



## Aberystwyth University

### *The Cornell Net Carbohydrate and Protein System*

Van Amburgh, M. E.; Collao-Saenz, E. A.; Higgs, R. J.; Ross, D. A.; Recktenwald, E. B.; Raffrenato, E.; Chase, L. E.; Overton, T. R.; Mills, J K; Foskolos, Andreas

*Published in:*  
Journal of Dairy Science

*DOI:*  
[10.3168/jds.2015-9378](https://doi.org/10.3168/jds.2015-9378)

*Publication date:*  
2015

*Citation for published version (APA):*  
Van Amburgh, M. E., Collao-Saenz, E. A., Higgs, R. J., Ross, D. A., Recktenwald, E. B., Raffrenato, E., Chase, L. E., Overton, T. R., Mills, J. K., & Foskolos, A. (2015). The Cornell Net Carbohydrate and Protein System: Updates to the model and evaluation of version 6.5. *Journal of Dairy Science*, 98(9), 6361-6380.  
<https://doi.org/10.3168/jds.2015-9378>

#### **General rights**

Copyright and moral rights for the publications made accessible in the Aberystwyth Research Portal (the Institutional Repository) are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the Aberystwyth Research Portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the Aberystwyth Research Portal

#### **Take down policy**

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

tel: +44 1970 62 2400  
email: [is@aber.ac.uk](mailto:is@aber.ac.uk)



J. Dairy Sci. 98:6361–6380

<http://dx.doi.org/10.3168/jds.2015-9378>

© 2015, THE AUTHORS. Published by FASS and Elsevier Inc. on behalf of the American Dairy Science Association®. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/3.0/>).

## The Cornell Net Carbohydrate and Protein System: Updates to the model and evaluation of version 6.5

M. E. Van Amburgh,<sup>\*1</sup> E. A. Collao-Saenz,<sup>†</sup> R. J. Higgs,<sup>\*</sup> D. A. Ross,<sup>\*</sup> E. B. Recktenwald,<sup>\*</sup> E. Raffrenato,<sup>‡</sup> L. E. Chase,<sup>\*</sup> T. R. Overton,<sup>\*</sup> J. K. Mills,<sup>§</sup> and A. Foskolos<sup>\*</sup>

<sup>\*</sup>Department of Animal Science, Cornell University, Ithaca, NY 14850

<sup>†</sup>Department of Animal Science, Federal University of Goiás, Jataí, Brazil 75800-970

<sup>‡</sup>Department of Animal Sciences, Stellenbosch University, Stellenbosch, South Africa 7600

<sup>§</sup>Elanco Animal Health, Canastota, NY 13032

### ABSTRACT

New laboratory and animal sampling methods and data have been generated over the last 10 yr that had the potential to improve the predictions for energy, protein, and AA supply and requirements in the Cornell Net Carbohydrate and Protein System (CNCPS). The objectives of this study were to describe updates to the CNCPS and evaluate model performance against both literature and on-farm data. The changes to the feed library were significant and are reported in a separate manuscript. Degradation rates of protein and carbohydrate fractions were adjusted according to new fractionation schemes, and corresponding changes to equations used to calculate rumen outflows and postrumen digestion were presented. In response to the feed-library changes and an increased supply of essential AA because of updated contents of AA, a combined efficiency of use was adopted in place of separate calculations for maintenance and lactation to better represent the biology of the cow. Four different data sets were developed to evaluate Lys and Met requirements, rumen N balance, and milk yield predictions. In total 99 peer-reviewed studies with 389 treatments and 15 regional farms with 50 different diets were included. The broken-line model with plateau was used to identify the concentration of Lys and Met that maximizes milk protein yield and content. Results suggested concentrations of 7.00 and 2.60% of metabolizable protein (MP) for Lys and Met, respectively, for maximal protein yield and 6.77 and 2.85% of MP for Lys and Met, respectively, for maximal protein content. Updated AA concentrations were numerically higher for Lys and 11 to 18% higher for Met compared with

CNCPS v6.0, and this is attributed to the increased content of Met and Lys in feeds that were previously incorrectly analyzed and described. The prediction of postruminal flows of N and milk yield were evaluated using the correlation coefficient from the BLUP ( $R^2_{BLUP}$ ) procedure or model predictions ( $R^2_{MDP}$ ) and the concordance correlation coefficient. The accuracy and precision of rumen-degradable N and undegradable N and bacterial N flows were improved with reduced bias. The CNCPS v6.5 predicted accurate and precise milk yield according to the first-limiting nutrient (MP or metabolizable energy) with a  $R^2_{BLUP} = 0.97$ ,  $R^2_{MDP} = 0.78$ , and concordance correlation coefficient = 0.83. Furthermore, MP-allowable milk was predicted with greater precision than metabolizable energy-allowable milk ( $R^2_{MDP} = 0.82$  and 0.76, respectively, for MP and metabolizable energy). Results suggest a significant improvement of the model, especially under conditions of MP limitation.

**Key words:** Cornell Net Carbohydrate and Protein System, update, evaluation, dairy cattle

### INTRODUCTION

A description of the Cornell Net Carbohydrate and Protein System (CNCPS) was first published in 1992 and 1993 in a series of 4 papers (Fox et al., 1992; Russell et al., 1992; Sniffen et al., 1992; O'Connor et al., 1993). The principal objective of the CNCPS was to serve as a tool for both research development and feed formulation for cattle (Russell et al., 1992). To fulfill these goals, the CNCPS has been evolving by incorporation of new research data and descriptions of rumen function and metabolism into mathematical equations and quantitative representations with the primary objective of field application and diet formulation. As a consequence, several updated versions have been released over the last 15 yr (Fox et al., 2000, 2004; Tytlutki et al., 2008).

Received January 24, 2015.

Accepted May 15, 2015.

<sup>1</sup>Corresponding author: [mev1@cornell.edu](mailto:mev1@cornell.edu)

One of the objectives of the CNCPS modeling process has been to incorporate enhanced knowledge in the platform to further explain differences in cattle productivity compared with expectations and to account for more of the unexplained variation in the predictions of ME and MP supply and requirements. In many cases this includes incremental changes and error corrections, and in some situations, new feed definitions and characterizations or alterations in postdigestive efficiencies of use are required to improve the predictions of nutrient requirements.

Also, several implementations of the program are used by the industry to evaluate and formulate diets, and accordingly, any improvements in the predictions of supply and requirements can immediately translate into application and improved on-farm benefits. The latest CNCPS versions 6.0 and 6.1 (Tylutki et al., 2008, Van Amburgh et al., 2010) are used as a formulation and evaluation platform by AMTS.Cattle (Agricultural Modeling and Training Systems LLC, Cortland, NY), NDS (Ruminant Management and Nutrition, Reggio Emilia, Italy), DinaMilk (Fabermatica, Ostriano, Italy), and Dalex (Dalex Livestock Solutions, Los Angeles, CA).

Since the last publication (Tylutki et al., 2008) several updates and modifications have been incorporated into the model. The objective of this paper was to describe these updates and modifications and to present a general evaluation of model performance against both literature and on-farm data. One of the major updates, a reedited feed library with contemporary AA values, is described in a companion paper (Higgs et al., 2015), and the evaluation of the library updates are described herein.

The updates to the CNCPS described here represent changes that have been made to CNCPS v6.0 (Tylutki et al., 2008) resulting in CNCPS v6.5. Updates have been made to predictions of nutrient requirements and supply, which are discussed in the following sections, but also to the feed library, which is described in a companion paper (Higgs et al., 2015). One other additional change in the description of feed chemistry that affects nutrient supply, the application of unavailable NDF as determined by a 240-h in vitro digestibility, is described in Raffrenato (2011).

## MATERIALS AND METHODS

### *Model Updates*

**Maintenance Requirements.** Previous versions of the CNCPS made adjustments to the maintenance requirements of growing cattle based on changes in BCS. The adjustment was based on data from the INRA

system for lactating beef cattle on pasture (Petit and Agabriel, 1989). The calculations made an association between previous levels of nutrient intake, BCS, and maintenance requirements by increasing or decreasing  $NE_M$  by 5%, above or below BCS 5 on a 1-to-9 scale (Fox et al., 2004). As cattle achieved greater BCS, theoretically, they consumed more energy and thus had larger organ mass, which resulted in more energy partitioned to maintenance and less to growth. Therefore, as BCS was increased, maintenance requirements also increased and vice versa. This adjustment was evaluated for growing Holstein heifers with known composition and energy balance using a fixed diet and varying the BCS from 1 to 5 on a dairy scale (adjusted from a 1–9 scale for beef as described in Fox et al., 2004) to evaluate the accuracy of the ME-allowable gain compared with measured data.

Adjustments have also been made to the calculation of surface area. Surface area is used within the CNCPS to adjust maintenance requirements for cold stress (Fox et al., 2004). The equation used to calculate surface area in the CNCPS, up to v6.0, was from Mitchell (1928). The equation from Mitchell ( $0.09 \times BW^{0.67}$ ) was derived from sheep weighing from 14 to 38 kg. Brody (1945) developed an equation ( $0.14 \times BW^{0.57}$ ) using Holstein cattle ( $n = 50$ ) weighing from 41 to 617 kg, and this equation was evaluated by Berman (2003) using a thermal balance model. Compared with Brody's equation, the Mitchell equation underestimated surface area by 7 to 10% at 30 to 50 kg of BW and overestimated surface area by 18% at 650 kg of BW, affecting the calculations of evaporative heat loss (Berman, 2003). Therefore, the equation of Brody was adopted for the calculation of surface area in v6.5.

**Feed Fractionation and Digestion Rates.** The feed fractionation scheme used in v6.5 was maintained in the format described by Tylutki et al. (2008) with the exception of the soluble protein pool that contains previously defined as NPN, and now redefined as ammonia (Higgs et al., 2015). This change was made in recognition of the AA content of the NPN fraction (Krishnamoorthy et al., 1982) and the contribution of this fraction to post-ruminal N flows (Choi et al., 2002a; Reynal et al., 2007). Nomenclature changes were also made to the protein fractions, where all soluble fractions are now prefaced with the letter A and insoluble fractions with the letter B. A full description of these changes is given in Higgs et al. (2015). The outcomes of these changes are a better description of the rumen ammonia balance and also the MP supply, given that MP is being supplied by the soluble fractions of feeds, and before these updates this protein fraction contributed primarily to rumen ammonia because of improper characterization and passage rates.

**Table 1.** Feed chemical pools, the variables, and degradation rates (kd, %/h) used for carbohydrates and proteins

Component <sup>1</sup>	Variable	kd, %/h
CA1	kdCA1	0
CA2	kdCA2	7
CA3	kdCA3	5
CA4	kdCA4	40–60
CB1	kdCB1	20–40
CB2	kdCB2	20–40
CB3	kdCB3	1–18
PA1	kdPA1	200
PA2	kdPA2	10–40
PB1	kdPB1	3–20
PB2	kdPB2	1–18

<sup>1</sup>CA1 = acetic, propionic, and butyric acids; CA2 = lactic acid; CA3 = other organic acids; CA4 = sugars; CB1 = starch; CB2 = soluble fiber; CB3 = available NDF; PA1 = ammonia; PA2 = soluble true protein; PB1 = moderately degradable protein; PB2 = slowly degradable protein, bound in NDF.

The digestion rates (**kd**) of protein and carbohydrate fractions were reviewed and updated to be consistent with literature reports and to be more biologically realistic. Previous versions of the CNCPS assumed NPN use was instantaneous with a kd of 10,000%/h. This implied a rumen retention time of 0.6 min and suggested any addition of urea would be dissolved and captured by rumen bacteria in 36 s or be converted to ammonia and leave the rumen in a similar period of time—an unrealistic expectation. In the original work to describe the NPN rate, the value was designed to represent the rate of solubilization and not necessarily microbial uptake. In v6.5, kd of protein pool A1 (PA1, ammonia N) was reduced to 200%/h for all feeds based on the bacterial ammonia metabolism data from Wallace (1979), Schaefer et al. (1980), and Wallace et al. (1998; Table 1).

Furthermore, the kd of soluble true protein (PA2) had previously ranged from 130 to 300%/h. Literature values are typically much lower (Broderick, 1987; Peltkova and Broderick, 1996; NRC, 2001; Hedqvist and Udén, 2006; Lanzas et al., 2007) and indicate the rate of protein degradation of the larger soluble proteins is slower than originally considered in the CNCPS (Sniffen et al., 1992). Furthermore, other data on AA and peptide uptake by bacteria indicate that peptide formation in the rumen is relatively rapid (Mahadevan et al., 1980; Chen et al., 1987); however, peptide uptake appears to be a rate-limiting step (Chen et al., 1987; Broderick and Wallace, 1988). Thus, any peptides that solubilize but are not recovered in a trichloroacetic acid or tungstic acid precipitation (Licitra et al., 1996) previously were apportioned to the NPN fraction and were calculated to degraded to ammonia and not have the opportunity to escape from the rumen and provide MP to the animal as described by Reynal et al. (2007). Sub-

sequently, the PA2 kd have been adjusted in v6.5 to be consistent with literature reports and now range from 5 to 50%/h (Table 1), and this provides predictions of MP supply from the soluble protein pool consistent with the data of Choi et al. (2002a) and Reynal et al. (2007).

Also, the original values for sugar kd were derived from in vitro fermentation studies using pure cultures of *Streptococcus bovis* grown on glucose (Russell and Hino, 1985; Russell, 1990). As might be expected, the kd of glucose in this situation is rapid (200–300%/h) but probably does not reflect the kd of sugar fermentation in the rumen for a mixed microbial system and rarely available soluble glucose. For example, a kd of 300%/h implies a rumen retention time of 12 min, a value greater than the mean growth rate of rumen bacteria. More recent data generated using gas production techniques indicate the kd of sugar by mixed rumen bacteria to range between 40 and 60%/h (Doane et al., 1998; Molina, 2002). Thus, values in v6.5 have been adjusted to fit within this lower range (Table 1).

To estimate the digestible fraction of NDF, the CNCPS has used a fixed value to describe the indigestible NDF of forages and feeds. This value was published by Chandler et al. (1980) and described as (lignin × 2.4)/NDF, and the approach was consistent with that described by Weiss et al. (1992), who used a surface area relationship between NDF and lignin to estimate the unavailable NDF. More recent data using both long fermentation times in situ and in vitro data demonstrate a fixed value is not consistent with observations and that the relationship between lignin and digestibility is dynamic and an outcome of agronomic conditions such as water, heat, and light and relates more to cross-linking between the lignin and hemicellulose than lignin concentration (Besle et al., 1994; Huhtanen et al., 2006; Raffrenato, 2011). In this update, the static estimation of unavailable NDF was replaced by the unavailable NDF as estimated by in vitro digestion of NDF after 240 h of incubation (Raffrenato, 2011). The unavailable NDF identified by this procedure captures the variable differences in the available NDF pool size based on growing conditions and genetics and appears to represent a fraction that relates to rumen function in a more robust manner (Huhtanen et al., 2006; Cotanch et al., 2014).

**Passage-Rate Assignments.** The single-pool, first-order approach used to estimate rumen digestion in the CNCPS [digestion = kd/(kd + kp)] makes estimating not only kd but also kp fundamental in predicting the extent of rumen digestion. Lanzas et al. (2007) evaluated the protein fractionation schemes for both the CNCPS and NRC (2001) and noted that the soluble fractions of both carbohydrate and protein

fractions were assigned to flow with the solids passage rate in the CNCPS structure. Given that liquid passage is 5- to 10-times faster than the solids passage rates (Seo et al., 2006), and soluble fractions generally have faster rates of digestion, most of the soluble components in the diet were predicted to degrade in the rumen. However, several studies have demonstrated that the soluble fraction of feed N can contribute 5 to 15% of the total AA flow to the duodenum of the cow (Hristov et al., 2001; Choi et al., 2002a,b; Reynal et al., 2007). To improve the capacity and robustness of the model to predict the escape of soluble components and to more appropriately reflect the interaction of the feed proteins in the rumen, the soluble pools (CA1–4 and PA1–2) were reassigned to flow with the liquid passage rate in v6.5. The effect of this change resulted in an increased flow of soluble components out of the rumen such as sugar and soluble true protein, thus decreasing microbial yield and ammonia production. This results in greater digestion of substrates such as sugar and soluble protein in the small intestine and subtle changes in rumen N requirements due to the lower microbial yields but consistent with the lower ammonia production.

**FA Intestinal Digestibility.** The original versions of the CNCPS (Sniffen et al., 1992) through v5.0 (Fox et al., 2004) treated dietary fat as a single entity with the assumption that all fat escapes the rumen undegraded and 95% is digested in the small intestine. The model of Moate et al. (2004) was incorporated into v6.0 of the CNCPS (Tylutki et al., 2008), which computed individual FA intake, predicted de novo synthesis of FA by rumen microbes, biohydrogenation of MUFA and PUFA in the rumen, passage of individual FA to the small intestine, and a global intestinal digestibility of 95%. In that publication, it was recognized that a global FA digestibility was not appropriate, but modifications were not evaluated. Since that time much work has been conducted to better estimate or describe individual FA digestibility. The intestinal digestibility of individual FA now implemented are in Table 2 and are based on data and review from Moate et al. (2004) and Lock et al. (2005, 2006).

**Tissue AA Composition and Postabsorptive Utilization.** The tissue AA composition used within the CNCPS was evaluated and updated using tissues from a serial slaughter experiment (Diaz et al., 2001). Representative samples of carcass; head, hide, feet, and tail; blood; and organs were obtained from 40 calves at 65 and 105 kg of BW and subjected to AA analysis. For the analysis of AA, sample aliquots (2 mg of N) were hydrolyzed at 110°C for 21 h in a block heater (Gehrke et al., 1985) with 5 mL of 6 M HCl after flushing with N<sub>2</sub> gas. Norleucine (50 µL; 125 mM) was used as an internal standard. Hydrolysates were filtered on

Whatman 541 filters and diluted to 50 mL with water. Aliquots (0.5 mL) were evaporated, redissolved in 1 mL of water, evaporated again, which was repeated 2 more times to remove the acid, and dissolved in 2 mL of sample buffer for analysis. Additional aliquots (2 mg of N) were preoxidized with 1 mL of performic acid (4.5 mL of 88% formic acid, 0.5 mL of 30% hydrogen peroxide, 25 mg of phenol) for 16 h on ice before acid hydrolysis for analysis of Met and Cys. Then, AA were separated on a lithium cation exchange column using a 3-buffer step gradient and column temperature gradient. Detection was at 560 nm following ninhydrin postcolumn derivation on an HPLC System Gold with 32 Karat software (Beckman-Coulter Inc., Fullerton, CA). Standards (250 nmol/mL) for Asp, Thr, Ser, Glu, Gly, Ala, Val, Met, Ile, Leu, Tyr, Phe, NH<sub>3</sub>, Lys, His, Arg, and Cys (125 nmol/mL) were prepared by diluting a purchased stock (AA standard H, #20088; Pierce Chemical, Rockford, IL) with the sample buffer. Internal standards (250 nmol/mL) norleucine for non-aromatic AA and 5-methyl-Trp for tryptophan were prepared in sample buffer and combined with the other standards. The volume of samples and standards loaded on the column was 50 µL. Tryptophan was measured in a separate analysis using fluorescence detection (excitation = 285 nm; emission = 345 nm) according to the procedure of Landry and Delhaye (1992). Briefly, samples (2 mg of N) were hydrolyzed using 1.2 g of Ba(OH)<sub>2</sub> at 110°C for 16 h on a block heater and subsequently cooled on ice to precipitate barium ions. An aliquot of the hydrolysate (3 µL) was added to 1 mL of acetate buffer (0.07 M sodium acetate; pH 4.5) and analyzed by HPLC. The tissue composition identified

**Table 2.** Post-ruminal FA digestibility constants used in the Cornell Net Carbohydrate and Protein System v6.5, adopted from Moate et al. (2004) and Lock et al. (2006)

FA <sup>1</sup>	%
C12:0	95.4
C14:0	75.1
C16:0	72.5
C16:1	72.0
C18:0	72.8
C18:1 <i>trans</i>	80.0
C18:1 <i>cis</i>	80.0
C18:2	83.0
C18:3	77.6
Other	58.7

<sup>1</sup>C12:0 = dodecanoic acid (lauric acid); C14:0 = tetradecanoic acid (myristic acid); C16:0 = hexadecanoic acid (palmitic acid); C16:1 = hexadecanoic acid (palmitoleic acid); C18:0 = octadecanoic acid (stearic acid); C18:1 *cis* = octadecenoic acid *cis* isomers (includes oleic acid and other positional isomers); C18:1 *trans* = octadecenoic acid *trans* isomers (includes elaidic acid, vaccenic acid, and other positional isomers); C18:2 = octadecadienoic acid (includes linoleic acid, conjugated linoleic acid, and other positional isomers); C18:3 = octadecatrienoic acid (includes α-linolenic and γ-linolenic acids); Other = long-chain FA other than those listed above and with more than 12 carbon atoms.

in this analysis was averaged with those from Williams and Hewitt (1979) and incorporated into the CNCPS.

Furthermore, the CNCPS uses a factorial approach to predict AA supply and requirements as described by O'Connor et al. (1993) and Fox et al. (2004). The efficiencies of use for absorbed AA in the CNCPS are different for maintenance, pregnancy, lactation, and growth and were most recently updated by Fox et al. (2004). Lapierre et al. (2007) discussed the biological correctness of having different efficiencies for maintenance and lactation. When considering the distribution of enzymes for AA catabolism and the dominate role the liver plays in the modifying peripheral AA supply, using a combined efficiency of AA utilization better reflects the utilization of AA by the cow. Doepel et al. (2004) conducted a meta-analysis of 40 published papers involving abomasal, duodenal, or intravenous infusions of casein or free AA and estimated the optimum efficiency of use for each essential AA. Lapierre et al. (2007) extended this work and estimated the optimum efficiencies for each EAA at various levels of MP supply. For application within the structure of the CNCPS, the efficiencies derived at 100% of MP supply were used because those efficiencies were apparently derived when the AA supply was in balance with the ME supply, or at least similar. The CNCPS uses fixed efficiencies, and the most correct representation of AA efficiencies should be on an energy-neutral basis. Also, the model does not account for efficiency changes due to over- or underfeeding AA because it assumed the user will formulate close to the predicted requirement. A comparison of the individual and combined efficiencies of use are in Table 3.

**Nitrogen Excretion and Methane Production.**

The CNCPS is designed to be used in the field to predict

nutrient excretion as part of a nutrient-management decision-making process. Through evaluation, the partitioning of urine and fecal N excretion was determined to be inconsistent with N balance data, thus a study was undertaken to improve this partitioning (Higgs et al., 2012). In part, this was done to help refine N feeding and excretion in relation to milk. Because urinary urea N is the most volatile form of excreted N and also represents the true excess N, better predictions of urinary N might help nutritionists formulate to decrease this form of N excretion. The equations developed by Higgs et al. (2012) were able to accurately partition between urinary and fecal N along with total manure N and have been implemented in v6.5.

Integrating prediction equations for greenhouse gas (GHG) emissions into field-usable models could provide a tool for producers and nutritionists to consider GHG emissions during the diet-formulation process; therefore, equations were implemented to predict methane production. Two extant equations were used, one for dairy cattle (Mills et al., 2003) and one for beef cattle (Ellis et al., 2007). The equation from Mills et al. (2003), used for dairy cattle, includes an exponential function that describes the effect of ME intake and the ratio of starch-to-ADF on methane production. The equation is described as follows:

$$CH_4 \text{ (MJ/d)} = 45.98 - (45.98e^{-1 \times [(-0.0011 \times \text{starch/ADF}) + 0.0045 \times \text{ME intake}]})$$

where starch and ADF are expressed as kilograms consumed per day and ME is expressed in megajoules consumed per day.

The equation from Ellis et al. (2007), used for beef cattle, was chosen because it had the lowest root mean square prediction error (14.4%) and the highest R<sup>2</sup> (0.85) of the evaluated equations and is described as follows:

$$CH_4 \text{ (MJ/d)} = 2.94 + 0.0585 \times \text{ME intake (MJ/d)} + 1.44 \times \text{ADF (kg/d)} - 4.16 \times \text{lignin (kg/d)}$$

To enhance the ability of the model to provide robust GHG emission predictions, CO<sub>2</sub> emissions were evaluated using 2 extant equations to determine the capacity of each equation to predict observed CO<sub>2</sub> from independent data sets. An equation from Casper and Mertens (2010),

$$CO_2 \text{ (g/d)} = 821.3 + 126.0 \times \text{DMI (kg/d)} - 1.18 \times \text{milk (kg/d)}$$

**Table 3.** The original efficiencies (%) of AA utilization as published by O'Connor et al. (1993) and the combined efficiencies (%) of AA utilization for both maintenance and lactation adapted from Doepel et al. (2004) and Lapierre et al. (2007)

AA	CNCPS <sup>1</sup> v6.0		CNCPS v6.5
	Maintenance	Lactation	Combined efficiency <sup>2</sup>
Met	85	100	66
Lys	85	82	69
Arg	85	35	58
Thr	85	78	66
Leu	66	72	61
Ile	66	66	67
Val	66	62	66
His	85	96	76
Phe	85	98	57
Trp	85	85	65

<sup>1</sup>CNCPS = Cornell Net Carbohydrate and Protein System.

<sup>2</sup>From Doepel et al. (2004) and Lapierre et al. (2007).

**Table 4.** Descriptive statistics of the rumen evaluation data set

Item	Mean	SD	Minimum	Maximum
Diet composition, % of DM				
CP	16.1	2.55	9.9	20.7
RDP	10.2	1.81	6.2	14.5
RUP	5.9	1.33	2.9	9.2
NDF	34.6	9.02	22.7	59.5
Starch	23.8	11.66	1.1	44.1
Fat	4.0	0.84	2.6	6.2
Omasal flows, g/d				
NAN	481	176.8	87	778
Bacterial nitrogen (BactN)	316	123.8	78	480
Rumen-degraded nitrogen (RDN)	337	126.2	50	539
Rumen-undegraded nitrogen (RUN)	164	65.1	7	326

was evaluated along with an equation by Kirchgessner et al. (1991),

$$\text{CO}_2 = [-1.4 + (0.42 \times \text{DMI}) + (0.045 \times \text{BW}^{0.75})]/0.27,$$

where  $\text{CO}_2$  is expressed in kilograms per day, DMI in kilograms per day, and BW in kilograms. The predictions from both equations were very similar, and the decision was made to implement the equation from Casper and Mertens (2010) because it was easy to implement and included milk yield as a factor, which provided a greater range in predictions related to metabolism compared with differences in BW as described by Kirchgessner et al. (1991).

### Model Evaluation: Data-Set Development

Four separate data sets were developed using literature studies and data from commercial farms provided by regional nutritionists. The first data set (AA data set) was used to estimate the optimum concentration of Lys and Met relative MP to maximize milk protein yield and milk protein concentration. Dose-response studies (Appendix) were used where the supply of Lys (8 studies; 43 treatment means) or Met (11 studies; 50 treatments means) was increased either by postruminal infusion (42% of studies) or by feeding rumen-protected sources (58% of studies). Digestible Lys and Met were estimated from Lys and Met content and bioavailability data provided either by the manufacturer or experimentally estimated and reported. The optimum AA concentrations were estimated according to the procedure described by Rulquin et al. (1993). Reference values used in the calculations were 6.80 and a 2.43% of MP 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 (%) and the predicted concentrations of Lys and Met (% of MP) were evaluated by regression procedures.

The second data set (rumen data set) was used to evaluate the predicted flows of bacterial, feed, and total N from the rumen and was compiled from studies where postruminal N flows were measured at the omasum (Huhtanen et al., 1997; Ahvenjärvi et al., 2000; Reynal and Broderick, 2005). In total, 20 studies (Appendix) with 74 treatments were included. All studies reported rumen-degradable N, rumen-undegradable N (RUN), NAN, and bacterial N (BactN). The data set represented a wide range diet ingredients and nutrient compositions. Descriptive statistics for the data set are in Table 4.

The third data set (lactation data set) was used to evaluate the ability of the model to predict milk yield from the supply of ME, MP, or both and was compiled from studies published in the *Journal of Dairy Science* between 2001 and 2012 (Appendix). Lactation trials were used with cows in different stages of lactation (early, mid, and late). Studies that used crossover designs (Latin square, Box-Behnken, and so on) or that had <6 experimental units per treatment were excluded. In total, 103 studies were preselected, of which 55 (200 treatments means) met the criteria for incorporation into the data set. The criteria for inclusion required each study to report (a) a description of the ingredients and chemical analysis of the ration fed for each treatment, (b) measured DMI, (c) milk yield and milk composition for each treatment, and (d) a description of the animal and environmental conditions where the study was completed. Additional data from commercial farms was supplied by nutritionists in the Northeast United States (15 farms; 50 diets). This data set was also used to evaluate the ME- and MP-allowable milk using the individual FA intestinal digestibility compared with the global 95% FA digestibility used in previous versions.

**Table 5.** Descriptive statistics of animal and production characteristics for the lactation data set

Item	Mean	SD	Minimum	Maximum
Diet composition, % of DM				
CP	16.9	2.35	9.4	29.5
RUP	7.2	1.55	3.3	16.7
RDP	9.7	1.38	6.1	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 BW, kg	623	44.4	525	737
Final BW, 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, <sup>1</sup> kg/d	32.3	6.18	14.9	47.2
Milk protein, %	3.02	0.194	2.51	3.60
Milk fat, %	3.67	0.479	2.06	5.06

<sup>1</sup>Tyrrell and Reid (1965).

Descriptive statistics of the data set used are in Table 5.

The fourth data set was compiled from studies that reported CO<sub>2</sub> and CH<sub>4</sub> production from animals in metabolic chambers and had adequate dietary information to run an evaluation in the CNCPS (van Dorland et al., 2007; Moate et al., 2011; Liu et al., 2012; Hammond et al., 2014; Reynolds et al., 2014). The carbon dioxide equation of Casper and Mertens (2010) was compared with another published and used equation from Kirchgessner et al. (1991) to verify that the predictions were similar and provided some assurance of a lack of bias.

A spreadsheet version of the CNCPS was used to conduct the model simulations. Information on feed chemistry required by the CNCPS to run a simulation was used as reported by the study. Often, limited information was presented on the chemical composition of the dietary components. In this situation, information reported by the study was used, and uncertain values were predicted using an extension of the method described in a companion paper (Higgs et al., 2015). Briefly, it was assumed that the feeds used in different treatments in the same study had the same chemical composition. The procedure optimized each chemical component in each feed to be within a likely range, to be internally consistent (chemical components sum to 100% DM), and to allow the compiled diet to match the reported composition when all feeds reported in the study had the same composition. Once entered into the model, the simulations were performed and the predicted and observed data were compared. Animal information required to run a simulation in the CNCPS included a description of housing conditions, BW, BW change over the period studied, BCS, BCS change over

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 reported, the final BCS (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). Empty BW was calculated from BW using the following equations: EBW = 0.851 × SBW, and SBW = 0.96 × BW, where SBW is shrunk BW. Therefore, EBW = 0.81696 × BW.

### Statistical Analysis

Statistical analysis was conducted using SAS (SAS Institute Inc., 2010). A broken-line model with a plateau was used to establish the dose-response relationship between Met or Lys and milk protein concentration and yield. According to the NRC (2001), this model was equal or superior to other models for establishing optimum Met and Lys supply. The model consisted of a linear regression 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 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, and  $C$  = the break point.

For the lactation and rumen data sets, a mixed effects model using the restricted maximum likelihood



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 level  $j$  of the continuous variable  $X$  in study  $i$  (or farm for the lactation data set),  $\beta_0$  = the overall intercept across all studies (or farms for the lactation data set),  $s_i$  = the random effect of study (or farm for the lactation data set)  $i$ ,  $\beta_1$  = the overall slope of  $Y$  on  $X$  across all studies (or farms for the lactation data set),  $b_{1i}$  = the random effect of study  $i$  (or farm for the lactation data set) on the slope of  $Y$  on  $X$ ,  $X_{ij}$  = the model predicted data associated with level  $j$  of the continuous variable  $X$  in study  $i$  (or farm for the lactation data set), and  $\varepsilon_{ij}$  = random variation.

Squared sample correlation coefficients reported were based on either the BLUP ( $\mathbf{R}^2_{\text{BLUP}}$ ) or model-predicted estimates ( $\mathbf{R}^2_{\text{MDP}}$ ). Conditional residuals were used and examined for bias as well as 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 were used to indicate accuracy. A decomposition of the mean square prediction error ( $\mathbf{MSPE}$ ) 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 ( $\mathbf{CCC}$ ) were used to simultaneously account for accuracy and precision. Concordance correlation coefficients can vary from zero to one, with a value of one indicating that no deviation from the  $Y = X$  line has occurred.

For the gas emissions, predictions data were analyzed using a mixed model where study was included as a random variable and the model included the specific gas,  $\text{CO}_2$  or  $\text{CH}_4$ , study, and error.

## RESULTS AND DISCUSSION

### Maintenance Calculations

Evaluations of this adjustment in growing animals were conducted, and the evaluations demonstrated that the changes in maintenance requirements were significantly overestimated (Van Amburgh et al., 1998; Guioy et al., 2001), thus the calculation for growing cattle was removed. The outcome was a difference of almost 0.4 kg/d in ME-allowable growth as the BCS ranged from 1 to 5 (Table 6). This resulted in the potential to overfeed energy to heifers given the model would predict less ME-allowable gain than was truly available at an average BCS. Thus, using BCS to adjust

**Table 6.** The energy-allowable gain of a 250-kg Holstein heifer as modified by the change in BCS from 1 to 5, independent of any diet or other inputs

BCS, 1–5	ME-allowable gain, kg/d
1	0.84
3	0.70
5	0.55

<sup>1</sup>This adjustment was removed in Cornell Net Carbohydrate and Protein System v6.5.

the maintenance requirements of growing cattle was removed.

### Tissue AA Content and Lysine and Methionine Requirements

The tissues were analyzed by compartment and then summarized on a whole-animal basis, and those values are in Table 7. The values analyzed in this study were similar to those of Williams and Hewitt (1979). The tissue AA values used previously were not analyzed using methods to protect the sulfur AA and were thus underestimating the sulfur AA contents of tissues (Ainslie et al., 1993). The updated AA values are used for tissue requirements for growing animals and can also, if desired, be applied to cattle that are mobilizing body reserves and associated body protein during periods of negative energy balance (Fox et al., 2004). Body protein mobilization will be proportional to the profile of the tissue that was last deposited and will contain a modest level of protein and available AA. When establishing the new efficiencies of use of absorbed AA for lactation, body mobilization of AA or any energy reserves were not considered directly, and it would be expected that an apparent increase in efficiency would occur because of the presence of mobilized AA available for milk protein synthesis and not consumed. To fully model this change in efficiency would require very specific data on the amount of each specific AA mobilized and the amount taken up by the mammary gland, and those data and modeling approach were not considered in this update.

To maximize milk protein yield, the calculated estimates using the breakpoint analysis for Lys and Met (% of MP) were 7.00 and 2.60% of MP, respectively (Figure 1), and to maximize milk protein content, 6.77 and 2.85 (% of MP), respectively (Figure 2). These data are similar to previous estimates for Lys using v6.0 of the CNCPS (6.74 and 6.68% of MP for protein yield and content, respectively) but 11 and 18% higher than previous estimates for Met (2.31 and 2.40% of MP for protein yield and content, respectively; Whitehouse et al., 2013). This is partly due to the reorganization

**Table 7.** The AA content of 4 component tissues (carcass; organs and blood; liver; and head, hide, feet, and tail) from 40 Holstein calves from the study of Diaz et al. (2001) at 65 and 105 kg of harvest weight on a gram per 100 g of CP basis and averaged to represent the AA content of the whole body

AA	Carcass		Organs and blood		Liver		Head, hide, feet, and tail		Whole-animal AA content (weighted average)	
	g/100 g of CP	% Total weight <sup>1</sup>	g/100 g of CP	% Total weight	g/100 g of CP	% Total weight	g/100 g of CP	% Total weight	g/100 g of CP	SD
Methionine	2.01	0.63	1.56	0.17	2.35	0.03	1.14	0.18	1.79	0.14
Lysine	6.51	0.63	7.59	0.17	5.39	0.03	4.26	0.18	6.26	0.10
Histidine	2.38	0.63	3.61	0.17	2.34	0.03	1.38	0.18	2.41	0.06
Phenylalanine	3.41	0.63	5.21	0.17	4.52	0.03	2.79	0.18	3.65	0.03
Tryptophan	1.21	0.63	1.64	0.17	1.82	0.03	0.57	0.18	1.18	0.21
Threonine	3.77	0.63	4.76	0.17	4.46	0.03	3.08	0.18	3.83	0.15
Leucine	6.45	0.63	9.86	0.17	8.59	0.03	5.73	0.18	6.96	0.21
Isoleucine	3.12	0.63	2.65	0.17	3.85	0.03	2.43	0.18	2.94	0.13
Valine	3.83	0.63	5.56	0.17	5.29	0.03	4.24	0.18	4.28	0.15
Arginine	6.68	0.63	6.08	0.17	6.17	0.03	7.81	0.18	6.75	0.18

<sup>1</sup>The percent total weight is the proportion of the component as a percentage of the whole animal averaged among the 2 harvest weights.

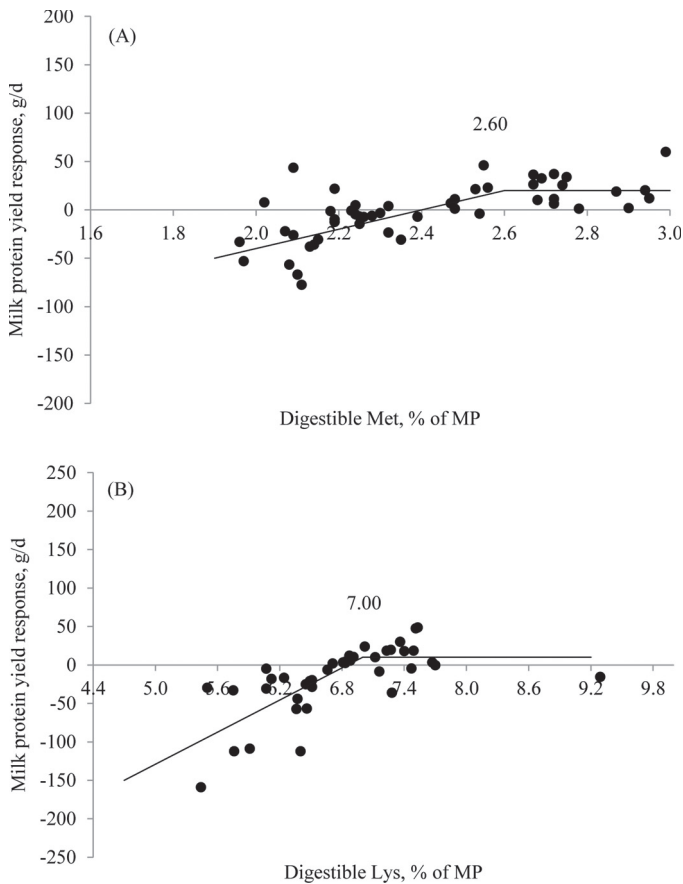
of the protein pools, which has increased predicted MP supply, but also due to the updated AA profiles (Higgs et al., 2015). The methods used to analyze AA for the original feed library (O'Connor et al., 1993) did not preoxidize and thus protect sulfur AA and were not adequate to correctly quantify those AA (Higgs et al., 2015). Consequently, the AA profiles in the new feed library are, in many cases, considerably higher in Met. It is important to note, although the recommendations for Met supply in v6.5 are higher, similar animal data were used to derive the recommendations as previous versions of the CNCPS (Whitehouse et al., 2013) and the NRC (2001); therefore, the changes represents a recalibration and are largely due to the updated AA profiles, rather than a suggestion to feed 18% more supplemental Met. It is also important to note that these recommendations are model specific and are not relevant to previous versions of the CNCPS or CPM Dairy and can only be used with the new efficiencies for postabsorptive AA use.

**Efficiency of AA Use**

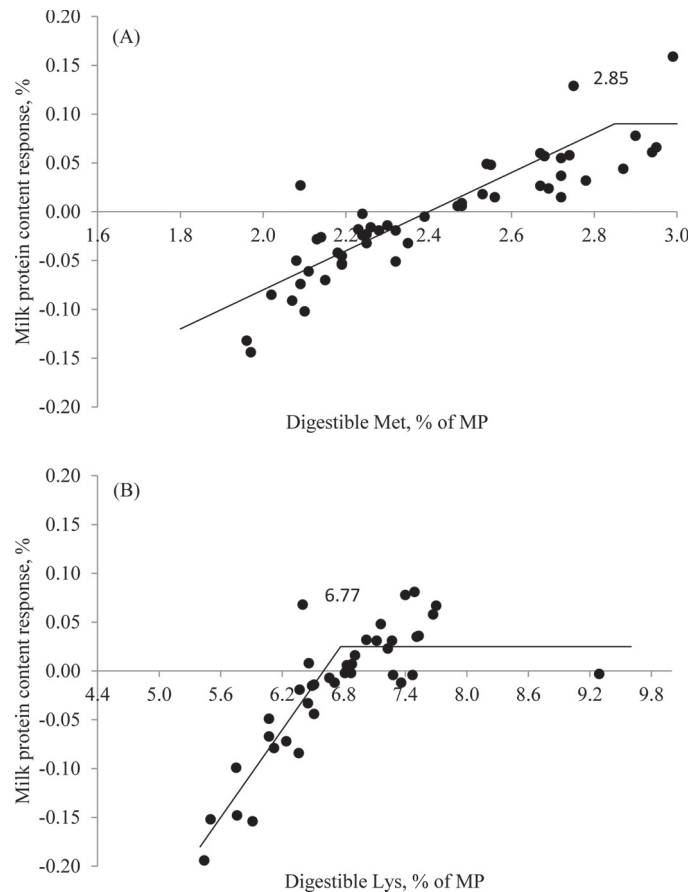
To evaluate the updated efficiency of AA use included in the CNCPS, the AA data set used to determine the optimum proportion of Met and Lys in MP was used to perform a regression of model-predicted AA balance (g of Met/d) against the concentration of Met in the diet (Met % of MP). Using the new efficiencies (Table 3), the regression line intercepted the y-axis at approximately 2.60% dietary Met relative to total MP (Figure 3), similar to the breakpoint derived in Figure 1A. The studies used to perform this analysis were specifically designed to be both sufficient and limited in Met supply 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 (% of MP), and no diets are predicted to have negative Met balance, contrary to expectations (Figure 3). Using the updated efficiencies, there is a balance of both positive and negative Met balance among the data set. This suggests that the new efficiencies of use allow the model to more adequately represent the true requirements of EAA, especially under conditions when ME is not first limiting.

**Rumen Nitrogen Flows**

Model-predicted N flows were compared with those measured using omasal sampling. The omasal sampling technique described by Huhtanen et al. (1997) has advantages over sampling in other compartments (abomasum or duodenum) that include less contamination



**Figure 1.** Milk protein yield response as a function of digestible Met (A;  $y = -219 + 92.7 \times \text{Met}$  and  $y = -219 + 92.7 \times 2.6$  for the linear and the plateau part of the model, respectively;  $R^2 = 0.48$ ) and Lys (B;  $y = -478 + 70.0 \times \text{Lys}$  and  $y = -478 + 70.0 \times 7.0$  for the linear and the plateau part of the model, respectively;  $R^2 = 0.55$ ) with the updated feed-library values for AA described by Higgs et al. (2015).



**Figure 2.** Milk protein content response as a function of digestible Met (A;  $y = -0.46 + 0.19 \times \text{Met}$  and  $y = -0.46 + 0.19 \times 2.85$  for the linear and the plateau part of the model, respectively;  $R^2 = 0.77$ ) and Lys (B;  $y = -0.99 + 0.15 \times \text{Lys}$  and  $y = -0.99 + 0.15 \times 6.77$  for the linear and the plateau sections of the model, respectively;  $R^2 = 0.78$ ) with the updated feed-library values for AA described by Higgs et al. (2015).

with endogenous material and potential confounding due to the markers used and the inability to define adequate marker recovery from incomplete marker recovery among studies, which affects all evaluations of duodenally cannulated-cattle studies (NRC, 2001; Seo et al., 2006). All studies in the current data set measured digesta flow using a triple marker approach (France and Siddons, 1986), which has been shown to be more representative of digesta flows than single markers such as  $\text{Cr}_2\text{O}_3$  that are often used in studies that have sampled at the duodenum (Firkins et al., 2007; Huhtanen et al., 2010).

The random effect of study accounted for >81% of the variation in the prediction of rumen-degradable N, BactN, and NAN and approximately 67% in the prediction of RUN resulting in high  $R^2_{\text{BLUP}}$  values (Table 8). Overall, CCC values were >0.81, indicating model

predictions were both precise and accurate, although the model overestimated RUN ( $\beta_1 = 0.73$ ; Figure 4). Lanzas et al. (2008) completed a similar analysis with v6.0 of the CNCPS using a data set of 5 studies that sampled at the omasum and found the CNCPS overestimated RDP and underestimated RUN flow, contrary to the findings of the current study. This shift can be explained by the reorganization of the N pools in v6.5 and indicates further work is required to correct biases within the model. However, when compared with the evaluation of Lanzas et al. (2008), updates to the CNCPS have improved accuracy and precision of rumen-degradable N and RUN (CCC = 0.81 and 0.63, respectively).

The flow of BactN was predicted accurately and precisely ( $R^2_{\text{BLUP}} = 0.97$ ; root mean square error = 24.6; CCC = 0.87), which is in agreement with previous

**Table 8.** Model adequacy statistics for the prediction of postruminal flow of rumen-degraded nitrogen (RDN), rumen-undegraded nitrogen (RUN), NAN, and bacterial nitrogen (BactN) and of the first-limiting MP- or ME-allowable, or both, milk<sup>1</sup>

Item	n	R <sup>2</sup> <sub>BLUP</sub>	R <sup>2</sup> <sub>MDP</sub>	RMSPE	Variance component <sup>2</sup>			CCC	MSPE	MSPE partitioned (%)		
					Study	Slope	Residual			U <sup>M</sup>	U <sup>S</sup>	U <sup>R</sup>
Rumen data set												
RDN	74	0.98	0.79	19.4	88.2	0.0	11.8	0.89	3,568	1.8	1.6	96.6
RUN	74	0.92	0.65	21.7	66.9	0.0	33.1	0.81	1,455	0.1	12.7	87.2
BactN	74	0.97	0.84	24.6	81.9	0.0	18.1	0.87	3,038	0.1	1.5	98.4
NAN	74	0.98	0.88	25.1	83.5	0.0	16.4	0.93	3,751	0.4	2.3	97.3
Lactation data set												
MP or ME	250	0.97	0.78	1.6	77.7	0.5	21.8	0.83	12.8	0.1	21.8	78.2
ME	177	0.95	0.76	1.8	67.0	0.6	32.4	0.84	11.8	0.1	16.7	83.2
MP	73	0.98	0.82	1.1	91.5	0.4	8.1	0.83	14.2	0.5	26.9	72.6

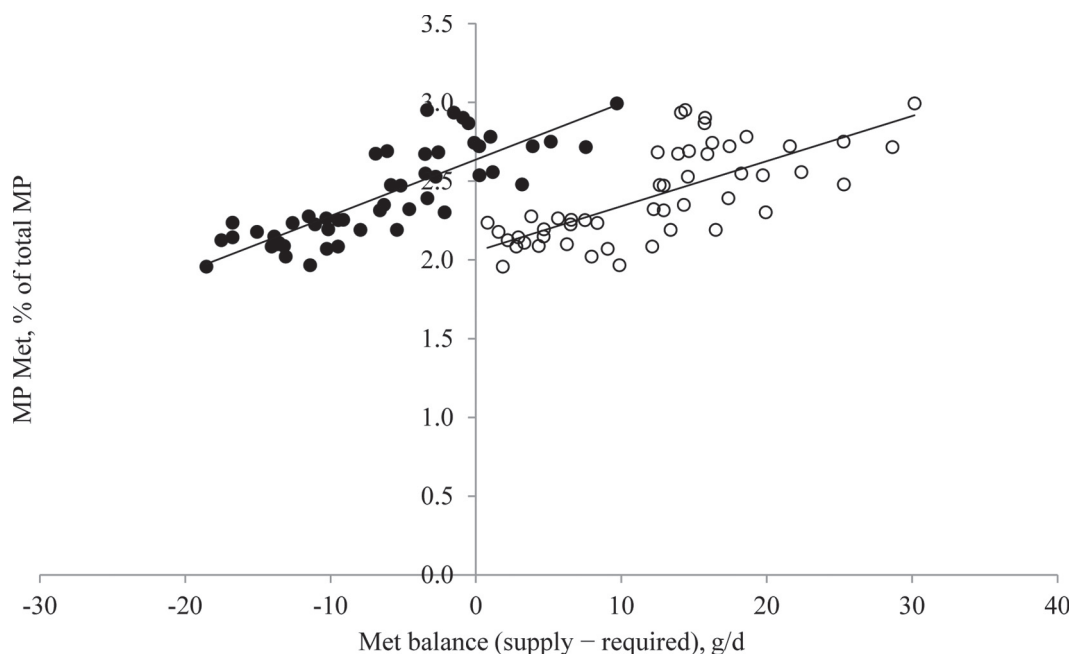
<sup>1</sup>R<sup>2</sup><sub>BLUP</sub> = correlation coefficient based on BLUP; R<sup>2</sup><sub>MDP</sub> = correlation coefficient based on model predictions using a mean study effect; RMSPE = root mean square prediction error; CCC = concordance correlation coefficient; MSPE = mean square prediction error; U<sup>M</sup> = percentage of error due to mean bias, U<sup>S</sup> = percentage of error due to systematic bias, U<sup>R</sup> = percentage of error due to random variation (U<sup>M</sup> + U<sup>S</sup> + U<sup>R</sup> = 100).

<sup>2</sup>Percentage of variance related to the effect of study and random variation (mixed model).

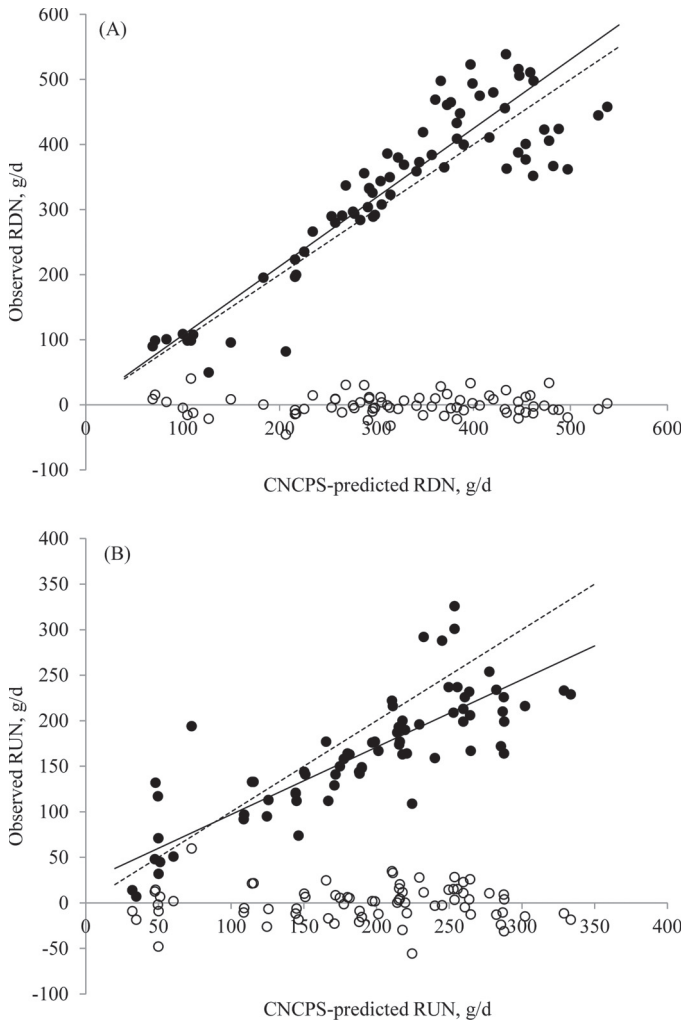
evaluations (Offner and Sauvant, 2004; Pacheco et al., 2012). Offner and Sauvant (2004) compared v5.0 of the CNCPS, Molly (Baldwin et al., 1987); and the model of Lescoat and Sauvant (1995) using duodenal flow data and found the CNCPS to have the most precise predictions of the 3 models. When considered together, biases in BactN and RUN offset, resulting in NAN predictions that are close to the unity line (Figure 5B) with an R<sup>2</sup><sub>BLUP</sub> = 0.98 and CCC = 0.93 and indicate the model can accurately predict total N flows from the rumen.

### Milk Yield Prediction

Previous evaluations of the CNCPS were conducted using specific experimental data sets of studies conducted at Cornell University (Fox et al., 2004; Tylutki et al., 2008). Model-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 coefficient of

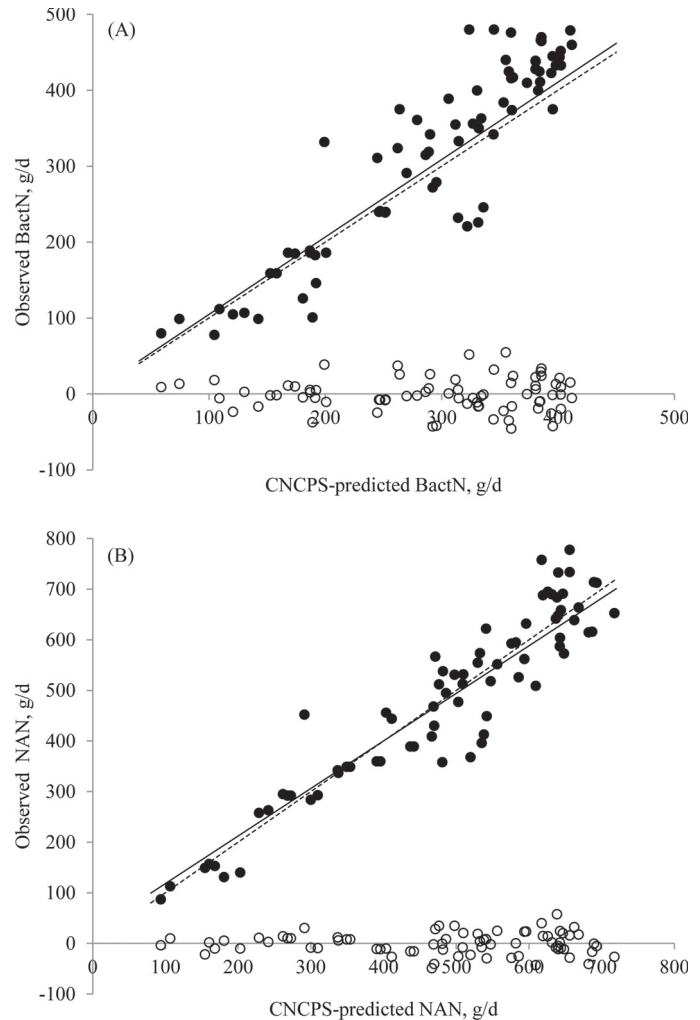


**Figure 3.** Model-predicted Met balance (Met supply less requirement) against dietary Met using Cornell Net Carbohydrate and Protein System (CNCPS) v6.5 with the updated feed-library values for AA described by Higgs et al. (2015; ●; slope = 0.0004; intercept = 0.0264; R<sup>2</sup> = 0.68) or CNCPS v6.1 (○; slope = 0.0003; intercept = 0.02054; R<sup>2</sup> = 0.51).



**Figure 4.** Observed versus Cornell Net Carbohydrate and Protein System (CNCPS) predictions (●) for (A) rumen-degradable nitrogen (RDN;  $y = 1.06x + 0.85$ ) and (B) rumen-undegradable nitrogen (RUN;  $y = 0.74x + 23.14$ ). Mixed-model residuals are also shown on the graph (○).

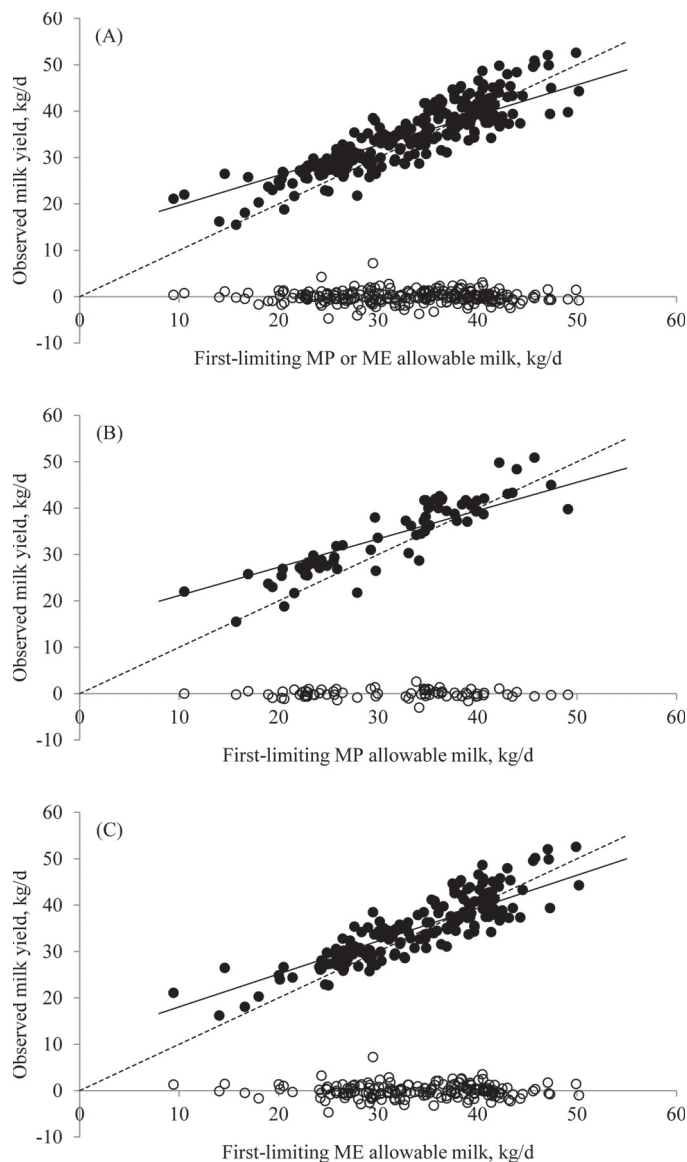
determination ( $R^2$ ) = 0.89 and CCC = 0.94 (Tylutki et al., 2008). The current evaluation, using a large data set with 250 treatments from 55 studies and 15 farms, reinforced the ability of the latest version to predict the most limiting nutrient; MP- or ME-allowable milk yield was predicted with an  $R^2_{BLUP} = 0.97$ ,  $R^2_{MDP} = 0.78$ , and root mean square error = 1.6 (Figure 6). Moreover, the low MSPE indicated the high accuracy of the model, and the decomposition of MSPE suggested that random variation (78.2% of MSPE) followed by systematic bias (21.8% of MSPE) are the main elements to explain bias (Table 8). The variance component analysis of the mixed model indicated that 77.7% of the variation was attributed to the random effect of study or farm. Furthermore, the overall accuracy and precision of the



**Figure 5.** Observed versus Cornell Net Carbohydrate and Protein System (CNCPS) predictions (●) for (A) bacterial nitrogen (BactN;  $y = 0.93x + 43.50$ ) and (B) NAN ( $y = 0.94x + 24.24$ ). Mixed-model residuals are also shown on the graph (○).

model to predict the first-limiting nutrient was high as indicated by the CCC (0.83).

The development of a large data set 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. Results of the evaluation of ME- and MP-allowable milk yield are presented in Figure 6 and Table 8. Both MP- and ME-allowable milk were predicted reasonably well as indicated by the high  $R^2_{MDP}$  and CCC and the low root mean square prediction error. In this evaluation, MP-allowable milk was predicted with greater precision than ME-allowable milk. An early attempt to evaluate CNCPS v6.0 when MP was the first-limiting nutrient resulted in low precision ( $R^2 = 0.29$ ; Tylutki et al., 2008). Part of this low performance of the model



**Figure 6.** Observed versus Cornell Net Carbohydrate and Protein System predictions (●) for (A) first-limiting MP- or ME-allowable milk ( $y = 0.65x + 13.17$ ), (B) MP first-limiting milk ( $y = 0.61x + 15.06$ ), and (C) ME first-limiting milk ( $y = 0.71x + 10.92$ ). Mixed-model residuals are also shown on the graph (○).

can be explained by the method used to evaluate the model. Dairy cows were used as the statistical unit, incorporating variation of the animal, and the studied range of CP of the diets was very low including only low CP diets based on the study by Recktenwald et al. (2014). However, results indicated that modifications to describe ruminal and postruminal protein requirements and supply, especially when low CP diets are fed, were needed. Current updates of protein fractionation and the corresponding adjustments of their degradation rates as well as the new AA profiles and utilization constants have made MP predictions more sensitive than

previous versions, resulting in this significant improvement of CNCPS to predict milk yield when MP is the limiting nutrient.

The sensitivity of the model to predict a MP limitation is partially a function of the overall efficiency of use of MP to net protein of 0.67, the same value used in the 2001 Dairy NRC (NRC, 2001; Tylutki et al., 2008). Data from recent studies in lactating cattle call into question the use of static efficiencies for either overall MP or specific AA, and this makes sense given the possible roles certain AA have in metabolism (Doepel et al., 2004; Pacheco et al., 2006; Metcalf et al., 2008).

Metcalf et al. (2008) challenged the use of a static efficiency and observed a range in efficiency of use of 0.77 to 0.50 as MP supply was increased. In that publication, they further determined using a best fit of data that the optimal efficiency of use of MP to net protein was between 0.62 and 0.64 for the average cow. This is lower than the current value but is consistent with the data of Doepel et al. (2004). Taking the simple mean of the efficiencies from the Doepel et al. (2004), the average efficiency of use of the essential AA was 62.2%, again lower than the value currently being using in the model but consistent with the data of Metcalf et al. (2008). Most likely, any change in efficiency of use of MP or AA will be associated in a change in ME utilization, thus the absolute differences within one nutrient will be hard to detect or manipulate. For this reason, we updated the efficiencies of use of absorbed AA but did not make modifications to the overall MP efficiency assuming the overall change in efficiency is more affected by ME for milk yield and not MP supply.

Although not shown, error fixes were made to the calculations for metabolic fecal nitrogen. A double-accounting error existed that resulted in underestimation of endogenous protein losses. Because this directly affects maintenance protein requirements, MP maintenance has increased slightly.

Furthermore, the performance of the model to predict milk yield when MP is limiting compared with ME limiting diets can be attributed to characteristics of the data sets. Within the data sets evaluated, it is more difficult to evaluate energy balance because information on BCS change and BW change are typically 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, and body-water versus body-fat changes, and changes in 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 where the majority of the error is random and due to study and not systematic within the model

(Table 8). This is a general problem with evaluating the predictions of energy supply and requirements using data from published studies because rarely is adequate information published that would allow for adequate representation of true energy exchanges and transformations in high-producing lactating cattle, thus we rely on the data generated by balance and chamber studies as the base of these calculations.

Individual FA digestibility constants reduced the amount of digestible energy originating from fat sources (6.3 vs. 9.0% of digestible energy for CNCPS v6.5 using individual vs. global FA digestibility), and this resulted into a 2.0-kg of ME-allowable milk difference (34.7 vs. 36.7 kg of ME-allowable milk for CNCPS v6.5 using individual vs. global FA digestibility). In terms of model prediction, the individual FA digestibility constants resulted in higher precision reducing the root mean square error (1.66 vs. 1.59 for MP- or ME-allowable milk, respectively). By implementing individual FA digestibilities, differences in predicted ME from both commercial products and also alternative fat sources become more apparent because of differences in the FA composition of various fat sources, and this improves the ability of the model to estimate the most limiting nutrient.

### Nitrogen, Methane, and Carbon Dioxide Predictions

The implementation of prediction equations for urinary and fecal N excretion allowed for an evaluation of the behavior of urinary N, milk N, and fecal N excretion under conditions where ME balance was similar among studies or treatments and in every case, first limiting (Kauffman and St-Pierre, 2001; Broderick, 2003; Hristov and Ropp, 2003; Groff and Wu, 2005; Recktenwald, 2007; Recktenwald et al., 2014). In the treatments chosen for evaluation, because energy was first limiting, it allowed for the evaluation of urinary N excretion under conditions of decreasing N intake (Figure 7). This approach was taken to better understand the behavior of intake N when energy supply limits the opportunity for increased milk protein synthesis or protein yield. Thus, overall N efficiency, assuming the cow made a metabolic decision that most of the urea N excreted in urine was excess N not required for recycling back to the gastrointestinal tract, was increased as N intake was decreased (Marini and Van Amburgh, 2003; Recktenwald et al., 2014).

Predicted CO<sub>2</sub> emissions using the works by Casper and Mertens (2010) or Kirchgessner et al. (1991) were similar (Table 9). We chose to use the equation from Casper and Mertens (2010) because it was easily integrated into the CNCPS and the studies used to develop

**Table 9.** Comparison of carbon dioxide (CO<sub>2</sub>) emission predictions from dairy cows between the equations of Casper and Mertens (2010) and Kirchgessner et al. (1991)<sup>1</sup>

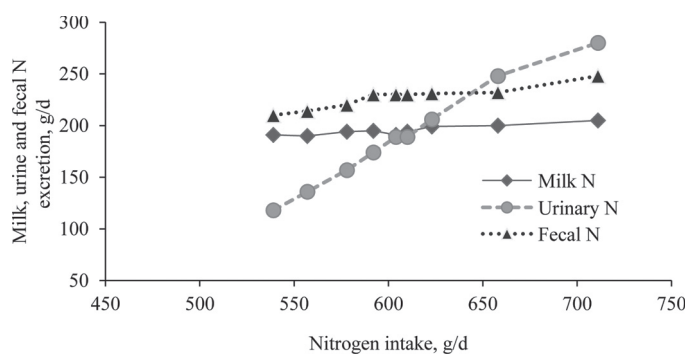
Item	CO <sub>2</sub> , g/cow per day	
	Casper and Mertens (2010)	Kirchgessner et al. (1991)
Mean	14,281	14,775
SD	1,181	1,244
Minimum	9,172	9,059
Maximum	16,429	17,187

<sup>1</sup>The emissions predictions were not significantly different between equations.

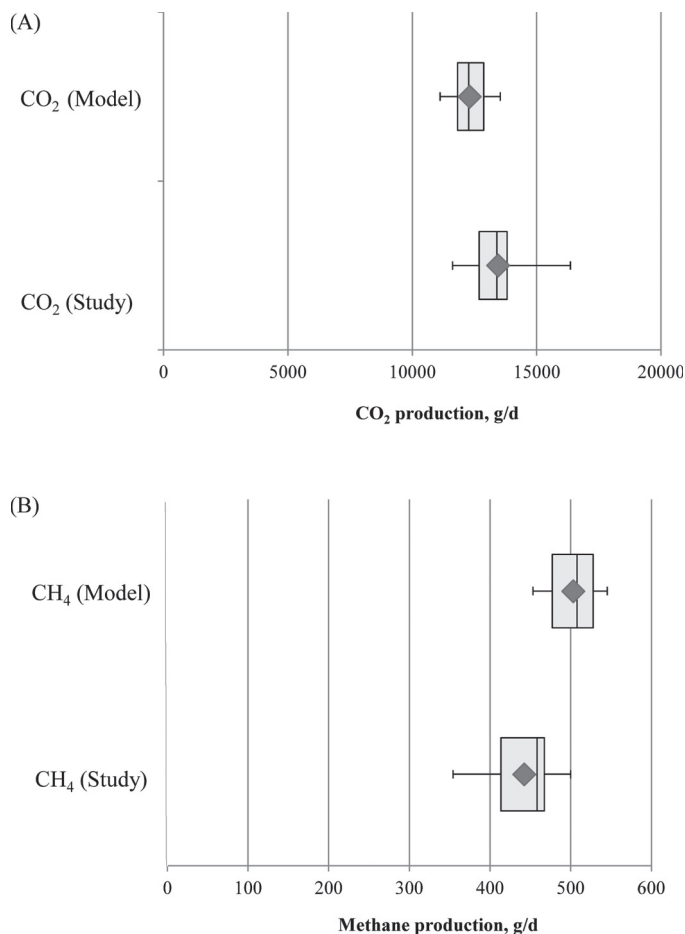
the equation encompassed a wide range of DMI and milk yields from 1,252 individual cattle respiration calorimetric trials and were the foundation of the energy metabolism system used in the United States. The observed and model-predicted (13,449 ± 1,228 and 12,306 ± 685; 503 ± 29 and 442 ± 37) CO<sub>2</sub> and CH<sub>4</sub> (mean g/d ± SD), respectively, were not significantly different ( $P > 0.05$ ), indicating the equations used could provide reliable estimates of GHG production as long as adequate dietary information was available (Figures 8A and 8B). These data demonstrate the potential for nutritionists to consider GHG production as part of diet formulation in a field-usable model to further reduce the environmental impact of dairy production.

### Summary

The most significant changes described in this update are related to the ability of the model to better partition



**Figure 7.** Nitrogen excretion in milk, feces, and urine based on nitrogen intake in lactating dairy cattle under controlled conditions of energy as first limiting. Cattle from studies and treatments selected were producing approximately 40 kg of milk and consuming approximately 25 kg of DM per day with diets ranging from 14 to 19% CP. Data were taken from studies by Kauffman and St-Pierre (2001), Broderick (2003), Hristov and Ropp (2003), Groff and Wu (2005), Recktenwald (2007), and Recktenwald et al. (2014).



**Figure 8.** Comparison of model-predicted and measured CO<sub>2</sub> and CH<sub>4</sub> emissions in dairy cattle using the equations of Casper and Mertens (2010; A) and Mills et al. (2003; B) using data from studies by van Dorland et al. (2007), Moate et al. (2011), Liu et al. (2012), Hammond et al. (2014), and Reynolds et al. (2014). Predictions were not significantly different from published values.

ruminal and postruminal N supply and requirements, the updated feed chemistry and feed library, and the changes made to improve the ability of the model to predict the AA supply and requirements of lactating dairy cattle. These updates improved the capacity of the model to detect the most limiting nutrient, which allows the user to refine diet formulation to improve the productive efficiency of cattle. Furthermore, the model is now able to provide estimates of the GHG emissions that add a dimension to diet formulation that better meets the needs of the industry and consumers in the 21st century. Further updates to the model are available; however, the current mathematical and framework structure is more than 30 yr old and accordingly requires a reimagining to a more dynamic system to fully implement and evaluate. Thus, the changes could not be fully implemented in this update.

## ACKNOWLEDGMENTS

The authors thank Charlie Sniffen (Fencrest, Holderness, NH) and Tom Tylutki (AMTS, Groton, NY) and many others for helpful suggestions and feedback and to the industry supporters who provided the financial support for A. Foskolos and E. A. Collao-Saenz, specifically Church & Dwight (Ewing, NJ), Kemin (Des Moines, IA), and Perdue AgSolutions (Salisbury, MD), and those that provided the feed amino acid data: Adisseo (Commentry, France) and Evonik/Degussa (Hanau-Wolfgang, Germany) that was necessary for updating the feed library and model.

## 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.
- Ainslie, S. J., D. G. Fox, T. C. Perry, D. J. Ketchen, and M. C. Barry. 1993. Predicting amino acid adequacy of diets fed to Holstein steers. *J. Anim. Sci.* 71:1312–1319.
- Baldwin, R. L., J. H. Thornley, and D. E. Beaver. 1987. Metabolism of the lactating cow. II. Digestive elements of a mechanistic model. *J. Dairy Res.* 54:107–131.
- Berman, A. 2003. Effects of body surface area estimates on predicted energy requirements and heat stress. *J. Dairy Sci.* 86:3605–3610.
- Besle, J.-M., A. Cornu, and J.-P. Jouany. 1994. Roles of structural phenylpropanoids in forage cell wall digestion. *J. Sci. Food Agric.* 64:171–190.
- Broderick, G. A. 1987. Determination of protein degradation rates using a rumen in vitro system containing inhibitors of microbial nitrogen metabolism. *Br. J. Nutr.* 58:463–475.
- Broderick, G. A. 2003. Effects of varying dietary protein and energy levels on the production of lactating dairy cows. *J. Dairy Sci.* 86:1370–1381.
- Broderick, G. A., and R. J. Wallace. 1988. Effects of dietary nitrogen source on concentrations of ammonia, free amino acids and fluorescamine-reactive peptides in the sheep rumen. *J. Anim. Sci.* 66:2233–2238.
- Brody, S. 1945. *Bioenergetics and Growth with Special Reference to the Energetic Efficiency Complex in Domestic Animals*. Reinhold Publ. Corp., New York, NY.
- Casper, D. P., and D. R. Mertens. 2010. Carbon dioxide, a greenhouse gas sequestered by dairy cattle. *J. Dairy Sci.* 93(E-Suppl. 1):843–844.
- Chandler, J. A., W. J. Jewell, J. M. Gossett, P. J. Van Soest, and J. B. Robertson. 1980. Predicting methane fermentation biodegradability. *Biotechnol. Bioeng. Symp.* 10:93–107.
- Chen, G., J. B. Russell, and C. J. Sniffen. 1987. A procedure for measuring peptides in rumen fluid and evidence that peptide uptake can be a rate-limiting step in ruminal protein degradation. *J. Dairy Sci.* 70:1211–1219.
- Choi, C. W., S. Ahvenjärvi, A. Vanhatalo, V. Toivonen, and P. Huhtanen. 2002a. Quantitation of the flow of soluble non-ammonia nitrogen entering the omasal canal of dairy cows fed grass silage based diets. *Anim. Feed Sci. Technol.* 96:203–220.
- Choi, C. W., A. Vanhatalo, S. Ahvenjärvi, and P. Huhtanen. 2002b. Effects of several protein supplements on flow of soluble non-ammonia nitrogen from the forestomach and milk production in dairy cows. *Anim. Feed Sci. Technol.* 102:15–33.
- Cotanch, K. W., R. J. Grant, M. E. Van Amburgh, A. Zontini, M. Fustini, A. Palmonari, and A. Formigoni. 2014. Applications of uNDF in ration modeling and formulation. Pages 114–131 in *Proc. Cornell Nutr. Conf., Dept. Anim. Sci., Cornell Univ., Ithaca, NY*.



- Diaz, M. C., M. E. Van Amburgh, J. M. Smith, J. M. Kelsey, and E. L. Hutten. 2001. Composition of growth of Holstein calves fed milk replacer from birth to 105-kilogram body weight. *J. Dairy Sci.* 84:830–842.
- Doane, P. H., A. N. Pell, and P. Schofield. 1998. Ensiling effects of the ethanol fractionation of forages using gas production. *J. Anim. Sci.* 76:888–895.
- Doepel, L., D. Pacheco, J. J. Kennelly, M. D. Hanigan, I. F. Lopez, and H. Lapiere. 2004. Milk protein synthesis as a function of amino acid supply. *J. Dairy Sci.* 87:1279–1297.
- Ellis, J. L., E. Kebreab, N. E. Odongo, B. W. McBride, E. K. Okine, and J. France. 2007. Prediction of methane production from dairy and beef cattle. *J. Dairy Sci.* 90:3456–3466.
- Firkins, J. L., Z. Yu, and M. Morrison. 2007. Ruminant nitrogen metabolism: Perspectives for integration of microbiology and nutrition for dairy. *J. Dairy Sci.* 90:E1–E16.
- 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. Technol.* 112:29–78.
- Fox, D. G., T. P. Tylutki, M. E. Van Amburgh, L. E. Chase, A. N. Pell, T. R. Overton, L. O. Tedeschi, C. N. Rasmussen, and V. M. Durbal. 2000. The Net Carbohydrate and Protein System for Evaluating Herd Nutrition and Nutrient Excretion. CNCPS Version 4.0: Model Documentation. Dept. Anim. Sci., Cornell Univ., Ithaca, NY.
- 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.
- France, J., and R. Siddons. 1986. Determination of digesta flow by continuous market infusion. *J. Theor. Biol.* 121:105–119.
- Gehrke, C. W., L. Wall Sr., J. Absheer, F. Kaiser, and R. Zumwalt. 1985. Sample preparation for chromatography of amino acids: Acid hydrolysis of proteins. *J. AOAC* 68:811–821.
- Groff, E. B., and Z. Wu. 2005. Milk production and nitrogen excretion of dairy cows fed different amounts of protein and varying proportions of alfalfa and corn silage. *J. Dairy Sci.* 88:3619–3632.
- Guiroy, P. J., D. G. Fox, L. O. Tedeschi, M. J. Baker, and M. D. Cravey. 2001. Predicting individual feed requirements of cattle fed in groups. *J. Anim. Sci.* 79:1983–1995.
- Hammond, K. J., D. J. Humphries, L. A. Crompton, P. Kirton, C. Green, and C. K. Reynolds. 2014. Methane emissions from lactating and dry dairy cows fed diets differing in forage source and NDF concentration. *J. Dairy Sci.* 97(E-Suppl. 1):322. (Abstr.)
- Hedqvist, H., and P. Udén. 2006. Measurement of soluble protein degradation in the rumen. *Anim. Feed Sci. Technol.* 126:1–21.
- Higgs, R. J., L. E. Chase, D. A. Ross, and M. E. Van Amburgh. 2015. Updating the CNCPS feed library and analyzing model sensitivity to feed inputs. *J. Dairy Sci.* 98:6340–6360. <http://dx.doi.org/10.3168/jds.2015-9379>.
- Higgs, R. J., L. E. Chase, and M. E. Van Amburgh. 2012. 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.
- Hristov, A. N., P. Huhtanen, L. M. Rode, S. N. Acharya, and T. A. McAllister. 2001. Comparison of the ruminal metabolism of nitrogen from <sup>15</sup>N-labeled alfalfa preserved as hay or as silage. *J. Dairy Sci.* 84:2738–2750.
- Hristov, A. N., and J. K. Ropp. 2003. Effect of dietary carbohydrate composition and availability on utilization of ruminal ammonia nitrogen for milk protein synthesis in dairy cows. *J. Dairy Sci.* 86:2416–2427.
- Huhtanen, C. N., J. Nousiainen, and M. Rinne. 2006. Recent developments in forage evaluation with special reference to practical applications. *Agric. Food Sci.* 15:31.
- 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.
- Kauffman, A. J., and N. R. St-Pierre. 2001. The relationship of milk urea nitrogen to urine nitrogen excretion in Holstein and Jersey cows. *J. Dairy Sci.* 84:2284–2294.
- Kirchgessner, M., W. Windisch, H. L. Muller, and K. Kreuzer. 1991. Release of methane and of carbon dioxide by dairy cattle. *Agribiol. Res.* 44:91–102.
- Krishnamoorthy, U., T. V. Muscato, C. J. Sniffen, and P. J. Van Soest. 1982. Nitrogen fractions in selected feedstuffs. *J. Dairy Sci.* 65:217–225.
- Landry, J., and S. Delhaye. 1992. Simplified procedure for the determination of tryptophan of foods and feedstuffs from barytic hydrolysis. *J. Agric. Food Chem.* 40:776–779.
- 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.
- Lapiere, H., G. E. Lobley, D. R. Quillet, 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 Nutr. Conf., Dept. Anim. Sci., Cornell Univ., Ithaca, 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.
- Licitra, G., T. M. Hernandez, and P. J. Van Soest. 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. *Anim. Feed Sci. Technol.* 57:347–358.
- Liu, Z., W. Powers, B. Oldick, J. Davidson, and D. Meyer. 2012. Gas emissions from dairy cows fed typical diets of midwest, south, and west regions of the United States. *J. Environ. Qual.* 41:1228–1237.
- Lock, A. L., K. J. Harvatine, J. K. Drackley, and D. E. Bauman. 2006. Concepts in fat and fatty acid digestion in ruminants. Pages 85–100 in *Proc. Intermountain Nutr. Conf., Utah State University, Logan, UT.*
- Lock, A. L., K. J. Harvatine, I. R. Ipharraguerre, M. E. Van Amburgh, J. K. Drackley, and D. E. Bauman. 2005. The dynamics of fat digestion in lactating dairy cows: What does the literature tell us? Pages 83–106 in *Proc. Cornell Nutr. Conf., Dept. Anim. Sci., Cornell Univ., Ithaca, NY.*
- Mahadevan, S., J. D. Erfle, and F. D. Sauer. 1980. Degradation of soluble and insoluble proteins by *Bacteroides amylophilus* protease and by rumen microorganisms. *J. Anim. Sci.* 50:723–728.
- Marini, J. C., and M. E. Van Amburgh. 2003. Nitrogen metabolism and recycling in Holstein heifers. *J. Anim. Sci.* 81:545–552.
- Metcalfe, J. A., R. J. Mansbridge, J. S. Blake, J. D. Oldham, and J. R. Newbold. 2008. The efficiency of conversion of metabolizable protein into milk true protein over a range of metabolizable protein intakes. *Animal* 2:1193–1202.
- Mills, J. A. N., E. Kebreab, C. M. Yates, L. A. Crompton, S. B. Cammell, M. S. Dhanoa, R. E. Agnew, and J. France. 2003. Alternative approaches to predicting methane emissions from dairy cows. *J. Anim. Sci.* 81:3141–3150.
- Mitchell, H. H. 1928. Check formulas for surface area of sheep. Pages 155–158 in *A Year's Progress in Solving Problems in Illinois. Illinois Agric. Stn. Annu. Rep., Urbana, IL.*
- Moate, P. J., W. Chalupa, T. C. Jenkins, and R. C. Boston. 2004. A model to describe ruminal metabolism and intestinal absorption of long chain fatty acids. *Anim. Feed Sci. Technol.* 112:79–105.
- Moate, P. J., S. R. O. Williams, C. Grainger, M. C. Hannah, E. N. Ponnampalam, and R. J. Eckard. 2011. Influence of cold-pressed canola, brewers grains and hominy meal as dietary supplements

- suitable for reducing enteric methane emissions from lactating dairy cows. *Anim. Feed Sci. Technol.* 166–167:254–264.
- Molina, D. O. 2002. Prediction in intake of lactating cows in the tropics and of energy value of organic acids. PhD Diss. Cornell Univ., Ithaca, NY.
- NRC. 2001. *Nutrient Requirements of Dairy Cattle*. 7th rev. ed. Natl. Acad. Press, Washington, DC.
- 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 Moly, CNCPS, and LES rumen models. *Anim. Feed Sci. Technol.* 112:107–130.
- Pacheco, D., R. A. Patton, C. Parys, and H. Lapierre. 2012. Ability of commercially available dairy ration programs to predict duodenal flows of protein and essential amino acids in dairy cows. *J. Dairy Sci.* 95:937–963.
- Pacheco, D., C. G. Schwab, R. Berthiaume, G. Raggio, and H. Lapierre. 2006. Comparison of net portal absorption with predicted flow of digestible amino acids: Scope for improving current models? *J. Dairy Sci.* 89:4747–4757.
- Peltekova, V. D., and G. A. Broderick. 1996. In vitro ruminal degradation and synthesis of protein on fractions extracted from alfalfa hay and silage. *J. Dairy Sci.* 79:612–619.
- Petit, M., and J. Agabriel. 1989. Beef cows. Pages 93–108 in *Ruminant Nutrition: Recommended Allowances and Feed Tables*. R. Jarrige, ed. John Libbey Eurotext, Paris, France.
- Raffrenato, E. 2011. Physical, chemical and kinetics factors associated with fiber digestibility in ruminants and models describing these relations. PhD Diss. Cornell Univ., Ithaca, NY.
- Recktenwald, E. B. 2007. Effect of feeding corn silage based diets predicted to be deficient in either ruminal nitrogen or metabolizable protein on nitrogen utilization and efficiency of use in lactating cows. MS Thesis. Cornell Univ., Ithaca, NY.
- Recktenwald, E. B., D. A. Ross, S. W. Fessenden, C. J. Wall, and M. E. Van Amburgh. 2014. Urea-N recycling in lactating dairy cows fed diets with 2 different levels of dietary crude protein and starch with or without monensin. *J. Dairy Sci.* 97:1611–1622.
- 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.
- Reynolds, C. K., D. J. Humphries, P. Kirton, M. Kindermann, S. Duval, and W. Steinberg. 2014. Effects of 3-nitrooxypropanol on methane emission, digestion, and energy and nitrogen balance of lactating dairy cows. *J. Dairy Sci.* 97:3777–3789.
- Rulquin, H., P. M. Pisulewski, R. Vérité, and J. Guinard. 1993. Milk production and composition as a function of post-ruminal lysine and methionine supply: A nutrient-response approach. *Livest. Prod. Sci.* 37:69–90.
- Russell, J. B. 1990. Low-affinity, high-capacity system of glucose transport in the ruminal bacterium *Streptococcus bovis*: Evidence for a mechanism of facilitated diffusion. *Appl. Environ. Microbiol.* 56:3304–3307.
- Russell, J. R., and T. Hino. 1985. Regulation of lactate production in *Streptococcus bovis*: A spiraling effect that contributes to rumen acidosis. *J. Dairy Sci.* 68:1712–1721.
- Russell, J. B., J. D. O'Connor, D. G. Fox, P. J. Van Soest, and C. J. Sniffen. 1992. A net carbohydrate and protein system for evaluating cattle diets: I. Ruminal fermentation. *J. Anim. Sci.* 70:3551–3561.
- SAS Institute Inc. 2010. JMP. SAS Inst. Inc., Cary, NC.
- Schaefer, D. M., C. L. Davis, and M. P. Bryant. 1980. Ammonia saturation constants for predominant species of rumen bacteria. *J. Dairy Sci.* 63:1248–1263.
- Seo, S., L. O. Tedeschi, C. Lanzas, C. G. Schwab, and D. G. Fox. 2006. Development and evaluation of empirical equations to predict feed passage rate in cattle. *Anim. Feed Sci. Technol.* 128:67–83.
- Sniffen, C. J., J. D. O'Connor, P. J. Van Soest, D. G. Fox, and J. B. Russell. 1992. A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. *J. Anim. Sci.* 70:3562–3577.
- 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. *Agric. Syst.* 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. Technol.* 143:174–202.
- Tyrrell, H. F., and J. T. Reid. 1965. Prediction of the energy value of cow's milk. *J. Dairy Sci.* 48:1215–1223.
- 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. Cornell Nutr. Conf.*, Dept. Anim. Sci., Cornell Univ., Ithaca, NY.
- Van Amburgh, M. E., D. G. Fox, D. M. Galton, D. E. Bauman, and L. E. Chase. 1998. Evaluation of National Research Council and Cornell Net Carbohydrate and Protein Systems for predicting requirements of Holstein heifers. *J. Dairy Sci.* 81:509–526.
- van Dorland, H. A., H. R. Wettstein, H. Leuenberger, and M. Kreuzer. 2007. Effect of supplementation of fresh and ensiled clovers to ryegrass on nitrogen loss and methane emission of dairy cows. *Livest. Sci.* 111:57–69.
- Wallace, R. J. 1979. Effect of ammonia concentration on the composition, hydrolytic activity and nitrogen metabolism of the microbial flora of the rumen. *J. Appl. Bacteriol.* 47:443–455.
- Wallace, R. J., R. Onodera, and M. A. Cotta. 1998. Metabolism of nitrogen-containing compounds. Pages 283–328 in *The Rumen Microbial Ecosystem*. P. N. Hobson and C. S. Stewart, ed. Blackie Acad. Prof., London, UK.
- Weiss, W. P., H. R. Conrad, and N. R. St. Pierre. 1992. A theoretically-based model for predicting total digestible nutrient values of forages and concentrates. *Anim. Feed Sci. Technol.* 39:95–110.
- 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(E-Suppl. 1):253. (Abstr.)
- Williams, A. P., and D. Hewitt. 1979. The amino acid requirements of the preruminant calf. *Br. J. Nutr.* 41:311–319.

## APPENDIX LIST OF PUBLICATIONS USED IN THE DEVELOPMENT OF DATA SETS

### *Amino Acid Data Set*

- Armentano, L. E., S. J. Bertics, and G. A. Ducharme. 1997. Response of lactating cows to methionine or methionine plus lysine added to high protein diets based on alfalfa and heated soybeans. *J. Dairy Sci.* 80:1194–1199.
- Brake, D. W., E. C. Titgemeyer, M. J. Brouk, C. A. Macgregor, J. F. Smith, and B. J. Bradford. 2013. Availability to lactating dairy cows of methionine added to soy lecithins and mixed with a mechanically extracted soybean meal. *J. Dairy Sci.* 96:3064–3074.
- Casper, D. P., and D. J. Schingoethe. 1988. Protected methionine supplementation to a barley-based diet for cows during early lactation. *J. Dairy Sci.* 71:164–172.

- Guinard, J., and H. Rulquin. 1994. Effects of graded amounts of duodenal infusions of lysine on the mammary uptake of major milk precursors in dairy cows. *J. Dairy Sci.* 77:3565–3576.
- Guinard, J., H. Rulquin, and R. Vérité. 1994. Effect of graded levels of duodenal infusions of casein on mammary uptake in lactating cows. 1. Major nutrients. *J. Dairy Sci.* 77:2221–2231.
- Illg, D. J., J. L. Sommerfeldt, and D. J. Schingoethe. 1987. Lactational and systemic responses to the supplementation of protected methionine in soybean meal diets. *J. Dairy Sci.* 70:620–629.
- Lee, C., A. N. Hristov, T. W. Cassidy, K. S. Heyler, H. Lapierre, G. A. Varga, M. J. de Veth, R. A. Patton, and C. Parys. 2012. Rumen-protected lysine, methionine, and histidine increase milk protein yield in dairy cows fed a metabolizable protein-deficient diet. *J. Dairy Sci.* 95:6042–6056.
- Papas, A. M., C. J. Sniffen, and T. V. Muscato. 1984. Effectiveness of rumen-protected methionine for delivering methionine post- ruminally in dairy cows. *J. Dairy Sci.* 67:545–552.
- Pisulewski, P. M., H. Rulquin, J. L. Peyraud, and R. Verite. 1996. Lactational and systemic responses of dairy cows to post-ruminal infusions of increasing amounts of methionine. *J. Dairy Sci.* 79:1781–1791.
- Rogers, J. A., U. Krishnamoorthy, and C. J. Sniffen. 1987. Plasma amino acids and milk protein production by cows fed rumen-protected methionine and lysine. *J. Dairy Sci.* 70:789–798.
- Rulquin, H., and L. Delaby. 1997. Effects of the energy balance of dairy cows on lactational responses to rumen-protected methionine. *J. Dairy Sci.* 80:2513–2522.
- Rulquin, H., C. Hurtaud, and L. Delaby. 1994. Effects of graded levels of rumen-protected lysine on milk production in dairy cows. *Ann. Zootech.* 43:246.
- Rulquin, H., P. M. Pisulewski, R. Vérité, and J. Guinard. 1993. Milk production and composition as a function of post-ruminal lysine and methionine supply: A nutrient-response approach. *Livest. Prod. Sci.* 37:69–90.
- Schingoethe, D. J., D. P. Casper, C. Yang, D. J. Illg, J. L. Sommerfeldt, and C. R. Mueller. 1988. Lactational response to soybean meal, heated soybean meal, and extruded soybeans with ruminally protected methionine. *J. Dairy Sci.* 71:173–180.
- Schwab, C. G., C. K. Bozak, N. L. Whitehouse, and M. M. A. Mesbah. 1992a. Amino acid limitation and flow to duodenum at 4 stages of lactation. 1. Sequence of lysine and methionine limitation. *J. Dairy Sci.* 75:3486–3502.
- Schwab, C. G., C. K. Bozak, N. L. Whitehouse, and V. M. Olson. 1992b. Amino acid limitation and flow to the duodenum at 4 stages of lactation. 2. Extent of lysine limitation. *J. Dairy Sci.* 75:3503–3518.
- Schwab, C. G., L. D. Satter, and A. B. Clay. 1976. Response of lactating dairy cows to abomasal infusion of amino acids. *J. Dairy Sci.* 59:1254–1270.
- Socha, M. T., D. E. Putnam, B. D. Garthwaite, N. L. Whitehouse, N. A. Kierstead, C. G. Schwab, G. A. Ducharme, and J. C. Robert. 2005. Improving intestinal amino acid supply of pre- and post-partum dairy cows with rumen-protected methionine and lysine. *J. Dairy Sci.* 88:1113–1126.
- Socha, M. T., C. G. Schwab, D. E. Putnam, N. L. Whitehouse, B. D. Garthwaite, and G. A. Ducharme. 2008. Extent of methionine limitation in peak-, early-, and mid-lactation dairy cows. *J. Dairy Sci.* 91:1996–2010.
- omasal digesta flow and milk production in lactating dairy cows. *Acta Agric. Scand. A-An.* 49:179–189.
- Brito, A. F., and G. A. Broderick. 2006. Effect of varying dietary ratios of alfalfa silage to corn silage on production and nitrogen utilization in lactating dairy cows. *J. Dairy Sci.* 89:3924–3938.
- Brito, A. F., G. A. Broderick, J. J. O. Colmenero, and S. M. Reynal. 2007a. Effects of feeding formate-treated alfalfa silage or red clover silage on omasal nutrient flow and microbial protein synthesis in lactating dairy cows. *J. Dairy Sci.* 90:1392–1404.
- Brito, A. F., G. A. Broderick, and S. M. Reynal. 2006. Effect of varying dietary ratios of alfalfa silage to corn silage on omasal flow and microbial protein synthesis in dairy cows. *J. Dairy Sci.* 89:3939–3953.
- Brito, A. F., G. A. Broderick, and S. M. Reynal. 2007b. Effects of different protein supplements on omasal nutrient flow and microbial protein synthesis in lactating dairy cows. *J. Dairy Sci.* 90:1828–1841.
- Brito, A. F., G. F. Tremblay, H. Lapierre, A. Bertrand, Y. Castonguay, G. Bélanger, R. Michaud, C. Benchaar, D. R. Ouellet, and R. Berthiaume. 2009. Alfalfa cut at sundown and harvested as baleage increases bacterial protein synthesis in late-lactation dairy cows. *J. Dairy Sci.* 92:1092–1107.
- Broderick, G. A., N. D. Luchini, S. M. Reynal, G. A. Varga, and V. A. Ishler. 2008. Effect on production of replacing dietary starch with sucrose in lactating dairy cows. *J. Dairy Sci.* 91:4801–4810.
- Broderick, G. A., and S. M. Reynal. 2009. Effect of source of rumen-degraded protein on production and ruminal metabolism in lactating dairy cows. *J. Dairy Sci.* 92:2822–2834.
- Choi, C. W., S. Ahvenjärvi, A. Vanhatalo, V. Toivonen, and P. Huhtanen. 2002. Quantitation of the flow of soluble non-ammonia nitrogen entering the omasal canal of dairy cows fed grass silage based diets. *Anim. Feed Sci. Technol.* 96:203–220.
- Colmenero, J. J., and G. A. Broderick. 2006. Effect of dietary crude protein concentration on ruminal nitrogen metabolism in lactating dairy cows. *J. Dairy Sci.* 89:1694–1703.
- Korhonen, M., A. Vanhatalo, and P. Huhtanen. 2002. Effect of protein source on amino acid supply, milk production, and metabolism of plasma nutrients in dairy cows fed grass silage. *J. Dairy Sci.* 85:3336–3351. [http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list\\_uids=12512607&dopt=Abstract](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=12512607&dopt=Abstract)
- Owens, D., M. McGee, and T. Boland. 2008a. Effect of grass regrowth interval on intake, rumen digestion and nutrient flow to the omasum in beef cattle. *Anim. Feed Sci. Technol.* 146:21–41.
- Owens, D., M. McGee, T. Boland, and P. O'Kiely. 2008b. Intake, rumen fermentation and nutrient flow to the omasum in beef cattle fed grass silage fortified with sucrose and/or supplemented with concentrate. *Anim. Feed Sci. Technol.* 144:23–43.
- Owens, D., M. McGee, T. Boland, and P. O'Kiely. 2009. Rumen fermentation, microbial protein synthesis, and nutrient flow to the omasum in cattle offered corn silage, grass silage, or whole-crop wheat. *J. Anim. Sci.* 87:658–668.
- Reynal, S. M., and G. A. Broderick. 2003. Effects of feeding dairy cows protein supplements of varying ruminal degradability. *J. Dairy Sci.* 86:835–843.
- 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., G. A. Broderick, S. Ahvenjärvi, and P. Huhtanen. 2003. Effect of feeding protein supplements of differing degradability on omasal flow of microbial and undegraded protein. *J. Dairy Sci.* 86:1292–1305.
- Vanhatalo, A., K. Kuoppala, S. Ahvenjärvi, and M. Rinne. 2009. Effects of feeding grass or red clover silage cut at 2 maturity stages in dairy cows. 1. Nitrogen metabolism and supply of amino acids. *J. Dairy Sci.* 92:5620–5633.

### Omasal Data Set

- Ahvenjärvi, S., E. Joki-Tokola, A. Vanhatalo, S. Jaakkola, and P. Huhtanen. 2006. Effects of replacing grass silage with barley silage in dairy cow diets. *J. Dairy Sci.* 89:1678–1687.
- Ahvenjärvi, S., A. Vanhatalo, and P. Huhtanen. 2002. Supplementing barley or rapeseed meal to dairy cows fed grass-red clover silage: I. Rumen degradability and microbial flow. *J. Anim. Sci.* 80:2176–2187.
- Ahvenjärvi, S., A. Vanhatalo, P. Huhtanen, and T. Varvikko. 1999. Effects of supplementation of a grass silage and barley diet with urea, rapeseed meal and heat-moisture-treated rapeseed cake on

### Lactation Data Set

- Arieli, A., G. Adin, and I. Bruckental. 2004. The effect of protein intake on performance of cows in hot environmental temperatures. *J. Dairy Sci.* 87:620–629.

- Barlow, J. S., J. K. Bernard, and N. A. Mullis. 2012. Production response to corn silage produced from normal, brown midrib, or waxy corn hybrids. *J. Dairy Sci.* 95:4550–4555.
- Bell, J. A., J. M. Griinari, and J. J. Kennelly. 2006. Effect of safflower oil, flaxseed oil, monensin, and vitamin E on concentration of conjugated linoleic acid in bovine milk fat. *J. Dairy Sci.* 89:733–748.
- Bernard, J. K., J. J. Castro, N. A. Mullis, A. T. Adesogan, J. W. West, and G. Morantes. 2010. Effect of feeding alfalfa hay or Tifton 85 bermudagrass haylage with or without a cellulase enzyme on performance of Holstein cows. *J. Dairy Sci.* 93:5280–5285.
- Bernard, J. K., J. W. West, and D. S. Trammell. 2002. Effect of replacing corn silage with annual ryegrass silage on nutrient digestibility, intake, and milk yield for lactating dairy cows. *J. Dairy Sci.* 85:2277–2282.
- Broderick, G. A. 2004. Effect of low level monensin supplementation on the production of dairy cows fed alfalfa silage. *J. Dairy Sci.* 87:359–368.
- Broderick, G. A., N. D. Luchini, S. M. Reynal, G. A. Varga, and V. A. Ishler. 2008. Effect on production of replacing dietary starch with sucrose in lactating dairy cows. *J. Dairy Sci.* 91:4801–4810.
- Broderick, G. A., D. R. Mertens, and R. Simons. 2002. Efficacy of carbohydrate sources for milk production by cows fed diets based on alfalfa silage. *J. Dairy Sci.* 85:1767–1776.
- Broderick, G. A., and W. J. Radloff. 2004. Effect of molasses supplementation on the production of lactating dairy cows fed diets based on alfalfa and corn silage. *J. Dairy Sci.* 87:2997–3009.
- Burke, F., J. J. Murphy, M. A. O'Donovan, F. P. O'Mara, S. Kavanagh, and F. J. Mulligan. 2007. Comparative evaluation of alternative forages to grass silage in the diet of early lactation dairy cows. *J. Dairy Sci.* 90:908–917.
- Carvalho, L. P. F., A. R. J. Cabrita, R. J. Dewhurst, T. E. J. Vicente, Z. M. C. Lopes, and A. J. M. Fonseca. 2006. Evaluation of palm kernel meal and corn distillers grains in corn silage-based diets for lactating dairy cows. *J. Dairy Sci.* 89:2705–2715.
- Chen, Z. H., G. A. Broderick, N. D. Luchini, B. K. Sloan, and E. Devillard. 2011. Effect of feeding different sources of rumen-protected methionine on milk production and N-utilization in lactating dairy cows. *J. Dairy Sci.* 94:1978–1988.
- Chow, L. O., V. S. Baron, R. Corbett, and M. Oba. 2008. Effects of planting date on fiber digestibility of whole-crop barley and productivity of lactating dairy cows. *J. Dairy Sci.* 91:1534–1543.
- Cooke, K. M., J. K. Bernard, and J. W. West. 2008. Performance of dairy cows fed annual ryegrass silage and corn silage with steam-flaked or ground corn. *J. Dairy Sci.* 91:2417–2422.
- Cooke, K. M., J. K. Bernard, C. D. Wildman, J. W. West, and A. H. Parks. 2007. Performance and ruminal fermentation of dairy cows fed whole cottonseed with elevated concentrations of free fatty acids in the oil. *J. Dairy Sci.* 90:2329–2334.
- Delahoy, J. E., L. D. Muller, F. Bargo, T. W. Cassidy, and L. A. Holden. 2003. Supplemental carbohydrate sources for lactating dairy cows on pasture. *J. Dairy Sci.* 86:906–915.
- Donkin, S. S., S. L. Koser, H. M. White, P. H. Doane, and M. J. Cecava. 2009. Feeding value of glycerol as a replacement for corn grain in rations fed to lactating dairy cows. *J. Dairy Sci.* 92:5111–5119.
- Ebling, T. L., and L. Kung Jr. 2004. A comparison of processed conventional corn silage to unprocessed and processed brown midrib corn silage on intake, digestion, and milk production by dairy cows. *J. Dairy Sci.* 87:2519–2526.
- Ferraretto, L. F., R. D. Shaver, M. Espineira, H. Gencoglu, and S. J. Bertics. 2011. Influence of a reduced-starch diet with or without exogenous amylase on lactation performance by dairy cows. *J. Dairy Sci.* 94:1490–1499.
- Gencoglu, H., R. D. Shaver, W. Steinberg, J. Ensink, L. F. Ferraretto, S. J. Bertics, J. C. Lopes, and M. S. Akins. 2010. Effect of feeding a reduced-starch diet with or without amylase addition on lactation performance in dairy cows. *J. Dairy Sci.* 93:723–732.
- Grainger, C., R. Williams, T. Clarke, A. D. G. Wright, and R. J. Eckard. 2010. Supplementation with whole cottonseed causes long-term reduction of methane emissions from lactating dairy cows offered a forage and cereal grain diet. *J. Dairy Sci.* 93:2612–2619.
- Ipharraguerre, I. R., and J. H. Clark. 2005. Varying protein and starch in the diet of dairy cows. II. Effects on performance and nitrogen utilization for milk production. *J. Dairy Sci.* 88:2556–2570.
- Kononoff, P. J., S. K. Ivan, W. Matzke, R. J. Grant, R. A. Stock, and T. J. Klopfenstein. 2006. Milk production of dairy cows fed wet corn gluten feed during the dry period and lactation. *J. Dairy Sci.* 89:2608–2617.
- Kung, L., Jr., P. Williams, R. J. Schmidt, and W. Hu. 2008. A blend of essential plant oils used as an additive to alter silage fermentation or used as a feed additive for lactating dairy cows. *J. Dairy Sci.* 91:4793–4800.
- Law, R. A., F. J. Young, D. C. Patterson, D. J. Kilpatrick, A. R. G. Wylie, and C. S. Mayne. 2009. Effect of dietary protein content on animal production and blood metabolites of dairy cows during lactation. *J. Dairy Sci.* 92:1001–1012.
- Lee, C., A. N. Hristov, K. S. Heyler, T. W. Cassidy, M. Long, B. A. Corl, and S. K. Karnati. 2011. Effects of dietary protein concentration and coconut oil supplementation on nitrogen utilization and production in dairy cows. *J. Dairy Sci.* 94:5544–5557.
- Lerch, S., A. Ferlay, D. Pomies, B. Martin, J. A. Pires, and Y. Chilliard. 2012. Rapeseed or linseed supplements in grass-based diets: Effects on dairy performance of Holstein cows over 2 consecutive lactations. *J. Dairy Sci.* 95:1956–1970.
- McCormick, M. E., J. D. Ward, D. D. Redfearn, D. D. French, D. C. Blouin, A. M. Chapa, and J. M. Fernandez. 2001. Supplemental dietary protein for grazing dairy cows: Effect on pasture intake and lactation performance. *J. Dairy Sci.* 84:896–907.
- Mena, H., J. E. P. Santos, J. T. Huber, M. Tarazon, and M. C. Calhoun. 2004. The effects of varying gossypol intake from whole cottonseed and cottonseed meal on lactation and blood parameters in lactating dairy cows. *J. Dairy Sci.* 87:2506–2518.
- Misciattelli, L., V. F. Kristensen, M. Vestergaard, M. R. Weisbjerg, K. Sejrsen, and T. Hvelplund. 2003. Milk production, nutrient utilization, and endocrine responses to increased postprandial lysine and methionine supply in dairy cows. *J. Dairy Sci.* 86:275–286.
- Mjoun, K., K. F. Kalscheur, A. R. Hippen, and D. J. Schingoethe. 2010a. Performance and amino acid utilization of early lactation dairy cows fed regular or reduced-fat dried distillers grains with solubles. *J. Dairy Sci.* 93:3176–3191.
- Mjoun, K., K. F. Kalscheur, A. R. Hippen, D. J. Schingoethe, and D. E. Little. 2010b. Lactation performance and amino acid utilization of cows fed increasing amounts of reduced-fat dried distillers grains with solubles. *J. Dairy Sci.* 93:288–303.
- Moallem, U., G. Altmark, H. Lehrner, and A. Arieli. 2010. Performance of high-yielding dairy cows supplemented with fat or concentrate under hot and humid climates. *J. Dairy Sci.* 93:3192–3202.
- Moreira, V. R., L. D. Satter, and B. Harding. 2004. Comparison of conventional linted cottonseed and mechanically delinted cottonseed in diets for dairy cows. *J. Dairy Sci.* 87:131–138.
- O'Neill, B. F., M. H. Deighton, B. M. O'Loughlin, F. J. Mulligan, T. M. Boland, M. O'Donovan, and E. Lewis. 2011. Effects of a perennial ryegrass diet or total mixed ration diet offered to spring-calving Holstein-Friesian dairy cows on methane emissions, dry matter intake, and milk production. *J. Dairy Sci.* 94:1941–1951.
- Odongo, N. E., M. M. Or-Rashid, R. Bagg, G. Vessie, P. Dick, E. Kebreab, J. France, and B. W. McBride. 2007. Long-term effects of feeding monensin on milk fatty acid composition in lactating dairy cows. *J. Dairy Sci.* 90:5126–5133.
- Ordway, R. S., S. E. Boucher, N. L. Whitehouse, C. G. Schwab, and B. K. Sloan. 2009. Effects of providing 2 forms of supplemental methionine to periparturient Holstein dairy cows on feed intake and lactational performance. *J. Dairy Sci.* 92:5154–5166.
- Ouellet, D. R., H. Lapierre, and J. Chiquette. 2003. Effects of corn silage processing and amino acid supplementation on the performance of lactating dairy cows. *J. Dairy Sci.* 86:3675–3684.
- Petit, H. V., M. Ivan, and P. S. Mir. 2005. Effects of flaxseed on protein requirements and nitrogen excretion of dairy cows fed diets with 2 protein concentrations. *J. Dairy Sci.* 88:1755–1764.
- Ranathunga, S. D., K. F. Kalscheur, A. R. Hippen, and D. J. Schingoethe. 2010. Replacement of starch from corn with nonforage

- fiber from distillers grains and soyhulls in diets of lactating dairy cows. *J. Dairy Sci.* 93:1086–1097.
- Randby, A. T., M. R. Weisbjerg, P. Norgaard, and B. Heringstad. 2012. Early lactation feed intake and milk yield responses of dairy cows offered grass silages harvested at early maturity stages. *J. Dairy Sci.* 95:304–317.
- Ruiz, R., G. L. Albrecht, L. O. Tedeschi, G. Jarvis, J. B. Russell, and D. G. Fox. 2001. Effect of monensin on the performance and nitrogen utilization of lactating dairy cows consuming fresh forage. *J. Dairy Sci.* 84:1717–1727.
- Ruiz, R., L. O. Tedeschi, J. C. Marini, D. G. Fox, A. N. Pell, G. Jarvis, and J. B. Russell. 2002. The effect of a ruminal nitrogen (N) deficiency in dairy cows: Evaluation of the Cornell Net Carbohydrate and Protein System ruminal N deficiency adjustment. *J. Dairy Sci.* 85:2986–2999.
- Samuelson, D. J., S. K. Denise, R. Roffler, R. L. Ax, D. V. Armstrong, and D. F. Romagnolo. 2001. Response of Holstein and Brown Swiss cows fed alfalfa hay-based diets to supplemental methionine at 2 stages of lactation. *J. Dairy Sci.* 84:917–928.
- Schroeder, J. W. 2003. Optimizing the level of wet corn gluten feed in the diet of lactating dairy cows. *J. Dairy Sci.* 86:844–851.
- Shingfield, K. J., C. K. Reynolds, G. Hervás, J. M. Griinari, A. S. Grandison, and D. E. Beever. 2006. Examination of the persistence of milk fatty acid composition responses to fish oil and sunflower oil in the diet of dairy cows. *J. Dairy Sci.* 89:714–732.
- Socha, M. T., D. E. Putnam, B. D. Garthwaite, N. L. Whitehouse, N. A. Kierstead, C. G. Schwab, G. A. Ducharme, and J. C. Robert. 2005. Improving intestinal amino acid supply of pre- and postpartum dairy cows with rumen-protected methionine and lysine. *J. Dairy Sci.* 88:1113–1126.
- Steinshamm, H., S. Purup, E. Thuen, and J. Hansen-Møller. 2008. Effects of clover-grass silages and concentrate supplementation on the content of phytoestrogens in dairy cow milk. *J. Dairy Sci.* 91:2715–2725.
- Sullivan, H. M., J. K. Bernard, H. E. Amos, and T. C. Jenkins. 2004. Performance of lactating dairy cows fed whole cottonseed with elevated concentrations of free fatty acids in the oil. *J. Dairy Sci.* 87:665–671.
- Thomas, E. D., P. Mandebvu, C. S. Ballard, C. J. Sniffen, M. P. Carter, and J. Beck. 2001. Comparison of corn silage hybrids for yield, nutrient composition, in vitro digestibility, and milk yield by dairy cows. *J. Dairy Sci.* 84:2217–2226.
- Vander Pol, M., A. N. Hristov, S. Zaman, and N. Delano. 2008. Peas can replace soybean meal and corn grain in dairy cow diets. *J. Dairy Sci.* 91:698–703.
- Wang, C., H. Y. Liu, Y. M. Wang, Z. Q. Yang, J. X. Liu, Y. M. Wu, T. Yan, and H. W. Ye. 2010. Effects of dietary supplementation of methionine and lysine on milk production and nitrogen utilization in dairy cows. *J. Dairy Sci.* 93:3661–3670.
- Wang, C., J. X. Liu, Z. P. Yuan, Y. M. Wu, S. W. Zhai, and H. W. Ye. 2007. Effect of level of metabolizable protein on milk production and nitrogen utilization in lactating dairy cows. *J. Dairy Sci.* 90:2960–2965.
- Weiss, W. P., and J. M. Pinos-Rodríguez. 2009. Production responses of dairy cows when fed supplemental fat in low- and high-forage diets. *J. Dairy Sci.* 92:6144–6155.
- Wickersham, E. E., J. E. Shirley, E. C. Titgemeyer, M. J. Brouk, J. M. DeFrain, A. F. Park, D. E. Johnson, and R. T. Ethington. 2004. Response of lactating dairy cows to diets containing wet corn gluten feed or a raw soybean hull-corn steep liquor pellet. *J. Dairy Sci.* 87:3899–3911.