# Changes in the Cornell Net Carbohydrate and Protein Model: How low is too low when feeding crude protein?

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### INTRODUCTION

The increased public concern on environmental issues in combination with the considerable environmental impact of dairy production and the increasing cost of protein fed to dairy cattle are challenging the dairy industry to improve the efficiency of use of nitrogen (N). Despite the importance of N efficiency, levels of crude protein (CP) in dairy rations are often high, overfeeding dairy cows, reducing milk N efficiency (MNE), and consequently increasing risk for environmental pollution with N. Ration crude protein concentration as high as 22% DM with an average of 18 %DM have been reported for the USA (Huhtanen and Hristov, 2009). Overfeeding N has been intentionally practiced to provide a safety margin against uncertain protein concentration of forages and feed (Satter et al., 2002, Firkins and Reynolds, 2005). Thus, better tools to manage protein supply at a farm level are needed.

An early attempt to evaluate CNCPSv6.0 when low CP diets were fed to dairy cows showed low precision [ $R^2 = 0.29$ ; Tylutki et al. (2008)]. However, it highlighted that modifications to the model describing how N was utilized by both the microbes and the cow, especially when low N diets are fed, were needed. Since the last version of CNCPS (v6.0; Tylutki et al., 2008) several updates and modifications have been incorporated into the model, resulting in version 6.5. The objective of this paper is to describe these updates and modifications of the new version and to present a general evaluation of model performance against both literature and on-farm data.

# UPDATES TO PROTEIN CHEMISTRY

#### **Feed Library**

Feed library consistency is one of the main prerequisites of any feed evaluation model where major inputs for the model, such as degradation rates (kd), are defined for each feedstuff. The CNCPS feed library includes more than 800 different feedstuffs like forages, silages, concentrates, commercial feed ingredients, minerals, vitamins etc. Both carbohydrate and protein fractions of feeds are used in CNCPS to describe the degradation processes in the rumen and the digestibility of nutrients in the lower digestive tract. Associating these with appropriate passage rates for the different "pools" in the rumen, nutrient flows and absorption can be calculated and effects on animal production predicted.

**Protein Fractionation and Digestion Rates.** The CNCPS feed library was recently reviewed and updated using large datasets from commercial laboratories by Higgs et al. (2012a). 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 runnial degradation, outflow and intestinal digestion are shown 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

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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. (2012a) shifted a considerable amount of true protein from the PA1 to the PA2 pool. In CNCPS, the PA1 pool does not contribute MP to the animal, whereas the PA2 pool can contribute up to 15% of total amino acid flow to the small intestine (Volden et al., 2002, Reynal et al., 2007). Hence, this new configuration increased the predicted MP supply considerably – and in doing so allowed for a parallel decrease in the amount required and fed. 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%/h and 3-20%/h for the PA2 and PB1 pool, respectively, and are consistent with literature reports (Lanzas et al., 2007).

**Table 1.** Equations to compute amounts, rumen degradation and passage for feed protein fractions

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Variables	Description	Equations <sup>1</sup>
PA1 $_j$	Ammonia	ammonia <sub><i>j</i></sub> × (SolCP <sub><i>j</i></sub> /100) x (CP <sub><i>j</i></sub> /100)
$PA2_{j}$	Soluble protein	$SolCP_j \times CP_j/100 - PA1$
$PC_{j}$	Unavailable protein	$ADFIP_j \times CP_j/100$
$PB2_{j}$	Slowly degradable protein	$(NDFIP_j - ADFIP_j) \times CP_j/100$
$PB1_i$	Moderately degradable protein	$CP_i - PA1_i - PA2_i - PB2_i - PC_i$
$RDPA1_{j}$	Ruminally degraded PA1	$\mathbf{DMI}_j \times \mathbf{PA1}_j$
$RDPA2_{i}$	Ruminally degraded PA2	$DMI_i \times PA2_i \times (kdPA2_i / (kdPA2_i + kp_i))$
$RDPB1_{i}$	Ruminally degraded PB1	$DMI_i \times PB1_i \times (kdPB1_i / (kdPB1_i + kp_i))$
$RDPB2_{i}$	Ruminally degraded PB2	$DMI_i \times PB2_i \times (kdPP2_i / (kdPB2_i + kp_i))$
RDPEP <sub>i</sub>	Ruminally degraded peptides	$RDPA2_i + RDPB1_i + RDPB2_i$
REPA2 $_{i}$	Ruminally escaped PA2	$DMI_i \times PA2_i \times (kp_i / (kdPA2_i + kp_i))$
<b>REPB1</b> $i$	Ruminally escaped PB1	$DMI_i \times PB1_i \times (kp_i / (kdPB1_i + kp_i))$
REPB2 $_{i}$	Ruminally escaped PB2	$DMI_i \times PB2_i \times (kp_i / (kdPB2_i + kp_i))$
REPC $j$	Ruminally escaped PC	$DMI_j \times PC_j$
1	6 1 1 1 1 1 C 10D 1 1 1	in 1.1

 $_{j}^{1}$  = for each feed in the diet; SolCP = soluble protein; kd = degradation rate; kp = passage rate [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 many feeds were 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 and commercial feeds, were analyzed using correct methodology which led to inconsistencies throughout the library.

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

**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 % buffer insoluble residue. Values from the new library are expressed as % CP from the whole feed.

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 (Commentry, 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 % CP basis (equivalent to NRC, 2001). This differs from previous versions of the CNCPS where AA were expressed as a % 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 un-degraded (Reynal and Broderick, 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. (1992). 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 that 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 and better represents how the cow metabolizes AA for productive uses.

**Table 3.** Combined efficiencies of amino acid utilization for both maintenance and lactation [(adapted from Doepel et al. (2004) and Lapierre et al. (2007)]

	Amino Acids <sup>1</sup>									
	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Val	Trp
Efficiency,%	58	76	67	61	69	66	57	66	66	65
<sup>1</sup> Arg - argining: Hig - highlight llg - isolouging: Law - louging: Lyg - lyging: Mat - methioning: Pho -										

<sup>1</sup> Arg = arginine; His = histidine; Ile = isoleucine; Leu = leucine; Lys = lysine; Met = methionine; Phe = phenylalanine; Thy = threonine; Val = valine;

### **EVALUATION OF CNCPSv6.5**

#### Dataset Development.

Three different datasets were developed from both the literature and 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 dataset), 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 metabolizable Lys (8 studies forming 43 treatments) and Met (11 studies forming 50 treatments) in metabolizable protein were calculated for control and treatment groups. A dose-response approach was used to estimate required Lys and Met concentrations in MP for maximal milk protein synthesis according to Rulquin et al. (1993). Reference values of 6.80 and a 2.43 % of MP were identified intermediate to the lowest and highest concentrations 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/d) or content (%) against the predicted concentrations of Lys and Met (% of MP) were evaluated by regression procedures.

The second dataset (rumen dataset) was compiled from studies where post-ruminal N flows were assessed with the omasal sampling technique (Huhtanen et al., 1997, Ahvenjärvi et al., 2000, Reynal and Broderick, 2005). In total, 22 peer-review studies with 74 treatments were included. Studies in the compiled dataset reported measures of RDN, RUN, non-ammonia N (NAN) and bacterial N (BactN) flows. The dataset represented a wide range of diets and nutrient compositions. Diet's CP, NDF, starch and fat averaged 16.1, 34.6, 23.8 and 4.0 % DM, respectively. Omasal flows of RDN and RUN ranged from 50 to 539 and from 7 to 326 g/d, respectively. Similarly, flows of NAN and Bact N reflected the wide range of diets and averaged 481 and 316 g/d, respectively. The third dataset (lactation dataset) was compiled from studies published in the Journal of Dairy Science between 2001 and 2012. Lactation trials were included for dairy cows at different stages of lactation (early, mid and late). Studies employing cross over designs (Latin square, Box-Behnken, etc.) and limited experimental units per treatment (n < 6) were excluded from the data set. In total, 103 lactation studies were selected, from which 55 with 200 treatments met the criteria for incorporation into the data set. The criteria dictated that each study should have: (a) description and chemical analysis of the ration fed for each treatment, (b) percent of each feed included into the ration, (c) existence of all feed ingredients in the CNCPS feed library, (d) information on actual DMI, and (e) 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 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 calculated based on CNCPS feed library with minor adjustments as described previously (Higgs et al., 2012b). Animal information required to run a simulation in the CNCPS included a description of housing conditions, BW, BW change for period studied, BCS, BCS change during the period studied, stage of lactation, and stage of pregnancy. If stage of pregnancy, BW, and BCS were not provided then 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 empty BW (EBW) changes on average 13.7% for each unit of BCS change (Fox et al., 1999, NRC, 2001).To calculate EBW from BW the following equations were used:

EBW = 0.851 \* SBW, and SBW = 0.96 \* BW; Therefore, EBW = 0.817 \* BW

#### Statistical Analysis

Statistical analysis was conducted with JMP (SAS Inst. Inc., Cary, NC). 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 from a linear regression line to a break point followed by a plateau:

 $Yij = \beta 0 + \beta 1Xij$ , when  $X \le C$ 

 $Yij = \beta 0 + \beta 1C$ , when X > C

Where, Yij = the expected outcome for the dependent variable Y observed at level j of the continuous variable X in study i,  $\beta 0$  = the overall intercept across all studies,  $\beta 1$  = the overall slope of Y on X across all studies, 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):

 $Yij = \beta 0 + \beta 1Xij + si + b1iXij + \varepsilon ij,$ 

Where, Yij = the expected outcome for the dependent variable Y observed at level j of the continuous variable X in study i (or farm for the lactation dataset),  $\beta 0$  = the overall intercept across all studies (or farms for the lactation dataset), si = the random effect of study (or farm for the lactation dataset) i,  $\beta 1$  = the overall slope of Y on X across all studies (or farms for the lactation dataset), bli = the random effect of study i (or farm for the lactation dataset) on the slope of Y on X, Xij = the model predicted data associated with level j of the continuous variable X in study i (or farm for the lactation dataset), and  $\epsilon i j$  = 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 predicted estimates ( $R^2_{MP}$ ). The conditional residuals that use the estimated BLUP of the random effects 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). Root mean square prediction errors (RMSPE) 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**

## Lysine and Methionine Requirements

The plots of model predicted concentrations of Lys and Met (% MP), the corresponding responses of milk protein yield (g/d), and milk protein content (%) are presented in Fig. 1. The breakpoint estimates for Lys and Met for maximal milk protein yield were 7.00 and 2.60 % MP, respectively and those for maximal milk protein content were 6.77 and 2.85 % MP, respectively. Similar break points were reported for NRC (2001) and the previous version of CNCPS. The CNCPSv6.0 estimated Lys breaking point at 6.74 and 6.68 % MP for milk protein yield and content, respectively and that of Met at 2.31 and 2.40 % MP for milk protein yield and content, respectively (Whitehouse et al., 2013). Current estimations result in 11 and 18% higher

metabolizable Met target concentrations for maximal protein yield and content, respectively. A small increase was also observed for Lys (3 and 1 % higher supplementation for maximal protein yield and content, respectively). This increase is partly explained by the new fractionation of protein and the more accurate degradation rates for PA1 and PA2 that make CNCPS more sensitive than previous versions. The methods used to analyze AA for the original feed library (O'Connor et al., 1993) were not adequate to correctly quantify sulfur AA and often represented only one sample. Feed concentration of Met in the old feed library was lower than the contemporary version and as a consequence Met recommendations are higher for CNCPSv6.5.



**Figure 1.** Responses of milk protein yield responses as a function of digestible methionine (A;  $\diamond$ ) (Met; y = -219 + 92.65\*Met and y = -219 + 92.65\*2.60 for the linear

and the plateau part of the model, respectively;  $r^2 = 0.48$ ) and lysine (B;  $\Delta$ ) (Lys; y = -

478 + 70.02\*Lys and y = -478 + 70.02\*7.00 for the linear and the plateau sections of the model, respectively;  $r^2 = 0.55$ ), and of milk protein content as a function of digestible methionine (A;  $\diamond$ ) (Met; y = -0.46 + 0.191\*Met and y = -0.46 + 0.191\*2.85 for the linear

and the plateau part of the model, respectively;  $r^2 = 0.77$ ) and lysine (B;  $\Delta$ ) (Lys; y = -

0.99 + 0.150\*Lys and y = -0.99 + 0.150\*6.77 for the linear and the plateau sections of the model, respectively; r<sup>2</sup> = 0.78), and

## Efficiency of AA use

To evaluate the updated efficiency of AA use included in the CNCPS, the AA dataset 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 2.60 % dietary Met relative to total MP (Fig. 2), similar to the breakpoint derived in Fig. 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 2.00 % dietary Met (% MP) and no diets are predicted to have negative Met balance, contrary to expectations. Using the new efficiencies, there is a more equal partition of both positive and negative Met balances in the data set. This suggests that the new efficiencies of use allow the model to more adequately represent the true g per d requirements of essential amino acids. Further, the intercept at 0 Met balance occurs at approximately 2.6% Met (%MP) which is consistent with the breakpoint analysis, and a mathematically different approach resulting in a similar outcome.



Met balance (Supply - required), g/d

**Figure 2.** Model predicted methionine (Met) balance (Met supply less requirement) against dietary Met using CNCPSv6.5 ( $\circ$ ; the regression has slope = 0.0004 and intercept = 0.03 with R<sup>2</sup> = 0.68) or CNCPS6.1 (×; the regression has slope = 0.0003 and intercept = 0.021 with R<sup>2</sup> = 0.51)

# **Rumen Function**

A recent meta-analyses of omasal sampling and flow data indicated that the method is a reliable alternative to measuring nutrient flows via duodenal cannula (Broderick et al., 2010, Huhtanen et al., 2010). Moreover, the use of a triple marker system is more robust and reduces variation caused by the multiple and diverse markers used with postruminally cannulated animals. Therefore, to avoid inducing variation due to cannula position and the variety of marker use, we included only studies using the omasal sampling technique.

The random effect of study in the mixed model analysis accounted for greater than 81% of the variation in predicted RDN, BactN, andNAN, and approximately 67% in predicted RUN. Total variation was, therefore, explained with a great deal of precision as indicted by the high  $R^2_{BLUP}$  values (Table 4). Overall CCC values were greater than 0.81, suggesting a precise and accurate prediction of tested variables. With the current rates and pools size descriptions, the model overestimates RUN (slope = 0.73; Fig. 3). The decomposition of MSPE suggested that random bias and systematic bias are the main elements to explain variation and not the mean bias. The overestimation of RUN is a function of at least one primary offsetting condition: the rates of degradation of protein fractions are not well characterized and standardized methods are not currently available; so any model comparisons are currently somewhat random with regards to protein flows due to this lack of information. However, the offset is with bacterial flow and RDN flow in which one study by study basis, proportionately compensated for the RUN prediction providing a reasonably good prediction of NAN flow.



**Figure 3.** Observed versus CNCPS predicted values assessed with a mixed effects model of: (A) rumen degradable nitrogen (RDN;  $\Box$ ) and conditional residuals (+); the regression has slope = 1.06 and intercept = 0.85, and (B) rumen undegradable nitrogen (RUN;  $\Diamond$ ) and conditional residuals (×); the regression has slope = 0.74 and intercept = 23.14, (C) bacterial nitrogen (BactN;  $\Box$ ) and conditional residuals (+); the regression has slope = 0.93 and intercept = 43.50, and (D) non ammonia nitrogen (RUN;  $\Diamond$ ) and conditional residuals (×); the regression has slope = 0.94 and intercept = 24.24.

Lanzas et al. (2008) using a dataset of five studies with omasal sampling reported that the old CNCPS overestimated RDP and underestimated RUN flow. Moreover, CCC reported (0.81 and 0.63 for RDN and RUN, respectively) were lower than the ones currently presented, suggesting an increased accuracy and precision of CNCPS with the current updates. Offner and Sauvant (2004) using 115 treatments from 32 studies conducted with duodenal cannulated cattle compared the old version of CNCPS with Molly (Baldwin et al., 1987) and the model of Lescoat and Sauvant (1995) reported

					Variance Component <sup>4</sup>					MSPE partitioned <sup>7</sup> (%)			
	n	$R^2_{BLUP}^1$	$R^2_{MP}^2$	RMSPE <sup>3</sup>	Study	Slope	Residual	$CCC^5$	MSPE <sup>6</sup>	$U^M$	U <sup>S</sup>	U <sup>R</sup>	
Rumen dataset													
RDN	74	0.98	0.79	19.4	88.18	0.01	11.81	0.89	3,568	1.77	1.62	96.61	
RUN	74	0.92	0.65	21.7	66.94	0.01	33.05	0.81	1,455	0.02	12.73	87.25	
BactN	74	0.97	0.84	24.6	81.88	0.00	18.12	0.87	3,038	0.05	1.54	98.41	
NAN	74	0.98	0.88	25.1	83.50	0.01	16.40	0.93	3,751	0.42	2.26	97.32	
Lactation dataset													
MPorME	250	0.97	0.78	1.6	77.70	0.50	21.80	0.83	12.8	0.05	21.75	78.20	
ME	177	0.95	0.76	1.8	67.00	0.60	32.40	0.84	11.8	0.01	16.68	83.31	
MP	73	0.98	0.82	1.1	91.50	0.40	8.10	0.83	14.2	0.45	26.91	72.64	

Table 4. Model adequacy statistics for the prediction of RDN and post ruminal flow of RUN, non-ammonia nitrogen (NAN) and bacterial nitrogen (BactN) and of the first limiting MP or/and ME allowable milk.

 ${}^{1}R^{2}_{BLUP}$  = correlation coefficient based on BLUP  ${}^{2}R^{2}_{MP}$  = correlation coefficient based on model predictions using a mean study effect  ${}^{3}Root$  mean square error

<sup>4</sup> Percentage of variance related to the effect of study and random variation (mixed model) <sup>5</sup> Concordance correlation coefficient

<sup>6</sup> Mean square prediction error.

<sup>7</sup>  $U^{M}$  = 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  $(U^{M} + U^{S} + U^{R} = 100)$ 

that CNCPS predicted better microbial flow ( $r^2 = 0.93$ ). Similarly, the new version of CNCPS predicted accurately and precise Bact N omasal flow ( $R^2_{BLUP} = 0.97$ ; RMSE = 24.6; CCC = 0.87). Again there is a uniform offset which provides a prediction of NAN that is robust with little bias ( $R^2_{BLUP} = 0.98$ ; RMSE = 25.99).

### Milk Yield Predictions

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 CNCPS predicted milk yield (allowable milk yield) according to 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 with  $r^2 =$ 0.89 and CCC = 0.94 (Tylutki et al., 2008). The current evaluation using a large dataset with 250 treatments from 55 studies and 15 farms reinforced the ability of the latest version to precisely predict the most limiting nutrient: MP or ME allowable milk yield predicted with an  $R^{2}_{BLUP} = 0.97$ ,  $R^{2}_{MP} = 0.78$  and RMSE = 1.6 (Fig. 4). Moreover, the low MSPE indicated the high accuracy of the model while the decomposition of MSPE suggested that random variation (78.20 % of MSPE) followed by systematic bias (21.75 % of MSPE) are the main elements to explain the bias (Table 4). The variance components analysis of the mixed model indicated that 77.7 % of the variation was attributed to the random effect of study or farm. Further, the overall accuracy and precision of the model to predict the first limiting nutrient was high as indicated by the CCC (0.83).

The development of a large dataset provided the opportunity to evaluate the model over a wide range of production and dietary conditions, but also to evaluate separately allowable milk for each limiting nutrient. Both MP and ME allowable milk were predicted reasonably well as indicated by the high  $R^2_{MP}$ , CCC, and the low RMSPE. In this evaluation, MP allowable milk was predicted with greater precision than ME allowable milk. An early attempt to evaluate CNCPSv6.0 when MP was the first limiting nutrient resulted in low precision [ $R^2 = 0.29$ ; Tylutki et al. (2008)]. Current updates of protein fractionation, the corresponding adjustments of their degradation rates, and as well the new AA profiles and utilization constants have made MP predictions more sensitive than previous versions; thus resulting in a significant improvement of CNCPS to predict milk yield when MP is the limiting nutrient.

# CONCLUSIONS

Nutritional models evolve over time. CNCPSv6.5 is the latest evolution along 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 crude protein 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 analysis 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 for each AA appears to provide a more consistent approach between AA supply and requirements that should improve the ability of the model to predict limiting AAs and be more adept in suggesting a solution to overcome the limitation.



**Figure 4**. Observed milk yield versus CNCPS predicted values, assessed with a mixed effects model of: (A) first limiting MP or ME allowable milk ( $\Box$ ) and conditional residuals (+); the regression has slope = 0.65 and intercept = 13.17, (B) MP limiting

allowable milk ( $\Delta$ ) and conditional residuals ( $\circ$ ); the regression has slope = 0.61 and

intercept = 15.06, and (C) ME limiting allowable milk ( $\diamond$ ) and conditional residuals (×); the regression has slope = 0.71 and intercept = 10.92.

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#### REFERENCES

- Ahvenjärvi, S., A. Vanhatalo, P. Huhtanen, and T. Varvikko. 2000. Determination of reticulo-rumen and whole-stomach digestion in lactating cows by omasal canal or duodenal sampling. Br. J. Nutr. 83:67-77.
- Baldwin, R. L., J. France, and M. Gill. 1987. Metabolism of the lactating cow. I. Animal elements of a mechanistic model. J. Dairy Res. 54:77-105.
- Broderick, G. A., P. Huhtanen, S. Ahvenjärvi, S. M. Reynal, and K. J. Shingfield. 2010. Quantifying ruminal nitrogen metabolism using the omasal sampling technique in cattle—A meta-analysis. J. Dairy Sci. 93:3216-3230.
- Doepel, L., D. Pacheco, J. J. Kennelly, M. D. Hanigan, I. F. Lopez, and H. Lapierre. 2004. Milk protein synthesis as a function of amino acid supply. J. Dairy Sci. 87:1279-1297.
- Firkins, J. L. and C. A. Reynolds. 2005. Whole animal nitrogen balance in cattle. Pages 167-186 in Nitrogen and phosphorus nutrition of cattle. E. Pfeffer and A. N. Hristov, ed. CABI Publishing, Cambridge, UK.
- Fox, D. G., C. J. Sniffen, J. D. O'Connor, J. B. Russell, and P. J. Van Soest. 1992. A net carbohydrate and protein system for evaluating cattle diets: III. Cattle requirements and diet adequacy. J. Anim. Sci. 70:3578-3596.
- Fox, D. G., L. O. Tedeschi, T. P. Tylutki, J. B. Russell, M. E. Van Amburgh, L. E. Chase, A. N. Pell, and T. R. Overton. 2004. The Cornell Net Carbohydrate and Protein System model for evaluating herd nutrition and nutrient excretion. Anim. Feed Sci. Tech. 112:29-78.
- Fox, D. G., M. E. Van Amburgh, and T. P. Tylutki. 1999. Predicting requirements for growth, maturity, and body reserves in dairy cattle. J. Dairy Sci. 82:1968-1977.
- Higgs, R., L. Chase, D. Ross, and M. Van Amburgh. 2012a. Evaluating and refining the cncps feed library using commercial laboratory feed databases. Pages 146-156 in Proc. 74<sup>th</sup> Cornell Nutrition Conference For Feed Manufacturers. Cornell University, Syracuse, NY.
- Higgs, R. J., L. E. Chase, and M. E. Van Amburgh. 2012b. Development and evaluation of equations in the Cornell Net Carbohydrate and Protein System to predict nitrogen excretion in lactating dairy cows. J. Dairy Sci. 95:2004-2014.
- Huhtanen, P., S. Ahvenjärvi, G. A. Broderick, S. M. Reynal, and K. J. Shingfield. 2010. Quantifying ruminal digestion of organic matter and neutral detergent fiber using the omasal sampling technique in cattle—A meta-analysis. J. Dairy Sci. 93:3203-3215.
- Huhtanen, P., P. G. Brotz, and L. D. Satter. 1997. Omasal sampling technique for assessing fermentative digestion in the forestomach of dairy cows. J. Anim. Sci. 75:1380-1392.
- Huhtanen, P. and A. N. Hristov. 2009. A meta-analysis of the effects of dietary protein concentration and degradability on milk protein yield and milk N efficiency in dairy cows. J. Dairy Sci. 92:3222-3232.

- Lanzas, C., G. A. Broderick, and D. G. Fox. 2008. Improved feed protein fractionation schemes for formulating rations with the Cornell Net Carbohydrate and Protein System. J. Dairy Sci. 91:4881-4891.
- Lanzas, C., L. O. Tedeschi, S. Seo, and D. G. Fox. 2007. Evaluation of protein fractionation systems used in formulating rations for dairy cattle. J. Dairy Sci. 90:507-521.
- Lapierre, H., G. E. Lobley, D. R. Quellet, L. Doepel, and D. A. Pacheco. 2007. Amino acid requirements for lactating dairy cows: Reconciling predictive models and biology. Pages 39-60 in Proc. Cornell Nutrition Conference For Feed Manufacturers Cornell University, Syracuse, NY.
- Lescoat, P. and D. Sauvant. 1995. Development of a mechanistic model for rumen digestion validated using the duodenal flux of amino acids. Reprod. Nutr. Dev. 35:45-70.
- NRC. 2001. Nutrient requirements of dairy cattle. 7th rev. ed. National Academy Press, Washington, D.C.
- O'Connor, J. D., C. J. Sniffen, D. G. Fox, and W. Chalupa. 1993. A net carbohydrate and protein system for evaluating cattle diets: IV. Predicting amino acid adequacy. J. Anim. Sci. 71:1298-1311.
- Offner, A. and D. Sauvant. 2004. Comparative evaluation of the Molly, CNCPS, and LES rumen models. Anim. Feed Sci. Tech. 112:107-130.
- Reynal, S. M. and G. A. Broderick. 2005. Effect of dietary level of rumen-degraded protein on production and nitrogen metabolism in lactating dairy cows. J. Dairy Sci. 88:4045-4064.
- Reynal, S. M., I. R. Ipharraguerre, M. Lineiro, A. F. Brito, G. A. Broderick, and J. H. Clark. 2007. Omasal flow of soluble proteins, peptides, and free amino acids in dairy cows fed diets supplemented with proteins of varying ruminal degradabilities. J. Dairy Sci. 90:1887-1903.
- Rulquin, H., P. M. Pisulewski, R. Vérité, and J. Guinard. 1993. Milk production and composition as a function of postruminal lysine and methionine supply: A nutrient-response approach. Liv. Prod. Sci. 37:69-90.
- Satter, L. D., T. J. Klopfenstein, and G. E. Erickson. 2002. The role of nutrition in reducing nutrient output from ruminants. J. Anim. Sci. 80:E143-E156.
- St-Pierre, N. R. 2001. Invited review: Integrating quantitative findings from multiple studies using mixed model methodology. J. Dairy Sci. 84:741-755.
- Tedeschi, L. O. 2006. Assessment of the adequacy of mathematical models. Agricult. Sys. 89:225-247.
- Tylutki, T. P., D. G. Fox, V. M. Durbal, L. O. Tedeschi, J. B. Russell, M. E. Van Amburgh, T. R. Overton, L. E. Chase, and A. N. Pell. 2008. Cornell Net Carbohydrate and Protein System: A model for precision feeding of dairy cattle. Anim. Feed Sci. Tech. 143:174-202.
- Van Amburgh, M., L. Chase, T. Overton, D. Ross, E. Recktenwald, R. Higgs, and T. Tylutki. 2010. Updates to the Cornell Net Carbohydrate and Protein System v6. 1 and implications for ration formulation. Pages 144-159 in Proc. 72<sup>nd</sup> Cornell Nutrition Conference For Feed Manufacturers. Cornell University, Syracuse, NY.
- Van Amburgh, M. E., A. Recktenwald, D. A. Ross, T. R. Overton, and E. S. Chase. 2007. Achieving better nitrogen efficiency in lactating dairy cattle: Updating field usable tools to improve nitrogen efficiency. Pages 25-37 in Proc. 69<sup>th</sup> Cornell Nutrition Conference For Feed Manufacturers. Cornell University, Syracuse, NY

- Volden, H., L. T. Mydland, and V. Olaisen. 2002. Apparent ruminal degradation and rumen escape of soluble nitrogen fractions in grass and grass silage administered intraruminally to lactating dairy cows. J. Anim. Sci. 80:2704-2716.
- Whitehouse, N. L., C. G. Schwab, T. P. Tylutki, and B. K. Sloan. 2013. Optimal lysine and methionine concentrations in metabolizable protein for milk protein production as determined with the latest versions of dairy NRC 2001 and AMTS.Cattle J. Dairy Sci. 96.