 | <1 ± 0.9 | <1 ± 0.3 | <1 ± 0.5 |
| Slope bias, % of MSPE | | | | <1 ± 0.7 | <1 ± 0.1 | <1 ± 0.4 | <1 ± 0.2 |
| CCC | | | | 0.30 ± 0.05 | 0.46 ± 0.02 | 0.55 ± 0.04 | 0.63 ± 0.03 |

[1]Model evaluation criteria included root mean squared prediction error as a percent of observed mean (RMSPE), mean and slope bias as a percent of mean squared prediction error (MSPE), RMSPE as a proportion of observed standard deviation (RMSPE/SD), and concordance correlation coefficient (CCC) for the NRC (2001). Evaluation criteria for derived equations included root mean squared prediction error (RMSE), mean and slope bias as a percent of mean squared error (MSE), RMSE as a proportion of observed standard deviation (RMSE/SD), CCC, corrected Akaike information criterion (AICc), variance from study ($\sigma_s$) and residual error ($\sigma_e$), and RMSE and CCC unadjusted for study effects.

[2]Fit method refers to the model derivation approach with NLME indicating that mixed-effect regression was used (with study effect), and NLS indicating that nonlinear least squares regression was used (no study effect).

[3]Parameter names are as referenced in each equation, and parameter estimates are presented with significance values in parentheses.

[4]Cross-validation (±SD of the variables) was performed using 500 iterations of a repeated random sampling approach, in which 60% of the data was used for derivation and 40% used as an independent evaluation.

that are not utilized by microbes and includes nutrients digested postruminally. The NRC (2016) for beef cattle utilized fat-free TDN to better account for true substrate available for microbes; however, they retained a structure with both fat-free TDN and RDP. These measures likely result in double accounting of available substrate because RDP is a component of TDN. To more thoroughly address available energy and protein for microbial N production, ruminally available N and CHO should be considered as distinct substrates. We attempted to move toward this goal by applying a nonlinear, biologically interpretable equation form, which assessed how microbial N responds to specific rumen-degraded carbohydrates (starch, nonstarch NFC, and NDF are the main substrates for ruminal microbes). Although RMSE showed minimal difference, this function (Eq. [20]) had improved CCC when compared with Eq. [16] (0.60 vs. 0.45; Table 5). Because a NLME derivation of this equation resulted in nonsignificant parameter estimates, only NLS derivation is shown. An additional kilogram of RDP supply increased potential microbial protein synthesis by 574 g of microbial protein (coefficient *b*; Eq. [20]; Table 5), and microbial protein increases from ruminally degradable starch were greater than those from ruminally degradable NDF (coefficients *d* and *c*, respectively; Eq. [20]; Table 5). Peptides typically have been prioritized for growth of amylolytic bacteria rather than fibrolytics (Fox et al., 2004). Roman-Garcia et al. (2016) found that microbial N was increased by rumen-degraded starch and the ratio of rumen-degraded starch to rumen-degraded NDF. Figure 1 depicts the increased responsiveness to starch compared with NDF as substrates, although caution should be exerted when evaluating these panels independently because the original equations depict increasing availability of starch suppressing NDF digestibility in the rumen. Although the ranges of the 3 driving dietary characteristics (RDP, ruminally degradable starch, and ruminally degradable NDF) were within the bounds allowed in this study, it is important to keep in mind that in most cases these inputs will co-vary to some degree and this covariation should be accounted for when testing the equation.

***Cross-Validation and Model Selection.*** Cross-validation of the microbial N equations showed slight improvement in the RMSPE of equations predicted from the NLS TDN models (Table 5) compared with the NLME models (Table 4), although differences in CCC did not always agree. Equation [20] (derived using NLS) had the lowest RMSPE (24%; Table 5) and highest CCC (0.63; Table 5) of any microbial equation evaluated (Table 4; Table 5), and therefore it was used in all downstream calculations. Coincidently, Eq. [20] also arguably described the response in a more accurate and biological sense because it related microbial N to ruminally degraded carbohydrate fractions and to RDP intake.

### *Remaining Errors in Predicting NANMN and Microbial N*

The substantial residual error in predicting NANMN and microbial N may support the movement toward a chemically derived feed database for use in characterizing passage or degradation of proteins (Higgs et al., 2015), rather than a database constructed from in situ observations. A chemically derived feed database is attractive from a model development standpoint because it might support improved uniformity in characterizing feeds used (when reporting for publication). Additionally, it would allow for more specific customization of model inputs during on-farm application based on the feasibility of using a chemical feed assay compared with incubating individual feeds over time in situ. That said, a tremendous amount of variability in feed ingredients and animal performance data should be gathered to properly validate such a database to ensure that the intent of the chemical assay is realized in practice. Although a logical approach forward, the practicality of switching to a new system presents a circular problem. Specifically, there will be no incentive to pay for an analysis unless it is a required model input; however, the chemical component will not be a required model input until sufficient data are available that link performance to shifts in that chemical component. The current calculation approach provides a simpler solution that only needs to allow washout (fraction A) and extent of disappearance reached at a suitable time point (at least 48 h for concentrates and at least 72 h for forages) while also better simulating the rumen environment in situ. As such, until a robust chemically derived database and model can be developed, the RUP/RDP models proposed herein may present a reasonable compromise.

## CONCLUSIONS

Mean and slope biases were evident in the NRC (2001) modeled estimates of postruminal N flows, which were quantitatively related to poorly specified predictions of TDN and RDP. Attempts to reduce bias in estimating NANMN by fitting adjustment equations for $K_d$ values or re-deriving Kp were not successful. Therefore, NRC (2001) estimates of Kp and $K_d$ were eliminated from the protein system, and a new system was derived based on prediction of postruminal appearance rates of A, B, and C protein fractions for different feed types. This new protein system had better statistical fit than a re-derived version of the NRC (2001) model when

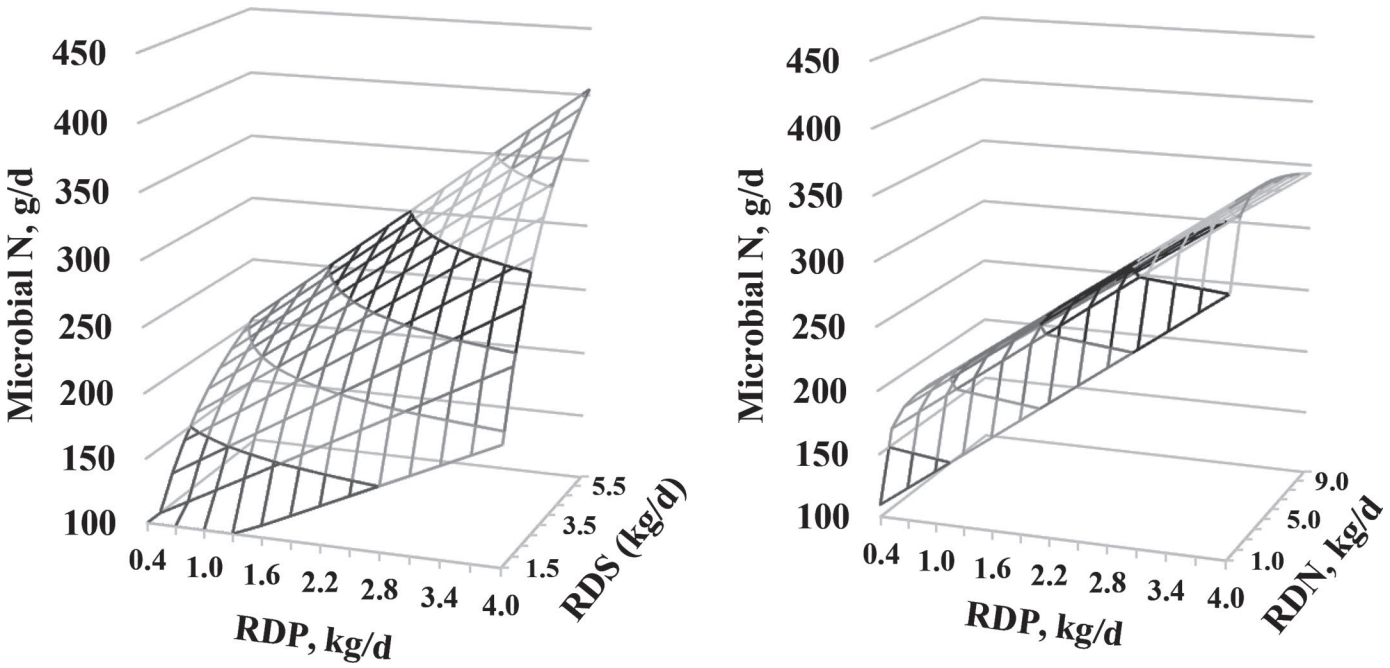


**Figure 1**. Predicted microbial N responses to increasing intakes of rumen-degraded starch (RDS), rumen-degraded NDF (RDN), and RDP using Eq. [20], which was derived using nonlinear least squares regression. The equation form predicted that microbial N increased asymptotically (saturated) as a function of increasing RDS and RDN with the horizontal asymptote expressed as a linear function of RDP. Covariation among intakes of RDS, RDN, and RDP was not considered in developing these graphs but must be considered when applying the equations.

both were cross-validated, suggesting that Kp and $K_d$ were a primary source of bias in the old system. A new prediction equation for microbial N was also identified and related microbial N to ruminally degradable NDF and starch and intake of RDP. Although the new equations had slight improvements in fit compared with the re-derived NRC (2001) models, substantial unexplained variation in microbial N and NANMN remained.

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