

Updates to the CNCPS v6.5 and a Perspective on the Future

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INTRODUCTION

The first complete version of the Cornell Net Carbohydrate and Protein System (CNCPS) was released in 1991, and was first published in 1992 and 1993 in a series of four papers (Fox et al., 1992; O'Connor et al., 1993, Russell et al., 1992; Sniffen et al., 1992). The principal objective of CNCPS was to serve as a tool for both research development and feed formulation for cattle (Russell et al., 1992). In order to fulfill these goals, the CNCPS has been continuously under development by incorporating research outcomes into mathematical equations. As a consequence, several updated versions have been released over the last 20 yr (Fox et al., 2000; Fox et al., 2004; Tylutki et al., 2008). Moreover, several implementations of the program have been used by the industry to evaluate and formulate diets. Other updates to the model have included the refining of the feed library (Higgs et al., 2015) and an improvement in the equations to predict nitrogen excretion (Higgs et al., 2012). The latest version, CNCPSv6.5 (Van Amburgh et al., 2015), is used as a formulation and evaluation platform by AMTS.Cattle (Agricultural Modeling and Training Systems LLC; Cortland, NY), NDS (Ruminant Management & Nutrition; Reggio Emilia, Italy), DinaMilk (Fabermatica; Ostiano, Italy), and Dalex (Dalex Livestock Solutions; Los Angeles, CA).

More recently, development of the CNCPS has been focused on improving the prediction of amino acid (AA) requirements and supply for lactating dairy cattle. This has led to a number of changes within the model including updated AA profiles in the feed

library, re-characterization of protein fractionation and pool assignments, and the adoption of a combined efficiency of utilization for essential amino acids (EAA) used for maintenance and lactation.

The objective of this paper is to provide a description of changes made to CNCPS in the last few years, which resulted in v6.5, and also to provide some discussion about the future of the model and how the current development group has incorporated more mechanistic and improved understanding of cattle biology, primarily in rumen and gut function, and how it alters our approach to formulating diets. The new version (v7.0) has been developed and evaluated on lactating cattle and the outcome will be discussed.

MODEL UPDATES

Protein Fractionation and Digestion Rates

The information provided by the CNCPS feed library, including estimations of digestion kinetics of protein fractions within each feed, are as important as any other component of the model structure. The CNCPS feed library includes more than 800 different feeds and was recently reviewed and updated using large datasets from commercial laboratories by Higgs et al. (2015). Updates to the feed library included a re-characterization of the non-protein nitrogen (NPN) fraction (PA) to ammonia (PA1) and the soluble true protein fraction (PB1) to soluble non-ammonia CP (PA2). A summary of the changing nomenclature in the equations used to calculate ruminal

degradation, outflow, and intestinal digestion are in Table 1.

Degradation rates of protein fractions were previously updated as described by Van Amburgh et al. (2007) which, along with re-assigning the soluble protein pools to flow with the liquid passage rate, represented a considerable improvement in the sensitivity of MP predictions. In this update, the PB2 pool (fiber bound protein) was linked to the CHOB3 pool (digestible NDF) and the PA1 pool was lowered to 200 %/hr from 10,000 %/hr. The more recent re-characterization of the PA1 pool from NPN to ammonia described by Higgs et al. (2015) shifted a considerable amount of protein from the PA1 to the PA2 pool. In the

CNCPS, the PA1 pool does not contribute MP to the animal; whereas the PA2 pool can contribute up to 15 % of total AA flow to the small intestine (Reynal et al., 2007; Volden et al., 2002). Hence, this new configuration considerably increased the predicted MP supply. Van Amburgh et al. (2010) reported that MP predictions, prior to the most recent update, were in good agreement with observed milk. Therefore, the rates associated with PA2 and PB1 pools were re-calculated to ensure MP predictions were consistent with the previous predictions. The re-calculated rates are 10-40 %/hr and 3-20 %/hr for the PA2 and PB1 pool, respectively, and are consistent with literature reports (Lanzas et al., 2007b).

Table 1. Equations to compute pools, rumen degradation, and intestinal digestion for feed protein fractions.

Variables ¹	Description	Equations ^{2,3}
PA1 _j	Ammonia	$\text{ammonia}_j \times (\text{SolCP}_j/100) \times (\text{CP}_j/100)$
PA2 _j	Soluble non-ammonia CP	$\text{SolCP}_j \times \text{CP}_j/100 - \text{PA1}$
PC _j	Unavailable CP	$\text{ADIP}_j \times \text{CP}_j/100$
PB2 _j	Slowly degradable CP	$(\text{NDIP}_j - \text{ADIP}_j) \times \text{CP}_j/100$
PB1 _j	Moderately degradable CP	$\text{CP}_j - \text{PA1}_j - \text{PA2}_j - \text{PB2}_j - \text{PC}_j$
RDPA1 _j	Ruminally degraded PA1	$\text{DMI}_j \times \text{PA1}_j$
RDPA2 _j	Ruminally degraded PA2	$\text{DMI}_j \times \text{PA2}_j \times (\text{kdPA2}_j / (\text{kdPA2}_j + \text{kp}_j))$
RDPB1 _j	Ruminally degraded PB1	$\text{DMI}_j \times \text{PB1}_j \times (\text{kdPB1}_j / (\text{kdPB1}_j + \text{kp}_j))$
RDPB2 _j	Ruminally degraded PB2	$\text{DMI}_j \times \text{PB2}_j \times (\text{kdPP2}_j / (\text{kdPB2}_j + \text{kp}_j))$
RDPEP _j	Ruminally degraded peptides	$\text{RDPA2}_j + \text{RDPB1}_j + \text{RDPB2}_j$
REPA2 _j	Ruminally escaped PA2	$\text{DMI}_j \times \text{PA2}_j \times (\text{kp}_j / (\text{kdPA2}_j + \text{kp}_j))$
REPB1 _j	Ruminally escaped PB1	$\text{DMI}_j \times \text{PB1}_j \times (\text{kp}_j / (\text{kdPB1}_j + \text{kp}_j))$
REPB2 _j	Ruminally escaped PB2	$\text{DMI}_j \times \text{PB2}_j \times (\text{kp}_j / (\text{kdPB2}_j + \text{kp}_j))$
REPC _j	Ruminally escaped PC	$\text{DMI}_j \times \text{PC}_j$
DIGPA2 _j	Digestible PA2	$\text{IntDigPA2}_j \times \text{REPA2}_j$
DIGPB1 _j	Digestible PB1	$\text{IntDigPB1}_j \times \text{REPB1}_j$
DIGPB2 _j	Digestible PB2	$\text{IntDigPB2}_j \times \text{REPB2}_j$
DIGFP _j	Digestible feed protein	$\text{DIGPA2}_j + \text{DIGPB1}_j + \text{DIGPB2}_j$

¹ Subscript *j* means for the *j* th feed.

² SolCP: soluble crude protein ; CP: Crude protein; NDIP: neutral detergent insoluble protein ; ADIP: acid detergent insoluble protein ; NDF: neutral detergent fiber; ADF: acid detergent fiber; DMI: dry matter intake; IntDig: intestinal digestibility constants

³ Kp is either liquid (kpl), forage (kpf), or concentrate (kpc).

Amino Acid Profiles

Comparison of feed AA profiles in the original CNCPS feed library with profiles of other databases used in the industry showed that there were inconsistencies among the data. Much of this can probably be attributed to the analytical methods used to generate data for the original AA CNCPS feed library (O'Connor et al., 1993). Methods used on some feeds were not adequate to correctly quantify sulfur AA and often represented only one sample. Thus, methionine concentrations of some feeds are lower than reality and the sample size used to populate the library may not best represent what is most commonly used in the industry. However, other feeds added after the original library developments, including many proprietary feeds, were analyzed using correct methodology which has led to inconsistencies throughout the library.

To improve the consistency and accuracy of AA profiles in the CNCPS feed library, profiles were updated using datasets provided by Evonik Industries AG (Hanau, Germany), Adisseo (Commentary, France), and taken from the NRC (2001). Data provided were mean values from analyses completed in the respective companies' laboratories or published in the NRC (2001). In all cases, AA analyses were completed on the whole feed and are expressed in the CNCPS on a percent CP basis (equivalent to NRC, 2001). This differs from previous versions of the CNCPS where AA were expressed as a percent of the buffer insoluble residue (O'Connor et al., 1993). Analyzing AA on the buffer insoluble residue is analytically challenging and much larger databases exist for analyses of whole feed samples. Amino acids in the soluble fraction also contribute up to 15 % of the AA flowing out of the rumen undegraded

(Reynal et al., 2005) which are not present in the buffer insoluble residue. For these reasons the AA profiles were changed to being expressed on a whole feed basis.

To update the feed library, the most appropriate profile was assigned based on data availability and was used as received by the source without alteration. If profiles for specific feeds were not available in the datasets provided, current CNCPS values were retained. Proprietary feeds were not changed and were assumed to be analyzed using appropriate methods that provided adequate AA recoveries. Table 2 has examples of AA profiles from the old and new feed library.

Amino Acid Utilization

Another area of consideration has been the efficiency of AA utilization used by the CNCPS. Currently, AA requirements for maintenance and lactation are derived using two separate efficiencies of use as described by Fox et al. (2004). Lapierre et al. (2007) discussed the biological correctness of this assumption and suggested when considering the distribution of enzymes for AA catabolism and the dominant role the liver plays in modifying peripheral AA supply, using a combined efficiency of use makes more sense. Doepel et al. (2004) calculated a single efficiency of use for each essential AA using a meta-analysis of 40 published papers involving abomasal, duodenal, or intravenous infusions of casein or free AA (Table 3). In this version of the CNCPS, we adopted the efficiency that represented what was considered to be 100 % of MP supply from the work of Doepel et al. (2004) as described by Lapierre et al. (2007) and believe this to be a more representative efficiency that can be evaluated among variable ME allowable milk supply.

Table 2. Comparison of old and new amino acid profiles from selected feeds in the CNCPS feed library. Values from the old library are expressed as percent buffer insoluble residue. Values from the new library are expressed as percent CP from the whole feed.

Ingredient		Met	Lys	Arg	Thr	Leu	Ile	Val	His	Phe	Trp
Alfalfa hay, 17 CP 46 NDF 20 LNDF	Old	0.7	6.0	6.4	5.0	9.3	6.0	7.1	2.6	6.3	1.8
	New	1.3	4.8	4.2	4.0	6.7	3.9	5.0	1.9	4.6	1.4
Mixed hay, 13 CP 56 NDF 14 LNDF	Old	0.7	4.4	4.6	3.9	7.4	4.4	5.5	1.8	4.9	1.6
	New	1.4	4.3	4.5	4.0	6.8	3.8	4.9	1.8	4.3	1.4
Corn silage unprocessed, 35 DM 45 NDF coarse	Old	0.8	2.1	1.9	2.1	6.4	2.4	3.2	1.1	2.9	0.1
	New	1.6	2.8	2.3	3.4	8.5	3.4	4.5	1.7	3.9	0.7
Blood meal	Old	1.1	9.3	5.0	4.7	13.4	0.9	9.1	6.5	7.9	1.9
	New	1.2	8.7	4.3	4.6	12.3	1.1	8.2	5.9	6.8	1.4
Soybean meal, 47.5 % CP solvent	Old	1.3	6.5	7.7	4.8	8.7	4.0	4.4	2.7	5.2	1.4
	New	1.3	6.1	7.3	3.9	7.6	4.5	4.7	2.6	5.1	1.3
Canola meal, expelled	Old	1.4	6.7	6.8	4.9	8.0	4.9	6.4	4.0	4.7	1.2
	New	2.1	5.7	6.1	4.4	7.0	4.2	5.3	2.6	4.0	1.5
Corn distillers, light spirits	Old	1.2	2.1	4.2	3.1	9.1	2.8	5.2	1.8	4.2	1.6
	New	2.0	2.8	4.3	3.7	11.7	3.7	4.9	2.7	4.9	0.8
Corn gluten, feed dry	Old	2.1	1.2	3.2	2.9	16.2	4.3	5.0	2.5	6.5	0.4
	New	1.6	3.1	4.6	3.6	8.5	3.0	4.7	2.9	3.5	0.5

Table 3. Combined efficiencies of amino acid utilization for both maintenance and lactation (adapted from Doepel et al. (2004) and Lapierre et al. (2007)) based on values derived from the data set at 100 % of the metabolizable protein requirement.

	Amino Acid								
	Arg	His	Ile	Leu	Lys	Met	Phe	Thy	Val
Efficiency	0.58	0.76	0.67	0.61	0.69	0.66	0.57	0.66	0.66

EVALUATION

Evaluation Dataset Development

Three different data sets were developed from both the literature (references not provided here), and from farm data from regional nutritionists to evaluate lysine (Lys) and methionine (Met) requirements, supply, rumen N balance, and milk yield predictions.

The first dataset (AA set), was compiled from studies where Lys, Met, or both were increased either by intestinal infusion or by feeding in ruminally protected form. In total 19 studies were selected and concentrations of digestible Lys (8 studies forming 43 treatments) and Met (11 studies forming 50 treatments) in protein truly digested were calculated for control and treatment groups. A dose-response approach was used to define required Lys and Met concentrations in MP for maximal protein synthesis according to Rulquin et al. (1993). Reference values of 6.80 and 2.43 % were identified intermediate to the lowest and highest concentration values for Lys and Met in MP, respectively. Predicted concentrations of Lys in MP varied between 4.99 and 9.30 % of MP and for Met between 1.69 and 2.85 % of MP. Positive and negative values for production responses were calculated using the reference values for control and treatment groups. Responses of milk protein yield (g/day) and the predicted concentrations of Lys and Met (% of MP) were evaluated by regression procedures.

The second dataset (rumen set) was compiled from studies where post-ruminal N flows were assessed with the omasal sampling technique (Ahvenjärvi et al., 2000; Huhtanen et al., 1997; Reynal and Broderick, 2005). A recent meta-analysis (Broderick et al., 2010; Huhtanen et al., 2010) on omasal sampling suggested that it is a reliable alternative to measuring nutrient flows via duodenal cannula. Moreover, the use of a triple marker system is more robust and reduces variation caused by the multiple and diverse markers used with post-ruminally cannulated animals. Therefore, to avoid inducing variation due to cannula position and the variety of markers used we included only studies with the omasal sampling technique. In total, 19 peer-review studies with 74 treatments were included.

The third data set (lactation set) was compiled from studies published in the Journal of Dairy Science between 2001 and 2012. Lactation trials were included for dairy cows in different stages of lactation (early, mid, and late). Studies with cross over design (Latin square, Box-Behnken, etc.) and with few experimental units ($n < 6$) were excluded from the data set. In total, 103 lactation studies were pre-selected, by which 55 with 200 treatments met the criteria for incorporation into the data set. The criteria for each study were:

- a. description and chemical analysis of the ration fed for each treatment,
- b. inclusion of each feed included into the ration,

- c. information of actual dry matter intake (DMI), and
- d. information on milk yield and milk composition for each treatment.

This dataset was enhanced by incorporating farm data from nutritionists in the Northeast U.S. that were willing to share their data. From the regional nutritionists 15 farms with 50 different diets were included.

A spreadsheet version of the CNCPS was used to conduct the model simulations for this study. Information on feed chemistry required by the CNCPS to run a simulation was used as reported by the study. When incomplete information was presented, values were predicted using the procedures described by Higgs et al. (2015). Animal information required to run a simulation in the CNCPS included a description of housing conditions, body weight (BW) and BW change for period studied, body condition score (BCS) and BCS change during the period studied, stage of lactation, and stage of pregnancy. If stage of pregnancy, BW, and BCS were not provided, CNCPS default values were used. When BW change was available, but BCS change was not, the final BCS (in CNCPS as the target BCS) was calculated from BW change assuming that empty body weight (EBW) changes, on average, 13.7 % for each unit of BCS change (Fox et al., 1999; and NRC, 2001). To calculate EBW from BW the following equations were used:

$$\text{EBW} = 0.851 * \text{Shrunk BW (SBW)}, \text{ and}$$

$$\text{SBW} = 0.96 * \text{BW}$$

$$\text{Therefore, EBW} = 0.81696 * \text{BW}$$

Statistical Analysis

Statistical analysis was conducted with JMP (SAS). To describe the relationships

between increasing concentrations of Lys and Met in MP and protein yield responses, a broken line model with a plateau was used. According to the NRC (2001), this linear model was either equal to or superior to other models for describing protein content and protein yield responses to increasing amounts of both Lys and Met in MP. The model consisted of a linear regression line to a break point followed by a plateau:

$$Y_{ij} = \beta_0 + \beta_1 X_{ij}, \text{ when } X \leq C$$

$$Y_{ij} = \beta_0 + \beta_1 C, \text{ when } X > C$$

Where, Y_{ij} = the expected outcome for the dependent variable Y observed at repetition j of the continuous variable X in study i , β_0 = the overall intercept across all studies, β_1 = the overall slope of Y on X across all studies, and C = the break point.

For the lactation and rumen datasets, a mixed effects model using the restricted maximum likelihood (REML) procedure was used to analyze the data as proposed by St-Pierre (2001):

$$Y_{ij} = \beta_0 + \beta_1 X_{ij} + s_i + b_{1i} X_{ij} + \varepsilon_{ij},$$

Where, Y_{ij} = the expected outcome for the dependent variable Y observed at repetition j of the continuous variable X in study i , β_0 = the overall intercept across all studies, s_i = the random effect of study i , β_1 = the overall slope of Y on X across all studies, b_{1i} = the random effect of study i on the slope of Y on X, X_{ij} = the data associated with repetition j of the continuous variable X in study i , and ε_{ij} = random variation.

To evaluate the performance of the model several statistics were calculated. The squared sample correlation coefficients reported were based on either the BLUP (R^2_{BLUP}) or model predictions using a mean

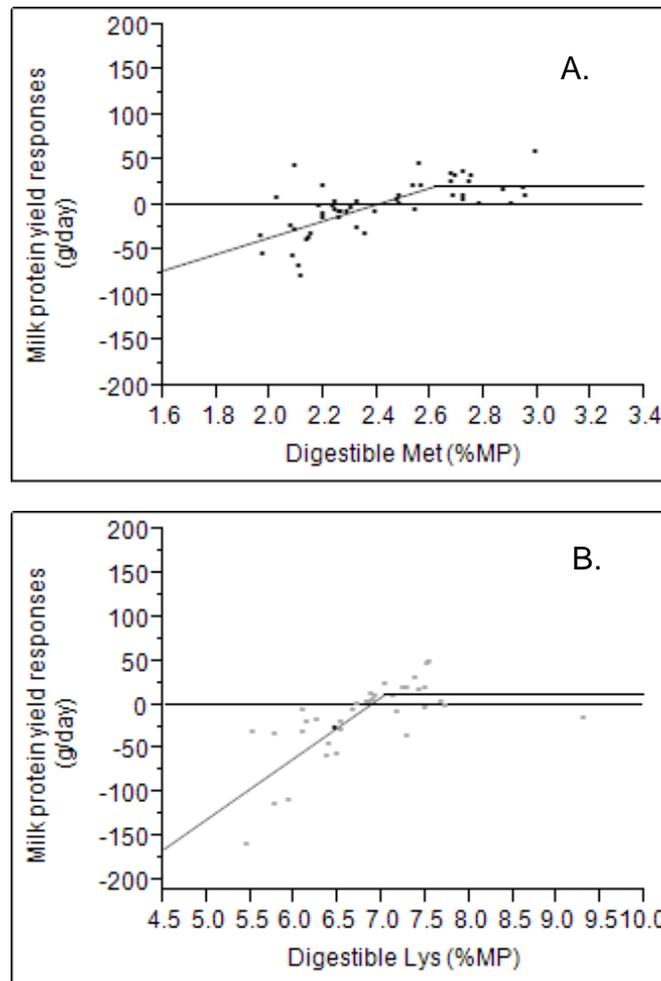


Figure 1. Milk protein yield responses as a function of digestible methionine (A) (Met; $y = -219 + 92.65 \cdot \text{Met}$ and $y = -219 + 92.65 \cdot 2.60$ for the linear and the plateau part of the model, respectively) and lysine (B) (Lys; $y = -478 + 70.02 \cdot \text{Lys}$ and $y = -478 + 70.02 \cdot 7.00$ for the linear and the plateau sections of the model, respectively).

study effect (R^2_{MP}). The Bayesian information criterion (BIC) was used as the statistical criterion to indicate the goodness of model fit, where lower values indicate a better fit. The residuals (predicted – observed) were visually examined for any patterns as well as for any potentially confounding factors. Additional model adequacy statistics were calculated to give further insight into the accuracy, precision, and sources of error in each model (Tedeschi, 2006). Mean square prediction errors

(MSPE) were used to indicate accuracy. A decomposition of the MSPE was also performed to give an estimation of the error due to central tendency (mean bias), regression (systematic bias), and random variation. Concordance correlation coefficients (CCC) were used to simultaneously account for accuracy and precision. Concordance correlation coefficients can vary from 0 to 1, with a value of 1 indicating that no deviation from the $Y = X$ line has occurred.

RESULTS AND DISCUSSION

Lys and Met Requirements

The plots of model predicted concentrations of Lys and Met (%MP) and the corresponding responses of milk protein yield are presented in Figure 1. The breakpoint estimates for Lys and Met for maximal milk protein yield were 7.00 and 2.60 % of MP, respectively. Similar breakpoint estimates were reported for NRC (2001) and the previous version of CNCPS. The CNCPSv6.1 estimated Lys breaking point at 6.93 % of MP and that of Met at 2.34 % of MP (Whitehouse et al., 2013). Current estimations require slightly higher Lys, and 11 % higher Met supply to optimize protein yield responses, which can be attributed to the updated AA profiles in the feed library.

Efficiency of AA Use

To evaluate the updated efficiency of AA use included in the CNCPS, the data set used to determine the optimum proportion of Met and Lys in MP was used to perform a regression of model predicted AA balance (g Met/d) against the concentration of Met in the diet (Met % MP). Using the new efficiencies (Table 3), the regression line intercepted the Y axis at approximately 2.6 % dietary Met relative to total MP (Figure 2), similar to the breakpoint derived in Figure 1 A. The studies used to perform this analysis were specifically designed to be both sufficient and limited in Met supply in order to observe a dose response. Hence, one would expect the model to predict both positive and negative Met balance. Using the old efficiencies of AA use, the regression line intercepts the Y axis at 2.0 %

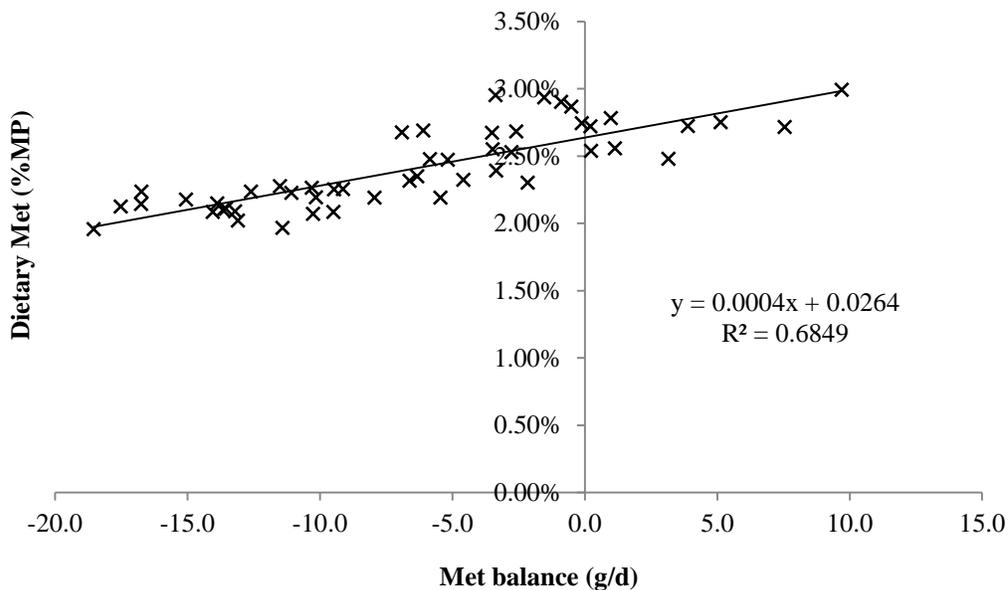


Figure 2. Model predicted Met balance (MP Met supply less requirement; g Met/d) versus dietary Met (% MP) with updated efficiencies of use of absorbed amino acids.

dietary Met (% MP) and no diets are predicted to have negative Met balance, contrary to expectations. Using the new efficiencies (Figure 2), there is a balance of both positive and negative Met balance among the data set. This suggests the new efficiencies of use allow the model to more adequately represent the true gram per day requirements of EAA.

Rumen Degradation

Updates to the digestion rates, passage rate assignments (Van Amburgh et al., 2010), and pool characterization (Higgs et al. 2015; and Lanzas et al., 2007a) have made MP predictions by the CNCPS more sensitive than previous versions of the model (Van Amburgh et al., 2010). The ability of the model to predict the various nitrogen fractions leaving the rumen was evaluated against omasal flow data. Studies in the compiled dataset reported measures of ruminal undegraded N (RUN), non-ammonia N (NAN) and bacterial N (BactN) flows. The dataset represented a wide range of diets and nutrient compositions (Table 4). The omasal flow of BactN and RUN ranged from 78 to 480 and from 7 to 326 g/d, respectively (Figure 3). The model predicted post-ruminal flows of NAN ($R^2 = 0.97$; RMSE = 24.57) and RUN ($R^2 = 0.91$; RMSE = 21.93) well, but with

the current rates and pools size descriptions, underestimates BactN ($\beta_1 = 1.55$) and overestimates RUN ($\beta_1 = 0.73$). However, there is a uniform offset which provides a prediction of NAN that is robust with little bias (NAN; $R^2 = 0.98$; RMSE = 26.77; $\beta_1 = 1.17$). The variance component analysis indicated that most of the variance is attributed to the study effect and not residuals, even though residual influence was higher for BactN (Table 6).

Milk Yield Prediction

Diets with a wide range of nutrients were included in the evaluation data set (Table 5). Previous evaluations of the CNCPS were conducted using specific experimental datasets of a few studies conducted at Cornell University (Fox et al., 2004; Tylutki et al., 2008). The first limiting nutrient (MP or ME) was regressed on the observed milk yield, and results demonstrated the capability of CNCPS to predict the first limiting nutrient. The current evaluation reinforced the ability of the latest version to accurately predict the most limiting nutrient: the first limiting nutrient (MP or ME) was predicted with an $R^2 = 0.95$ and a RMSE = 1.77. Further, the development of a large dataset provided the opportunity to evaluate the model over a wide range of production and dietary conditions.

Table 4. Input variables used for the rumen sub-model evaluation dataset.

	Mean	SD	Min	Max
Diet Composition (% DM)				
CP	16.1	2.55	9.9	20.7
RUP	5.9	1.33	2.9	9.2
RDP	10.2	1.81	6.2	14.5
NDF	34.6	9.02	22.7	59.5
Starch	23.8	11.66	44.1	1.1
Fat	4	0.84	2.6	6.2
Omasal flows (g/d)				
Non ammonia nitrogen (NAN)	481	176.8	87	778
Bacterial nitrogen (BactN)	316	123.8	78	480
Rumen undegraded nitrogen (RUN)	164	65.1	7	326

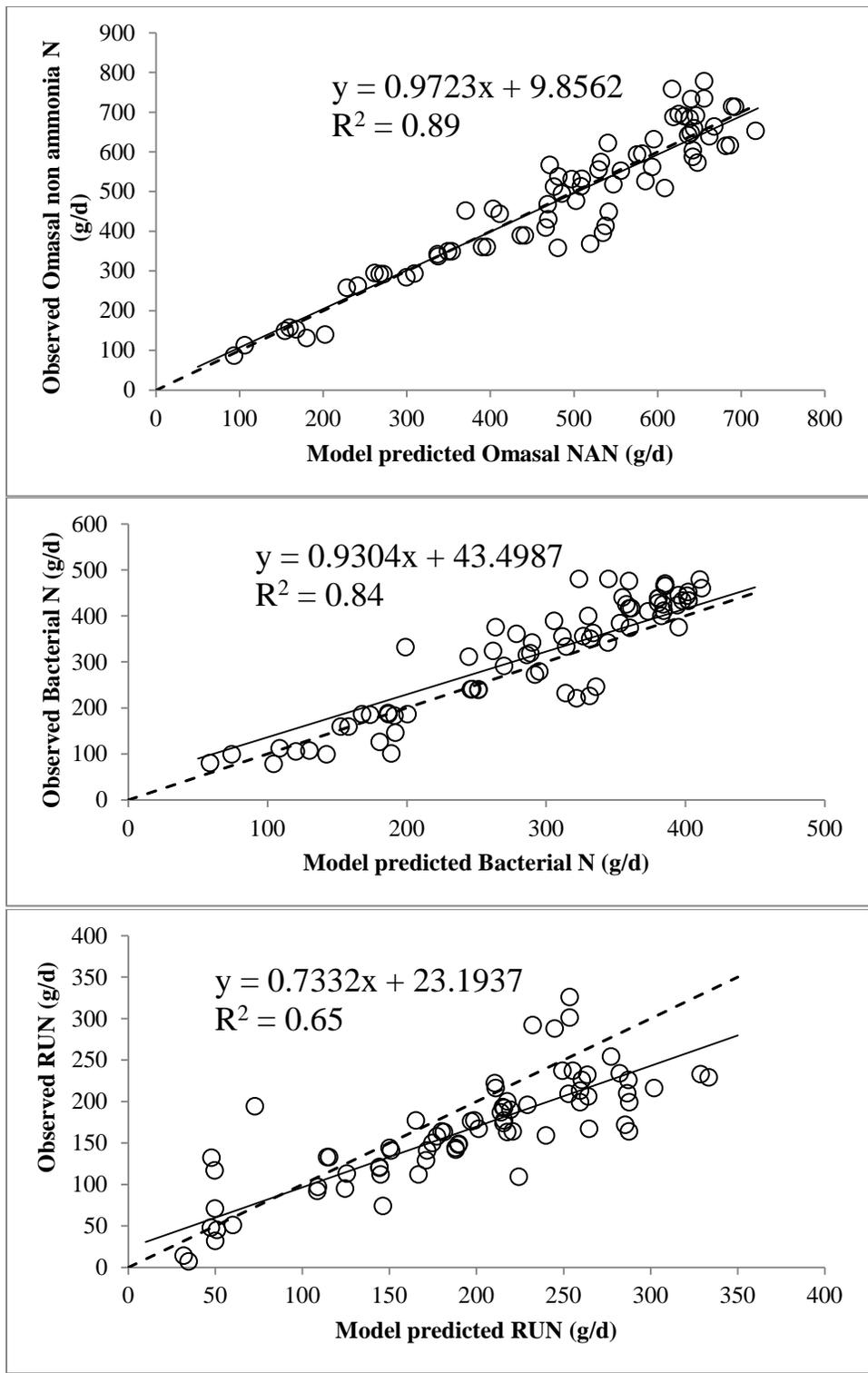


Figure 3. Observed versus model predicted values of: (A) non-ammonia nitrogen (NAN), (B) bacterial nitrogen (BactN) and (C) rumen undegradable nitrogen (RUN), assessed with a mixed effects model.

Results of the evaluation of ME and MP allowable milk yield are presented in Figure 4 and Table 6. Both MP and ME allowable milk were predicted reasonably well with an overall R^2 of 0.76 and a RMSE of 1.59 kg. In this evaluation, MP allowable milk was predicted with greater accuracy than ME allowable milk ($R^2 = 0.82$ and RMSE = 1.12 kg; $R^2 = 0.76$ and RMSE = 1.96 kg, respectively). An early attempt to evaluate CNCPSv6.0 when MP was the first limiting nutrient resulted in low precision ($R^2 = 0.29$; Van Amburgh et al., 2007). Since then, several updates to the model have been made (Higgs et al., 2012b; Van Amburgh et al., 2010; Van Amburgh et al., 2007) and among them, the updates to the protein fractionation and degradation rates have resulted in

improved predictions and sensitivity of the model.

Within the data sets evaluated, it is more difficult to evaluate energy balance because typically information on BCS change and BW change are not reported. Also, BW change, depending on stage of lactation, is not a good indicator of energy balance due to changes in rumen fill and DMI; body water vs body fat changes; and physiological state (e.g. pregnancy related BW changes). Thus, the ability to describe ME allowable milk or ME balance among published data sets is more difficult and that outcome is reflected in the partitioning of error in the MSPE (Table 6), where the majority of the error is random and due to study and not systematic within the model.

Table 5. Cattle and production characteristics for the lactation evaluation dataset.

	Mean	SD	Min	Max
Diet Composition (%DM)				
CP	16.9	2.35	9.4	29.5
RUP	7.2	1.55	3.3	16.7
RDP	9.7	1.38	6.08	14.6
NDF	33.8	5.4	25.3	52.7
Starch	23.1	7.2	2.1	37.8
Fat	4.8	1.3	2.0	13.1
Animal Inputs				
Initial body weight, kg	623	44.4	525	737
Final body weight, kg	632	46.1	532	748
Initial BCS, 1-5 scale	2.92	0.374	1.1	3.6
Final BCS, 1-5 scale	2.96	0.384	1.2	4.4
DMI, kg	22.3	2.73	13.5	29.1
Production inputs				
Milk Yield, kg/d	34.6	7.14	15.5	52.6
ECM ¹ , kg/d	32.3	6.18	14.9	47.15
Milk protein, %	3.02	0.194	2.51	3.61
Milk fat, %	3.67	0.479	2.06	5.06

¹ECM: energy corrected milk (Tyrrell and Reid, 1965)

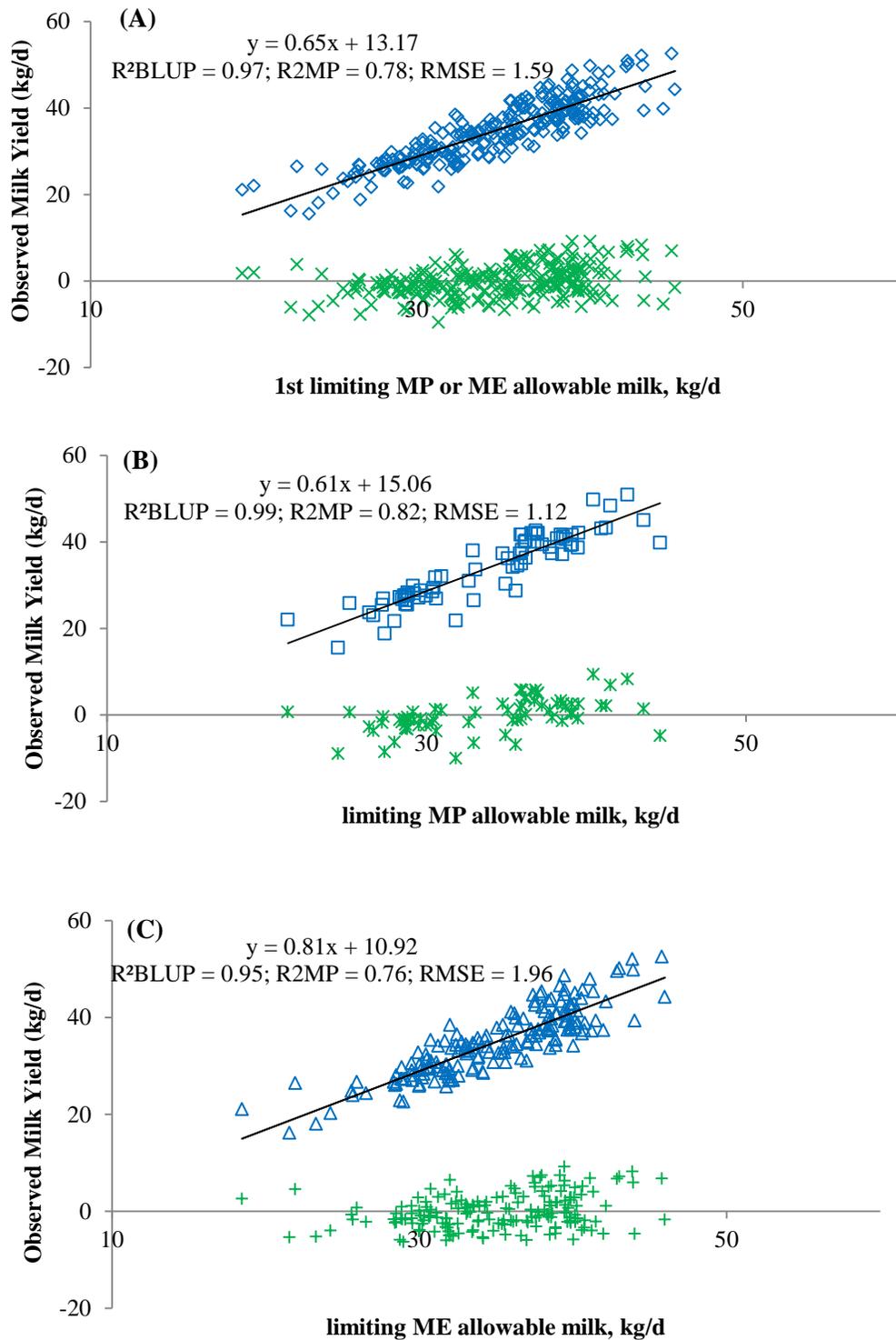


Figure 4. Observed versus model predicted values of: (A) first limiting MP or ME (\diamond ;) and residuals (\times), (B) MP limiting (\square) and residuals ($*$) and (C) ME limiting (\triangle) and residuals ($+$), assessed with a mixed effects model.

Table 6. Model adequacy statistics for the prediction of the first limiting nutrient (metabolizable protein or/and metabolizable energy; MP and ME, respectively) and of post ruminal flow of non-ammonia nitrogen (NAN), bacterial nitrogen (BactN) and rumen undegradable nitrogen (RUN).

	n	RMSE ¹	BIC ²	Variance Component ³			CCC ⁴	MSPE ⁵	MSPE partitioned ⁶ (%)		
				Study	Slope	Residual			U ^M	U ^S	U ^R
Lactation											
MP or ME	250	1.56	1192	77.7	0.5	21.8	0.83	12.8	0.05	21.75	78.20
ME	177	1.77	870	67.0	0.6	32.4	0.84	11.8	0.55	16.33	83.12
MP	73	1.12	360	91.5	0.4	8.1	0.83	14.2	0.45	26.91	72.64
Post-ruminal flow (g/d)											
NAN	74	24.97	767	84.6	NS	15.4	0.68	14011	83.35	3.52	13.13
BactN	74	24.55	743	86.1	NS	13.9	0.31	17762	91.08	6.16	2.76
RUN	74	21.73	726	66.9	NS	33.1	0.71	1141.6	24.41	7.68	67.91

¹ Root mean square error

² Bayesian information criterion

³ Percentage of variance related to the effect of study and random variation

⁴ Concordance correlation coefficient.

⁵ Mean square prediction error.

⁶ U^M = percentage of error due to mean bias, U^S = percentage of error due to systematic bias, U^R = percentage of error due to random variation (U^M + U^S + U^R = 100).

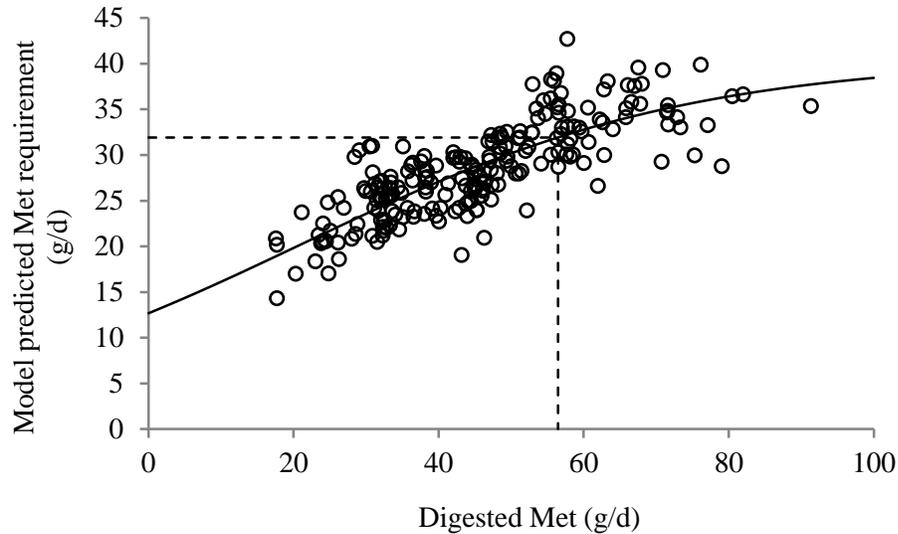


Figure 6. Logistic fit of model predicted Met requirement and Met supply. The dashed line represents the optimum ratio of Met requirement and Met supply.

To evaluate the model, 64 high producing dairy cows (100 ± 31 DIM) were randomly assigned to one of 4 treatments:

- 1) Base - limited in Met, MP, and rumen N,
- 2) Base+M - adequate in Met, but limited MP and rumen N,
- 3) Base+MU - adequate in Met and rumen N, but limited MP, and
- 4) Positive - adequate in MP and rumen N, while balanced for all EAA on a g/Mcal ME basis.

The chemical composition and ingredients used in each diet are in Table 8. Model predicted (CNCPS v7.0) dietary MP balance was -231 , -310 , -142 , and 33 g/d for the Base, Base+M, Base+MU, and Positive treatments, respectively.

Milk yield was not significantly different among the treatments, despite CP levels in the 13.5-13.6 % range. However, as the

grams of AA per Mcal of ME approached the optimum, energy corrected milk yield increased (Table 9). The predictions of grams of AA increased in two ways, first by meeting the N requirements of the rumen and in the Positive control, by adding ingredients to meet the AA requirements. The Base+MU treatment was designed to ensure adequate ruminal N availability and this treatment was considered not necessary during the formulation of treatment diets; however, due to the significant shift in the protein content of the corn silage (from 9 % to 7 %), the treatment became quite useful to help us evaluate the ability of the model to predict rumen ammonia levels and microbial yield, which in turn impacted the grams of AA supplied from the rumen. The model predicted the depression in microbial yield due to the low rumen N status (Table 10) and this prediction coincided with plasma urea N concentrations below 6 mg/dL. A review of most studies where data are available would indicate that after the PUN

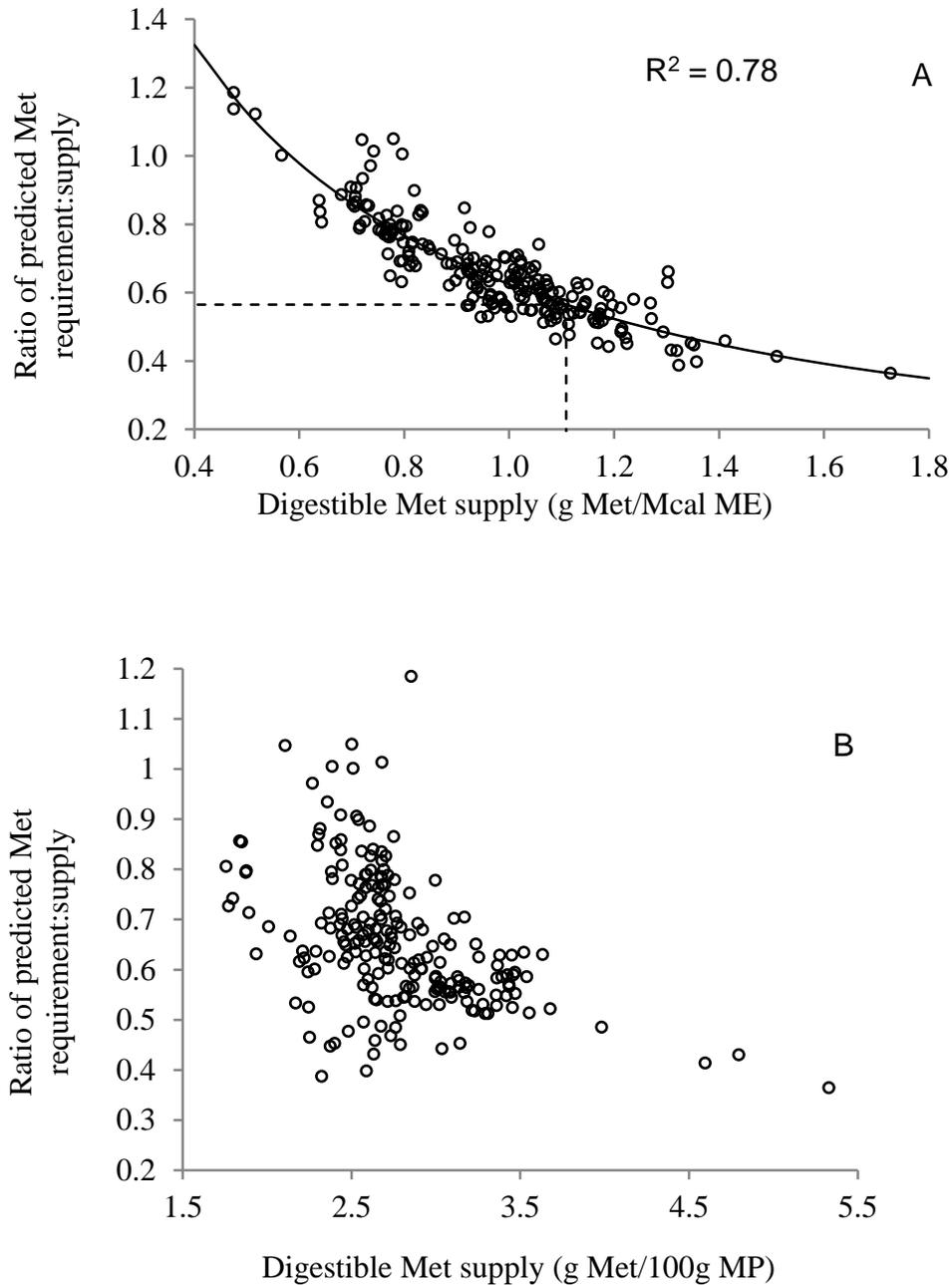


Figure 7. Relationship between model predicted Met requirement: supply and Met supply relative to ME (A) or MP (B). The dashed line in (A) represents the Met supply at the optimum ratio of model predicted Met requirement and supply. No significant relationship was determined in (B).

concentrations drop below 6 mg/dL, the blood pool of urea and urea production are not high enough to recycle adequate urea N to the gastrointestinal tract; thus the rumen goes into negative N balance and NDF

digestibility is decreased. This data and the latest version of the model all coincide with these observations (Table 10). It is important to note that as the PUN decreased, there was a discrepancy between the MUN

Table 7. The predicted AA supply for each treatment compared with the calculated optimum supply (g digested AA/Mcal ME).

AA	Optimum	Base ¹	Base+M	Base+MU	Positive
Arg	2.04	1.85	1.86	1.96	2.15
His	0.91	1.01	1.01	1.05	1.19
Ile	2.16	1.83	1.83	1.94	2.00
Leu	3.42	3.64	3.65	3.81	4.15
Lys	3.03	2.83	2.82	2.98	3.09
Met	1.14	0.93	1.13	1.17	1.25
Phe	2.15	2.12	2.12	2.22	2.42
Thr	2.14	2.16	2.16	2.27	2.43
Trp	0.59	0.60	0.60	0.63	0.69
Val	2.48	2.33	2.33	2.45	2.62
Lys:Met	2.66	3.04	2.51	2.54	2.47

¹Base = balanced for ME (assuming 45 kg ECM), but limited in MP and rumen N;
 Base+M = balanced for ME and MP Met but limited in MP and rumen N;
 Base+MU = balanced for ME, MP Met, with adequate rumen N, but limited in MP;
 Positive = balanced for ME, MP, all EAA and adequate rumen N.

Table 8. Ingredient and nutrient profile of the Base diet, Base plus Met, Base plus Met and urea and Positive control diets.

Ingredient, % DM	Base	Base+M	Base+MU	Positive
Corn Silage	46.98	46.49	46.75	46.13
Grass Hay	8.53	8.53	8.42	8.46
Corn grain ground fine	15.73	15.84	15.66	15.12
Corn gluten feed	8.69	8.75	8.66	7.07
Soybean meal	6.21	6.25	6.18	7.89
Soyhulls	2.07	2.08	2.06	2.10
SoyPLUS	2.07	2.08	2.06	4.11
Molasses Dried	2.07	2.08	2.06	1.20
NutraCor	1.90	1.92	1.90	1.64
Urea	0.08	0.08	0.52	0.12
AjiPro-L	0.10	0.10	0.09	0.00
Smartamine M	0.00	0.08	0.08	0.09
Blood meal	1.66	1.67	1.65	2.18
Minerals and vitamins	3.92	4.05	3.91	3.88
Chemical components				
CP	13.5	13.6	14.6	15.6
SP, % CP	38.8	38.6	38.8	37.8
Starch	31.9	31.9	31.5	30.9
NDF	29.7	29.6	29.3	29.3
Ash	7.3	7.4	7.3	7.3
EE	4.7	4.7	4.6	4.4

Table 9. Dry matter intake, energy corrected milk (ECM) yield, milk yield, and milk components.

Item, kg/d	Base	Base+M	Base+MU	Positive	P-Value
Dry matter intake	24.1	24.5	24.8	24.7	0.717
ECM yield	38.5 ^a	39.3 ^a	40.0 ^a	41.8 ^b	0.005
Milk yield	40.0	40.6	40.7	41.8	0.288
True protein yield	1.13 ^a	1.18 ^{ab}	1.18 ^{ab}	1.22 ^b	0.009
Fat yield	1.30 ^a	1.28 ^a	1.34 ^{ab}	1.41 ^b	0.047
Lactose yield	1.93	1.94	1.95	2.00	0.344
Milk composition					
True protein, %	2.88 ^a	2.93 ^{ab}	2.96 ^b	2.98 ^b	0.009
Fat, %	3.31	3.20	3.34	3.51	0.078
Lactose, %	4.84	4.85	4.85	4.86	0.799

and the PUN concentrations, and we see this as PUN concentrations are measured below about 8 mg/dL. This suggests to us that below a certain range, most mid-infrared units are not calibrated properly and are insensitive to some of the changes in N metabolism that might be useful for diet formulation and diagnostics.

SUMMARY

Nutritional models can be evolutionary. The CNCPSv6.5 is the latest evolution in the CNCPS path and the final update for this version. Among the analytical improvements, error corrections, and new research implemented within the CNCPS framework, model accuracy has been improved. These changes allow the nutrition professional to reduce dietary CP levels while maintaining or improving production and profitability. More importantly, the feed descriptions for AA in the feed library are now current and in a form that allows any user to make updates and additions with contemporary AA analyses methods. This step provides the next opportunity to continue to develop the model to better

predict the supply and requirements of AA for lactating and growing cattle. Further, the application of a combined efficiency of use of MP AA appears to provide a more consistent approach between AA supply and requirements that should improve the ability of the model to predict limiting AA and provide more sensitivity in determining a dietary approach to overcome the limitation. Finally, the model is being reprogrammed to incorporate more dynamic approaches to modeling and data analyses. Protozoal growth and yield, endogenous protein supply and digestibility, recycled urea N, and intestinal digestibility provided new insights into AA supply and were incorporated into the new model. Further, new estimates of AA requirements were developed on an energy basis, similar to monogastric animals and evaluated in lactating dairy cattle and with this approach and capability, dairy cattle were able to produce ~40 kg of milk on diets containing ~13.5 % CP and responded positively to improved AA balance on an ECM and ME basis.

Table 10. Nitrogen intake, milk and plasma urea N, N use efficiency, neutral detergent fiber digestibility, and bacterial growth depression due to predicted rumen ammonia N.

	Base	Base+M	Base+MU	Positive	P-Value
N intake, mg/dl	521.6 ^a	532.1 ^a	581.9 ^b	615.1 ^c	< 0.001
MUN, mg/dl	6.9 ^a	7.3 ^a	9.1 ^b	10.4 ^c	< 0.001
PUN, mg/dl	5.9 ^a	5.7 ^a	8.5 ^b	8.7 ^b	< 0.001
N use efficiency	0.37 ^a	0.38 ^a	0.35 ^b	0.34 ^b	< 0.001
NDF digestion %	40.8 ^{ab}	40.5 ^b	42.9 ^a	42.9 ^a	0.008
pd NDF digestion %	56.7 ^{ab}	55.2 ^b	59.0 ^a	59.2 ^a	0.011
Bacterial growth depression, %	16 %	17 %	4 %	2 %	

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