DEVELOPMENT OF A DYNAMIC RUMEN AND GASTRO-INTESTINAL MODEL IN THE CORNELL NET CARBOHYDRATE AND PROTEIN SYSTEM TO PREDICT THE NUTRIENT SUPPLY AND REQUIREMENTS OF DAIRY CATTLE

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DEVELOPMENT OF A DYNAMIC RUMEN AND GASTRO-INTESTINAL MODEL IN THE CORNELL NET CARBOHYDRATE AND PROTEIN SYSTEM TO PREDICT THE NUTRIENT SUPPLY AND REQUIREMENTS OF DAIRY CATTLE

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The high value of milk protein, increasing feed costs, and growing concern for the environment has made nitrogen utilization a central component in ration balancing on dairy farms. The Cornell Net Carbohydrate and Protein System (CNCPS) is a nutritional model that enables the formulation of diets that closely match predicted animal requirements. The CNCPS includes a library of approximately 800 different ingredients which provide the platform for describing the chemical composition of the diet. The objectives of this research were 1) to review and update the chemical composition of feeds in the feed library, 2) develop new capability within the model to predict nitrogen and amino acid supply and requirements and, 3) investigate the potential to improve nitrogen utilization in high producing dairy cows through using the new model to formulate diets precisely to animal requirements. The feed library was updated using a procedure that combined linear regression, matrix regression and genetic algorithm optimization to predict uncertain values. Each feed was evaluated and updated where required to be consistent with data from commercial laboratories. Amino acid profiles were also updated using contemporary datasets. A new, dynamic version of the rumen and gastro-intestinal (GIT) sub-model was constructed in the system dynamics modeling software Vensim®. The new model included, among other things, estimations of protozoal growth, endogenous N transactions along the entire GIT and a new system to estimate N digestion in the small intestine. Relative to

measured data, the model was able to predict the flows of microbial, un-degraded feed, and total non-ammonia N with a high degree of accuracy and precision (R^2 = 0.97, 0.90 and 0.98, respectively). Lactating dairy cows fed diets formulated to be adequate in rumen N and EAA supply using the model were able to produce >40 kg milk on diets <15 % CP, utilize N with 38% efficiency and, partition 1.7 times more N to milk than urine. The study demonstrates that high levels of animal performance can be achieved, N utilization can be improved and the environmental impact of dairy production reduced through more precise predictions of N and AA requirements and supply.

BIOGRAPHICAL SKETCH

Ryan John Higgs grew up on a farm in the small Waikato town of Ohaupo, New Zealand. He attended Ohaupo Primary School from 1989-1996, after which he attended Hamilton Boys High School (1997-2002). A strong interest in agriculture and the dairy industry led him to pursue Bachelor of Applied Science (honors) with a major in agriculture at Massey University from 2003-2007. During his time at Massey, Ryan became interested in the use of models to aid decision making on dairy farms. In particular, he became interested in the Cornell Net Carbohydrate and Protein System (CNCPS) model due to its reputation and wide use around the world. He applied for a Fulbright Ministry of Research Science and Technology Scholarship to complete graduate studies in the U.S. In February of 2007 he was offered the Fulbright Scholarship and was accepted into Masters Program in the Department of Animal Science at Cornell University. He moved to Ithaca, NY in August 2007 and began his Masters with Dr. Larry Chase. The title of his Masters thesis was: Nitrogen use efficiency and sustainable nitrogen management in high producing dairy farms. On completion of his Masters, he was accepted into a PhD program at Cornell with Dr. Mike Van Amburgh to continue working on the development of the CNCPS.

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CHAPTER 1: INTRODUCTION

1.1 Overview

Ruminants have a unique system of protein digestion and metabolism that has evolved to enable subsistence in relatively poor nutritional conditions. Dietary N sources support the requirements of both the animal, and rumen microbes. However, the extensive recycling between body, gut, and lumen pools, and interactions between the animal and microbes, make determining the net supply of protein to the small intestine complex. The high value of milk protein, increasing feed costs, and growing concerns for the environment has made N utilization a central component in ration balancing on dairy farms.

The Cornell Net Carbohydrate and Protein System (CNCPS) is a mathematical model designed to evaluate the nutrient requirements of cattle over a wide range of environmental, dietary, management and production situations (Fox et al., 2004, Tylutki et al., 2008, Van Amburgh et al., 2013). The CNCPS was first described in a series of publications outlining carbohydrate and protein digestion (Sniffen et al., 1992), microbial growth (Russell et al., 1992), amino acid supply (O'Connor et al., 1993) and animal requirements (Fox et al., 1992). The model uses estimations of carbohydrate and protein degradation and passage rates to predict the extent of ruminal fermentation, microbial growth, and the absorption of metabolizable energy and protein throughout the digestive tract (Fox et al., 2004). Predictions also encompass differing physiological states and body reserves meaning a diverse range of situations can be evaluated (Fox et al., 2004, Tylutki et al., 2008). The CNCPS has been developed for field application with care taken to ensure model inputs are routinely available on most farms (Fox et al., 2004). Ration formulations systems such as the CNCPS and the NRC (2001) are important tools that allow

nutritionists to formulate diets that are close to animal requirements and reduce nutrient loss to the environment. Refining the ability of the CNCPS to predict N and AA supply and requirements in lactating dairy cows could enable further improvements in the efficiency of N utilization.

1.2 Protein digestion and availability in the CNCPS

1.2.1 Fractionation of dietary protein

To estimate protein digestion and flows along the digestive tract, the CNCPS uses chemically determined N fractions to calculate N pools within the model (Table 1.1). The pool structure was established based on the behavior of the various protein fractions in feeds during digestion (Sniffen et al., 1992). Proteins can vary in size, shape, function, solubility and AA composition which influence how they behave in the digestive tract and their nutritional value to the animal (NRC, 2001). Examples include globular proteins like albumins, globulins, glutelins, prolamines or histones which are common to all feedstuffs, and fibrous proteins such as collagens, elastins and keratins which are of animal or marine origin (NRC, 2001). Each protein fraction in the CNCPS has a specific digestion rate which reflects the inherent properties of the fraction and is assigned to flow with either the liquid or solid phase out of the rumen. These kinetic parameters are what determine the amount of protein that is degraded (RDP) or escapes (RUP) the rumen and, thus, the RDP and RUP supply from each feed to the animal, and the subsequent rumen N availability and MP supply.

Table 1.1 Protein fractions used in the CNCPS (% CP)

Fraction	Description	Calculation[2]
PA*j*[1]	Non-protein N (NPN)	NPN × SP
PB1*j*	Rapidly degraded protein	SP*j* × CP*j* / 100 – PA*j*
PB2*j*	Intermediately degraded protein	CP*j* - (PA*j* – PB2*j* – PB3*j* - PC*j*)
PB3*j*	Slowly degraded protein	(NDICP*j* - ADICP*j*) × CP*j* / 100
PC*j*	Unavailable protein	ADICP*j* × CP*j* / 100

[1] subscript j represents the jth feedstuff

[2] NPN = non protein N (% SP); SP = soluble protein (% CP); ADICP = acid detergent insoluble CP (% CP); NDICP = neutral detergent insoluble CP (% CP).

1.2.2 Microbial protein synthesis

Microbial protein synthesis in the rumen is the other major source of protein considered by the CNCPS and is central to understanding AA supply from the diet (Schwab et al., 2005). The CNCPS uses a mechanistic approach to estimate bacterial growth in the rumen (Russell et al., 1992). In this system bacteria are characterized as fermenting either fiber carbohydrates (CHO) or non-fiber CHO and microbial yield is determined by the rate and extent of CHO digestion in the rumen. Protozoal predation is accommodated in the CNCPS by reducing the theoretical maximum growth yield of bacteria from 0.5 to 0.4 g cells per g CHO fermented (Russell et al., 1992). However, other dynamics of protozoal metabolism, including their contribution to rumen N supply, organic matter digestion or contribution to microbial protein supply (Firkins et al., 2007) are not considered.

1.2.3 Digestion of protein in the small intestine

Protein escaping the rumen as either un-degraded feed, or microbial protein, is digested and absorbed in the small intestine based on fixed digestibility coefficients (Sniffen et al., 1992). Microbial protein is partitioned into either cell wall protein, which is considered completely

indigestible, or non-cell wall protein, which is considered completely digestible (Russell et al., 1992). The intestinal digestion coefficients of RUP are 100, 100, 100, 80 and 0% for the A, B1, B2, B3 and C fractions, respectively which are based on data summarized by Van Soest (1982). Any protein that is not digested in the small intestine is considered unavailable by the model and will appear in the feces.

1.2.4 Amino acid supply

The original system for calculating AA supply in the CNCPS was described by O'Connor et al. (1993) and has been used in all subsequent versions of the model (Fox et al., 2004, Tylutki et al., 2008, Van Amburgh et al., 2013). In this system, an AA profile is applied to the RUP fraction of each feed which, in turn, determines the daily appearance of AA in the small intestine. The amino acid profiles of feeds were determined on the insoluble fraction as this was thought to best represent the material escaping the rumen (Macgregor et al., 1978). The same system is used to estimate AA from bacteria with the AA profiles used based on a review by (Clark et al., 1992).

1.3 Evolution of the CNCPS

Since the original publications, updates have continually been made to improve the models capability (Fox et al., 2004, Tylutki et al., 2008, Van Amburgh et al., 2010, Van Amburgh et al., 2007). Important updates that have affected protein and AA supply since version 5 of the model (Fox et al., 2004) include an expansion of the feed carbohydrate fractionation scheme which refined microbial protein predictions (Lanzas et al., 2007a), a reduction the digestion rates of A and B1 protein fractions (Table 1.1) to be more consistent with literature reports (Van Amburgh

et al., 2007) and a re-organization of the passage rate assignments of the various protein fractions to better reflect the phase in which each fraction would flow out of the rumen (Van Amburgh et al., 2007). These changes resulted in a model that was more sensitive in predicting the level of milk production that could be supported by the most limiting nutrient in a diet (ME or MP) and provided a platform that could be used to reduce dietary protein levels without impacting animal performance (Van Amburgh et al., 2010). Given the improvements in the sensitivity of the CNCPS in predicting total MP supply, efforts have since been shifted to refining predictions of individual amino acids.

1.4 Strategies for improving amino acid predictions in the CNCPS

Amino acids flowing to the duodenum in ruminants encompass three major fractions: 1) Un-degraded feed, 2) microbial and 3) endogenous AA (Lapierre et al., 2006). Combined, these fractions represent the gross AA supply, potentially available to the animal. However, the endogenous fraction, and its contribution to the microbial pool make establishing the net AA supply complex (Ouellet et al., 2002). Free endogenous N and the contribution of endogenous N to the microbial pool represent a recycling of previously absorbed AA that cannot be considered a new supply (Lapierre et al., 2006). Further, the AA profiles of components not currently considered by the CNCPS vary (Table 1.2) and can contribute meaningful amounts to total AA flow. For example, protozoal protein in high producing cows can represent 5-10% of total microbial protein (Sylvester et al., 2005) and AA of endogenous origin can contribute 15-20% of the total AA flow (Ouellet et al., 2010, Ouellet et al., 2002). Given the variation in AA profiles of sources not considered by the model (Table 1.2), future updates to the CNCPS should include these sources. Van Amburgh et al. (2010) also suggested a refinement in the characterization of

the protein fractions described in Table 1.1 to account for AA currently associated with the NPN fraction which have the potential to escape rumen fermentation and supply AA to the animal. A more detailed discussion of each of these areas is provided below.

Table 1.2. Amino acid profiles of endogenous and microbial protein components in ruminants (g/1000g AA)

AA	Rumen Epithelia[1]	Abomasal Juice[2]	Pancreatic Juice[3]	Cow Bile[1]	Bovine Bile[4]	Bacteria[5]	Protozoa[5]
Ala	53	60	60	10	21	71	54
Arg	75	52	41	3	11	50	48
Asp	100	98	127	12	10	124	133
Cys	17	34	31	5	5	15	16
Glu	154	133	105	19	12	137	145
Gly	59	68	63	892	870	55	47
His	26	38	34	5	5	24	23
Ile	49	50	53	5	3	67	71
Leu	99	51	89	9	8	83	81
Lys	80	78	62	5	28	80	104
Met	22	16	16	2	1	25	24
Phe	47	50	43	4	6	55	55
Pro	51	67	45	7	0	42	41
Ser	62	70	89	8	7	49	47
Thr	47	70	66	6	6	55	52
Val	59	65	76	8	7	68	59

[1] (Larsen et al., 2000)

[2] (Ørskov et al., 1986)

[3] (Hamza, 1976)

[4] (Gabel and Poppe, 1986)

[5] (Jensen et al., 2006)

1.4.5 Protein fractions

Non-protein N is defined as the N passing into the filtrate after precipitation with protein specific reagent (tungstic or tricholoracetic acid; (Licitra et al., 1996) and represents the A pool in the model (Table 1.1). Non-protein N is typically assumed to be completely degraded in the rumen (Lanzas et al., 2007b). However, small peptides and free AA not precipitated by the

chemical method are still metabolically relevant to the animal if they escape rumen degradation and flow through to the small intestine (Givens and Rulquin, 2004). Choi et al. (2002) suggested 10% of the AA flowing through to the small intestine originated from dietary NPN sources which under the current system are unaccounted for. Likewise, Velle et al. (1997) infused free AA into the rumen at various rates and showed up to 20% could escape degradation and flow through to the small intestine. Van Amburgh et al. (2010) suggested it may be more appropriate to redefine the protein A pool from NPN as described by Licitra et al. (1996) to ammonia. This would shift small peptides and free AA previously associated with the A pool into the B1 pool (Table 1.1) where they could contribute to MP supply. Ammonia also has the advantage of being easily measured and available from most commercial laboratories.

1.4.6 Endogenous flows

The contribution of endogenous AA to total AA flows were recognized by O'Connor et al. (1993), but at the time, it was deemed there was not enough quantitative information available to include them in the CNCPS. There is agreement in the literature that endogenous flows must be accounted for in order to predict true net AA supply, however, data used to estimate these flows is varied (Lapierre et al., 2006). Endogenous secretions occur at various places along the gastro-intestinal tract. Important sources include saliva, gastric juices, bile, pancreatic secretions, sloughed epithelial cells and mucin (Tamminga et al., 1995). Digestive secretions containing enzymes such as proteases, nucleases, lipases and amylases in monogastrics are influenced by the composition of the diet (Harmon, 1993). Ruminants, in contrast, have a much more constant, and consistent digesta flow than monogastrics due to the extensive pre-gastric fermentation and selective retention mechanism of the reticular-rumen (Tamminga et al., 1995). Consequently,

secretions are less variable, and are probably more closely related to digesta flow than diet composition *per se* (Tamminga et al., 1995). The implication of this when trying to predict endogenous contributions to the small intestine is that simple relationships based on intake or flow may be adequate rather than more complex relationships that account for dietary differences. An important difficulty encountered when measuring endogenous secretions is distinguishing the origin of the various proteins (Tamminga et al., 1995). Different approaches have been used, with those having the most relevance to dairy cows including protein-free diets (Larsen et al., 2000, Ørskov et al., 1986), regression techniques (Marini et al., 2008), or stable isotope methods (Ouellet et al., 2010, Ouellet et al., 2002). The NRC (2001) adopted a value of 1.9 g endogenous N/ kg DMI based on work with N free diets (Ørskov et al., 1986) and diets with very low protein supply and degradability (Hannah et al., 1991, Hart and Leibholz, 1990, Lintzenich et al., 1995). However, these conditions are somewhat artificial compared to what might be expected in typical production systems. Ouellet et al. (2002) conducted an experiment using ^{15}N-leucine infused over an 8-day period and measured the enrichment of protein flows at the duodenum at differing fiber levels (high and low). The effect of fiber was not significant, however, endogenous flows were estimated to be 4.4 g N/kg DMI, over twice that used by the NRC (2001). Approximately half (2.3 g N/kg DMI) was 'free', and the balance incorporated in bacterial protein (Ouellet et al., 2002). Marini et al. (2008) generated similar results using a meta-analytical approach and estimated free endogenous flows at the duodenum to be approximately 3.29 g N/kg OMI. Endogenous protein in bacteria were calculated to contribute approximately 2.25 g N/kg OMI based on the assumptions that bacteria don't discriminate between feed and endogenous N, and that urea N and other endogenous sources contribute equally to bacterial N (Ouellet et al., 2002). The close agreement of Ouellet et al. (2002) and

Marini et al. (2008) despite the different approaches used, and the more typical feeding environments used in generating these data suggests they may be the most relevant estimations to use when predicting endogenous flows and that adequate data are now available to incorporate estimations of endogenous AA transactions in the CNCPS.

1.4.7 Protozoa

Protozoa are currently accommodated in the CNCPS by reducing the theoretical maximum growth yield from 0.5 to 0.4 g cells per g CHO fermented (Russell et al., 1992) but do not contribute to digestion or microbial protein production. Protozoa have important effects not only on bacterial yield, but also nutrient digestion and cycling within the rumen (Firkins et al., 2007, Hristov and Jouany, 2005) and can make 40% to 50% of the total microbial biomass (Hristov and Jouany, 2005). Further, protozoa can contribute 5-10% of the microbial flow in high producing dairy cows, and given their AA profile differs to that of bacteria, particularly in Lys (Table 1.2), a more mechanistic approach is warranted to fully capture these effects in the CNCPS.

1.4.8 Protein digestion in small intestine

The CNCPS currently uses static library values for digestion of nitrogen fractions in the small intestine (Sniffen et al., 1992). However, numerous *in situ* and *in vitro* procedures have been developed to directly measure the digestion of feeds in the small intestine (Boucher et al., 2009, Calsamiglia and Stern, 1995, Gargallo et al., 2006). Ross et al., (2013) modified and extended previous methods to an *in vitro* technique designed specifically to provide an input into the CNCPS and with a focus on practical application in commercial laboratories. Data presented by

Ross et al., (2013) show important differences in the digestibility of commonly fed feeds like blood meal and soybean meal which cannot be adequately captured using static digestibility values. As models improve in their ability to predict nitrogen flows to the small intestine, more scrutiny will be placed on quantifying digestion in the small intestine to improve predictions of metabolizable protein and AA supply. Therefore, updating the CNCPS to accommodate data generated from the procedure of Ross (2013) could help refine predictions of AA availability to the animal.

1.4.9 Amino acid requirements

Requirements for each individual EAA in the CNCPS are predicted for processes that are quantified by the model (maintenance, lactation, pregnancy, growth) and subsequently divided by the efficiency of transfer to that process to give the total AA requirement (Fox et al., 2004, O'Connor et al., 1993). Previous versions of the CNCPS have treated different physiological functions separately with the original values coming from a range of sources outlined in O'Connor et al. (1993). Lapierre et al. (2007) suggested using a single factor to calculate total AA requirement for maintenance and milk production makes more biological sense as it is difficult to localize the large number of processes that are encompassed by the efficiency of transfer. Recommendations for v6.1 of the CNCPS were presented by Lapierre et al. (2007) and have been implemented in the most recent update of the model v6.5 (Van Amburgh et al., 2013). Recommendations for dietary Lys and Met supply are well established (NRC, 2001, Rulquin et al., 1993, Schwab, 1996) and numerous studies have demonstrated improvements in animal productivity when the balance of Lys and Met is improved (Armentano et al., 1997, Chen et al.,

2011, Noftsger and St-Pierre, 2003). Further investigation into the optimum AA supply when using the CNCPS will be warranted as updates are made to the model.

1.5 Summary

Mathematical models provide an advanced method of strategically improving N utilization and animal performance using inputs that are easy to collect, and economically measured. Models such as the CNCPS are continually being updated and improved as new data become available and the understanding of biological mechanisms improves. Recent updates to the model have focused on improving predictions of MP supply to enable the formulation of diets that closely match animal requirements. Efforts are now being focused on improving the models ability to predict AA supply and requirements. Areas of opportunity include refining the characterization of feed proteins and the addition of N components into the CNCPS that have been previously omitted such as protozoa and endogenous secretions. New techniques have also been developed to estimate protein digestion in the small intestine and new recommendations are available to predict AA requirements. Incorporation of these areas into the CNCPS could provide improved capability to formulate rations that maximize animal performance and minimize environmental impact.

1.6 Objectives

The objectives of this dissertation are:

1) Review and update the chemical composition of feeds in the CNCPS feed library and investigate opportunities to re-classify the protein fractions to refine predictions of AA supply

2) Develop new capability within the CNCPS to predict nitrogen and amino acid supply and requirements

3) Investigate the potential to improve nitrogen utilization in high producing dairy cows by formulating diets that more closely match animal requirements

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CHAPTER 2: UPDATING THE CNCPS FEED LIBRARY AND ANALYZING MODEL SENSITIVITY TO FEED INPUTS

2.1 Abstract

The Cornell Net Carbohydrate and Protein System (CNCPS) is a nutritional model that evaluates the environmental and nutritional resources available in an animal productions system and enables the formulation of diets that closely match the predicted animal requirements. The model includes a library of approximately 800 different ingredients which provide the platform for describing the chemical composition of the diet to be formulated. Each feed in the feed library was evaluated against data from two commercial laboratories and updated where required to enable more precise predictions of dietary energy and protein supply. A multi-step approach was developed to predict uncertain values using linear regression, matrix regression and optimization. The approach provided an efficient and repeatable way of evaluating and refining the composition of a large number of different feeds against commercially generated data similar to that used by CNCPS users on a daily basis. The protein A fraction in the CNCPS, formally classified as non-protein nitrogen, was re-classified to ammonia for ease and availability of analysis and to provide a better prediction of the contribution of metabolizable protein (MP) from free amino acids and small peptides. Amino acid profiles were updated using contemporary datasets and now represent the profile of AA in the whole feed rather than the insoluble residue. Model sensitivity to variation in feed library inputs was investigated using Monte Carlo simulation. Results showed that the prediction of metabolizable energy was most sensitive to variation in feed chemistry, whereas predictions of MP were most sensitive to variation in digestion rates. Regular laboratory analysis of samples taken on-farm remains the recommended

approach to characterizing the chemical components of feeds in a ration. However, updates to the CNCPS feed library provide a database of ingredients that are consistent with current feed chemistry information and laboratory methods and can be used as a platform to formulate rations and improve the biology within the model.

2.2 Introduction

Obtaining useful outputs from any biological model is very dependent on the quality of the information being used to perform a simulation (Haefner, 2005). The feed library in the Cornell Net Carbohydrate and Protein System (**CNCPS**) contains information not routinely available from commercial laboratories such as AA profiles, FA profiles, digestion rates (**kd**) and intestinal digestibility (Tylutki et al., 2008). The feed library also provides commonly analyzed fractions that can be used as they are, or updated by the user. Correct estimation of these chemical components is critical in enabling the CNCPS to best predict the metabolizable energy (**ME**), and protein (**MP**) and other specific nutrients available from a given ration (Lanzas et al., 2007a, Lanzas et al., 2007b, Offner and Sauvant, 2004). Regular laboratory analysis of feeds will reduce the variation in model inputs to that derived from the sampling process, sample handling, preparation, and the variation of the assay itself (Hall and Mertens, 2012). However, in some situations this is not possible and feed library values have to be relied on. In other situations, feed compositions are very consistent, meaning library values provide a reasonable estimation without laboratory analysis. The CNCPS feed library consists of approximately 800 ingredients including forages, concentrates, vitamins, minerals and commercial products and serves as the reference database for describing the chemical composition of a diet. The objective of this study was to evaluate and revise the CNCPS feed library to ensure it is consistent with values being generated

and used as inputs from commercial laboratories. A multi-step approach was designed and used to combine current feed library information with new information and predict uncertain values. The intended methods for analyzing each major chemical component for use in the CNCPS are reported as well as a sensitivity analysis of model outputs to variation in feed library inputs.

2.3 Materials and Methods

2.3.1 Feed chemistry

The chemical components considered in this study were those routinely analyzed by commercial laboratories and required by the CNCPS for evaluation and formulation of nutrient adequacy and supply. These include: DM, CP, soluble protein (**SP**), ammonia, acid detergent insoluble CP (**ADICP**), neutral detergent insoluble CP (**NDICP**), acetic acid, propionic acid, butyric acid, lactic acid, other organic acids, sugar, starch, ADF, NDF, lignin, ash, ether extract (**EE**) and soluble fiber. Amino acids were also reviewed and updated. A list of the expected analytical procedures for measuring each chemical component and the units required by the CNCPS v6.5 are described in Table 2.1. Fractionation of chemical components from Table 2.1 into the pool structure of the CNCPS are described by Tylutki et al. (2008) and summarized in Table 2.2.

Table 2.1. Expected wet chemistry methods for analyzing feeds used in CNCPS v6.1

			Expected wet chemistry method for use in the CNCPS v6.5	
Chemical component	Abbreviations	Units	Base reference[1]	Brief description
Dry Matter	DM	%	AOAC 934.01	Gravimetric difference between dry and wet sample weights.
Crude Protein	CP	% DM	AOAC 968.06	Nitrogen measured using a combustion N analyzer and multiplied by a factor of 6.25.
Soluble protein	SP	% CP	(Licitra et al., 1996) Procedure 3.	Crude protein soluble in borate-phosphate buffer including sodium azide. Non-protein nitrogen is not subtracted. This is corrected within the framework of the model.
Ammonia	Ammonia	CPE (% SP)	AOAC 941.04	Nitrogen measured by Kjeldahl on fresh feed samples and multiplied by a factor of 6.25 to convert to crude protein equivalents (CPE).
Acid detergent insoluble crude protein	ADICP	% CP	(Licitra et al., 1996) Procedure 4.	Residual nitrogen measured by combustion or Kjeldahl after completing the ADF procedure described below.
Neutral detergent insoluble crude protein	NDICP	% CP	(Licitra et al., 1996) Procedure 4.	Residual nitrogen measured by combustion or Kjeldahl after completing the NDF procedure described below.
Volatile fatty acids, lactic acid and other organic acids	Acetic, propionic, butyric, isobutyric, lactic and other OAs	% DM	(Siegfried et al., 1984)	A fresh sample (25g) is weighed into an Erlenmeyer flask with 200ml of distilled water, mixed, and refrigerated overnight. The sample is then blended and filtered through a 25 µm filter. The extract is then analyzed according to Siegfried et al. (1984).
Sugar	Sugar	% DM	(Hall, 2014)	Water soluble carbohydrates analyzed using a phenol-sulfuric acid assay after a water extraction for 1 h at 40°C.

Table 2.1. *(Continued)*

			Expected wet chemistry method for use in the CNCPS v6.5	
Chemical component	Abbreviations	Units	Base reference[1]	Brief description
Starch	Starch	% DM	(Hall, 2009)	Enzymatic analysis after gelatinization with acetate buffer.
Acid detergent fiber	ADFom	% DM	AOAC 973.18	Acid detergent fiber, excluding ash, measured gravimetrically after an extraction with acid detergent and filtration on a 1.5 µm glass filter.
Neutral detergent fiber	aNDFom	% DM	(Mertens, 2002)	Neutral detergent fiber, excluding ash, measured gravimetrically after an extraction with neutral detergent, heat stable amylase, sodium sulfite and filtration on a 1.5 µm glass filter.
Lignin	Lignin	% NDF	AOAC 973.18[2]	Acid detergent lignin (ADL) applied to the fiber residue after completing an ADF extraction. Measured gravimetrically on an ash free basis.
Undigested neutral detergent fiber	uNDFom	% NDF	(Raffrenato, 2011)	Undigested aNDFom after completing a 240 h in vitro NDF digestibility and filtration on a 1.5 µm glass filter.
Ether extract	EE	% DM	AOAC 920.39	Measured gravimetrically after extraction with diethyl ether.
Soluble fiber	Soluble fiber	% DM	N/A	Calculated by difference within the model.
Ash	Ash	% DM	AOAC 942.05	Gravimetric difference between dry sample weight and dry sample weight after ashing.

Table 2.1. *(Continued)*

			Expected wet chemistry method for use in the CNCPS v6.5	
Chemical component	Abbreviations	Units	Base reference[1]	Brief description
Essential amino acids excluding methionine and tryptophan	Arg, His, Ile, Leu, Lys, Phe, Thr, Val	% CP	AOAC 994.12	Sample is hydrolyzed with 6N HCL for 21 h. An internal standard is added and HCL is evaporated. Hydrolysates are diluted with lithium citrate buffer and individual amino acids are measured by ion exchange chromatography.
Methionine	Met	% CP	AOAC 988.15	Sample is oxidized with performic acid for 16 h to form methionine sulfone, then hydrolyzed with 6N HCL for 21 h and analyzed by ion exchange chromatography.
Tryptophan	Trp	% CP	(Landry and Delhaye, 1992)	Sample is hydrolyzed with barium hydroxide for 16 h using 5-Methyltryptophan as an internal standard and analyzed by chromatography with fluorescence detection.

[1] AOAC methods were taken from AOAC International. (2005).

[2] Raffrenato and Van Amburgh (2011) provide details on improving recovery during filtration.

Table 2.2. Equations used by the CNCPS to calculate carbohydrate and protein fractions

Fraction[1]	Description	Equations[2,3]	
CHO*j*	Carbohydrates	100 - CP*j* - EE*j* – Ash*j*	(1)
CC*j*	Indigestible fiber	(aNDFom*j* × (Lignin*j* × aNDFom*j*) × 2.4) / 100 or, aNDFom*j* × uNDFom*j*	(2)
CB3*j*	Digestible fiber	aNDFom*j* – CC*j*	(3)
NFC*j*	Non-fiber CHO	CHO*j* – aNDFom*j*	(4)
CB2*j*	Soluble fiber	NFC*j* - CA1*j* - CA2*j* - CA3*j* - CA4*j* - CB1*j*	(5)
CA1*j*	Volatile fatty acids	Acetic*j* + Propionic*j* + (Butyric + Isobutyric)*j*	(6)
CA2*j*	Lactic acid	Lactic*j*	(7)
CA3*j*	Other organic acids	Organic acids*j*	(8)
CA4*j*	Sugar	Sugars*j*	(9)
CB1*j*	Starch	Starch*j*	(10)
PA1*j*[4]	Ammonia	Ammonia*j* × (SP*j*/100) × (CP*j*/100)	(11)
PA2*j*	Soluble true protein	SP*j* × CP*j* / 100 – PA1*j*	(12)
PB1*j*	Insoluble true protein	CP*j* - (PA1*j* – PA2*j* – PB2*j* - PC*j*)	(13)
PB2*j*	Fiber bound protein	(NDICP*j* - ADICP*j*) × CP*j* / 100	(14)
PC*j*	Indigestible protein	ADICP*j* × CP*j* / 100	(15)

[1] Subscript *j* means for the *j*th feed in the library.

[2] SP = soluble protein; ADICP = acid detergent insoluble CP; NDICP = neutral detergent insoluble CP.

[3] Chemical components are expressed as % DM except: SP = % CP; ADICP = % CP; NDICP = % CP; Ammonia = % SP; Lignin = % NDF; uNDFom = % NDF.

[4] Previous versions of the CNCPS feed library use non-protein nitrogen for the PA1 fraction. This has been replaced with ammonia.

2.3.2 Calculation procedure

To complete the analysis, datasets were provided by two commercial laboratories (Cumberland Valley Analytical Services Inc, Maugansville, MD, USA and Dairy One Cooperative Inc, Ithaca, NY, USA). The compiled dataset included 90 different ingredients and >100,000 individual samples. Additional means and SD of individual feeds were sourced from the laboratory websites. The online resource for both labs includes >10 years of data and an extensive collection of different ingredients. Each feed was evaluated for internal consistency, and consistency against laboratory data. Internal consistency required each feed to adhere to the

fractionation scheme summarized in Table 2.2. Briefly, Eq. (1) provides the relationship between carbohydrates (**CHO**), CP, EE and Ash. Carbohydrates are decomposed by Eq. (4) and (5) to NDF, acetic, propionic, butyric, isobutyric, lactic, other organic acids, sugar, starch and soluble fiber. From Eq. (1), (4) and (5), equation 16 can be derived for the j^{th} feed in the library:

$$100 = CP_j + EE_j + ash_j + NDF_j + acetic_j + propionic_j + butyric_j + isobutyric_j + lactic_j + other\ organic\ acis_j + sugars_j + starch_j + solubl\ fiber_j \quad (16)$$

Soluble fiber (CB2) is calculated in the CNCPS by difference (Eq. 5). This means any error in the estimation of the CA1, CA2, CA3, CA4 or CB1 fractions will result in an over- or under-estimation of soluble fiber. Also, error in the estimation of CP, EE, Ash or NDF will cause error in soluble fiber through the calculation of CHO (Eq. (1)) and the subsequent calculation of non-fiber carbohydrates (NFC; Eq. (4)). Overestimation of components in Eq. (16) can cause a situation where soluble fiber is forced to 0 and the sum of the equation is greater than 100 % DM which, theoretically, is chemically impossible. Feeds that didn't adhere to the assumptions of Eq. (16) were updated. This rule can be problematic when the N content of protein deviates from 16% in which a factor of 6.25 was used to convert the amount of N to an equivalent weight of protein (Van Soest, 1994). The mass of all proteins in the CNCPS are calculated as N × 6.25 despite the proper factor varying according to feed type (Van Soest, 1994). Therefore, for feeds high in NPN (urea, ammonium salts), Eq. 16 was overlooked. This is a legacy issue with the CNCPS and other formulation systems and would require considerable recoding to a nitrogen (N) basis to overcome. However, future versions of the model will address this problem. Likewise, NDF in the datasets provided were not ash corrected as recommended in Table 2.1 as

these data were not available at time the analysis was done. Using aNDFom in future updates is recommended to reduce variation. Evaluation against laboratory data compared each individual feed in the feed library to the mean and SD of the corresponding feed in the databases available from the commercial labs. Each component within each feed was required to fall within 1 SD of the mean value from the laboratory dataset, or the entire feed would be updated. The calculation procedure consisted of four steps:

Step 1 – Setting Descriptive Values

Chemical components used to differentiate different forms of the same feed were held constant during the re-calculation process. The CNCPS has multiple options for many of the feeds in the feed library to give users the flexibility to pick the feed that best matches what they are feeding on the farm. For example, the feed library has 24 different options for processed corn silage which are differentiated on the basis of DM and NDF. Therefore, in this example, DM and NDF were maintained as they were in the original library while other components were re-calculated.

Step 2 – Linear Regression

In the second step, the dataset provided was used to established relationships using linear regression ($Y = A + BX_1 + CX_2 + DX_3$). Regression was used if components could be robustly predicted by other components within a feed ($R^2 > 0.65$). Regression equations were derived using SAS (2010). Examples of some of the regression equations used are in Table 2.3.

Table 2.3. Predicting chemical components[1] of feeds using simple and multiple linear regression ($Y = A + BX_1 + CX_2 + DX_3$)

Feed name	Y	X_1	X_2	X_3	A	B	C	D	RMSE[2]	R^2
Barley silage	ADF	NDF	Lignin		-7.15	0.69	0.5		1.53	0.90
Corn Silage	ADF	NDF			-3.67	0.68			1.28	0.89
Corn Silage	Starch	NDF	CP		96.18	-1.18	-1.62		2.6	0.87
Fresh grass (High NDF)	ADF	NDF	Lignin	CP	0.47	0.54	0.75	-0.27	2.54	0.67
Fresh grass (Low NDF)	ADF	NDF	Lignin	CP	5.84	0.45	0.51	-0.17	2.11	0.83
Fresh legume	ADF	NDF	Lignin		-6.31	0.69	0.52		1.53	0.88
Grass hay	ADF	NDF			3.57	0.57			3.21	0.69
Grass silage	ADF	NDF	Lignin		-0.25	0.57	0.47		1.79	0.85

[1] Expressed as % DM except lignin which is expressed as % NDF.

[2] RMSE = Root mean square error.

Step 3 – Matrix Regression

In the third step, factors that couldn't be predicted using standard linear regression were calculated using a matrix of regression coefficients derived from data generated using a Monte Carlo simulation (Law and Kelton, 2000). The Monte Carlo simulation was completed using @Risk version 5.7 (Palisade Corporation, Ithaca, NY, USA). To complete the analysis, probability density functions were fit to each chemical component of each feed using the data provided by the commercial labs and the distribution fitting function in @Risk (Palisade, 2010a). Distributions were ranked on how well they fit the input data using the Chi-Squared goodness of fit statistic. Equiprobable bins were used to adjust bin size in the Chi-Square calculation to contain an equal amount of probability (Law and Kelton, 2000). The distribution with the lowest Chi-Square was assigned to each component. Examples of the distribution derived for each chemical component for a range of feeds are in (Table 2.6).

Components within each feed were then correlated to each other using laboratory data and the define correlation function in @Risk (Palisade, 2010a). If components were not correlated, they would change randomly relative to each other during the Monte Carlo simulation. Correlating the components meant that for each iteration, components changed in tandem relative to each other with the magnitude of the change depending on the assigned correlation coefficient (Law and Kelton, 2000). Spearman rank order correlations were used which determine the rank of a component relative to another by its position within the min-max range of possible values. Rank correlations can range between -1 and 1 with a value of 1 meaning components are 100% positively correlated, -1 meaning components are 100% negatively correlated and 0 meaning there is no relationship between components (Law and Kelton, 2000). The correlation coefficients derived for a range of feeds used in the Monte Carlo simulation are in (Table 2.7).

Once the probability density functions had been fit to each component, and components within each feed correlated, a Monte Carlo simulation was performed with 30,000 iterations. Various sampling techniques are available in @Risk to draw the sample from the probability density function (Palisade, 2010a). The Latin Hypercube technique was used which divides the distribution into intervals of equal probability and then randomly takes a sample from each interval forcing the simulation to represent the whole distribution (Shapiro, 2003). The raw data from the simulation was then used to construct a matrix of regression estimates in the arrangement shown below and according to the general form $Y_{ij} = A + BX_i$ where Y is the response variable and column vector for the *i*th component in the *j*th feed with *n* entries, A is the intercept arranged in an $n \times p$ matrix, B is the predictor variable arranged in an $n \times p$ matrix and X is the regression coefficient and row vector for the *i*th component with *n* entries:

$$Y = \begin{pmatrix} Y_1 \\ Y_2 \\ \vdots \\ Y_n \end{pmatrix} \quad A = \begin{pmatrix} A_{11} & \dots & A_{1p} \\ A_{21} & \cdots & A_{2p} \\ \vdots & \ddots & \vdots \\ A_{n1} & \cdots & A_{np} \end{pmatrix}, \quad B = \begin{pmatrix} B_{11} & \dots & B_{1p} \\ B_{21} & \cdots & B_{2p} \\ \vdots & \ddots & \vdots \\ B_{n1} & \cdots & B_{np} \end{pmatrix}, \quad X = \begin{pmatrix} X_1 \\ X_2 \\ \vdots \\ X_n \end{pmatrix}$$

In this arrangement $Y_n = X_n$ and, therefore, $A_{np} = 0$ and $B_{np} = 1$. For example, if Y_1 was the response variable CP, then the predictor variable X_1 would also be CP and the relationship would have an intercept of 0 and slope of 1. Therefore, equations where $Y_n = X_n$ were excluded from the matrix. The weighted mean of response variables were calculated across each row of the matrix. The coefficients used to correlate each probability density function for the Monte Carlo simulation (Table 2.7) were normalized to sum to 1 and then used as weights (W) in the weighted mean, i.e.

$$\sum_{i=1}^{n} W_i = 1 \quad \text{and, therefore,} \quad \overline{Y} = \sum_{i=1}^{n} W_i X_i.$$

Using correlation coefficients as weights meant components within a specific feed that were more highly correlated had more influence on the mean and *vice versa*.

Components calculated using this method varied depending on the data available for a specific feed. To avoid confounding, components within a feed that were calculated by the matrix were not used as predictor variables for other components in the matrix. Therefore, the number of components calculated using the matrix was limited to avoid running out of predictor variables. Typically, nitrogenous components (SP, Ammonia, NDICP, ADICP) not calculated in the preceding steps and not factors in Eq. (16) were calculated in this step.

Step 4 – Optimize to a Final Solution

Lastly, components that were not assigned values in any of the preceding steps were calculated using an optimization. RISKOptimizer version 5.7 (Palisade Corporation, Ithaca, NY, USA) was used to perform the optimization which uses a genetic algorithm simulation to find solutions when there is uncertainty around the values (Palisade, 2010b). Minimum and maximum boundaries for each component within a feed were set to constrain the optimizer to a likely range of values. The data used to calculate the range in each component was taken from the databases available online from the commercial laboratories. Each range was calculated as the mean plus or minus the SD of each component multiplied by global coefficient that was adjusted in order to allow the optimizer to converge. Typically the coefficient used was between 0.5 and 1.5 meaning the range for each component was the mean plus or minus 0.5 to 1.5 times the SD of each component. An example of the constraints used to optimize corn silage is in Table 2.4.

The second constraint applied to the optimization was the relationship described by Eq. (16). Components included in the optimization were, therefore, adjusted within the calculated range to the most likely values in which Eq. (16) summed to 100 % DM. The optimization step was completed last in the calculation process to ‘fit’ the components within each feed together within the described constraints. The process was dynamic in that the values calculated in the optimization fed back into the matrix and regression calculations described above. Typically, the optimizer had to be run numerous times before it would converge and stabilize. If insufficient data was available to perform any of the calculation steps described above, current CNCPS library values were retained. The approach was not acceptable for proprietary feeds due to a lack

of robust data of chemical components or the functional nature of some ingredients beyond the nutrient content. Current library values were retained in these circumstances. Approximately 75% of the feeds in the feed library were updated and 25% remained unchanged. Those remaining unchanged were primarily commercial products, minerals and vitamins along with unusual feeds with little information within the databases.

Table 2.4. Minimum and maximum boundaries used to constrain the chemical components of corn silage during optimization in step 4 of the procedure used to update the CNCPS feed library

			Optimizer boundaries (1.5 × SD)	
Chemical component[1]	Mean	SD	Minimum	Maximum
DM	33.8	10.3	18.3	49.2
CP	8.2	1.0	6.7	9.8
SP (% CP)	53.4	10.1	38.3	68.5
Ammonia (% SP)	13.4	6.2	4.1	22.7
ADICP (% CP)	7.5	1.8	4.8	10.2
NDICP (% CP)	15.2	3.8	9.6	20.9
Acetic	2.4	1.5	0.1	4.6
Propionic	0.3	0.3	0.0	0.9
Butyric	0.0	0.0	0.0	0.2
Lactic	4.7	2.2	1.4	8.1
Other OA	0.0	0.0	0.0	0.0
Sugar	2.1	1.3	0.2	4.0
Starch	31.3	7.5	20.0	42.6
ADF	26.1	4.1	20.0	32.2
NDF	44.1	6.0	35.1	53.1
Lignin (% NDF)	7.6	1.5	5.3	9.9
Ash	4.2	1.2	2.5	6.0
EE	3.3	0.5	2.6	4.0

[1] Expressed as % DM unless otherwise stated. SP = soluble protein; ADICP = acid detergent insoluble CP; NDICP = neutral detergent insoluble CP; Other OA = other organic acids; EE = ether extract.

2.3.3 Amino Acids

In addition to the chemical components described above, each feed in the CNCPS feed library includes a profile of the 10 essential AA. Amino acid profiles were updated using datasets

provided by Evonik Industries AG (Hanau, Germany), Adisseo (Commentary, France) and taken from the NRC (2001). Data provided were mean values from analyses completed in the respective companies' laboratories or published in the NRC (2001). In all cases, AA analyses were completed on the whole feed and are expressed in the CNCPS on a % CP basis. This differs from previous versions of the CNCPS where AA were expressed as a % of the buffer insoluble residue (O'Connor et al., 1993). The most appropriate profile was assigned based on data availability and was used as received by the source without alteration. If profiles for specific feeds were not available in the datasets provided, current CNCPS values were retained. Proprietary feeds were not changed.

2.3.4 Model sensitivity

The sensitivity of model outputs to variation in feed library inputs was also evaluated. The analysis was split into two parts. Part one looked at the likely range in six major chemical components in the diet: 1) CP; 2) Starch; 3) NDF; 4) Lignin; 5) Ash; 6) EE; and four model outputs: 1) ME allowable milk; 2) MP allowable milk; 3) MP from RUP; 4) MP from bacteria. To complete this part of the analysis, a reference diet was constructed in a spreadsheet version of the CNCPS (Van Amburgh et al., 2013). The diet was formulated using ingredients typically found in North American dairy cattle rations and was balanced to provide enough ME and MP for a mature, non-pregnant, 600 kg cow in steady state (0 energy balance) to produce 35 kg of milk containing 3.1% true protein and 3.5% fat (Table 2.5). Probability density functions were fit to chemical components within each feed in the reference diet (Table 2.7) and correlated to each other with Spearman Rank order correlations (Table 2.6) using @Risk version 5.7 (as previously described). Frequency distributions for model outputs were then generated using a Monte Carlo

simulation with 10,000 iterations to describe the range of possible outcomes for each output and the relative likelihood of occurrence.

Part two of the analysis investigated which feed library inputs for the feeds in the reference diet had the most influence on selected model outputs: 1) ME allowable milk; 2) MP allowable milk; 3) MP from RUP; 4) MP from bacteria. The feed library inputs investigated were those described in part one of the analysis, as well as kd for the carbohydrate and protein fractions summarized in Table 2.2. Probability density functions were fit to each chemical component within each feed as previously described. Program Evaluation and Review Technique (PERT) distributions (Cottrell, 1999) were used to describe the variation in kd. The PERT distribution is similar to a beta or triangular distribution and is useful to describe variation in a situation where there is limited data (Johnson, 1997). The PERT distribution requires three estimates: 1) the most likely result; 2) the minimum expected result; 3) the maximum expected result. Most likely results were set as CNCPS feed library values. Minimum and maximum values were set as the most likely value ± 2 SD to encompass approximately 95% of the expected data without including extreme results. Data on kd are scarce, and other than the CB3 fraction, are not routinely estimated for model input. Variation in kd changes proportionally to changes in mean values (Weiss, 1994). Therefore, in situations where data were not available, the proportional variation relative to the mean of other known feeds was used as a proxy to calculate the minimum and maximum values of unknown feeds. The CB3 kd was calculated for the forage feeds in the reference diet using the approach described by Van Amburgh et al. (2003) and the datasets provided. Variation in kd for fractions other than CB3 were estimated from literature values. CA and CB fractions were estimated from data in Offner et al. (2003). The PB2 fractions

(fiber bound protein) were set to equal the CB3 fractions as described by Van Amburgh et al. (2007), PB1 values were taken from the NRC (2001) and PA2 values were estimated from Broderick (1987). Correlation coefficients among components were not assigned for this part of the analysis as the interest was in understanding model sensitivity to individual components independent of correlated changes in composition. To complete the analysis, a Monte Carlo simulation with 10,000 iterations was performed. Changes in model outputs resulting from a 1 SD increase in model inputs were captured and are presented in Figures 2.5, 2.6 and 2.7.

Table 2.5. Diet ingredients, chemical composition and model predicted ME and MP for the reference diet used to analyze model sensitivity

Diet Ingredients (kg DM)	
Corn Silage	4.76
Alfalfa Silage	3.14
Grass Hay	4.03
Corn Grain Ground Fine	6.48
Soybean Meal Solvent Extracted	2.58
Blood Meal	0.20
Minerals and Vitamins	0.50
Total DMI	21.69
Diet composition[1]	
CP	16.7
SP (% CP)	35.3
ADICP (% CP)	6.4
NDICP (% CP)	15.6
Sugar	3.5
Starch	29.0
NDF	31.8
Lignin (% NDF)	11.5
EE	3.0
Ash	7.7
Model outputs	
ME (Mcals/d)	53.7
MP (g/d)	2385.4

[1] Expressed as % DM unless stated. SP = soluble protein; ADICP = acid detergent insoluble CP; NDICP = neutral detergent insoluble CP; EE = ether extract.

Table 2.6. Mean, SD, distribution and distribution parameters for each chemical component of each feed used to perform Monte Carlo simulations

Feed name and chemical components[1,2]	Mean	SD	Distribution	Distribution parameters[3]			
				A	B	C	D
Corn Silage							
CP	8.0	0.90	Loglogistic	0.6	7.4	14.1	
SP	56.4	9.61	BetaGeneral	75.0	7.2	-238.8	84.3
ADICP	7.7	1.86	Loglogistic	-0.1	7.6	7.1	
NDICP	14.0	3.24	Pearson5	16.9	214.0	0.6	
NDF	42.5	5.08	Loglogistic	14.5	27.8	9.4	
Lignin	7.1	1.00	Loglogistic	-5.4	12.5	21.6	
Starch	33.0	7.11	Weibull	10.1	65.7	-29.7	
Sugar	1.6	0.97	Pearson5	3.4	3.9	0.0	
EE	3.3	0.48	Logistic	3.3	0.3		
Ash	4.3	1.14	Extvalue	3.8	1.0		
Alfalfa Silage							
CP	21.7	2.83	Normal	21.7	2.9		
SP	60.0	9.07	Logistic	60.1	5.3		
ADICP	7.2	2.10	Loglogistic	1.9	5.0	4.3	
NDICP	14.6	4.95	Pearson5	13.2	224.6	-3.6	
NDF	42.5	5.24	Loglogistic	-17.0	59.3	19.5	
Lignin	17.2	2.34	Logistic	17.3	1.3		
Starch	1.9	0.88	Loglogistic	-0.6	2.4	4.8	
Sugar	3.4	1.95	Loglogistic	0.1	2.9	2.8	
EE	3.7	0.81	Lognorm	77.3	0.8	-73.6	
Ash	11.0	1.80	Loglogistic	4.8	6.0	5.9	
Grass Hay							
CP	10.9	3.46	Lognorm	15.0	3.7	-3.9	
SP	31.3	6.21	Loglogistic	-43.6	74.7	20.8	
ADICP	9.1	4.12	Pearson5	6.9	64.7	-1.5	
NDICP	32.6	7.68	Loglogistic	-22.3	54.5	12.2	
NDF	62.6	7.95	Logistic	62.6	4.6		
Lignin	8.7	2.37	Loglogistic	1.3	7.1	5.5	
Starch	2.2	1.27	Invgauss	3.3	17.7	-1.1	
Sugar	6.8	2.69	Loglogistic	-22.8	29.4	18.2	
EE	2.5	0.72	Pearson5	46.3	226.4	-2.5	
Ash	7.7	2.27	Logistic	7.7	1.3		

Figure 2.6. *(Continued)*

Feed name and chemical components[1,2]	Mean	SD	Distribution	Distribution parameters[3]			
				A	B	C	D
Corn Grain							
CP	8.6	0.70	Loglogistic	3.6	5.0	12.5	
SP	17.6	5.59	Logistic	17.6	3.3		
ADICP	7.1	2.28	Loglogistic	-0.5	7.4	5.4	
NDICP	11.9	3.81	Lognorm	13.0	4.1	-1.0	
NDF	11.4	1.30	Loglogistic	-0.8	12.2	16.4	
Lignin	15.7	4.73	Loglogistic	1.9	13.4	4.6	
Starch	72.1	1.49	Logistic	72.1	0.8		
Sugar	2.5	0.62	Loglogistic	-1.6	4.0	11.3	
EE	3.7	0.52	Logistic	3.7	0.3		
Ash	1.5	0.29	Loglogistic	0.7	0.8	5.2	
Soybean Meal							
CP	53.1	1.72	Logistic	53.1	1.0		
SP	24.3	6.75	Lognorm	61.6	7.0	-37.2	
ADICP	2.8	1.45	Loglogistic	-1.0	3.6	4.2	
NDICP	13.4	4.17	Logistic	13.0	2.8		
NDF	11.1	1.91	Pearson5	11.0	65.7	4.7	
Lignin	9.1	3.69	Logistic	9.1	2.5		
Starch	1.1	0.49	Loglogistic	-1.2	2.3	7.5	
EE	1.7	0.68	Loglogistic	-0.2	1.8	4.6	
Ash	7.6	0.77	Logistic	7.6	0.4		
Blood Meal[4]							
CP	104.5	3.57	Weibull	14.1	45.2	60.8	

[1] SP = soluble protein; ADICP = acid detergent insoluble CP; NDICP = neutral detergent insoluble CP; EE = ether extract.

[2] Chemical components are expressed as % DM except: SP = % CP; ADICP = % CP; NDICP = % CP; Lignin = % NDF.

[3] A, B, C and D are the parameters that define the characteristics of each distribution: BetaGeneral, A = Shape, B = Shape, C = Min value, D= Max value; ExtValue, A = Location, B = Scale; Invgauss, A = Mean, B = Variance, C = Shift; Logistic, A = Location, B = Scale, Loglogistic, A = Location, B = Scale, C = Shape; Lognorm, A = Mean, B = Variance, C = Shift; Normal, A = Mean, B = SD; Pearson5, A = Shape, B = Scale, C = Shift; Weibull, A = Shape, B = Scale, C = Shift.

[4] Blood meal CP can be > 100 % DM if nitrogenous components are > 16 % N.

Table 2.7. Spearman rank correlation coefficients for the chemical components of feeds used to perform Monte Carlo simulations. Rows are blank if there was insufficient data available to perform the analysis

	CP[1,2]	SP	ADICP	NDICP	NDF	Lignin	Starch	Sugar	EE	Ash
Corn Silage										
CP	1.00									
SP	0.11	1.00								
ADICP	-0.19	-0.27	1.00							
NDICP	-0.12	-0.55	0.39	1.00						
NDF	0.18	-0.10	0.41	0.46	1.00					
Lignin	0.08	-0.09	0.25	0.15	0.05	1.00				
Starch	-0.37	0.09	-0.39	-0.38	-0.91	-0.10	1.00			
Sugar	0.07	-0.30	0.09	0.11	0.09	-0.06	-0.25	1.00		
EE	0.18	0.37	-0.27	-0.27	-0.29	-0.01	0.30	-0.28	1.00	
Ash	0.35	-0.08	0.26	0.12	0.35	0.30	-0.50	0.07	-0.16	1.00
Alfalfa Silage										
CP	1.00									
SP	0.18	1.00								
ADICP	-0.52	-0.23	1.00							
NDICP	-0.31	-0.57	0.67	1.00						
NDF	-0.62	-0.18	0.54	0.56	1.00					
Lignin	0.27	0.13	0.23	-0.02	-0.21	1.00				
Starch	-0.25	-0.15	0.01	0.01	-0.08	-0.13	1.00			
Sugar	0.17	-0.62	-0.27	-0.14	-0.42	-0.10	0.18	1.00		
EE	0.27	0.45	-0.16	-0.14	-0.12	-0.16	-0.07	-0.56	1.00	
Ash	0.18	0.20	0.02	-0.16	-0.12	0.22	-0.18	-0.17	0.05	1.00
Grass Hay										
CP	1.00									
SP	0.07	1.00								
ADICP	-0.43	-0.21	1.00							
NDICP	-0.11	-0.42	0.48	1.00						
NDF	-0.51	-0.11	0.27	0.36	1.00					
Lignin	0.04	-0.03	0.55	0.25	-0.04	1.00				
Starch	-0.10	-0.07	0.10	-0.04	-0.24	0.10	1.00			
Sugar	0.09	0.24	-0.48	-0.46	-0.65	-0.31	0.13	1.00		
EE	0.51	-0.13	-0.27	-0.11	-0.60	0.05	0.09	0.34	1.00	
Ash	0.50	0.10	-0.16	-0.06	-0.55	-0.18	-0.01	0.01	0.23	1.00
Corn Grain										
CP	1.00									
SP	0.17	1.00								
ADICP	-0.10	-0.19	1.00							
NDICP	-0.18	-0.11	0.43	1.00						
NDF	0.05	0.02	0.10	0.34	1.00					
Lignin	0.19	-0.07	0.17	-0.07	-0.24	1.00				
Starch	-0.40	-0.16	0.13	0.00	-0.56	0.01	1.00			
Sugar	0.03	0.34	-0.11	-0.05	0.04	0.16	-0.20	1.00		
EE	0.21	0.22	-0.25	-0.16	0.24	0.14	-0.48	0.23	1.00	
Ash	0.15	0.23	0.00	0.03	-0.01	-0.02	-0.14	0.00	0.22	1.00

Figure 2.7. *(Continued)*

	CP[1,2]	SP	ADICP	NDICP	NDF	Lignin	Starch	Sugar	EE	Ash
Soybean Meal										
CP	1.00									
SP	-0.03	1.00								
ADICP	0.10	-0.62	1.00							
NDICP	-0.36	-0.39	0.14	1.00						
NDF	-0.15	-0.31	0.06	0.20	1.00					
Lignin	-0.03	-0.09	0.32	-0.35	-0.18	1.00				
Starch	-0.02	0.00	-0.16	-0.54	-0.18	0.27	1.00			
Sugar										
EE	0.08	-0.24	-0.03	0.44	0.21	-0.14	-0.19		1.00	
Ash	-0.26	-0.06	0.04	-0.01	-0.34	0.10	0.04		0.03	1.00

[1] SP = soluble protein; ADICP = acid detergent insoluble CP; NDICP = neutral detergent insoluble CP; EE = ether extract.

[2] Chemical components are expressed as % DM except: SP = % CP; ADICP = % CP; NDICP = % CP; Lignin = % NDF.

Table 2.8. Parameters used to specify PERT distributions (mean, minimum and maximum) and SD for the carbohydrate and protein fractions of feeds in the reference diet used to analyze model sensitivity

	Carbohydrate and protein fractions[1]												
	CA1	CA2	CA3	CA4	CB1	CB2	CB3	CC	PA1	PA2	PB1	PB2	PC
Corn Silage													
Mean	0.0	7.8	5.6	22.3	35.7	33.5	3.8	0.0	200.0	50.0	20.0	3.8	0.0
SD	0.0	3.5	2.5	10.0	16.1	15.1	0.7	0.0	15.1	6.6	5.2	0.7	0.0
Minimum	0.0	0.2	0.1	0.2	0.6	0.7	1.9	0.0	161.1	32.8	6.8	1.9	0.0
Maximum	0.0	18.2	13.0	52.4	82.8	78.6	5.6	0.0	238.4	66.8	33.4	5.7	0.0
Alfalfa Silage													
Mean	0.0	7.0	5.0	20.0	30.0	35.0	7.0	0.0	200.0	45.0	16.0	7.0	0.0
SD	0.0	1.4	1.0	4.0	6.0	7.0	1.4	0.0	15.1	6.0	5.0	1.4	0.0
Minimum	0.0	3.4	2.5	9.9	14.6	17.1	3.5	0.0	161.3	29.7	3.3	3.4	0.0
Maximum	0.0	10.5	7.6	30.1	45.2	52.8	10.5	0.0	238.9	60.2	28.6	10.5	0.0
Grass Hay													
Mean	0.0	7.0	5.0	40.0	30.0	30.0	4.5	0.0	200.0	20.0	14.0	4.5	0.0
SD	0.0	1.4	1.0	8.0	6.0	6.0	1.0	0.0	15.1	2.7	5.1	1.0	0.0
Minimum	0.0	3.5	2.4	19.8	14.6	14.8	1.9	0.0	161.4	13.2	0.7	1.9	0.0
Maximum	0.0	10.6	7.6	60.7	45.3	45.3	7.1	0.0	238.9	26.8	27.1	7.1	0.0
Corn Grain													
Mean	0.0	7.0	5.0	40.0	15.0	20.0	6.0	0.0	200.0	16.0	9.0	6.0	0.0
SD	0.0	2.4	1.7	14.0	5.2	7.0	1.2	0.0	15.1	2.1	2.8	1.2	0.0
Minimum	0.0	0.8	0.4	4.1	1.6	2.3	2.8	0.0	161.0	10.6	1.9	2.8	0.0
Maximum	0.0	13.2	9.5	76.7	28.6	38.0	9.2	0.0	238.8	21.4	16.1	9.1	0.0
Soybean Meal													
Mean	0.0	7.0	5.0	40.0	25.0	30.0	6.0	0.0	200.0	24.0	11.0	6.0	0.0
SD	0.0	2.2	1.6	12.5	7.8	9.4	1.2	0.0	15.1	3.2	2.7	1.2	0.0
Minimum	0.0	1.4	1.0	7.9	5.2	5.8	2.9	0.0	161.3	15.9	4.2	2.8	0.0
Maximum	0.0	12.5	9.0	71.9	45.3	53.9	9.1	0.0	238.8	32.1	17.8	9.2	0.0
Blood Meal													
Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	200.0	13.5	3.7	0.0	0.0
SD	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	15.1	1.8	1.9	0.0	0.0
Minimum	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	161.4	8.9	0.0	0.0	0.0
Maximum	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	238.4	18.1	9.7	0.0	0.0

[1] CA1 = acetic + propionic + butyric + isobutyric; CA2 = lactic; CA3 = other organic acids; CA4 = sugar; CB1 = starch; CB2 = soluble fiber; CB3 = digestible fiber; CC = indigestible fiber; PA1 = ammonia; PA2 = soluble true protein; PB1 = insoluble true protein; PB2 = fiber bound protein; PC = indigestible protein.

2.4 Results and Discussion

2.4.5 Analytical techniques and fractionation

The required procedures to most appropriately characterize the chemical components of feeds for version 6.5 of the CNCPS are described in Table 2.1. Chemical components and fractionation of feeds in the updated library were maintained in the format described by Tylutki et al. (2008) with the exception of the protein A1 fraction. Previously this has been classified as non-protein nitrogen (**NPN**) which is measured as the nitrogen passing into the filtrate after precipitation with protein specific reagent (tungstic or tricholoracetic acid; Licitra et al., 1996). The protein A1 fraction is typically assumed to be completely degraded in the rumen (Lanzas et al., 2007b). However, small peptides and free AA not precipitated by this method are still nutritionally relevant to the animal if they escape rumen degradation and flow through to the small intestine (Givens and Rulquin, 2004). Choi et al. (2002) suggested 10% of the AA flowing through to the small intestine originated from dietary NPN sources which under the previous approach within the CNCPS were unaccounted for. Likewise, Velle et al. (1997) infused free AA into the rumen at various rates and showed up to 20% could escape degradation and flow through to the small intestine which is in agreement with data from Volden et al. (1998). Van Amburgh et al. (2010) suggested it may be more appropriate to redefine the protein A1 fraction from NPN as described by Licitra et al. (1996) to ammonia. This would shift small peptides and free AA currently associated with the A1 fraction into the A2 fraction where they could contribute to MP supply and also refines the prediction of rumen N balance as less N is degraded in the rumen. Ammonia has the advantage of being easily measured and available from most commercial laboratories. Therefore, the NPN fraction in previous feed libraries has been updated to ammonia in version 6.5 (Van Amburgh et al., 2013).

Amino acid profiles from the original feed database (O'Connor et al., 1993) were determined on the insoluble protein residue and analyzed using a single acid hydrolysis with 6N HCL for 24 h (Macgregor et al., 1978, Muscato et al., 1983). During acid hydrolysis Met is partially converted to methionine sulfoxide, which cannot be quantitatively recovered, and Trp is completely destroyed (Allred and MacDonald, 1988). Methionine is typically considered one of the most limiting AA in dairy cattle diets (Armentano et al., 1997, Rulquin and Delaby, 1997, Schwab et al., 1992) and is frequently the target of supplementation (Schwab, 1996). Therefore, updating AA profiles in the feed library, particularly Met, was an important part of improving overall model predictions. The AA profiles used to update the feed library were analyzed on a whole feed basis, rather than on the insoluble protein residue. The insoluble protein residue was originally assumed to have a greater probability of escaping the rumen and was more likely to match the AA profile of the RUP fraction (Macgregor et al., 1978). However, Tedeschi et al. (2001) investigated this hypothesis and found no differences in AA profiles of feeds analyzed with, or without extraction of the soluble fraction. Further, the soluble fraction of feeds has been shown to contribute 10-20% to the flow of AA to the small intestine (Choi et al., 2002, Velle et al., 1997, Volden et al., 1998). Extracting the insoluble protein residue requires soaking samples in borate-phosphate buffer to remove the soluble fraction (Krishnamoorthy et al., 1982) and adds another step to AA analysis. Therefore, it was decided using AA profiles determined on a whole feed basis was simpler, more feasible for commercial laboratories, biologically more relevant and provided access to much larger datasets than using profiles from the insoluble residue.

2.4.6 Revision of the feed library

The process of evaluating and updating the feed library was designed specifically to pool data from various sources and combine it to estimate likely values. Although the dataset used in this analysis encompassed a large number of samples from a wide range of situations, information on environmental and management factors implicit in the composition of individual samples were not available. Many external factors affect the nutrient composition of feeds both pre- and post-harvest. When considering forages, pre-harvest environmental factors such as temperature, light intensity, nitrogen availability, water and predation impact quality and composition (Van Soest et al., 1978). Post-harvest, management factors such as packing density, particle size, silo type, silo filling rate and the way in which the face of the silo is managed can impact ADF, non-fiber carbohydrates, ADICP, SP, ammonia, pH, surface temperature and aerobic instability (Ruppel et al., 1995). Furthermore, biological processes during ensiling such as plant respiration, plant enzymatic activity, clostridial activity and aerobic microbial activity will impact levels of rapidly fermentable CHO, AA, NPN and can lead to heating and Maillard reactions (Muck, 1988). Analytically, elevated levels of ADICP are indicative that Maillard reactions have occurred and are common in many heat dried feeds and fermented feeds where excessive heating occurred (Van Soest and Mason, 1991). Given the importance of external factors on the composition of different feeds, the process used in this project was not sensitive enough to accurately predict the composition of feeds on a sample by sample basis. However, it was capable of producing estimated compositions under average conditions in an efficient and repeatable manner which was useful for reviewing and updating a large database such as the CNCPS feed library.

Examples of the changes made to selected forages and concentrates are in Figures 2.1 and 2.2. The figures were constructed so that the 0 point on the Y axis represents the mean of the dataset

used to update the composition (given in brackets on the X axis) and the error bars represent ± 1 SD from the mean. The new and old values for each chemical component within the example feeds are presented relative to the mean and SD. For forage feeds, there were typically multiple options for each feed in the feed library. Therefore, some deviation from the mean could be expected as the variation is what makes the individual option unique (e.g. high NDF, low NDF). In contrast, the concentrate feeds typically had only one option. In this case, the composition could be expected to be similar to the mean (Figure 2.2). Noteworthy changes that reflect some of the relationships observed in the dataset include a reduction in starch for the corn silage in Figure 2.1A. Starch and NDF in corn silage have a strong reciprocal relationship (correlation coefficient = -0.91; Table 2.7) and NDF in the example is approximately 6 units greater than the mean. Based on the correlation, starch in this example should be a similar magnitude below the mean which is reflected by the updated composition. In another example, the composition of canola meal in the old feed library (Figure 2.2B) was similar to mean values for all components other than starch, which was considerably higher, and outside the expected range. In this case the recalculation procedure reduced starch to within 1 SD of the mean. Similar adjustments were made on a feed by feed basis for the entire feed library.

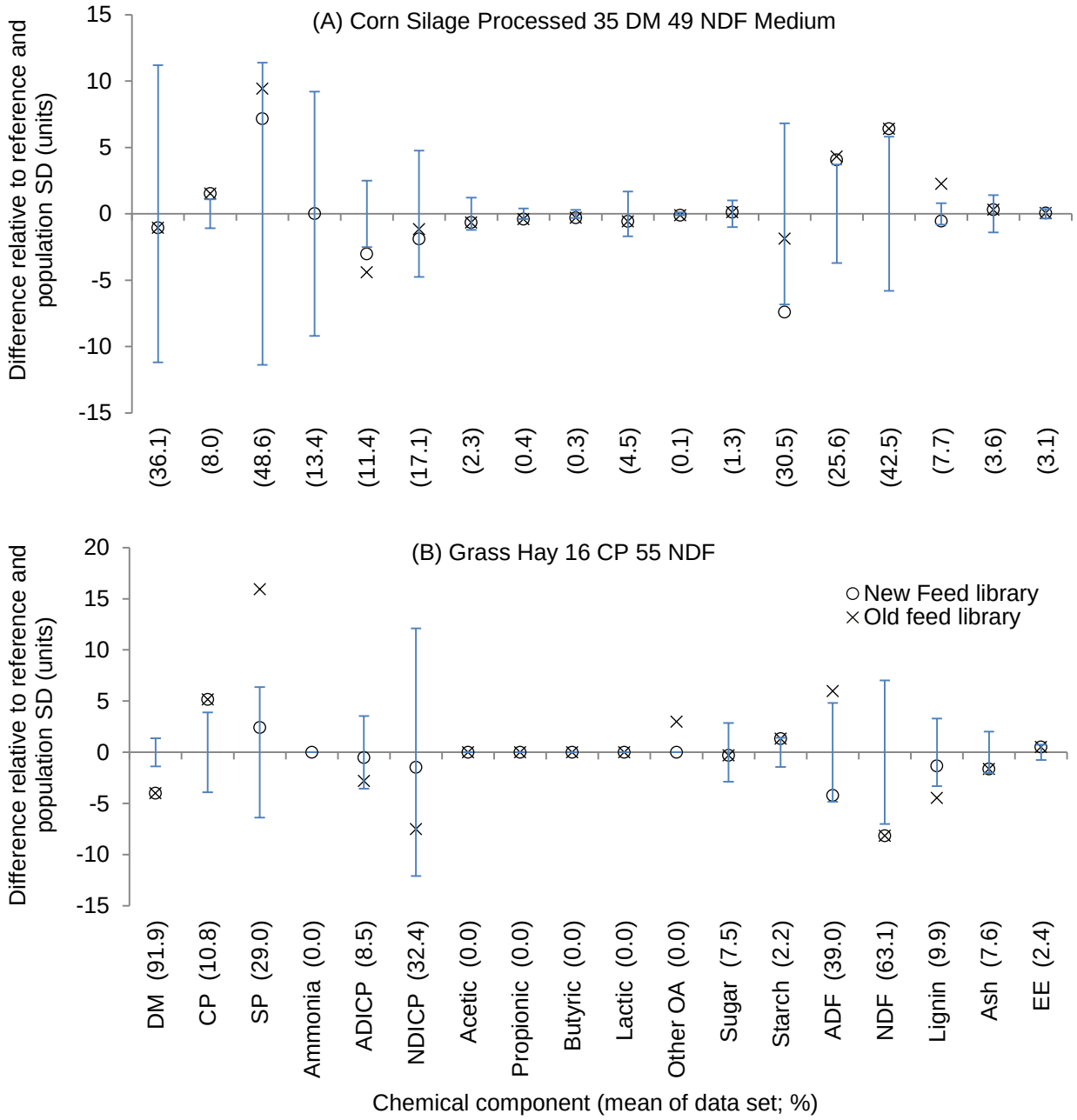


Figure 2.1.Comparison of the relative difference in chemical composition between the old (×) and new (○) CNCPS feed library for two forages (A = Corn Silage Processed 35 DM 49 NDF Medium; B = Grass Hay 16 CP 55 NDF) using the mean and SD of commercial laboratory data sets as a reference (Cumberland Valley Analytical Services Inc, Maugansville, MD, USA and Dairy One Cooperative Inc, Ithaca, NY, USA). All components are expressed as % DM with the exception of soluble protein (SP; % CP), Ammonia (% SP), acid detergent insoluble CP (ADICP; % CP), neutral detergent insoluble CP (NDICP; % CP) and lignin (% NDF).

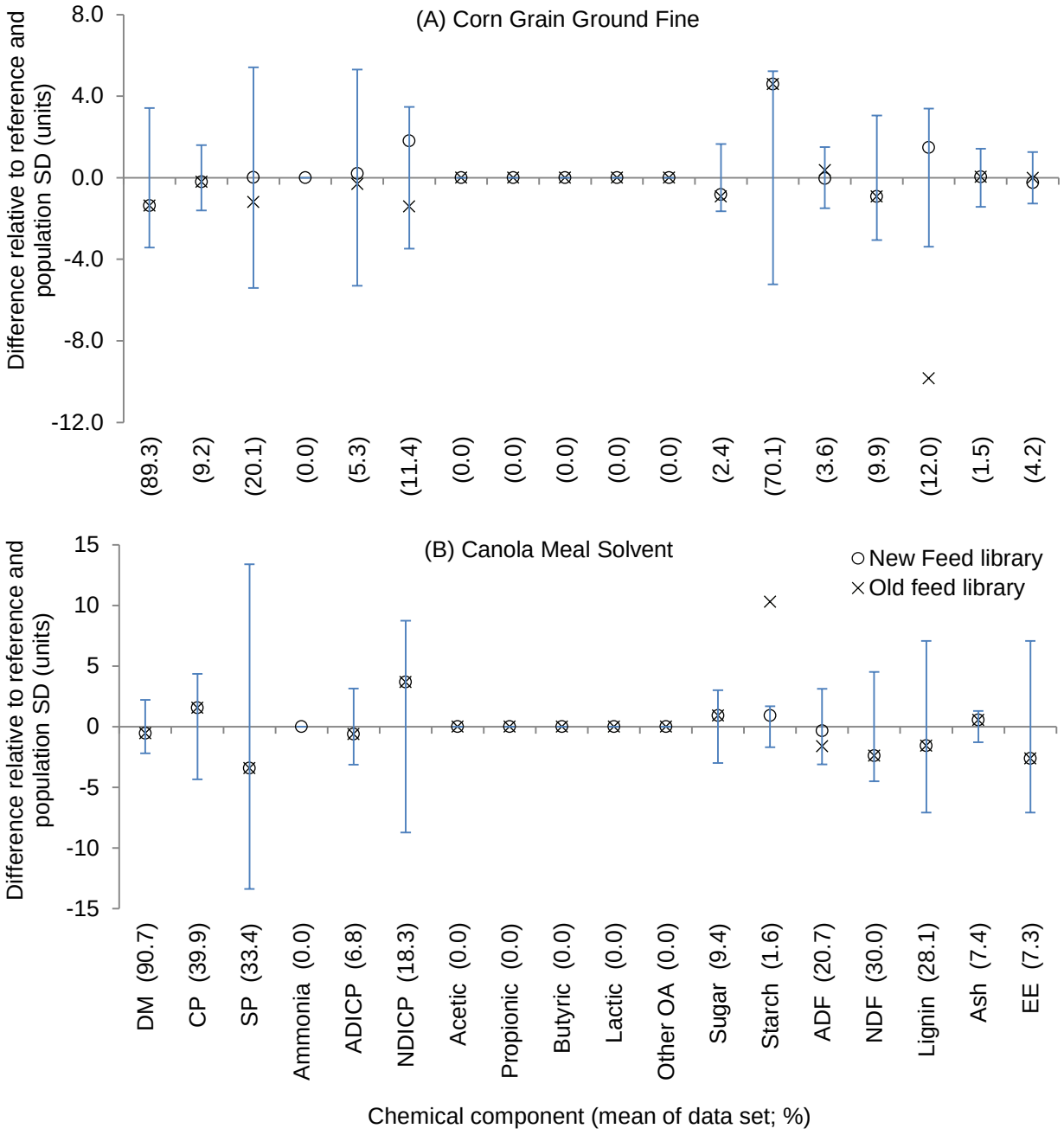
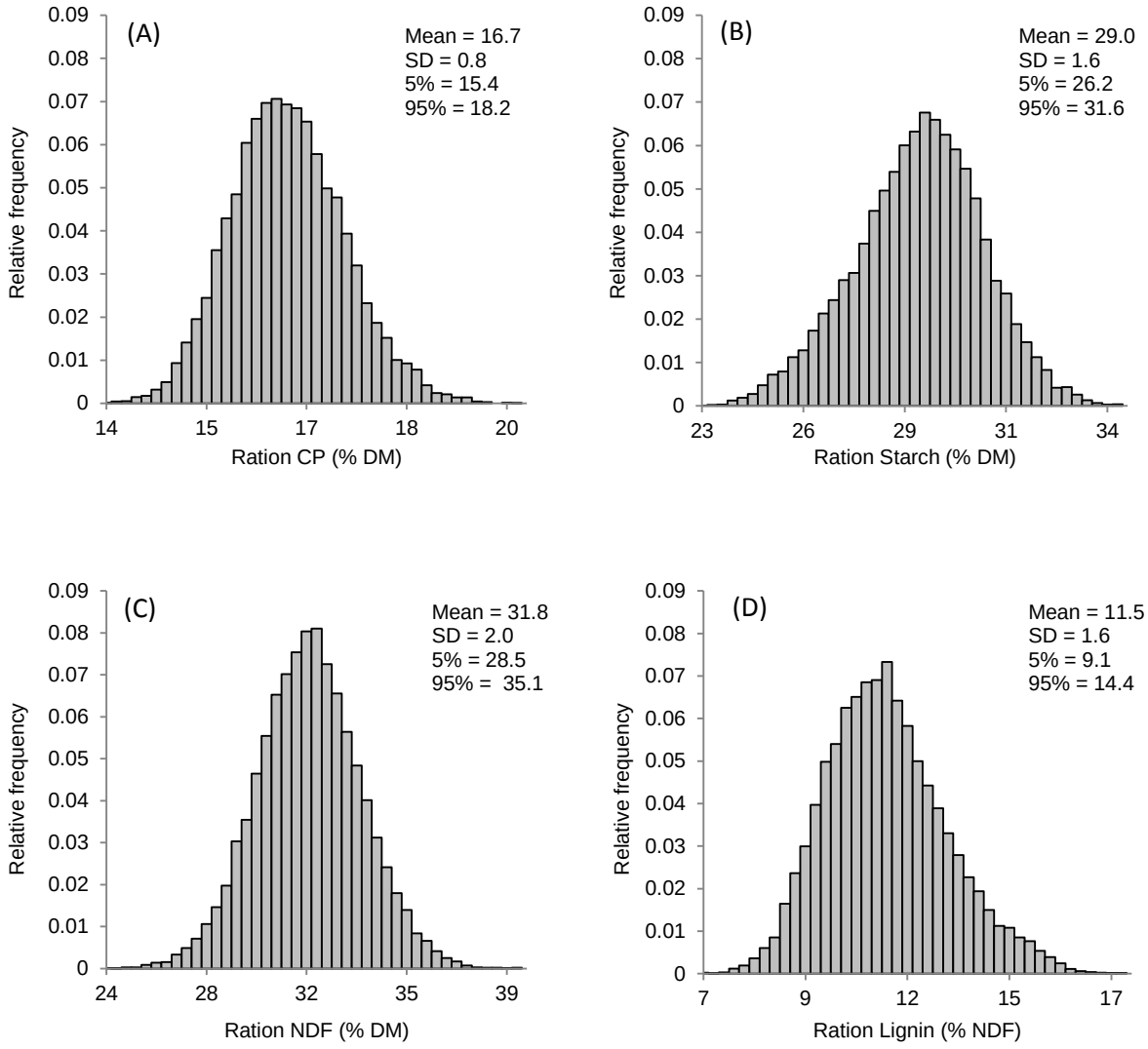


Figure 2.2. Comparison of the relative difference chemical composition between the old (×) and new (○) feed library of two concentrate feeds (A = Corn Grain Ground Fine; B = Canola Meal Solvent) using the mean and SD of the online laboratory data sets as a reference (Cumberland Valley Analytical Services Inc, Maugansville, MD, USA and Dairy One Cooperative Inc, Ithaca, NY, USA). All components are expressed as % DM with the exception of soluble protein (SP; % CP), ammonia (% SP), acid detergent insoluble CP (ADICP; % CP), neutral detergent insoluble CP (NDICP; % CP) and lignin (% NDF).

2.4.7 Model sensitivity to variation in feed chemistry and digestion kinetics

Analyzing model sensitivity to variation in inputs can help users understand where emphasis should be placed when requesting feed analyses and also help identify target areas for investigation if model outputs deviate from expected or observed outcomes. The variation in this study represents an entire population of samples for each feed analyzed over numerous growing seasons. Therefore, the variation encompassed is what might be expected if a user ran a simulation in the CNCPS using feeds from the feed library with no information on actual feed chemistry. The mean, SD and distribution for the components considered in this analysis are in Table 2.6 and are similar to other reports where the same components and feeds are presented (Kertz, 1998, Lanzas et al., 2007a, Lanzas et al., 2007b). Data rarely fit a normal distribution and were more commonly represented by a loglogistic distribution; similar to the findings of Lanzas et al. (2007a, 2007b). The data of some components were skewed and were better represented by distributions such as the Beta, Pearson or Weibull (Table 2.6). When data are skewed, the mean and SD are less appropriate in describing centrality and dispersion of a population (Law and Kelton, 2000). Outputs of deterministic models such as the CNCPS represent an average (Lanzas et al., 2007b), however, when input variation is accounted for, the mean value may no longer represent the most likely value. For example in Figure 2.4A the mean value for ME allowable milk is 34.1 kg/d, however, the most likely value based on frequency of occurrence is 36.3 kg/d. These types of considerations are particularly important when conducting model evaluations as studies rarely report adequate information to complete a robust model simulation (Higgs et al., 2012, Pacheco et al., 2012). Feed library defaults are typically used in place of reported data leading to the type of variation and bias reported in Figures 2.3 and 2.4. Presenting model outputs in the CNCPS as frequency distributions, similar to Figures 2.3 and 2.4, could be useful for aid users in managing risk, particularly when balancing rations close to animal requirements.

Estimating the variation associated with the sampling process, sample handling, preparation, and the variation of the assay itself could be challenging (Hall and Mertens, 2012).



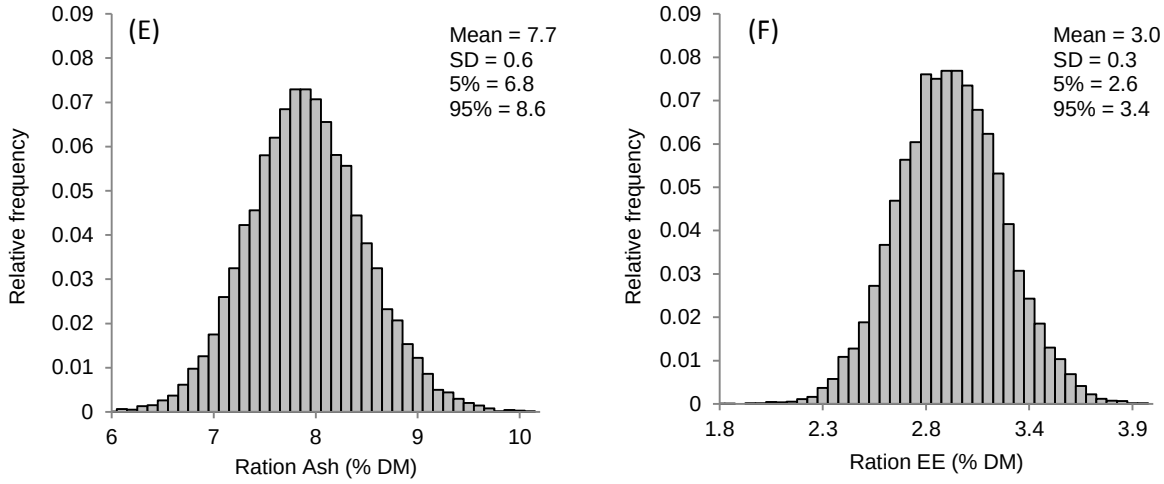


Figure 2.3. Frequency distributions generated from a Monte Carlo simulation for selected chemical components in the reference diet. Each graph displays the range of possible outcomes for each component and the relative likelihood of occurrence.

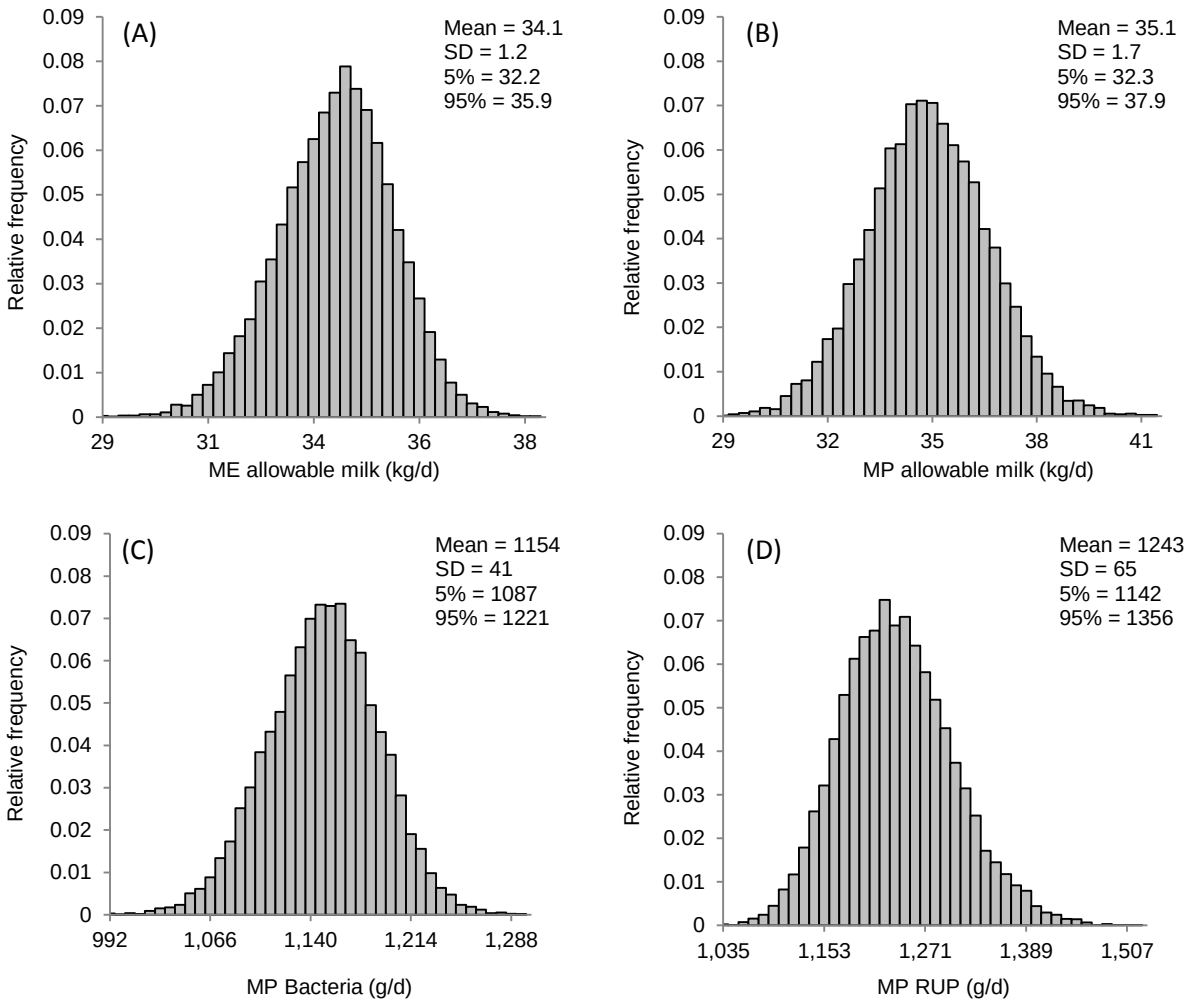


Figure 2.4. Frequency distributions generated from a Monte Carlo simulation for selected model outputs from the reference diet. Each graph displays the range of possible outcomes for each component and the relative likelihood of occurrence.

The relative importance of specific model inputs was also investigated. This part of the analysis included variation from both feed composition and the kd values for the CHO and protein fractions. For this analysis, correlations were not fit to chemical components meaning, during the simulation, values were drawn from probability density functions independently of each other. The rationale for treating components as independent was to understand model

behavior irrespective of biological relationships in feed composition. In doing this, insight can be gained into the lab analyses that are most critical to predict target model outputs.

The chemical components the model was most sensitive to differed among the outputs considered (Figure 2.5). Prediction of ME allowable milk was most sensitive to forage NDF, lignin and ash whereas MP allowable milk was most sensitive to CP along with CHO components and ash. Interestingly, ME allowable milk was negatively correlated to all the items it was most sensitive to with a 1 SD increase in grass hay NDF resulting in a 0.74 kg/d reduction in predicted milk (Figure 2.5A). This behavior can be attributed to aspects of the models internal structure; ME in the CNCPS is calculated using the apparent total digestible nutrient (**TDN**) system described by Fox et al. (2004) where the net energy derived from the diet is empirically calculated from an estimate of total tract nutrient digestion. In this system, carbohydrate intake is calculated by difference according to Eq. 1 in Table 2.2, and total tract nutrient digestion is calculated as the difference between nutrient intake and fecal output. Error in laboratory analysis that forces Eq. 16 to a sum > 100% DM leads to an overestimation of fecal appearance and an underestimation of apparent TDN. Further, because soluble fiber is also calculated by difference (Eq. 5; Table 2.2), an increase in the concentration of any component less digestible than soluble fiber i.e. NDF, results in an increase in fecal nutrient appearance and decrease in apparent TDN. For these reasons, ensuring laboratory results are internally consistent, and adhere to the framework of Eq. 16 is critical for the accurate prediction of ME. Metabolizable protein is derived from a combination of microbial protein and RUP (Sniffen et al., 1992). Predictions of microbial yield are directly related to ruminal CHO digestion (Russell et al., 1992). The prediction of microbial growth was most sensitive to components that affect the quantity and

digestibility of CHO in the rumen (Figure 2.5C). In contrast, sensitivity in RUP prediction was most affected by CP concentration and the concentration of ADICP which defines the indigestible protein fraction (Figure 2.5D).

Ruminal digestion of CHO and protein fractions in the CNCPS are calculated mechanistically according to the relationship originally proposed by Waldo et al. (1972) where: digestion = kd/(kd+kp). Estimations of kd are, therefore, fundamental in predicting nutrient digestion and subsequent model outputs. With the exception of the CB3 kd (Table 2.2), which can be calculated according to Van Amburgh et al. (2003), kd values are not routinely estimated during laboratory analysis. Various techniques exist to estimate kd (Broderick et al., 1988, Nocek, 1988), however, technical challenges restrict their application in commercial laboratories and, thus, library values are generally relied on. Compared to variation in chemical components, predictions of ME were less sensitive to variation in kd, and predictions of MP were more sensitive (Figure 2.6). Predictions of bacterial MP were most sensitive to the rate of starch digestion in both corn grain and corn silage (Figure 2.6C), whereas predictions of RUP were most sensitive to the PB1 kd in soybean meal, corn grain, and blood meal (Figure 2.6D) which agree with the findings of Lanzas et al. (2007a, 2007b). These data demonstrate the importance of kd estimates in the feed library, particularly for the prediction of MP. To improve MP prediction, methods that are practical for commercial laboratories to routinely estimate the kd of starch and protein fractions are urgently needed.

Overall, the prediction of ME allowable milk was more sensitive to variation in the chemical composition compared to MP allowable milk which was more sensitive to variation in kd (Figure

2.7). Model sensitivity to variation in forage inputs was generally higher than concentrates which can be attributed to the variation of the feed itself (Table 2.6), but also the higher inclusion of forage feeds in the reference diet (Table 2.5). The exception was corn grain, which despite having lower variability, had a high inclusion which inflated the impact of its variance. Both variability and dietary inclusion should be considered when deciding on lab analyses to request for input into the CNCPS. Regular laboratory analyses of samples taken on-farm remains the recommended approach to characterizing the components in a ration and reduce the likely variance in the outputs.

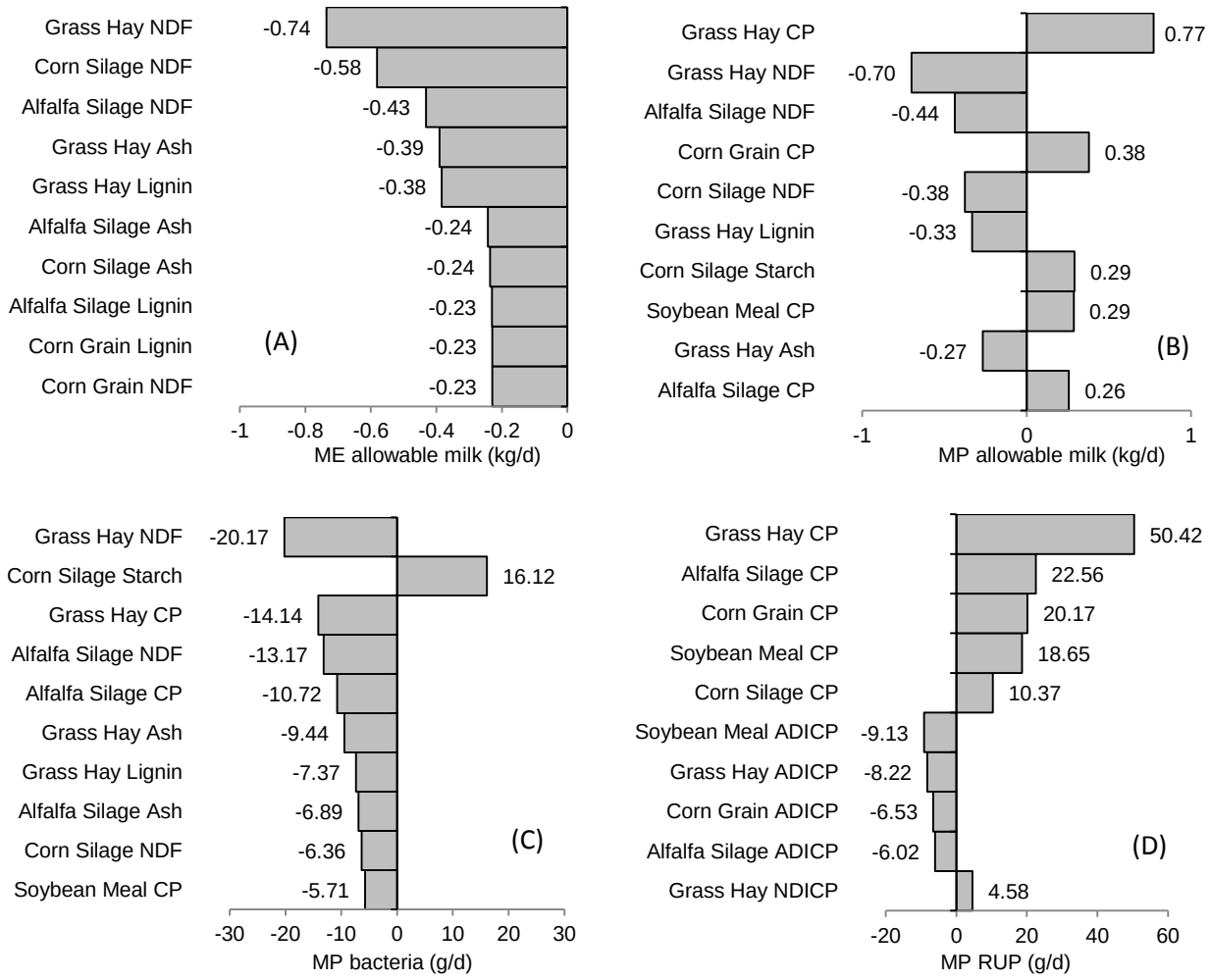


Figure 2.5. Change in model output from a 1 SD increase in the chemical components of feeds used in the reference diet ranked in order of importance.

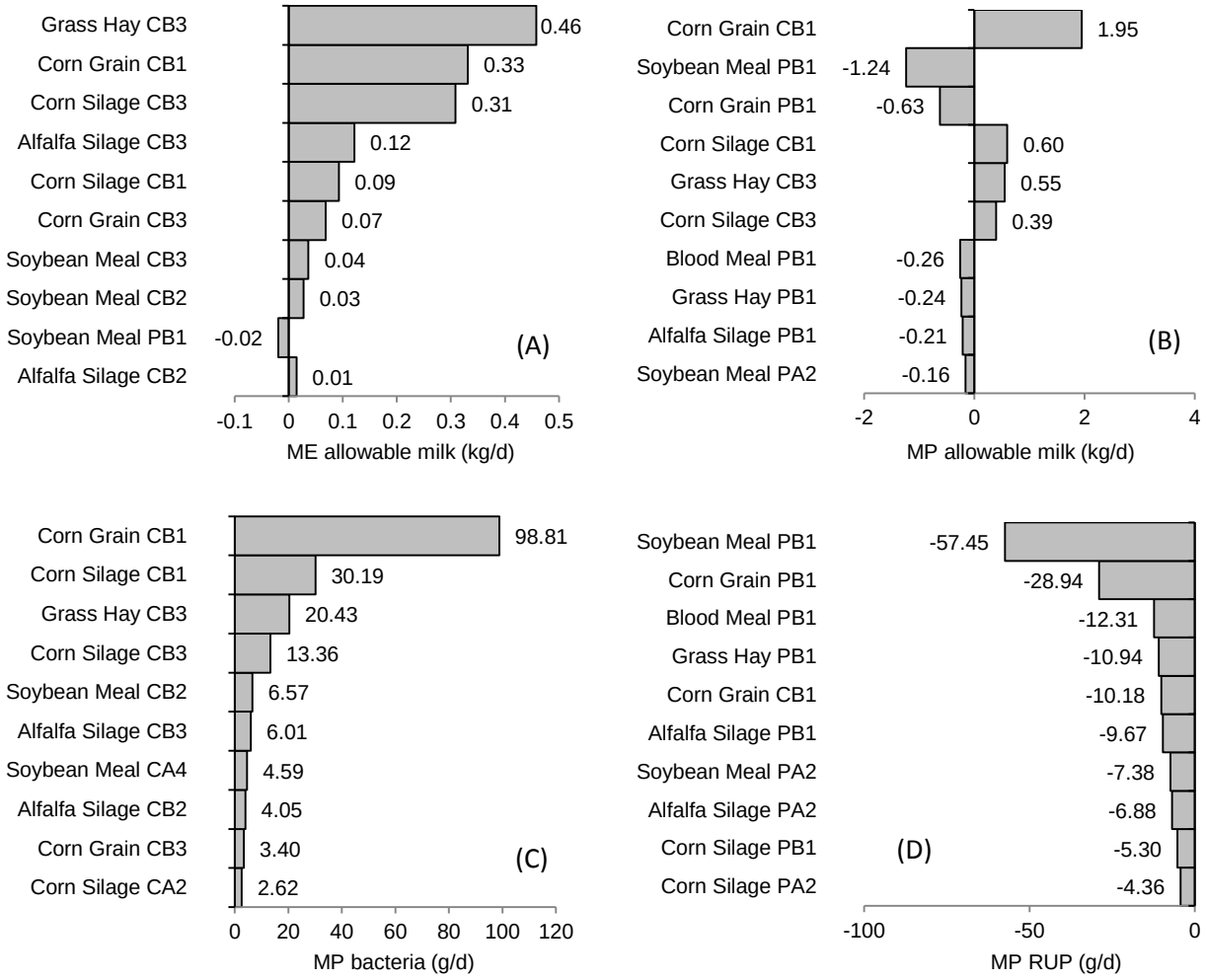


Figure 2.6. Change in model output from a 1 SD increase in the digestion rates of carbohydrate and protein fractions of feeds used in the reference diet ranked in order of importance.

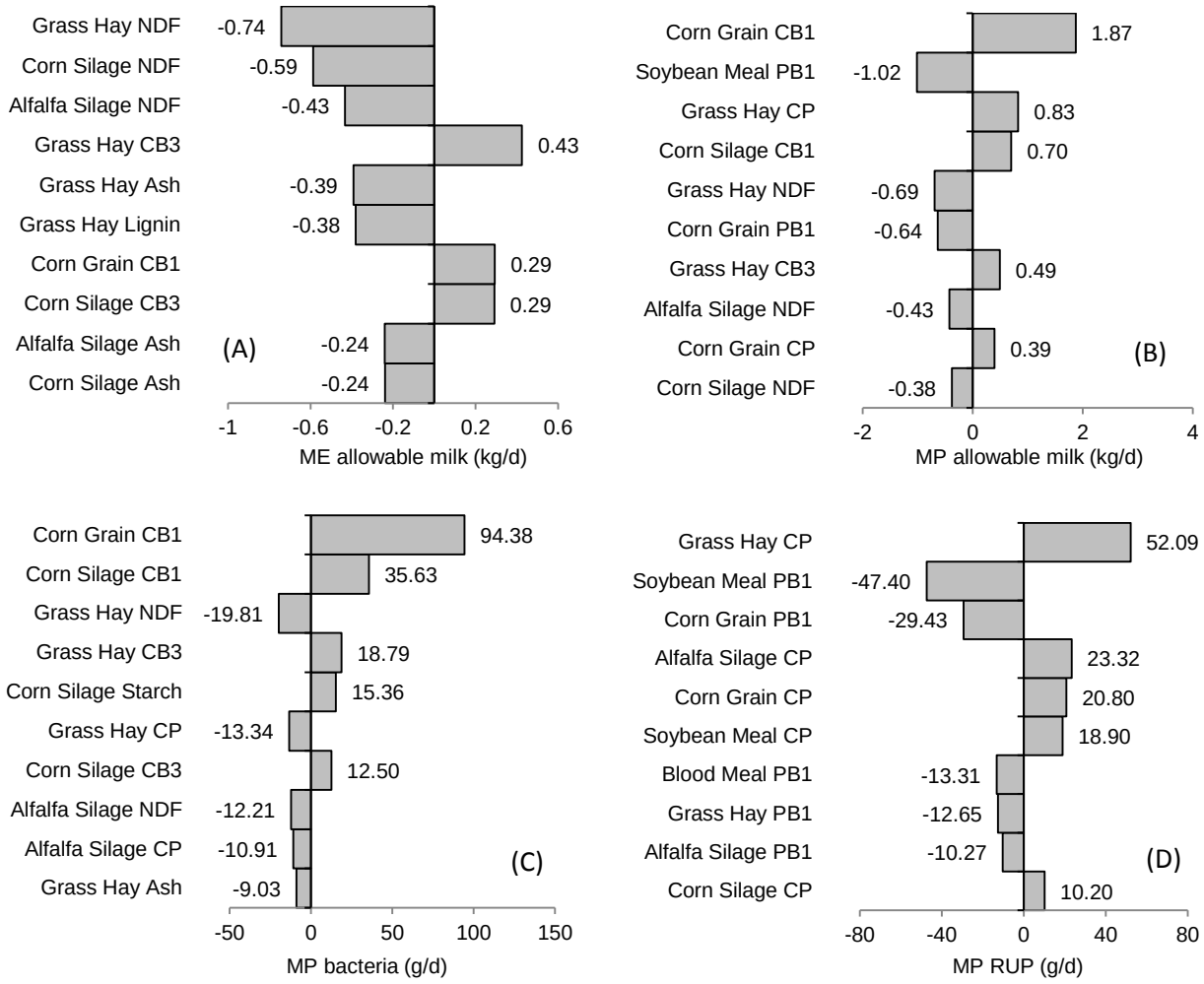


Figure 2.7. Change in model output from a 1 SD increase in both, the chemical components, and digestion rates of carbohydrate and protein fractions of feeds used in the reference diet. Items are ranked in order of importance.

2.5 Conclusion

Chemical components of feeds in the CNCPS feed library have been evaluated and refined using a multi-step process designed to pool data from various sources and optimize feeds to be both internally consistent, and consistent with current laboratory data. When predicting ME, the model is most sensitive to variation in chemical composition, whereas MP predictions are more sensitive to variation in kd. Methods that are practicable for commercial laboratories to routinely

estimate the kd of starch and protein fraction are necessary to improve MP predictions. When using the CNCPS to formulate rations, the variation associated with environmental and management factors, both pre- and post-harvest, should not be overlooked as they can have marked effects on the composition of a feed. Regular laboratory analysis of samples taken on-farm, therefore, remains the recommended approach to characterizing the components in a ration. However, updates to CNCPS feed library provide a database of ingredients that are consistent with current laboratory data and can be used as a platform to, both formulate rations and improve the biology within the model.

2.6 Acknowledgements

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CHAPTER 3: DEVELOPING A DYNAMIC VERSION OF THE CORNELL NET CARBOHYDRATE AND PROTEIN SYSTEM: CARBOHYDRATE AND NITROGEN DIGESTION

3.1 Abstract

The Cornell Net Carbohydrate and Protein System (CNCPS) is a mathematical model used to predict the nutrient supply and requirements of dairy and growing cattle. A new, dynamic version of the CNCPS rumen submodel was constructed in the system dynamics modeling software Vensim®. The new model uses a similar structure to previous versions of CNCPS, but rather than calculating statically, it calculates iteratively over time. The time unit used in the model is hour with integration every 6 minutes and a total simulation time of 300 hours. Carbohydrate and protein digestion in the rumen is estimated using the kinetic relationship between passage and degradation. The lower gut has been expanded from a single compartment with fixed digestion coefficients to a separate small and large intestine. The large intestine is fully mechanistic and follows the same principles of digestion and passage used in the rumen model. Digestion in the small intestine is partially static and partially mechanistic with the implementation of a new system for estimating intestinal digestion of feed protein for non-forage feeds. A new system for calculating urea recycling back to the gastrointestinal tract (GIT) was also constructed. The dynamic framework allows for different meal patterns to be modeled which impact rumen pool sizes of carbohydrate, microbes and nitrogen availability. While new capability is available within the model, the same basic output structure has been maintained to facilitate field application and outputs are generally expressed on a per day basis.

3.2 Introduction

Mathematical models are widely used in animal agriculture to estimate animal requirements and nutrient supply under a range physiological states and production systems (NRC, 2001). The integration of models into computer programs provides a convenient platform to apply biological principles on farms and has helped facilitate improved animal performance and lowered nutrient loss to the environment. The Cornell Net Carbohydrate and Protein System (**CNCPS**) is an example of a model that has integrated understanding of ruminant digestion, physiology and requirements under different environmental and management circumstances to aid farmers and nutritionists in optimizing animal performance (Fox et al., 2004).

The CNCPS was first described in a series of publications outlining carbohydrate and protein digestion (Sniffen et al., 1992), microbial growth (Russell et al., 1992), amino acid supply (O'Connor et al., 1993) and animal requirements (Fox et al., 1992). Since the original publications, updates have continually been made to improve the model capability (Fox et al., 2004, Tylutki et al., 2008, Van Amburgh et al., 2010) with the most recent updates resulting in version 6.5 of the CNCPS (Van Amburgh et al., 2013). This chapter describes a further evolution of the CNCPS into a dynamic framework with a focus on carbohydrate and protein digestion. Microbial growth, amino acid supply, and amino acid requirements are described in subsequent chapters.

3.3 Model description

3.3.1 General model structure

The model is constructed in the system dynamics modeling software Vensim (2010). Vensim uses a diagrammatic interface with embedded mathematical statements and calculates iteratively over time. The time unit used in the development of this model is hour, and the model simulates for 300 hours with integration every 6 minutes. The simulation time used was the shortest period needed for the model to reach dynamic equilibrium or 'steady state' (Sterman, 2000) across a range of diets. The diagrammatic interface of Vensim is convenient and allows for visual critique of the model which aids interpretation. Although acronyms were required given the size of the model (>1200 variables), an effort was made to avoid overly complicated mathematical notation and to make acronyms intuitive. A list of acronyms and abbreviations are in Table 3.1.

Digestion of nutrients in the original CNCPS (Sniffen et al., 1992) followed the system proposed by Waldo et al. (1972) where the kinetics of digestion and passage are integrated to predict substrate digestion. Assuming a single potentially digestible pool, the system can be described by the following equation:

$$\frac{dA}{dt} = -k_1 A - k_2 A$$

where:

A = the amount of potentially digestible substrate in the rumen,

k_1 = the digestion rate,

k_2 = the rate of passage,

t = time in hours.

The derivative of the previous equation gives:

$$R = Ae^{-(k1+k2)t}$$

where, assuming a single feeding:

R = the remaining potentially available substrate present in the rumen after *t* hours,

A = the amount of substrate fed.

Using this system, the ratio of $k_1/(k_1 + k_2)$ gives the fraction of substrate digested in the rumen from a single feeding and has been used to statically capture the dynamics of rumen digestion in both the CNCPS and the protein sub-model of the NRC (2001).

The new rumen sub-model follows the same general system previously used, but because the model is dynamic, rather than static, and calculates continuously, an intake term can be added to the model which allows the estimation of substrate pool size at steady state. The general form of the system is shown in Figure 3.1 and is represented by the equation:

$$\frac{dA}{dt} = k_1A - k_2A - k_3A$$

where:

A = the amount of potentially digestible substrate in the rumen,

k_1 = the rate of substrate intake,

k_2 = the digestion rate,

k_3 = the rate of passage,

t = time in hours.

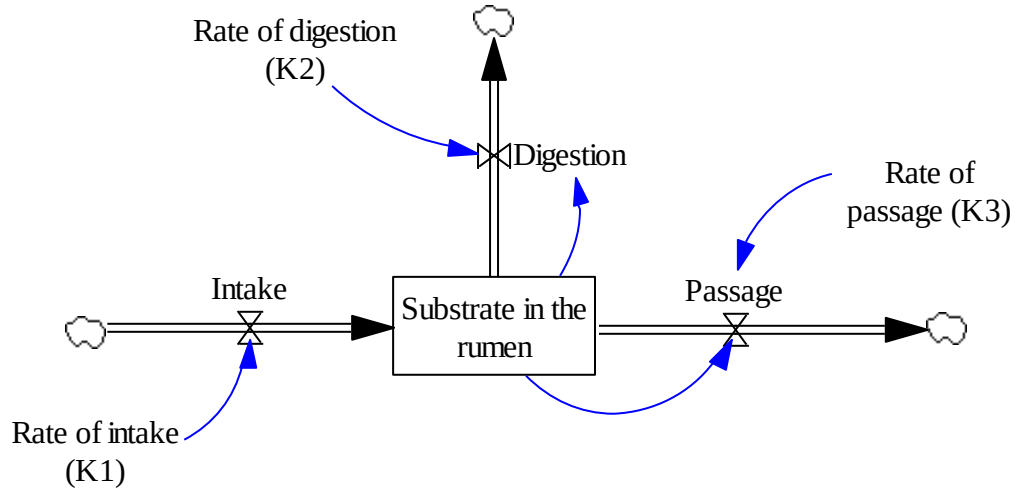

Figure 3.1. Diagram representing the dynamics of substrate digestion in rumen

In previous versions of the CNCPS, material that escapes rumen digestion and arrives in the lower GIT can either be digested or passed out in the feces (Sniffen et al., 1992). This is calculated using an intestinal digestibility coefficient that represents the entire lower GIT. In reality, digestion in the small intestine and large intestine occur by different processes with the small intestine being enzymatic and the large intestine fermentative (Van Soest, 1994). In the current model, digestion in these two compartments has been separated with digestion in the small intestine modeled using a single digestion coefficient, while the large intestine utilizes a mechanistic structure, similar to the rumen model.

Table 3.1.Abbreviations used in the model

Abbreviation	Description
A1 N	Feed ammonia
A1a CHO	Acetic acid
A1b CHO	Butyric acid
A1p CHO	Propionic acid
A2 CHO	Lactic acid
A2 N	Soluble non-ammonia feed N
A3 CHO	Other organic acids
A4 CHO	Water soluble CHO
AA	Amino acids
Ab	Absorbed
B1 CHO	Starch
B1 N	Insoluble non-ammonia feed N
B2 CHO	Soluble fiber
B2 N	Fiber bound non-ammonia feed N
B3 fast CHO	Rapidly degrading NDF
B3 slow CHO	Slowly degrading NDF
C CHO	Indigestible NDF
C N	Undegradable non-ammonia feed N
CHO	Carbohydrate
CW	Bacterial cell wall
Deg	Degradation in the rumen
End N	Endogenous N
EPZ	Entodiniomorphid protozoa
Escape	Escape from the rumen
FB	Fiber bacteria
HPZ	Holotrich protozoa
ID	Digestion in the small intestine
Kd	Rate of fermentation
LI	Large intestine
NA	Bacterial nucleic acids
NAN	Non-ammonia N
NFB	Non-fiber bacteria
NH3	Ammonia
OA	Omasum and abomasum
Out	Passage from the large intestine to the feces
PAA	Peptides and free AA
Pass	Passage from the small intestine to the large intestine
PDV	Portal drained viscera
PZ	Protozoa
R	Rumen
SI	Small intestine

3.3.2 Passage rates

In version 6.5 of the CNCPS, 3 different passage rate equations are used to estimate flows out of the rumen (Seo et al., 2006). Feed fractions are assigned to the most appropriate rate depending on the phase in which they would flow. All soluble fractions are assumed to flow with the liquid phase, while solids are categorized as either forages or concentrates, which have different rates of passage (Seo et al., 2006). The current model includes additional passage rates for NDF. Within the new model structure, all non-NDF material and soluble material use the rates described by Seo et al. (2006). However, NDF in forages and concentrates use equations from NorFor (2011) and are described as follows:

$$kpNDFconc = \left(2.504 + 0.1375 \times \frac{DMI \times 1000}{BW} - 0.02 \times \%\ diet\ conc\right) \times 0.43$$

where:

kpNDFconc = the passage rate of NDF out of the rumen from concentrate feeds (%/hr),

DMI = total dry matter intake (kg/d),

BW = body weight (kg),

% diet conc = proportion of diet DM that is made up of concentrate feeds.

$$kpNDFforage = 0.480 + \frac{1.511}{1 + \left(\frac{DMI \times NDF}{BW \times 7.484}\right)^{-3.198}}$$

where:

kpNDFforage = the passage rate of NDF out of the rumen from forage feeds (%/hr),

DMI = total dry matter intake (kg/d),

BW = body weight (kg),

NDF = diet NDF concentration (g/kg DM).

The expanded system allowed the model to better predict NDF pool sizes in the rumen and also total rumen volume which are likely important for further predictions of chewing and rumination and feed intake.

3.3.3 Carbohydrate digestion

Feeds are assumed to be composed of fat, protein, carbohydrates, ash and water. Carbohydrates and protein are further subdivided into fractions that have similar chemical and physical properties with uniform digestion behaviour (Sniffen et al., 1992). The carbohydrate fractions used in the CNCPS were first defined by Sniffen et al. (1992) and later expanded by Lanzas et al. (2007) to include soluble fiber, volatile fatty acids, lactic acid and other organic acids. The current model uses the same scheme as Lanzas et al. (2007) with an expansion of potentially digestible (**pd**) NDF from a single first order pool, to two pd pools, both first order, but with different rates of digestion. Mertens and Ely (1979) proposed this system as a more appropriate representation of NDF digestion which has been supported by numerous studies

(Ellis et al., 2005, Huhtanen et al., 2008, Van Milgen et al., 1991), and therefore, implemented in this model. The size of each pool and associated digestion rate can be calculated using the system described by Raffrenato and Van Amburgh (2010). If data are not available to estimate two pools of pdNDF the model will assume a single pool consistent with current model behaviour except for the use of uNDF in place of lignin * 2.4 as the estimate of unavailable NDF. The required CHO inputs are in Table 3.2 and the expected analytical methods to estimate the chemical fractions are defined in Chapter 2.

Other model inputs include fermentation rates and coefficients for intestinal digestibly. Typically, library values are used for these inputs with the exception of pdNDF (see Chapter 2). The feed library used by this model is the same as that described in Chapter 2 with the exception of the intestinal digestibility coefficients used for the digestion of the B2, B3 slow and B3 fast CHO fractions (Table 3.2). Mammals lack the carbohydrases needed to digest structural and soluble fiber components in the small intestine (Van Soest, 1994). Because in this model the lower gut has been separated into a small and large intestine, the intestinal digestion coefficients for the fiber fractions were set to 0 and any post-ruminal digestion estimated mechanistically in the large intestine.

The large intestine is modeled using a similar structure to the rumen where the extent of digestion is determined from the rate of digestion and the rate of passage through the compartment. Digestion rates in the large intestine were assumed to be the same as in the rumen given a similar population of bacteria exist in the large intestine (Van Soest, 1994). However, limited data exist to estimate the transit time through the large intestine. Version 6 of the CNCPS

assumes a fixed post ruminal fiber digestion of 20% which would occur exclusively in the large intestine (Fox et al., 2004). Using these assumptions, transit time can be estimated by rearranging the relationship described by Waldo et al. (1972) where:

$$Digestion = \frac{kd}{kd + kp}$$

becomes,

$$kp = \frac{kd}{Digestion} - kd \qquad or, \qquad Transit\ time = \frac{kd}{Post\ ruminal\ digestion} - kd$$

In general, pdNDF from corn silage has a mean digestion rate of approximately 3.5%/hr in the CNCPS feed library which implies a transit time of 14%/hr (Mean retention time (**MRT**) of 7.1 hours). In sheep, MRT in the large intestine ranges from >20 to <10 hours and decreases with level of intake (Coombe and Kay, 1965, Grovum and Hecker, 1973). Similar results have been found in dairy cattle where MRT can range from 22.5 to 7.2 hours for the lower gut as a whole (Colucci et al., 1982, Huhtanen and Kukkonen, 1995, Mambrini and Peyraud, 1997). Therefore, the value of 14%/hr extrapolated from version 6 of the CNPCS is probably reasonable for lactating cows. No difference has been observed in the MRT of solids and liquids past the duodenum which suggests a single transit factor is appropriate (Huhtanen and Kukkonen, 1995, Mambrini and Peyraud, 1997).

A generalized summary of CHO digestion in the model is in Figure 3.2 which shows entry into the rumen (CHO intake), followed by protozoal engulfment (CHO R Engulfment), bacterial

degradation (CHO R Deg) or escape (CHO Escape). Material engulfed by protozoa is either returned to the rumen pool as protozoa lyse (PZ CHO Engulfed Recycled), digested (PZ CHO Deg) or can escape within the protozoa as they flow out of the rumen (PZ CHO Escape). Once in the small intestine, material is either digested according to a static digestion coefficient (CHO ID) or passes through to the large intestine (CHO Pass). In the large intestine it will either pass out in the feces (CHO Out) or can be further digested by bacteria (CHO LI Deg). A complete list of model carbohydrate pools, organized by compartment is in Table 3.3 and a complete list of flows is in Table 3.4. The equations used to calculate the pools and flows are in Tables 3.8 and 3.9.

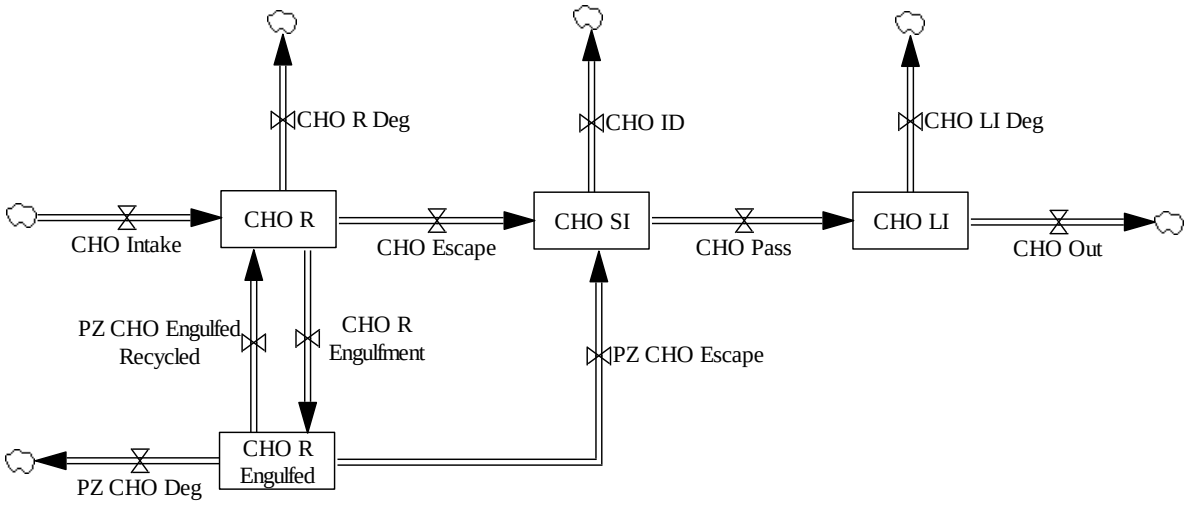

Figure 3.2. Generalized summary of carbohydrate digestion through each compartment of the model. Boxes represent pools and arrows represent flows. For definitions of abbreviations see Table 3.1.

Table 3.2. Model inputs for carbohydrate digestion.

Variable[1]	Units	Description
g A1a CHO*i*	g/d	Daily acetate intake
g A1b CHO*i*	g/d	Daily butyrate intake
g A1p CHO*i*	g/d	Daily propionate CHO intake
g A2 CHO*i*	g/d	Daily lactate CHO intake
g A3 CHO*i*	g/d	Daily intake of other organic acids
g A4 CHO*i*	g/d	Daily water soluble CHO intake
g B1 CHO*i*	g/d	Daily starch intake
g B2 CHO*i*	g/d	Daily soluble fiber intake
g B3 fast CHO*i*	g/d	Daily rapidly degrading NDF intake
g B3 slow CHO*i*	g/d	Daily slowly degrading NDF intake
g C CHO*i*	g/d	Daily indigestible NDF intake
Kd A2 CHO*i*	%/hr	Rate of A2 CHO fermentation
Kd A3 CHO*i*	%/hr	Rate of A3 CHO fermentation
Kd A4 CHO*i*	%/hr	Rate of A4 CHO fermentation
Kd B1 CHO*i*	%/hr	Rate of B1 CHO fermentation
Kd B2 CHO*i*	%/hr	Rate of B2 CHO fermentation
Kd B3 fast CHO*i*	%/hr	Rate of B3 fast CHO fermentation
Kd B3 slow CHO*i*	%/hr	Rate of B3 slow CHO fermentation
Kd C CHO*i*	%/hr	Proportion of C CHO digested in the SI
ID A1 CHO*i*	% CHO	Proportion of A1 CHO digested in the SI
ID A2 CHO*i*	% CHO	Proportion of A2 CHO digested in the SI
ID A3 CHO*i*	% CHO	Proportion of A3 CHO digested in the SI
ID A4 CHO*i*	% CHO	Proportion of A4 CHO digested in the SI
ID B1 CHO*i*	% CHO	Proportion of B1 CHO digested in the SI
ID B2 CHO*i*	% CHO	Proportion of B2 CHO digested in the SI
ID B3 fast CHO*i*	% CHO	Proportion of B3 fast CHO digested in the SI
ID B3 slow CHO*i*	% CHO	Proportion of B3 slow CHO digested in the SI
ID C CHO*i*	% CHO	Proportion of C CHO digested in the SI

[1] Subscript *i* refers to the i^{th} feed in the diet.

Table 3.3. Carbohydrate pools by compartment in the model. Units for all items are g of carbohydrate.

Compartment	Pool[1]	Description
Rumen		
	A1a CHO R*i*	A1a CHO in the rumen
	A1b CHO R*i*	A1b CHO in the rumen
	A1p CHO R*i*	A1p CHO in the rumen
	A2 CHO R*i*	A2 CHO in the rumen
	A3 CHO R*i*	A3 CHO in the rumen
	A4 CHO R*i*	A4 CHO in the rumen
	B1 CHO R*i*	B1 CHO in the rumen
	B2 CHO R*i*	B2 CHO in the rumen
	B3 fast CHO R*i*	B3 fast CHO in the rumen
	B3 slow CHO R*i*	B3 slow CHO in the rumen
	C CHO R*i*	C CHO in the rumen
Small Intestine		
	A1a CHO SI*i*	A1a CHO in the small intestine
	A1b CHO SI*i*	A1b CHO in the small intestine
	A1p CHO SI*i*	A1p CHO in the small intestine
	A2 CHO SI*i*	A2 CHO in the small intestine
	A3 CHO SI*i*	A3 CHO in the small intestine
	A4 CHO SI*i*	A4 CHO in the small intestine
	B1 CHO SI*i*	B1 CHO in the small intestine
	B2 CHO SI*i*	B2 CHO in the small intestine
	B3 fast CHO SI*i*	B3 fast CHO in the small intestine
	B3 slow CHO SI*i*	B3 slow CHO in the small intestine
	C CHO SI*i*	C CHO in the small intestine
Large intestine		
	A4 CHO LI*i*	A4 CHO in the large intestine
	B1 CHO LI*i*	B1 CHO in the large intestine
	B2 CHO LI*i*	B2 CHO in the large intestine
	B3 fast CHO LI*i*	B3 fast CHO in the large intestine
	B3 slow CHO LI*i*	B3 slow CHO in the large intestine
	C CHO LI*i*	C CHO in the large intestine

[1] Subscript *i* refers to the i^{th} feed in the diet.

Table 3.4. Carbohdrate flows in the model by compartment. Units for all flows are g CHO/hr.

Compartment	Flow[1]	Description
Flows into and within the rumen		
	A1a CHO Intake*i*	Intake of A1a CHO
	A1b CHO Intake*i*	Intake of A1b CHO
	A1p CHO Intake*i*	Intake of A1p CHO
	A2 CHO Intake*i*	Intake of A2 CHO
	A3 CHO Intake*i*	Intake of A3 CHO
	A4 CHO Intake*i*	Intake of A4 CHO
	B1 CHO Intake*i*	Intake of B1 CHO
	B2 CHO Intake*i*	Intake of B2 CHO
	B3 fast CHO Intake*i*	Intake of B3 fast CHO
	B3 slow CHO Intake*i*	Intake of B3 slow CHO
	C CHO Intake*i*	Intake of C CHO
	A4 CHO Engulfment*i*	A4 CHO engulfed by HPZ
	B1 CHO Engulfment*i*	B1 CHO engulfed by EPZ
	B2 CHO Engulfment*i*	B2 CHO engulfed by EPZ
	B3 fast CHO Engulfment*i*	B3 fast CHO engulfed by EPZ
	B3 slow CHO Engulfment*i*	B3 slow CHO engulfed by EPZ
	C CHO Engulfment*i*	C CHO engulfed by EPZ
	HPZ A4 Engulfed Recycled*i*	Engulfed A4 CHO released back to the rumen
	EPZ B1 Engulfed Recycled*i*	Engulfed B1 CHO released back to the rumen
	EPZ B2 Engulfed Recycled*i*	Engulfed B2 CHO released back to the rumen
	EPZ B3 fast Engulfed Recycled*i*	Engulfed B3 fast CHO released back to the rumen
	EPZ B3 slow Engulfed Recycled*i*	Engulfed B3 slow CHO released back to the rumen
	EPZ C Engulfed Recycled*i*	Engulfed C CHO released back to the rumen
Rumen disappearance		
	A1a CHO R Ab*i*	A1a CHO absorbed in the rumen
	A1b CHO R Ab*i*	A1b CHO absorbed in the rumen
	A1p CHO R Ab*i*	A1p CHO absorbed in the rumen
	A2 CHO R Deg*i*	A2 CHO degraded in the rumen
	A3 CHO R Deg*i*	A3 CHO degraded in the rumen
	A4 CHO R Deg*i*	A4 CHO degraded in the rumen
	B1 CHO R Deg*i*	B1 CHO degraded in the rumen
	B2 CHO R Deg*i*	B2 CHO degraded in the rumen
	B3 fast CHO R Deg*i*	B3 fast CHO degraded in the rumen
	B3 slow CHO R Deg*i*	B3 slow CHO degraded in the rumen
	A1a CHO Escape*i*	A1a CHO escaping from the rumen to the SI
	A1b CHO Escape*i*	A1b CHO escaping from the rumen to the SI
	A1p CHO Escape*i*	A1p CHO escaping from the rumen to the SI
	A2 CHO Escape*i*	A2 CHO escaping from the rumen to the SI
	A3 CHO Escape*i*	A3 CHO escaping from the rumen to the SI
	A4 CHO Escape*i*	A4 CHO escaping from the rumen to the SI
	B1 CHO Escape*i*	B1 CHO escaping from the rumen to the SI
	B2 CHO Escape*i*	B2 CHO escaping from the rumen to the SI
	B3 fast CHO Escape*i*	B3 fast CHO escaping from the rumen to the SI

Table 3.4 *(Continued)*

Compartment	Flow[1]	Description
	B3 slow CHO Escape*i*	B3 slow CHO escaping from the rumen to the SI
	C CHO Escape*i*	C CHO escaping from the rumen to the SI
	HPZ A4 Escape*i*	A4 CHO escaping in HPZ
	EPZ B1 Escape*i*	B1 CHO escaping in EPZ
	EPZ B2 Escape*i*	B2 CHO escaping in EPZ
	EPZ B3 fast Escape*i*	B3 fast CHO escaping in EPZ
	EPZ B3 slow Escape*i*	B3 slow CHO escaping in EPZ
	EPZ C Escape*i*	C CHO escaping in EPZ
Disappearance from the SI		
	A1a CHO ID*i*	A1a CHO digested in the SI
	A1b CHO ID*i*	A1b CHO digested in the SI
	A1p CHO ID*i*	A1p CHO digested in the SI
	A2 CHO ID*i*	A2 CHO digested in the SI
	A3 CHO ID*i*	A3 CHO digested in the SI
	A4 CHO ID*i*	A4 CHO digested in the SI
	B1 CHO ID*i*	B1 CHO digested in the SI
	B2 CHO ID*i*	B2 CHO digested in the SI
	B3 fast CHO ID*i*	B3 fast CHO digested in the SI
	B3 slow CHO ID*i*	B3 slow CHO digested in the SI
	C CHO ID*i*	C CHO digested in the SI
	A4 CHO Pass*i*	A4 CHO Passing from the SI to LI
	B1 CHO Pass*i*	B1 CHO Passing from the SI to LI
	B2 CHO Pass*i*	B2 CHO Passing from the SI to LI
	B3 fast CHO Pass*i*	B3 fast CHO Passing from the SI to LI
	B3 slow CHO Pass*i*	B3 slow CHO Passing from the SI to LI
	C CHO Pass*i*	C CHO Passing from the SI to LI
Disappearance from the LI		
	A4 CHO LI Deg*i*	A4 CHO degrading in the LI
	B1 CHO LI Deg*i*	B1 CHO degrading in the LI
	B2 CHO LI Deg*i*	B2 CHO degrading in the LI
	B3 fast CHO LI Deg*i*	B3 fast CHO degrading in the LI
	B3 slow CHO LI Deg*i*	B3 slow CHO degrading in the LI
	A4 CHO Out*i*	A4 CHO passing out in the feces
	B1 CHO Out*i*	B1 CHO passing out in the feces
	B2 CHO Out*i*	B2 CHO passing out in the feces
	B3 fast CHO Out*i*	B3 fast CHO passing out in the feces
	B3 slow CHO Out*i*	B3 slow CHO passing out in the feces
	C CHO Out*i*	C CHO passing out in the feces

[1] Subscript *i* refers to the i^{th} feed in the diet.

3.3.4 Nitrogen digestion

Protein digestion and metabolism in previous versions of the CNCPS (Fox et al., 2004, Sniffen et al., 1992, Tylutki et al., 2008), the NRC (2001) and throughout the literature are typically expressed on a CP basis. The concept of CP assumes all protein matter is 16% N and the mass of protein can be calculated by multiplying N by a factor of 6.25 (NRC, 1985). Nitrogen components in feeds comprise of AA, nitrates, phenolic compounds, ammonia and other by-products of the ensiling process (Van Soest, 1994) which vary greatly in the concentration of N on a molecular weight basis. For example ammonia is approximately 82% N whereas nitrate is 23% N (Nelson et al., 2008). Differences also exist among individual AA with Phe and Arg having 8% and 32% N, respectively (Nelson et al., 2008). Therefore, the mass of protein can vary depending on the relative contribution of the fractions that make up the protein. This variance is most important for calculations that require protein to be expressed on a mass basis. An example is the calculation of ME in the CNCPS using apparent total digested nutrients (**TDN**; Fox et al., 2004, NRC, 2001). The TDN system calculates the net disappearance of carbohydrates, protein and fat along the digestive tract by subtracting fecal output from what was consumed from the diet (Fox et al., 2004, NRC, 2001). Fecal protein is comprised of undigested feed, microbial debris from the rumen, microbes grown in the large intestine and endogenous secretions into the gut (Higgs et al., 2012, Marini et al., 2008). Considering only the bacterial fraction, cell wall material and true protein have mass factors of 14 and 6.67, respectively (Van Soest, 1994). Mason (1969) concluded up to 81% of the non-dietary fecal nitrogen was of bacterial origin, mostly originating from the rumen. True bacterial protein is considered highly digestible (Storm et al., 1983a), therefore, much of the bacterial N appearing in the feces would be bacterial cell wall. Consequently, using a factor of 6.25 to estimate the mass of fecal protein is inappropriate and will influence the prediction of ME. Complications also arise in predicting AA

supply. The CNCPS currently expresses AA relative to CP on a whole feed basis (see Chapter 2). This is essentially the same as expressing them relative to N as CP is a factor of N. However, the concentration of AA relative to N, in many cases, will be different in RUP to what was consumed (Ross, 2013). Therefore, using AA profiles expressed relative to CP (or N) to predict AA supply to the animal can introduce error. This is discussed in more detail in Chapter 6. However, to avoid the complications from using CP, protein digestion and supply in this model is calculated entirely on an N basis and is reconciled by compartment to ensure N balance through the model is consistent with the amount of N entering and leaving the compartment, thus conserving mass. This was not possible when using percentages of CP among fractions and moving through compartments, because using that procedure introduced bias as digestion occurred.

The required inputs into the model follow the same structure as described for carbohydrates with N intake being split into five chemically determined fractions. The fractionation of feed N follows the same general scheme outlined by Sniffen et al. (1992) with refinements outlined in Van Amburgh et al. (2007) and in Chapter 2 of this dissertation. Digestion rates and intestinal digestion coefficients are required for each fraction and are listed in Table 3.5.

Table 3.5. Model inputs for nitrogen digestion.

Inputs[1]	Units	Description
g A1 N*i*	g/d	Daily ammonia N intake
g A2 N*i*	g/d	Daily soluble non-ammonia N intake
g B1 N*i*	g/d	Daily insoluble available N intake (Total N – Soluble N – ND insoluble N)
g B2 N*i*	g/d	Daily fiber bound N intake (ND insoluble N – AD insoluble N)
g C N*i*	g/d	Daily unavailable N intake (AD insoluble N)
Kd Urea	%/hr	Rate of urea degradation
Kd PAA N R	%/hr	Rate of peptide and free AA degradation
Kd A1 N*i*	%/hr	Rate of A1 N degradation
Kd A2 N*i*	%/hr	Rate of A2 N degradation
Kd B1 N*i*	%/hr	Rate of B1 N degradation
Kd B2 N*i*	%/hr	Rate of B2 N degradation
Kd C N*i*	%/hr	Rate of C N degradation
ID A2 N*i*	%	Proportion of A2 N digested in the SI
ID B1 N*i*	%	Proportion of B1 N digested in the SI
ID B2 N*i*	%	Proportion of B2 N digested in the SI
ID C N*i*	%	Proportion of C N digested in the SI

[1] Subscript *i* refers to the i^{th} feed in the diet.

The digestion of feed N in the rumen follows the same kinetic principles outlined in Figure 3.1. Total nitrogen entering the rumen comes from a number of sources including feed, recycled urea and endogenous secretions (Lapierre et al., 2005). Complex N transactions exist within the rumen which are a result of microbial growth and the interactions among the various microbial populations (Firkins et al., 2007, NRC, 2001). A generalized summary of the rumen N pools and transactions represented in the current model are in Figure 3.3. Nitrogen pools are organized according to state and include undigested feed N (Feed N R), peptides and free AA (PAA N R), ammonia (NH3 N R), undegraded endogenous secretions (End N R), cellular N from non-fiber bacteria (NFB Cell N), fiber bacteria (FB Cell N), protozoa (PZ Cell N) and N engulfed by protozoa (PZ N Engulfed).

Nitrogen escapes the rumen in various forms with the rate of escape being linked to the phase in which the form would flow i.e. with the liquid, solids, or bound to fiber. Ammonia can escape

with the liquid (NH3 N R Escape) or be absorbed directly through the rumen wall (NH3 N R Ab). Feed protein can escape undegraded (Feed N Escape) or as peptides and free AA which flow with the liquid phase (PAA N R Escape). Peptides and free AA come from a variety of sources (feed, endogenous, protozoa or bacteria consumed and excreted by protozoa) which are individually tracked within the model. Microbial N escapes with the solids passage rate. Microbial transactions are explained in more detail in Chapter 4.

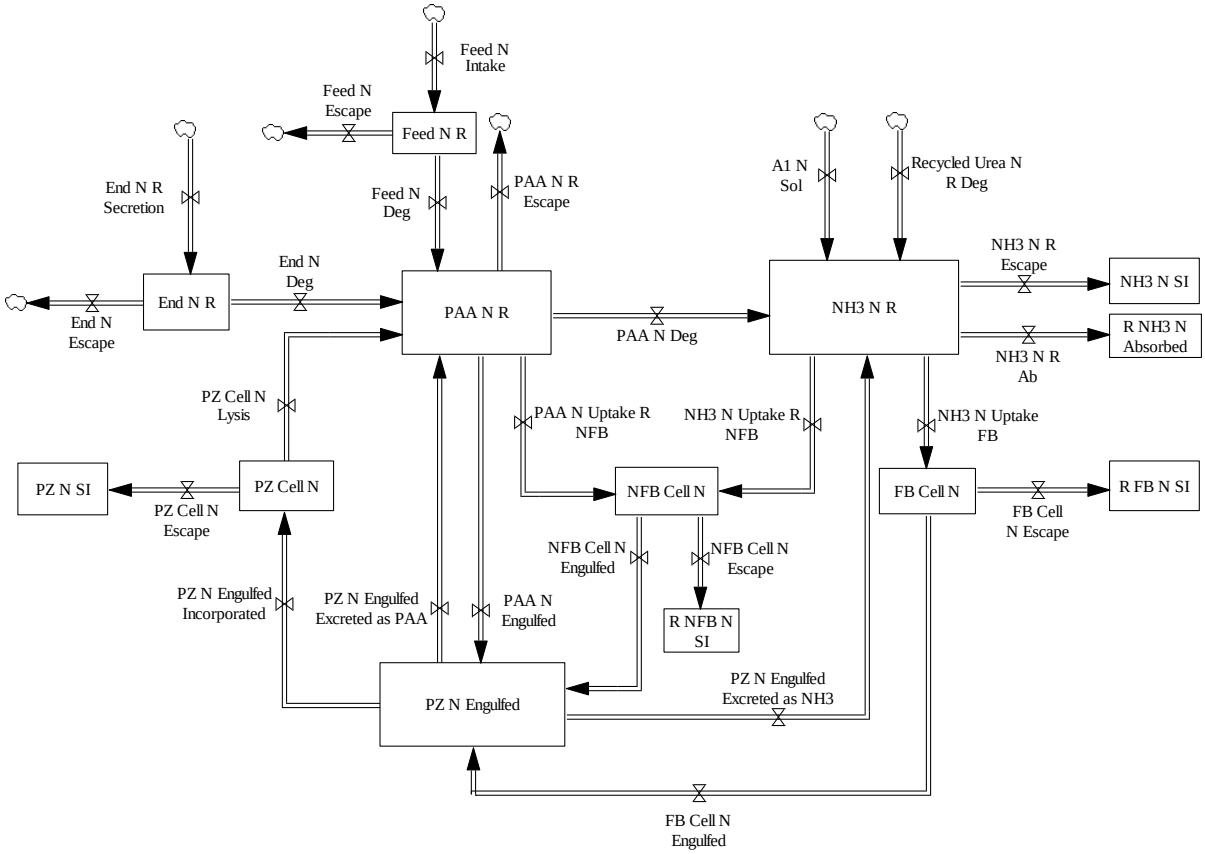

Figure 3.3. Nitrogen transactions in the rumen model. Boxes represent pools and arrows represent flows. For definitions of abbreviations see Table 3.1.

Nitrogen appearing in the small intestine can either be digested or passed through into the large intestine undegraded. The model has capability to calculate feed N digestion using two different systems:

System 1: Uses the same system originally described by Sniffen et al. (1992) and used by all subsequent versions of the CNCPS (Fox et al., 2004, Tylutki et al., 2008, Van Amburgh et al., 2010) where each nitrogen fraction has a fixed digestibility coefficient: 100, 100, 80 and 0 for the A2, B1, B2 and C fractions, respectively, which are used to estimate N absorption in the small intestine. The weighted mean of the proportional contribution of each fraction to the total feed N escaping the rumen and the respective digestibility coefficients gives the digestibility of undegraded feed N.

System 2: Calculates intestinal digestibility using an estimation of indigestible N from the assay developed by Ross (2013), and total model predicted feed N escaping the rumen, as summarized in the following equation:

$$Intestinal\ digestibility_i = 1 - \left(\frac{Indigestible\ N_i}{A2\ N_i + B1\ N_i + B2\ N_i + C\ N_i + PAA\ N_i}\right)$$

where:

i represents the ith feed in the diet,

indigestible N is estimated using the assay of Ross (2013),

A2 N, B1 N, B2 N and C N represent model predicted N escape for each fraction.

This system recognizes that variation in protein digestion in the small intestine exists which is not adequately captured using static digestibility coefficients (Calsamiglia and Stern, 1995, Ross, 2013, Stern et al., 1985, Waltz et al., 1989). The assay for estimating indigestible N was designed to mimic N digestion in three gastrointestinal compartments beginning with an in-vitro

rumen fermentation, followed by acidification and incubation with pepsin to mimic the abomasum, and finally a neutral incubation with trypsin, chymotrypsin, amylase and lipase, to mimic the small intestine (Ross, 2013). The assay was designed for application in a commercial setting to routinely generate model inputs and appears highly sensitive to variation among and within feeds (Ross, 2013).

Microbial N reaching the small intestine is partitioned into AA N, nucleic acid N and residual cell wall N. There is no clear consensus in the literature on the digestibility of individual microbial components. Some studies have indicated microbial cell wall N is largely indigestible (Mason, 1969, 1978) while others have suggested it is readily available (Bird, 1972, Hoogenraad and Hird, 1970). Bacterial cell wall comprises of both AA and glucosamines, similar to the shells of shellfish (Van Soest, 1994), so it seems reasonable to assume digestion of the glucosamine fraction would be limited. Russell et al. (1992) assumed 15% of cell N is nucleic acid N, 25% is cell wall N and 60% is N from true protein. Of these three fractions, nucleic acid and true protein N were assumed to be completely available and cell wall N completely unavailable (Russell et al., 1992). In the current model, the original system has been maintained with some modification: True protein N is now total AA N and is assumed to be 67% of total N as reported by Clark et al. (1992), nucleic acid N remains at 15% which is consistent with other literature reports (Czerkawski, 1976), and cell wall N is calculated by difference. The same digestibility coefficients were used for each fraction as in Russell et al. (1992). Using this system, the weighted mean of bacterial N digestion is approximately 80% which is similar to the measurements of Storm et al. (1983b) and Fonseca et al. (2014).

Transactions of N once absorbed are summarized in Figure 3.4. Non-ammonia N absorbed in the small intestine (NAN Ab to PDV) is assumed to have two general fates: 1) it is utilized for a function of maintenance or production (Liver NAN Utilized) or, 2) it is converted to urea in the liver (Liver NAN to Urea). Nitrogen requirements for maintenance or production include milk, growth, reserves, fetal growth, scurf, metabolic urinary losses and gut secretions. Absorbed NH_3-N is assumed to be completely converted to urea in the liver (PDV NH3 to Urea). Nitrogen converted to urea can either be returned to the gut (Urea N Liver Recycled to the Gut), or excreted in the urine (Urea N Liver Irreversible Loss). The proportion of urea returned to the GIT relative to urea production is remarkably uniform among experiments when animal are fed diets at, or in moderate excess of MP requirements (Lapierre et al., 2004, Ouellet et al., 2004, Recktenwald, 2007, Valkeners et al., 2007). However, recycling increases when N supply is limited (Reynolds and Kristensen, 2008, Valkeners et al., 2007) and decreases when N supply is greatly in excess (Lapierre et al., 2004, Reynolds and Kristensen, 2008). To estimate urea recycling in the model, the equations presented in Recktenwald et al. (2014) and Reynolds and Kristensen (2008) were used in combination. Recktenwald et al. (2014) showed a linear relationship between urea production and urea recycling in high producing cows fed diets ranging from 15% - 17% CP, while, Reynolds and Kristensen (2008) showed an increase in the proportion of urea recycled at very low N intakes. Therefore, using the equations in combination allowed for a wider range in dietary conditions to be represented.

Urea that is recycled can enter either the rumen, or the lower GIT (Lapierre and Lobley, 2001, NRC, 1985, Reynolds and Kristensen, 2008). The process by which urea enters the gut appears partly passive and party active (Huntington, 1986, Kennedy and Milligan, 1980), although the

exact mechanism of active transport is still unclear (Marini et al., 2004, Marini and Van Amburgh, 2003). Reports on the relative proportion of total recycled N entering the different gut compartments are variable and appear to differ by species (sheep vs cattle) and diet (Huntington, 1989, Parker et al., 1995, Theurer et al., 2002). Huntington (1989) measured an increase in blood urea removal by the rumen compared with the hindgut in steers fed high vs low concentrate diets, respectively, suggesting the site of removal is party determined by the relative requirement for N in each compartment (Firkins and Reynolds, 2005). Further, up to 48% of recycled urea enters the small intestine (Siddons et al., 1985), which is not an important site for microbial growth, and therefore, doesn't have a urea requirement *per se* (Hecker, 1971, Lapierre and Lobley, 2001). Urea concentration in ileal contents ranges from 50 to 100% of that in blood suggesting that entry into the small intestine is by diffusion with the flow of N from the terminal ileum providing an important source of N for microbial growth in the large intestine (NRC, 1985). To model these transactions, the active component of the transfer was assumed to be related to the N requirement in each compartment (rumen vs large intestine) and the diffusive component was assumed to be related to tissue mass which was estimated from (Reynolds et al., 2004). A weighting was then placed on the active and diffusive component to estimate N recycling to each GIT compartment. Because few direct estimates exist on the proportion of N recycled to lower GIT, the weighting was set so that the proportion of ammonia absorbed from the lower GIT was between 28% and 53% of total ammonia absorption (Reynolds and Kristensen, 2008). These transfers are summarized in Figure 3.4.

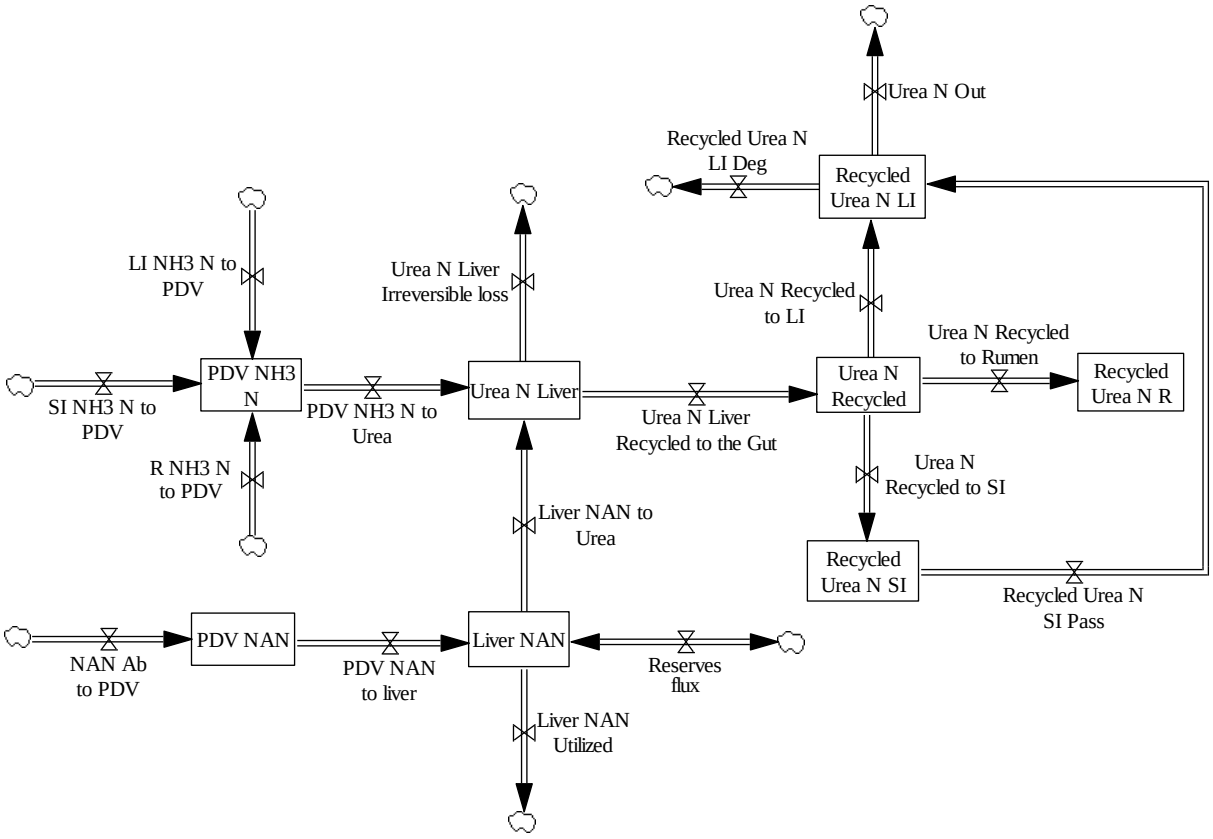

Figure 3.4. Post absorptive N transactions in the model. Boxes represent pools and arrows represent flows. For definitions of abbreviations see Table 3.1.

Feed N that passes from the small intestine to the large intestine is considered completely indigestible. There is little evidence to suggest that, after being exposed to microbial fermentation in the rumen and enzymatic digestion in the small intestine, any further digestion occurs (NRC, 1985). Likewise, microbial residues from the rumen are considered completely indigestible in the large intestine and flow through to the feces (Mason, 1984). Sources of N for microbial growth in the large intestine include urea passing from the small intestine, urea transferred across the gut wall, and endogenous proteins passing from the small intestine (Hecker, 1971). Fecal N is calculated by summing the 6 major components flowing through the large intestine: Rumen microbial N, microbial N grown in the large intestine, feed N,

endogenous N, urea N and NH_3-N. A complete list of model N pools, organized by compartment is in Table 3.6 and a complete list of flows is in Table 3.7. The equations used to calculate the pools and flows are in Tables 3.10 and 3.11.

Table 3.6. Nitrogen pools by compartment in the model. Units for all items are g of N.

Compartment	Pool[1,2]	Description
Rumen		
	A1 N R*i*	A1 N in the rumen
	A2 N R*i*	A2 N in the rumen
	B1 N R*i*	B1 N in the rumen
	B2 N R*i*	B2 N in the rumen
	C N R*i*	C N in the rumen
	End N R*j*	Endogenous N in the rumen
	NH3 N R	Ammonia in the rumen
	PAA N R	Peptides and free AA in the rumen
	FB Cell N	FB cell N in the rumen
	NFB Cell N	NFB cell N in the rumen
	PZ N Engulfed	N engulfed by PZ in the rumen
	PZ Cell N	PZ cell N in the rumen
Small Intestine		
	A2 N SI*i*	A2 N in the SI
	B1 N SI*i*	B1 N in the SI
	B2 N SI*i*	B2 N in the SI
	C N SI*i*	C N in the SI
	Feed PAA N SI*i*	Peptides and free AA from feed in the SI
	R FB N SI	FB cell N from the rumen in the SI
	R NFB N SI	NFB cell N from the rumen in the SI
	PZ N SI	PZ cell N from the rumen in the SI
	End N SI*j*	Endogenous N in the SI
	End N OA*j*	Endogenous N in the omasum and abomasum
	NH3 N SI	Ammonia N in the SI
	Urea N SI	Urea N in the SI

Table 3.6. *(Continued)*

Compartment	Pool[1,2]	Description
Post absorption		
	PDV NAN	Non-ammonia N in the PDV
	PDV NH3 N	Ammonia N in the PDV
	L*i*ver NAN	Non-ammonia N in the liver
	Urea N Liver	Urea N in the liver
	Urea N Recycled	Urea N recycled back to the gut
	Recycled Urea N R	Urea recycled back to the rumen
	Recycled Urea N LI	Urea recycled back to the LI
	Recycled Urea N SI	Urea recycled back to the SI
Large intestine		
	A2 N LI*i*	A2 N in the LI
	B1 N LI*i*	B1 N in the LI
	B2 N LI*i*	B2 N in the LI
	C N LI*i*	C N in the LI
	Feed PAA N LI*i*	Peptides and free AA from feed in the LI
	R FB AA N LI	AA N from rumen FB in the LI
	R FB NA N LI	Nucleic acid N from rumen FB in the LI
	R FB CW N LI	Cell wall N from rumen FB in the LI
	R NFB AA N LI	AA N from rumen NFB in the LI
	R NFB NA N LI	Nucleic acid N from rumen NFB in the LI
	R NFB CW N LI	Cell wall N from rumen NFB in the LI
	PZ AA N LI	AA N from rumen PZ in the LI
	PZ NA N LI	Nucleic acid N from rumen PZ in the LI
	PZ CW N LI	Cell wall N from rumen PZ in the LI
	LI FB Cell N	Cell N of FB grown in the LI
	LI NFB Cell N	Cell N of NFB grown in the LI
	End N LI*j*	Endogenous N in the LI
	PAA N LI	Peptides and free AA in the LI
	NH3 N LI	Ammonia N in the LI

[1] Subscript *i* refers to the i^{th} feed in the diet.

[2] Subscript *j* refers to the j^{th} endogenous component secreted into the GIT

Table 3.7. Nitrogen flows in the model by compartment. Units for all flows are g N/hr.

Compartment	Variable[1,2]	Description
Flows into and within the rumen	A1 N Intake*i*	Intake of A1 N
	A2 N Intake*i*	Intake of A2 N
	B1 N Intake*i*	Intake of B1 N
	B2 N Intake*i*	Intake of N2 N
	C N Intake*i*	Intake of C N
	End N R Secretion*j*	Secretion of endogenous N into the rumen
	Urea N Recycled to Rumen	Recycled urea entering the rumen
	A1 N Sol*i*	Solubilization of A1 N
	A2 N Deg*i*	Degradation of A2 N
	B1 N Deg*i*	Degradation of B1 N
	B2 N Deg*i*	Degradation of B2 N
	C N Deg*i*	Degradation of C N
	PAA N Deg	Degradation of peptides and free AA
	PAA N Uptake R NFB	Uptake of peptides and free AA by NFB
	PAA N Engulfed	Engulfment of peptides and free AA by protozoa
	NH3 N Uptake R NFB	Uptake of ammonia N by NFB
	NH3 N Uptake FB	Uptake of ammonia N by FB
	PZ N Engulfed Excreted as NH3	Excretion of ammonia by PZ
	PZ N Engulfed Incorporated	Incorporation of engulfed N into PZ cells
	PZ N Engulfed Excreted as PAA	Excretion of peptides and free AA by PZ
	PZ Cell N Lysis	Lysis of PZ cells
	NFB Cell N Engulfed	Engulfment of NFB cell N by PZ
	FB Cell N Engulfed	Engulfment of FB cell N by PZ
	End N R Deg*j*	Degradation of endogenous N
Rumen disappearance		
	Recycled Urea N R Deg	Degradation of urea
	NH3 N R Ab	Ammonia absorption through the rumen wall
	A2 N Escape*i*	Escape of A2 N to the SI
	B1 N Escape*i*	Escape of B1 N to the SI
	B2 N Escape*i*	Escape of B2 N to the SI
	C N Escape*i*	Escape of C N to the SI
	Feed PAA N Escape*i*	Escape of peptides and free AA originating from feed to the SI
	End PAA N Escape*j*	Escape of peptides and free AA originating from endogenous N to the SI
	FB PAA N Escape	Escape of peptides and free AA originating from FB cell N to the SI
	NFB PAA N Escape	Escape of peptides and free AA originating from NFB cell N to the SI
	PZ PAA N Escape	Escape of peptides and free AA originating from PZ cell N to the SI
	NH3 N R Escape	Escape of ammonia to the SI
	FB Cell N Escape	Escape of FB cell N to the SI
	NFB Cell N Escape	Escape of NFB cell N to the SI

Table 3.7 *(Continued)*

Compartment	Variable[1,2]	Description
	PZ Cell N Escape	Escape of PZ N to the SI
	End N Escape*j*	Escape of endogenous N to the SI
Post rumen N entry		
	End N OA Secretion*j*	Endogenous N secretions into the omasum and abomasum
	End N SI Secretion*j*	Endogenous N secretions into the SI
	End N OA Flow*j*	Endogenous N flow from the omasum and abomasum to the SI
	Urea N Recycled to SI	Recycled urea entering the SI
	Recycled Urea N SI to Lumen	Recycled urea moving the to lumen of the SI
Disappearance from the SI		
	A2 N ID*i*	Digestion of A2 N in the SI
	B1 N ID*i*	Digestion of B1 N in the SI
	B2 N ID*i*	Digestion of B2 N in the SI
	C N ID*i*	Digestion of C N in the SI
	Feed PAA N ID*i*	Digestion of peptide and free AA N originating from feed in the SI
	R FB AA N ID	Rumen FB AA N digested in the SI
	R FB NA N ID	Rumen FB nucleic acid N digested in the SI
	R FB CW N ID	Rumen FB cell wall N digested in the SI
	R NFB AA N ID	Rumen NFB AA N digested in the SI
	R NFB NA N ID	Rumen NFB nucleic acid N digested in the SI
	R NFB CW N ID	Rumen NFB cell wall N digested in the SI
	PZ AA N ID	PZ AA N digested in the SI
	PZ NA N ID	PZ FB nucleic acid N digested in the SI
	PZ CW N ID	PZ FB cell wall N digested in the SI
	End N ID*j*	Endogenous N digested in the SI
	Urea N SI Resorption	Desorption of recycled urea N in the SI
	A2 N Pass*i*	A2 N passing from the SI to the LI
	B1 N Pass*i*	B1 N passing from the SI to the LI
	B2 N Pass*i*	B2 N passing from the SI to the LI
	C N Pass*i*	C N passing from the SI to the LI
	Feed PAA N Pass*i*	Feed peptide and free AA N passing from the SI to the LI
	R FB AA N Pass	Rumen FB AA N passing from the SI to the LI
	R FB NA N Pass	Rumen FB nucleic acid N passing from the SI to the LI
	R FB CW N Pass	Rumen FB cell wall N passing from the SI to the LI
	R NFB AA N Pass	Rumen NFB AA N passing from the SI to the LI
	R NFB NA N Pass	Rumen NFB nucleic acid N passing from the SI to the LI
	R NFB CW N Pass	Rumen NFB cell wall N passing from the SI to the LI
	PZ AA N Pass	PZ AA N passing from the SI to the LI
	PZ NA N Pass	PZ nucleic acid N passing from the SI to the LI
	PZ CW N Pass	PZ cell wall N passing from the SI to the LI
	End N Pass*j*	Endogenous N passing from the SI to the LI
	Recycled Urea N SI Pass	Recycled urea N passing from the SI to the LI

Table 3.7 *(Continued)*

Compartment	Variable[1,2]	Description
Post absorptive N transactions		
	NAN Ab to PDV	Total non-ammonia N absorbed in the SI flowing to the PDV
	R NH3 N to PDV	Ammonia absorbed in the rumen flowing to the PDV
	SI NH3 N to PDV	Ammonia absorbed in the SI flowing to the PDV
	LI NH3 N to PDV	Ammonia absorbed in the LI flowing to the PDV
	PDV NH3 N to Urea	Ammonia from the PDV being converted to urea in the liver
	PDV NAN to liver	Non-ammonia N from the PDV flowing to the liver
	Liver NAN to Urea	Non-ammonia N in the liver being converted to urea
	Liver NAN Utilized	Utilization of non-ammonia N for N requirements
	Urea N Liver Irreversible loss	Irreversible loss of urea N produced in the liver to the urine
	Urea N Liver Recycled to the Gut	Recycling of urea produced in the liver to the gut
Post SI N entry		
	End N LI Secretion*j*	Endogenous secretions to the LI
	Urea N Recycled to LI	Recycled urea N entering the LI
Disappearance from the LI		
	End N LI Deg*j*	Degradation of endogenous N
	NH3 N LI Ab	Ammonia absorption in the LI
	NH3 N LI Uptake FB	Ammonia uptake by FB in the LI
	NH3 N LI Uptake NFB	Ammonia uptake by NFD in the LI
	PAA N LI Uptake NFB	Peptide and free AA N uptake by NFB in the LI
	PAA N LI Deg	Degradation of peptide and free AA N in the LI
	Recycled Urea N LI Deg	Degradation of recycled urea N in the LI
	A2 N Out*i*	A2 N passing out in the feces
	B1 N Out*i*	B1 N passing out in the feces
	B2 N Out*i*	B2 N passing out in the feces
	C N Out*i*	C N passing out in the feces
	Feed PAA N Out*i*	Peptide and free AA N originating from feed passing out in the feces
	R FB AA N Out	AA N from rumen FB passing out in the feces
	R FB NA N Out	Nucleic acid N from rumen FB passing out in the feces
	R FB CW N Out	Cell wall N from rumen FB passing out in the feces
	R NFB AA N Out	AA N from rumen NFB passing out in the feces
	R NFB NA N Out	Nucleic acid N from rumen NFB passing out in the feces
	R NFB CW N Out	Cell wall N from rumen NFB passing out in the feces
	PZ AA N Out	AA N from PZ passing out in the feces
	PZ NA N Out	Nucleic acid N from PZ passing out in the feces
	PZ CW N Out	Cell wall N from PZ passing out in the feces
	End N Out*j*	Endogenous N passing out in the feces

[1] Subscript *i* refers to the i^{th} feed in the diet.

[2] Subscript *j* refers to the j^{th} endogenous component secreted into the GIT

3.4 Model outputs

3.4.5 Differences between new and old model outputs

The CNCPS has historically been developed for field application with care taken to ensure model inputs are routinely available on most farms (Fox et al., 2004). This model adheres to the same fundamental principles, and while new capability is available within the model, ensuring the model would be field usable was a priority. Nutritionists generally balance rations for the average cow in a group on a per day basis. Although this model calculates continuously over time, and the unit used within the model is hour, the output from the model is expressed on a per day basis. To do this, the model is sampled for 24 hr after simulating for 276 hr (once it has reached steady state). Therefore, the formats of the outputs generated are similar to those from version 6.5. Important differences exist in the calculations of AA supply and requirement which are described in Chapter 6. Differences also exist in the estimations of microbial growth, largely due to the addition of protozoa to the model, which are explained further in Chapter 4. Other differences that impact model outcomes are discussed below.

3.4.6 Rumen pool sizes and intake dynamics

An important new capability of model is the addition of variable intake. The pattern of intake affects many aspects of the model including, but not limited to, microbial growth, rumen N supply and rumen pool sizes. To demonstrate the effects of variable intake, an example simulation was performed with a 600 kg cow producing 45 kg milk, eating 25 kg DM with a diet composition of 15.8 % CP, 29% Starch, 33.8 % NDF, 4.1 % EE and 7.9 % ash. All pools in the model start at 0 and accumulate to steady state. The accumulation of undigestible NDF (uNDF) and pdNDF in the rumen using continuous intake is in Figure 3.5. The uNDF pool takes the

longest to reach steady state of any pool in the model and typically stabilizes after 250 hours of simulation. For the example used, at steady state, the uNDF pool is approximately 4 kg and the pdNDF pool approximately 4.5 kg giving a total rumen NDF pool size of 8.5 kg (Figure 3.5).

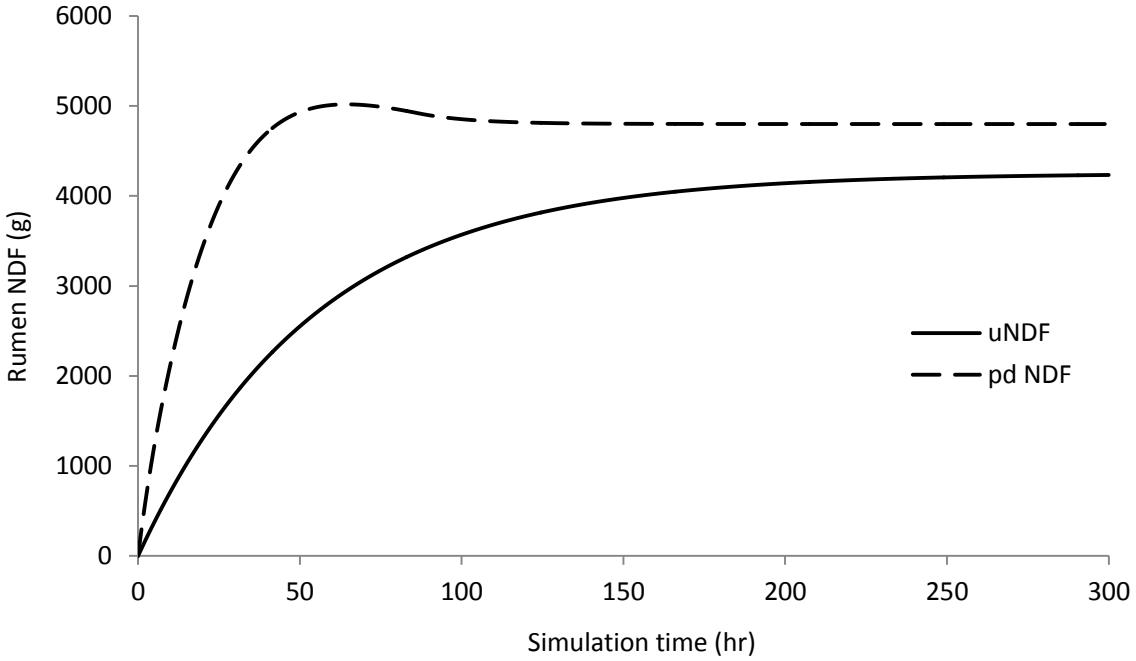

Figure 3.5. Model predicted accumulation of undigestible NDF (uNDF) and pd NDF in the rumen over 300 hours of simulation.

Changing the intake pattern from a constant influx to pulses, that represent meals, causes variation in the predicted rumen pools sizes (Figure 3.6). More frequent, smaller meals (Figure 3.6 – D) result in less variation than larger, less frequent meals (Figures 3.6 – B and C). Meal duration is also important with longer slower meals (Figure 3.6 – B) resulting in less variation than the same meal size over a shorter period of time (Figure 3.6 – C). The model could also accommodate unequal meal sizes allowing for assessment of true on-farm

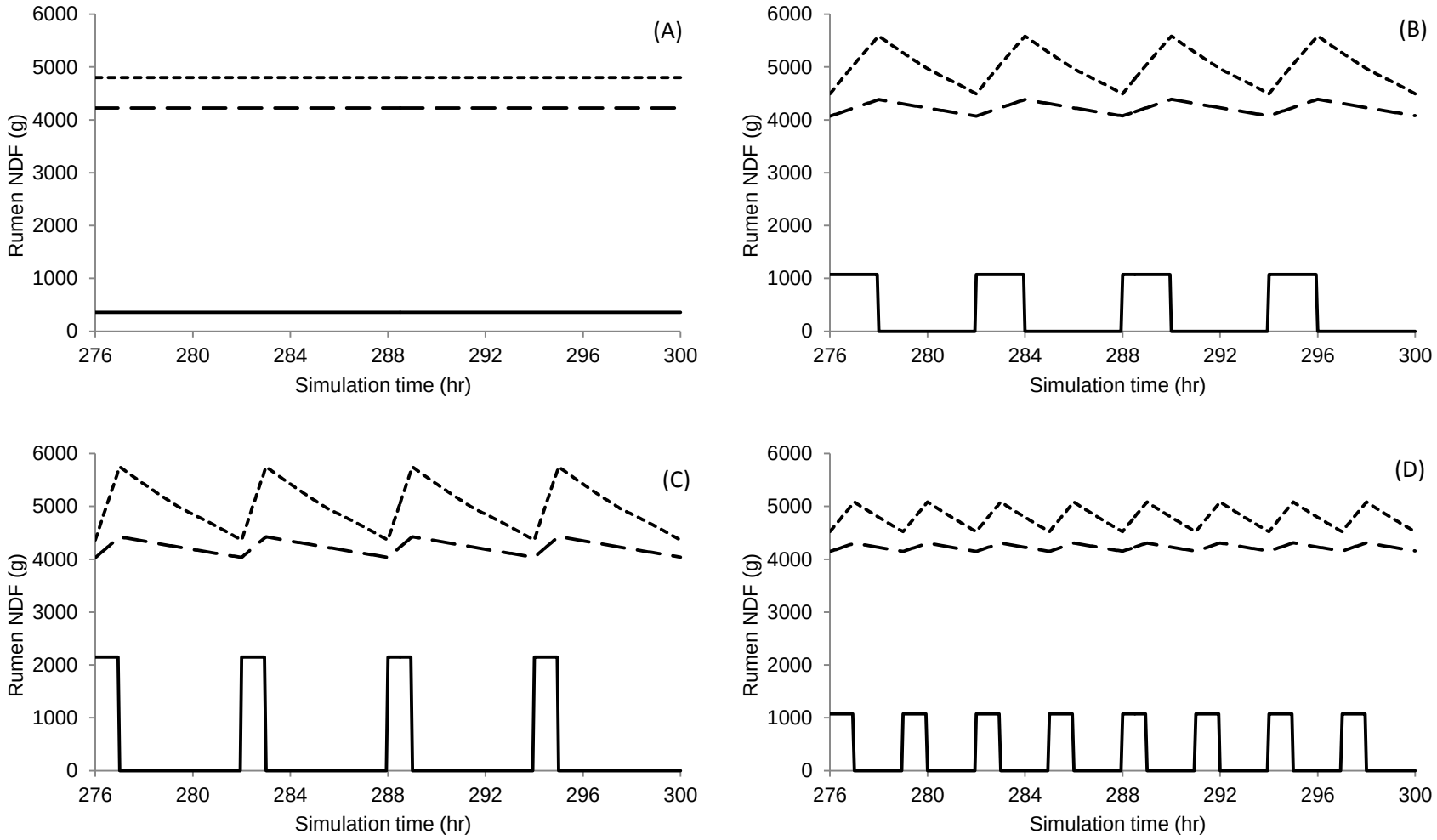

Figure 3.6. Comparison of NDF intake —— (g/hr) and rumen pools sizes for indigestible NDF — — (g) and rumen pd NDF - - - (g) over 24 hours of simulation using different meal intervals and sizes (A = continuous intake; B = 4, 2 hour meals; C = 4, 1 hour meals; D = 8, 1 hour meals).

3.4.7 Rumen nitrogen

Intake pattern strongly influences both the mean and variance of predicted rumen NH_3-N. Figure 3.7 shows a comparison of predicted NH_3-N using continuous intake, 4 meals/d and 8 meals/d. Microbial growth in the model becomes limited when rumen NH_3-N falls below 5.0 mg/dl (see Chapter 4). This interaction causes the uninform behavior observed when NH_3-N falls below 5.0 mg/dl when the meal pattern is 4 meal/d. The effect of N recycling within the model is evident as rumen NH_3-N slowly increases until the next meal is consumed. The same general pattern is presented by Schwab et al. (2005) using in-vivo data. With continuous feeding and with 8 meal/d rumen NH_3-N remains above 5.0 mg/dl demonstrating the importance of feeding pattern on rumen N supply. Having capability to vary intake patterns allows for the comparison of different systems (tie-stalls, free-stalls or grazing) and different management scenarios (over-crowding, slug feeding, etc.) and might help capture more on-farm variation.

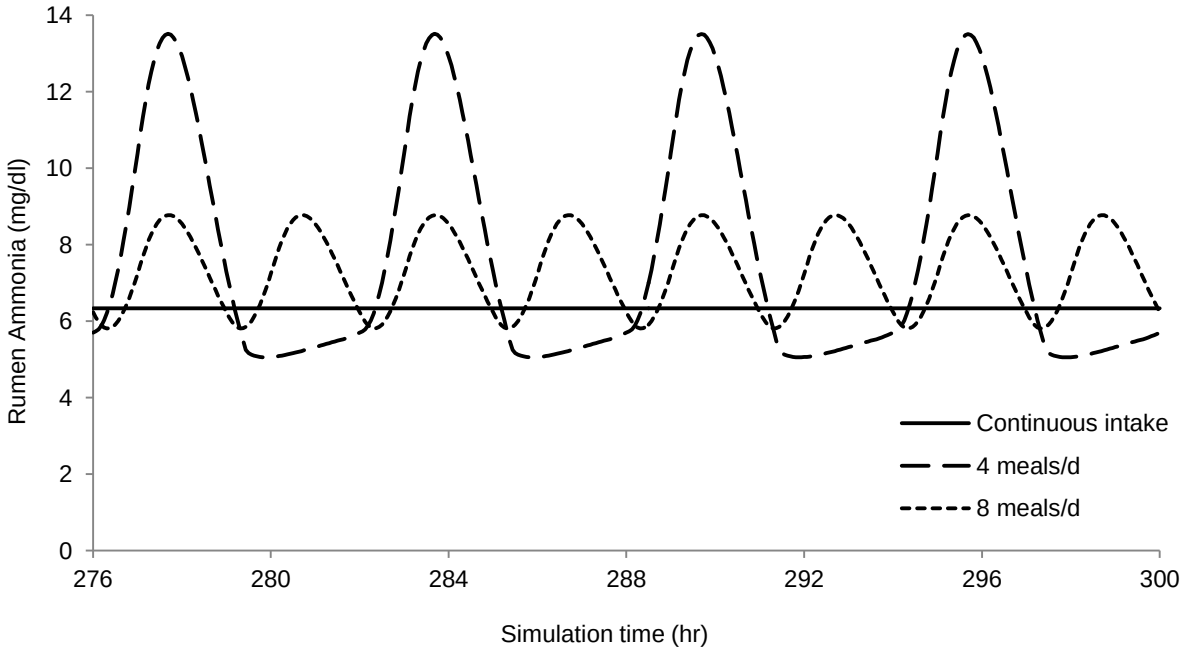

Figure 3.7. Variation in rumen NH_3-N (mg/dl) among three different meal distributions represented by continuous intake, four meals per day and eight meals per day.

3.4.8 Metabolizable energy

Metabolizable energy supply is estimated using the same general system described by Sniffen et al. (1992) with modifications by Tylutki et al. (2008) where crude fat was partitioned into individual fatty acids. In this system net energy and metabolizable energy are calculated from apparent TDN (NRC, 2001). Differences in the current model that affect the estimates of TDN include incorporation of new passage rates for the NDF fractions and the calculation of fecal protein using individual mass factors for each N component. The more mechanistic large intestine portion of the sub-model allows for more sensitivity in post-ruminal digestion, particularly of NDF.

3.4.9 Metabolizable protein

Like ME, estimations of MP follow the same general structure used in previous versions of the model with some refinement. The most notable difference is the estimation of individual endogenous components secreted along the GIT (see Chapter 5) which are subtracted off MP supply. The result is a lower net MP supply, but this is offset by lower predicted MP requirements which culminate in a similar MP balance between this model and version 6.5. Of greater consequence are the changes to the individual N components flowing to the small intestine and their contribution to AA supply which is described further in Chapter 6.

3.5 Implications

The version of the CNCPS presented in this chapter represents a structural shift from previous versions that calculated statically, to a dynamic framework. The new structure is able to more effectively capture the dynamics of carbohydrate and protein digestion, as well as post-absorptive N transactions and recycling. This provides new capability to understand variation in nutrient supply and can help refine ration formulation.

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3.7 Appendix

Table 3.8. Differential equations used to calculate carbohydrate pools. The equations follow the general form d/dt $pool_t = flow_t$

Pool[1]	Equation	
Rumen		
A1a CHO R*i*	A1a CHO Intake*i* - A1a CHO Escape*i* - A1a CHO R Ab*i*	(1.1)
A1b CHO R*i*	A1b CHO Intake*i* - A1b CHO Escape*i* - A1b CHO R Ab*i*	(1.2)
A1p CHO R*i*	A1p CHO Intake*i* - A1p CHO Escape*i* - A1p CHO R Ab*i*	(1.3)
A2 CHO R*i*	A2 CHO Intake*i* - A2 CHO R Deg*i* - A2 CHO Escape*i*	(1.4)
A3 CHO R*i*	A3 CHO Intake*i* - A3 CHO R Deg*i* - A3 CHO Escape*i*	(1.5)
A4 CHO R*i*	A4 CHO Intake*i* + HPZ A4 Engulfed Recycled*i* - A4 CHO R Deg*i* - A4 CHO Escape*i* - A4 CHO Engulfment*i*	(1.6)
B1 CHO R*i*	B1 CHO Intake*i* + EPZ B1 Engulfed Recycled*i* - B1 CHO R Deg*i* - B1 CHO Engulfment*i* - B1 CHO Escape*i*	(1.7)
B2 CHO R*i*	B2 CHO Intake*i* + EPZ B2 Engulfed Recycled*i* - B2 CHO R Deg*i* - B2 CHO Engulfment*i* - B2 CHO Escape*i*	(1.8)
B3 fast CHO R*i*	B3 fast CHO Intake*i* + EPZ B3 fast Engulfed Recycled*i* - B3 fast CHO Engulfment*i* - B3 fast CHO Escape*i* - B3 fast CHO R Deg*i*	(1.9)
B3 slow CHO R*i*	B3 slow CHO Intake*i* + EPZ B3 slow Engulfed Recycled*i* - B3 slow CHO Engulfment*i* - B3 slow CHO Escape*i* - B3 slow CHO R Deg*i*	(1.10)
C CHO R*i*	C CHO Intake*i* + EPZ C Engulfed Recycled*i* - C CHO Engulfment*i* - C CHO Escape*i*	(1.11)
Small Intestine		
A1a CHO SI*i*	A1a CHO Escape*i* - A1a CHO ID*i*	(1.12)
A1b CHO SI*i*	A1b CHO Escape*i* - A1b CHO ID*i*	(1.13)
A1p CHO SI*i*	A1p CHO Escape*i* - A1p CHO ID*i*	(1.14)
A2 CHO SI*i*	A2 CHO Escape*i* - A2 CHO ID*i*	(1.15)
A3 CHO SI*i*	A3 CHO Escape*i* - A3 CHO ID*i*	(1.16)
A4 CHO SI*i*	A4 CHO Escape*i* + HPZ A4 Escape*i* - A4 CHO ID*i* - A4 CHO Pass*i*	(1.17)
B1 CHO SI*i*	B1 CHO Escape*i* + EPZ B1 Escape*i* - B1 CHO ID*i* - B1 CHO Pass*i*	(1.18)
B2 CHO SI*i*	B2 CHO Escape*i* + EPZ B2 Escape*i* - B2 CHO ID*i* - B2 CHO Pass*i*	(1.19)
B3 fast CHO SI*i*	B3 fast CHO Escape*i* + EPZ B3 fast Escape*i* - B3 fast CHO ID*i* - B3 fast CHO Pass*i*	(1.20)
B3 slow CHO SI*i*	B3 slow CHO Escape*i* + EPZ B3 slow Escape*i* - B3 slow CHO ID*i* - B3 slow CHO Pass*i*	(1.21)
C CHO SI*i*	C CHO Escape*i* + EPZ C Escape*i* - C CHO ID*i* - C CHO Pass*i*	(1.22)
Large intestine		
A4 CHO LI*i*	A4 CHO Pass*i* - A4 CHO LI Deg*i* - A4 CHO Out*i*	(1.23)
B1 CHO LI*i*	B1 CHO Pass*i* - B1 CHO LI Deg*i* - B1 CHO Out*i*	(1.24)
B2 CHO LI*i*	B2 CHO Pass*i* - B2 CHO LI Deg*i* - B2 CHO Out*i*	(1.25)
B3 fast CHO LI*i*	B3 fast CHO Pass*i* - B3 fast CHO LI Deg*i* - B3 fast CHO Out*i*	(1.26)
B3 slow CHO LI*i*	B3 slow CHO Pass*i* - B3 slow CHO LI Deg*i* - B3 slow CHO Out*i*	(1.27)
C CHO LI*i*	C CHO Pass*i* - C CHO Out*i*	(1.28)

[1] Subscript *i* refers to the i^{th} feed in the diet

Table 3.9. Equations used to calculate the flow of carbohydrates between pools

Flow[1]	Equation	
A1a CHO Intake*i*	Meal pattern × g A1a CHO*i*	(2.1)
A1b CHO Intake*i*	Meal pattern × g A1b CHO*i*	(2.2)
A1p CHO Intake*i*	Meal pattern × g A1p CHO*i*	(2.3)
A2 CHO Intake*i*	Meal pattern × g A2 CHO*i*	(2.4)
A3 CHO Intake*i*	Meal pattern × g A3 CHO*i*	(2.5)
A4 CHO Intake*i*	Meal pattern × g A4 CHO*i*	(2.6)
B1 CHO Intake*i*	Meal pattern × g B1 CHO*i*	(2.7)
B2 CHO Intake*i*	Meal pattern × g B2 CHO*i*	(2.8)
B3 fast CHO Intake*i*	Meal pattern × g B3 fast CHO*i*	(2.9)
B3 slow CHO Intake*i*	Meal pattern × g B3 slow CHO*i*	(2.10)
C CHO Intake*i*	Meal pattern × g C CHO*i*	(2.11)
A4 CHO Engulfment*i*	A4 CHO R*i* × K A4 CHO engulfment*i*	(2.12)
B1 CHO Engulfment*i*	B1 CHO R*i* × K B1 CHO engulfment*i*	(2.13)
B2 CHO Engulfment*i*	B2 CHO R*i* × K B2 CHO engulfment*i*	(2.14)
B3 fast CHO Engulfment*i*	B3 fast CHO R*i* × K engulfment FC EPZ*i*	(2.15)
B3 slow CHO Engulfment*i*	B3 slow CHO R*i* × K engulfment FC EPZ*i*	(2.16)
C CHO Engulfment*i*	C CHO R*i* × K engulfment FC EPZ*i*	(2.17)
HPZ A4 Engulfed Recycled*i*	(HPZ A4 Cell Lysis × Ratio HPZ A4 Cells to HPZ A4 Engulfed) / (sum(HPZ A4 Engulfed*i*) × HPZ A4 Engulfed*i*)	(2.18)
EPZ B1 Engulfed Recycled*i*	(Ratio EPZ B1 engulfed to EPZ B1 Cells × EPZ B1 Cell Lysis) / (sum(EPZ B1 Engulfed*i*) × EPZ B1 Engulfed*i*)	(2.19)
EPZ B2 Engulfed Recycled*i*	(EPZ B2 Cell Lysis × Ratio EPZ B2 Cells to EPZ B2 Engulfed) / (sum(EPZ B2 Engulfed*i*) × EPZ B2 Engulfed*i*)	(2.20)
EPZ B3 fast Engulfed Recycled*i*	(EPZ Fiber Cell Lysis × Ratio of EPZ B3 fast engulfed to EPZ fiber Cells) / ((sum(EPZ B3 fast Engulfed*i*) × EPZ B3 fast Engulfed*i*) + (EPZ B3 fast Engulfed*i* × EPZ fiber excretion))	(2.21)
EPZ B3 slow Engulfed Recycled*i*	(EPZ Fiber Cell Lysis × Ratio of EPZ B3 slow engulfed to EPZ fiber Cells) / (sum(EPZ B3 slow Engulfed*i*) × EPZ B3 slow Engulfed*i*) + (EPZ B3 slow Engulfed*i* × EPZ fiber excretion))	(2.22)
EPZ C Engulfed Recycled*i*	(EPZ Fiber Cell Lysis × Ratio of EPZ C engulfed to EPZ fiber Cells) / (sum(EPZ C Engulfed*i*) × EPZ C Engulfed*i*) + (EPZ C Engulfed*i* × EPZ fiber excretion))	(2.23)
Rumen disappearance		
A1a CHO R Ab*i*	A1a CHO R*i*	(2.24)
A1b CHO R Ab*i*	A1b CHO R*i*	(2.25)
A1p CHO R Ab*i*	A1p CHO R*i*	(2.26)
A2 CHO R Deg*i*	A2 CHO R*i* × Kd A2 CHO*i*	(2.27)
A3 CHO R Deg*i*	A3 CHO R*i* × Kd A3 CHO*i*	(2.28)
A4 CHO R Deg*i*	A4 CHO R*i* × Kd A4 CHO*i*	(2.29)
B1 CHO R Deg*i*	B1 CHO R*i* × Kd B1 CHO*i*	(2.30)
B2 CHO R Deg*i*	B2 CHO R*i* × Kd B2 CHO*i*	(2.31)
B3 fast CHO R Deg*i*	((B3 fast CHO R*i* × Kd B3 fast CHO*i*) × ph Inhibition) × Rumen NH3 allowable growth	(2.32)
B3 slow CHO R Deg*i*	((B3 slow CHO R*i* × Kd B3 slow CHO*i*) × ph Inhibition) × Rumen NH3 allowable growth	(2.33)
A1a CHO Escape*i*	A1a CHO R*i* × Kp liquid	(2.34)

Table 3.9. *(Continued)*

Compartment[1]	Variable	
A1b CHO Escape*i*	A1b CHO R*i* × Kp liquid	(2.35)
A1p CHO Escape*i*	A1p CHO R*i* × Kp liquid	(2.36)
A2 CHO Escape*i*	A2 CHO R*i* × Kp liquid	(2.37)
A3 CHO Escape*i*	Kp liquid × A3 CHO R*i*	(2.38)
A4 CHO Escape*i*	A4 CHO R*i* × Kp liquid	(2.39)
B1 CHO Escape*i*	B1 CHO R*i* × Kp solids by feed*i*	(2.40)
B2 CHO Escape*i*	B2 CHO R*i* × Kp solids by feed*i*	(2.41)
B3 fast CHO Escape*i*	B3 fast CHO R*i* × Kp fiber by feed*i*	(2.42)
B3 slow CHO Escape*i*	B3 slow CHO R*i* × Kp fiber by feed*i*	(2.43)
C CHO Escape*i*	C CHO R*i* × Kp fiber by feed*i*	(2.44)
HPZ A4 Escape*i*	(HPZ A4 Cell Escape × Ratio HPZ A4 Cells to HPZ A4 Engulfed) / (sum(HPZ A4 Engulfed*i*) × HPZ A4 Engulfed*i*)	(2.45)
EPZ B1 Escape*i*	(Ratio EPZ B1 engulfed to EPZ B1 Cells × EPZ B1 Cell Escape) / (sum(EPZ B1 Engulfed*i*) × EPZ B1 Engulfed*i)*	(2.46)
EPZ B2 Escape*i*	(EPZ B2 Cell Escape × Ratio EPZ B2 Cells to EPZ B2 Engulfed) / (sum(EPZ B2 Engulfed*i*) × EPZ B2 Engulfed*i)*	(2.47)
EPZ B3 fast Escape*i*	(EPZ Fiber Cell Escape × Ratio of EPZ B3 fast engulfed to EPZ fiber Cells) / (sum(EPZ B3 fast Engulfed*i*) × EPZ B3 fast Engulfed*i)*	(2.48)
EPZ B3 slow Escape*i*	(EPZ Fiber Cell Escape × Ratio of EPZ B3 slow engulfed to EPZ fiber Cells) / (sum(EPZ B3 slow Engulfed*i*) × EPZ B3 slow Engulfed*i)*	(2.49)
EPZ C Escape*i*	(EPZ Fiber Cell Escape × Ratio of EPZ C engulfed to EPZ fiber Cells) / (sum(EPZ C Engulfed*i*) × EPZ C Engulfed*i)*	(2.50)
Disappearance from the SI		
A1a CHO ID*i*	A1a CHO SI*i* × ID A1 CHO*i*	(2.51)
A1b CHO ID*i*	A1b CHO SI*i* × ID A1 CHO*i*	(2.52)
A1p CHO ID*i*	A1p CHO SI*i* × ID A1 CHO*i*	(2.53)
A2 CHO ID*i*	A2 CHO SI*i* × ID A2 CHO*i*	(2.54)
A3 CHO ID*i*	A3 CHO SI*i* × ID A3 CHO*i*	(2.55)
A4 CHO ID*i*	A4 CHO SI*i* × ID A4 CHO*i*	(2.56)
B1 CHO ID*i*	B1 CHO SI*i* × ID B1 CHO*i*	(2.57)
B2 CHO ID*i*	B2 CHO SI*i* × ID B2 CHO*i*	(2.58)
B3 fast CHO ID*i*	B3 fast CHO SI*i* × ID B3 fast CHO*i*	(2.59)
B3 slow CHO ID*i*	B3 slow CHO SI*i* × ID B3 slow CHO*i*	(2.60)
C CHO ID*i*	C CHO SI*i* × ID C CHO*i*	(2.61)
A4 CHO Pass*i*	A4 CHO SI*i* × (1 - ID A4 CHO*i*)	(2.62)
B1 CHO Pass*i*	B1 CHO SI*i* × (1 - ID B1 CHO*i*)	(2.63)
B2 CHO Pass*i*	B2 CHO SI*i* × (1 - ID B2 CHO*i*)	(2.64)
B3 fast CHO Pass*i*	B3 fast CHO SI*i* × (1 - ID B3 fast CHO*i*)	(2.65)
B3 slow CHO Pass*i*	B3 slow CHO SI*i* × (1 - ID B3 slow CHO*i*)	(2.66)
C CHO Pass*i*	C CHO SI*i* × (1 - ID C CHO*i*)	(2.67)
Disappearance from the LI		
A4 CHO LI Deg*i*	A4 CHO LI*i* × Kd A4 CHO*i*	(2.68)
B1 CHO LI Deg*i*	B1 CHO LI*i* × Kd B1 CHO*i*	(2.69)
B2 CHO LI Deg*i*	B2 CHO LI*i* × Kd B2 CHO*i*	(2.70)
B3 fast CHO LI Deg*i*	B3 fast CHO LI*i* × Kd B3 fast CHO*i*	(2.71)
B3 slow CHO LI Deg*i*	B3 slow CHO LI*i* × Kd B3 slow CHO*i*	(2.72)
A4 CHO Out*i*	A4 CHO LI*i* × LI transit time	(2.73)

Table 3.9. *(Continued)*

Compartment[1]	Variable	
B1 CHO Out*i*	B1 CHO LI*i* × LI transit time	(2.74)
B2 CHO Out*i*	B2 CHO LI*i* × LI transit time	(2.75)
B3 fast CHO Out*i*	B3 fast CHO LI*i* × LI transit time	(2.76)
B3 slow CHO Out*i*	B3 slow CHO LI*i* × LI transit time	(2.77)
C CHO Out*i*	C CHO LI*i* × LI transit time	(2.78)

[1] Subscript *i* refers to the i^{th} feed in the diet

Table 3.10: Differential equations used to calculate nitrogen pools. The equations follow the general form d/dt $pool_t = flow_t$

Pool[1,2]	Equation	
Rumen		
A1 N R*i*	A1 N Intake*i* - A1 N Sol*i*	(3.1)
A2 N R*i*	A2 N Intake*i* - A2 N Deg*i* - A2 N Escape*i*	(3.2)
B1 N R*i*	B1 N Intake*i* - B1 N Deg*i* - B1 N Escape*i*	(3.3)
B2 N R*i*	B2 N Intake*i* - B2 N Deg*i* - B2 N Escape*i*	(3.4)
C N R*i*	C N Intake*i* - C N Deg*i* - C N Escape*i*	(3.5)
End N R*j*	End N R Secretion*j* - End N R Deg*j* - End N Escape*j*	(3.6)
NH3 N R	sum(A1 N Sol*i*) + PAA N Deg + PZ N Engulfed Excreted as NH3 + Recycled Urea N R Deg - NH3 N Uptake FB - NH3 N Uptake R NFB - NH3 N R Ab - NH3 N R Escape	(3.7)
PAA N R	sum(A2 N Deg*i*) + sum(B1 N Deg*i*) + sum(B2 N Deg*i*) + sum(C N Deg*i*) + sum(End N R Deg*j*) + PZ N Engulfed Excreted as PAA + PZ Cell N Lysis - PAA N Uptake R NFB - PAA N Deg - PAA N Engulfed - PAA N R Escape	(3.8)
FB Cell N	NH3 N Uptake FB - FB Cell N Escape - FB Cell N Engulfed	(3.9)
NFB Cell N	NH3 N Uptake R NFB + PAA N Uptake R NFB - NFB Cell N Engulfed - NFB Cell N Escape	(3.10)
PZ N Engulfed	NFB Cell N Engulfed + FB Cell N Engulfed + PAA N Engulfed - PZ N Engulfed Excreted as NH3 - PZ N Engulfed Excreted as PAA - PZ N Engulfed Incorporated	(3.11)
PZ Cell N	PZ N Engulfed Incorporated - PZ Cell N Lysis - PZ Cell N Escape	(3.12)
Small Intestine		
A2 N SI*i*	A2 N Escape*i* - A2 N ID*i* - A2 N Pass*i*	(3.13)
B1 N SI*i*	B1 N Escape*i* - B1 N ID*i* - B1 N Pass*i*	(3.14)
B2 N SI*i*	B2 N Escape*i* - B2 N Pass*i* - B2 N ID*i*	(3.15)
C N SI*i*	C N Escape*i* - C N Pass*i* - C N ID*i*	(3.16)
Feed PAA N SI*i*	Feed PAA N Escape*i* - Feed PAA N Pass*i* - Feed PAA N ID*i*	(3.17)
R FB N SI	FB Cell N Escape + FB PAA N Escape - R FB CW N Pass - R FB AA N ID - R FB AA N Pass - R FB CW N ID - R FB NA N ID - R FB NA N Pass	(3.19)
R NFB N SI	NFB Cell N Escape + NFB PAA N Escape - R NFB AA N ID - R NFB AA N Pass - R NFB CW N ID - R NFB NA N ID - R NFB NA N Pass - R NFB CW N Pass	(3.20)
PZ N SI	PZ Cell N Escape + PZ PAA N Escape - PZ AA N ID - PZ AA N Pass - PZ CW N ID - PZ CW N Pass - PZ NA N ID - PZ NA N Pass	(3.21)
End N SI*j*	End N OA Flow*j* + End N SI Secretion*j* - End N ID*j* - End N Pass*j*	(3.22)
End N OA*j*	End N Escape*j* + End N OA Secretion*j* + End PAA N Escape*j* - End N OA Flow*j*	(3.23)
NH3 N SI	NH3 N R Escape - SI NH3 absorption	(3.24)
Urea N SI	Recycled Urea N SI to Lumen - Urea N SI Resorption	(3.25)
Post absorption		
PDV NAN	AA infusion + NAN Ab to PDV - PDV NAN to liver	(3.26)
PDV NH3 N	LI NH3 N to PDV + R NH3 N to PDV + SI NH3 N to PDV - PDV NH3 N to Urea	(3.27)
L*i*ver NAN	PDV NAN to liver - Liver NAN to Urea - Liver NAN Utilized + Reserves flux	(3.28)
Urea N L*i*ver	Liver NAN to Urea + PDV NH3 N to Urea + Urea N SI Resorption - Urea N Liver Irreversible loss - Urea N Liver Recycled to the Gut	(3.29)
Urea N Recycled	Urea N Liver Recycled to the Gut - Urea N Recycled to LI - Urea N Recycled to Rumen - Urea N Recycled to SI	(3.30)
Recycled Urea N R	Urea N Recycled to Rumen - Recycled Urea N R Deg	(3.31)

Table 3.10. *(Continued)*

Pool[1,2]	Equation	
Recycled Urea N LI	Recycled Urea N SI Pass + Urea N Recycled to LI - Recycled Urea N LI Deg	(3.32)
Recycled Urea N SI	Urea N Recycled to SI - Recycled Urea N SI to Lumen - Recycled Urea N SI Pass	(3.33)
Large intestine		
A2 N LI*i*	A2 N Pass*i* - A2 N Out*i*	(3.34)
B1 N LI*i*	B1 N Pass*i* - B1 N Out*i*	(3.35)
B2 N LI*i*	B2 N Pass*i* - B2 N Out*i*	(3.36)
C N LI*i*	C N Pass*i* - C N Out*i*	(3.37)
Feed PAA N LI*i*	Feed PAA N Pass*i* - Feed PAA N Out*i*	(3.38)
R FB AA N LI	R FB AA N Pass - R FB AA N Out	(3.39)
R FB NA N LI	R FB NA N Pass - R FB NA N Out	(3.40)
R FB CW N LI	R FB CW N Pass - R FB CW N Out	(3.41)
R NFB AA N LI	R NFB AA N Pass - R NFB AA N Out	(3.42)
R NFB NA N LI	R NFB NA N Pass - R NFB NA N Out	(3.43)
R NFB CW N LI	R NFB CW N Pass - R NFB CW N Out	(3.44)
PZ AA N LI	PZ AA N Pass - PZ AA N Out	(3.45)
PZ NA N LI	PZ NA N Pass - PZ NA N Out	(3.46)
PZ CW N LI	PZ CW N Pass - PZ CW N Out	(3.47)
LI FB Cell N	NH3 N LI Uptake FB - LI FB N Out	(3.48)
LI NFB Cell N	NH3 N LI Uptake NFB + PAA N LI Uptake NFB - LI NFB N Out	(3.49)
End N LI*j*	End N Pass*j* + End N LI Secretion*j* - End N LI Deg*j* - End N Out*j*	(3.50)
PAA N LI	sum(End N LI Deg*j*) - PAA N LI Deg - PAA N LI Uptake NFB	(3.51)
NH3 N LI	Recycled Urea N LI Deg + PAA N LI Deg - NH3 N LI Uptake FB - NH3 N LI Uptake NFB - NH3 N LI Ab	(3.52)

[1] Subscript *i* refers to the i[th] feed in the diet

[2] Subscript *j* refers to the j[th] endogenous component secreted into the gut

Table 3.11. Equations used to calculate the flow of carbohydrates among pools

Flow[1,2]	Equation	
Flows into and within the rumen		
A1 N Intake*i*	Meal pattern × g A1 N*i*	(4.1)
A2 N Intake*i*	Meal pattern × g A2 N*i*	(4.2)
B1 N Intake*i*	Meal pattern × g B1 N*i*	(4.3)
B2 N Intake*i*	Meal pattern × g B2 N*i*	(4.4)
C N Intake*i*	Meal pattern × g C N*i*	(4.5)
End N R Secret*ion*j	Rumen end sec*j*	(4.6)
Urea N Recycled to Rumen	Urea N Recycled × Prop UER rumen	(4.7)
A1 N Sol*i*	A1 N R*i* × Kd A1 N*i*	(4.8)
A2 N Deg*i*	A2 N R*i* × Kd A2 N*i*	(4.9)
B1 N Deg*i*	B1 N R*i* × Kd B1 N*i*	(4.10)
B2 N Deg*i*	B2 N R*i* × Kd B2 N*i*	(4.11)
C N Deg*i*	C N R*i* × Kd C N*i*	(4.12)
PAA N Deg	PAA N R × Kd PAA N R	(4.13)
PAA N Uptake R NFB	PAA N R × NFB PAA Uptake	(4.14)
PAA N Engulfed	PAA consumption EPZ + PAA consumption HPZ	(4.15)
NH3 N Uptake R NFB	NFC bact N required - PAA N Uptake R NFB	(4.16)
NH3 N Uptake FB	FC N required	(4.17)
PZ N Engulfed Excreted as NH3	PZ N Engulfed × 0.25	(4.18)
PZ N Engulfed Incorporated	PZ N Engulfed × 0.5	(4.19)
PZ N Engulfed Excreted as PAA	PZ N Engulfed × 0.25	(4.20)
PZ Cell N Lysis	Total protozoal cell lysis × PZ N	(4.21)
NFB Cell N Engulfed	HPZ predation of NFB + EPZ predation of NFB	(4.22)
FB Cell N Engulfed	EPZ R FB N Engulfment	(4.23)
End N R Deg*j*	End N R*j* × Kd Rumen End N*j*	(4.24)
Rumen disappearance		
Recycled Urea N R Deg	Recycled Urea N R × Kd Urea	(4.25)
NH3 N R Ab	NH3 N R	(4.26)
A2 N Escape*i*	A2 N R*i* × Kp liquid	(4.27)
B1 N Escape*i*	B1 N R*i* × Kp solids by feed*i*	(4.28)
B2 N Escape*i*	B2 N R*i* × Kp solids by feed*i*	(4.29)
C N Escape*i*	C N R*i* × Kp solids by feed*i*	(4.30)
Feed PAA N Escape*i*	Feed PAA N escape / sum(Feed N Deg*i*) × Feed N Deg*i*	(4.31)
End PAA N Escape*j*	End PAA N escape / (sum(End N R Deg*j*) × End N R Deg*j*)	(4.32)
FB PAA N Escape	FB PAA N escape	(4.33)
NFB PAA N Escape	NFB PAA N escape	(4.34)
PZ PAA N Escape	PZ PAA N	(4.35)
NH3 N R Escape	NH3 N R × Kp liquid	(4.36)
FB Cell N Escape	FB Cell N × Kp solids mean	(4.37)
NFB Cell N Escape	NFB Cell N × Kp solids mean	(4.38)

Table 3.11. *(Continued)*

Flow[1,2]	Equation	
PZ Cell N Escape	PZ Cell N × PZ Kp	(4.39)
End N Escape*j*	End N R*j* × Kp solids mean	(4.40)
Post rumen N entry		
End N OA Secret*ion*j	OA end sec*j*	(4.41)
End N SI Secret*ion*j	SI end sec*j*	(4.42)
End N OA Flow*j*	End N OA*j*	(4.43)
Urea N Recycled to SI	Urea N Recycled × Prop UER SI	(4.44)
Recycled Urea N SI to Lumen	Recycled Urea N SI × Urea N diffusion rate	(4.45)
Disappearance from the SI		
A2 N ID*i*	A2 N SI*i* × ID A2 N*i*	(4.46)
B1 N ID*i*	B1 N SI*i* × ID B1 N*i*	(4.47)
B2 N ID*i*	B2 N SI*i* × ID B2 N*i*	(4.48)
C N ID*i*	C N SI*i* × ID C N*i*	(4.49)
Feed PAA N ID*i*	Feed PAA N SI*i* × ID Feed PAA*i*	(4.50)
R FB AA N ID	(R FB N SI × FB AA N) × ID FB AA N	(4.51)
R FB NA N ID	(R FB N SI × FB NA N) × ID FB NA N	(4.52)
R FB CW N ID	(R FB N SI × FB CW N) × ID FB CW N	(4.53)
R NFB AA N ID	(R NFB N SI × NFB AA N) × ID NFB AA N	(4.54)
R NFB NA N ID	(R NFB N SI × NFB NA N) × ID NFB NA N	(4.55)
R NFB CW N ID	(R NFB N SI × NFB CW N) × ID NFB CW N	(4.56)
PZ AA N ID	(PZ N SI × PZ AA N) × ID PZ AA N	(4.57)
PZ NA N ID	(PZ N SI × PZ NA N) × ID PZ NA N	(4.58)
PZ CW N ID	(PZ N SI × PZ CW N) × ID PZ CW N	(4.59)
End N ID*j*	End N SI*j* × ID End N*j*	(4.60)
Urea N SI Resorption	Urea N SI	(4.61)
A2 N Pass*i*	A2 N SI*i* × (1 - ID A2 N*i*)	(4.62)
B1 N Pass*i*	B1 N SI*i* × (1 - ID B1 N*i*)	(4.63)
B2 N Pass*i*	B2 N SI*i* × (1 - ID B2 N*i*)	(4.64)
C N Pass*i*	C N SI*i* × (1 - ID C N*i*)	(4.65)
Feed PAA N Pass*i*	Feed PAA N SI*i* × (1 - ID Feed PAA*i*)	(4.66)
R FB AA N Pass	(R FB N SI × FB AA N) × (1 - ID FB AA N)	(4.67)
R FB NA N Pass	(R FB N SI × FB NA N) × (1 - ID FB NA N)	(4.68)
R FB CW N Pass	(R FB N SI × FB CW N) × (1 - ID FB CW N)	(4.69)
R NFB AA N Pass	(R NFB N SI × NFB AA N) × (1 - ID NFB AA N)	(4.70)
R NFB NA N Pass	(R NFB N SI × NFB NA N) × (1 - ID NFB NA N)	(4.71)
R NFB CW N Pass	(R NFB N SI × NFB CW N) × (1 - ID NFB CW N)	(4.72)
PZ AA N Pass	(PZ N SI × PZ AA N) × (1 - ID PZ AA N)	(4.73)

Table 3.11. *(Continued)*

Flow[1,2]	Equation	
PZ NA N Pass	(PZ N SI × PZ NA N) × (1 - ID PZ NA N)	(4.74)
PZ CW N Pass	(PZ N SI × PZ CW N) × (1 - ID PZ CW N)	(4.75)
End N Pass*j*	End N SI*j* × (1 - ID End N*j*)	(4.76)
Recycled Urea N SI Pass	Recycled Urea N SI - Recycled Urea N SI to Lumen	(4.77)
Post absorptive N transactions		
NAN Ab to PDV	sum(A2 N ID*i*) + sum(B1 N ID*i*) + sum(B2 N ID*i*) + sum(C N ID*i*) + sum(End N ID*j*) + R FB N ID + R NFB N ID + PZ N ID + sum(Feed PAA N ID*i*)	(4.78)
R NH3 N to PDV	R NH3 N Absorbed	(4.79)
SI NH3 N to PDV	SI NH3 N Absorbed	(4.80)
LI NH3 N to PDV	LI NH3 N Absorbed	(4.81)
PDV NH3 N to Urea	PDV NH3 N	(4.82)
PDV NAN to liver	PDV NAN	(4.83)
Liver NAN to Urea	(PDV NAN to liver + Reserves flux) - Liver NAN Utilized	(4.84)
Liver NAN Utilized	Total N Requirement	(4.85)
Urea N Liver Irreversible loss	Urea N Liver × (1 - Fraction of UER recycled)	(4.86)
Urea N Liver Recycled to the Gut	Urea N Liver × Fraction of UER recycled	(4.87)
Post SI N entry		
End N LI Secretion*j*	LI end sec*j*)	(4.88)
Urea N Recycled to LI	Urea N Recycled × Prop UER LI	(4.89)
Disappearance from the LI		
End N LI Deg*j*	End N LI*j* × Kd LI End N*j*	(4.90)
NH3 N LI Ab	NH3 N LI × K Ab LI NH3	(4.91)
NH3 N LI Uptake FB	LI FC N requirement	(4.92)
NH3 N LI Uptake NFB	LI NFC N requirement - PAA N LI Uptake NFB	(4.93)
PAA N LI Uptake NFB	PAA N LI × LI PAA uptake	(4.94)
PAA N LI Deg	PAA N LI	(4.95)
Recycled Urea N LI Deg	Recycled Urea N LI × Kd Urea	(4.96)
A2 N Out*i*	A2 N LI*i* × LI transit time	(4.97)
B1 N Out*i*	B1 N LI*i* × LI transit time	(4.98)
B2 N Out*i*	B2 N LI*i* × LI transit time	(4.99)
C N Out*i*	C N LI*i* × LI transit time	(4.100)
Feed PAA N Out*i*	Feed PAA N LI*i* × LI transit time	(4.101)
R FB AA N Out	R FB AA N LI × LI transit time	(4.102)
R FB NA N Out	R FB NA N LI × LI transit time	(4.103)
R FB CW N Out	R FB CW N LI × LI transit time	(4.104)
R NFB AA N Out	R NFB AA N LI × LI transit time	(4.105)
R NFB NA N Out	R NFB NA N LI × LI transit time	(4.106)

Table 3.11. *(Continued)*

Flow[1,2]	Equation	
R NFB CW N Out	R NFB CW N LI × LI transit time	(4.107)
PZ AA N Out	PZ AA N LI × LI transit time	(4.108)
PZ NA N Out	PZ NA N LI × LI transit time	(4.109)
PZ CW N Out	PZ CW N LI × LI transit time	(4.110)
End N Out*j*	End N LI*j* × LI transit time	(4.111)

[1] Subscript *i* refers to the i[th] feed in the diet

[2] Subscript *j* refers to the j[th] endogenous component secreted into the gut

CHAPTER 4: DEVELOPING A DYNAMIC VERSION OF THE CORNELL NET CARBOHYDRATE AND PROTEIN SYSTEM: MICROBIAL GROWTH

4.1 Abstract

The Cornell Net Carbohydrate and Protein System (CNCPS) includes a mechanistic model to predict rumen fermentation and microbial growth. Previous versions of the CNCPS have included the effects of protozoa indirectly by reducing the theoretical maximum growth yield of bacteria to simulate predation. A new dynamic version of the CNPCS was constructed in the modeling software Vensim® and includes protozoa mechanistically within the model. Bacterial growth follows the same assumptions used in previous versions of the CNCPS where bacteria are characterized as fermenting either fiber or non-fiber CHO, growth is CHO driven and related to the rate of digestion and fermented substrates are used for the purposes of maintenance and growth. The model assumes protozoal growth is also CHO driven and that protozoa consume sugar, starch, soluble fiber, neutral detergent fiber and bacteria. Carbohydrate digestion by protozoa follows a sequence of engulfment then digestion followed by partitioning of the digested material between maintenance and growth. Engulfment is restricted when the ratio of engulfed CHO to cell mass exceeds 1.8 g per g cells and typically ranges from 0.46 to 0.97 g CHO g^{-1} protozoal cells hr^{-1} at steady state. Carbohydrate digestion is calculated relative to the size of the engulfed pool and is assumed to be half the rate of bacterial digestion for each CHO source. Typical digestion rates range from 0.16 – 0.30 g CHO g^{-1} protozoal cells hr^{-1}. Pool sizes of protozoa in the rumen are smaller when dry matter intake is high (25 kg DMI/d; 4 – 9% of microbial N) and larger when DMI is low (15 kg DMI/d; 10 – 25% of microbial N) and this behavior is linked to the rate of passage out of the rumen. Protozoa consume N at double the rate

required to meet their N requirements for growth and excrete half back to the rumen as ammonia which has a stabilizing effect on the rumen N supply. Bacteria contribute two-thirds of the protozoal N intake and the remainder is met by engulfment of dietary amino acids. Therefore, the rate of bacterial engulfment is proportional to the rate of protozoal growth. Integrating protozoal and bacterial growth in a dynamic framework provides the CNCPS with new capability in understanding rumen metabolism and the supply of microbial protein available to meet the metabolizable protein requirements of cattle.

4.2 Introduction

Microbial protein synthesis in the rumen provides a considerable contribution to the daily AA supply in ruminants and is central in understanding AA supply from the diet (Schwab et al., 2005). Previous versions of the CNCPS use a mechanistic approach to estimate bacterial growth in the rumen (Russell et al., 1992). In this system bacteria are characterized as fermenting either fiber carbohydrates (**CHO**) or non-fiber CHO. Protozoa are accommodated by reducing the theoretical maximum growth yield from 0.5 to 0.4 g cells per g CHO fermented (Russell et al., 1992) but do not contribute to digestion or microbial protein production. Protozoa have important effects not only on bacterial yield, but also nutrient digestion and cycling within the rumen (Firkins et al., 2007, Hristov and Jouany, 2005). Therefore, a more mechanistic approach is warranted to fully capture these effects in the CNCPS.

A new, dynamic version of the CNCPS was constructed in the system dynamics modeling software Vensim® to estimate carbohydrate and protein digestion (Chapter 3). The new model uses a similar structure to previous versions of CNCPS, but rather than calculating statically, it

calculates iteratively over time. This new framework was extended to include microbial growth in both the rumen and large intestine. Bacterial growth was based on the model described by Russell et al. (2009). A new mechanistic model of protozoal growth was also constructed. Mechanistic models of protozoal growth been previously published (Dijkstra, 1994, Dijkstra et al., 1992) and have improved the understanding of the dynamics of protozoal growth and their interactions with bacteria and different dietary components. The goal of the model described in this chapter was to improve estimations of microbial growth and their interactions within the structure of the CNCPS in a framework that was applicable for field use to improve the predictions of metabolizable protein and amino acid supply.

4.3 Model description

4.3.1 Bacterial growth

For the development of this model, bacterial growth was estimated using the approach described by Russell et al. (2009). The underlying principles used in this model are the same as the original version of the CNCPS (Russell et al., 1992) where the rate of bacterial growth (***μ***) is relative to the rate of CHO digestion (**kd**) and digested CHO is used for functions of maintenance (***m***) and growth. The model assumes that kd is an inherent property of a given feed and, given μ is relative to kd, the rumen operates in a substrate limited, enzyme excess environment (Russell et al., 1992). The maintenance function used in this and previous versions of the CNCPS was described by Pirt (1965) as the amount of energy required to sustain a mass of bacteria for a given period of time (g glucose g^{-1} bacteria h^{-1}). Maintenance can also be expressed as a constant (***a***) which is mathematically related to *m* according to the equation $a = m \times Y_G$ where Y_G is the theoretical maximum growth yield (g cells g^{-1} CHO; Russell et al., 2009).

Russell et al. (2009) integrated these concepts into a dynamic model to describe cellulose digestion and microbial growth in the rumen. The model assumed digested CHO had 3 fates: 1) generating ATP for maintenance; 2) generating ATP for growth; 3) the carbon is used to synthesize cells. The rate and extent of CHO digestion is the product of the digestion rate and passage rate (Waldo et al., 1972). Once digested, the model partitions CHO to either maintenance or growth using the equation: $m\mu = (kd - a) - a$ (% h^{-1}). Carbohydrate used for growth is then partitioned to either generate ATP to grow, or to synthesize cell dry matter using the equation: $(1/Y_G) - 1$ (% h^{-1}). This system was extrapolated into the current model and used to estimate microbial growth from all CHO sources.

The CNCPS categorizes bacteria as fermenting either fiber or non-fiber CHOs (Russell et al., 1992). Non-fiber bacteria have higher maintenance coefficients than fiber bacteria (Russell and Baldwin, 1979) which are assumed as 0.15 and 0.05 g CHO g^{-1} bacteria h^{-1}, respectively. Theoretical maximum growth coefficients were assumed to be 0.4 g cells g^{-1} CHO which are lower than the 0.5 g cells g^{-1} CHO reported by Isaacson et al. (1975) to account for protozoal predation (Russell et al., 1992). Similar assumptions are used in the current model where fiber bacteria (**FB**) were assumed to grow more slowly and utilize ammonia as an N source for protein synthesis. Non-fiber bacteria (**NFB**) were assumed to grow more rapidly and utilize either ammonia or peptides and free AA as an N source. Maintenance '*a*' coefficients were set at 0.01 and 0.03 g CHO g^{-1} bacteria h^{-1} for FB and NFB, respectively (Russell et al., 2009, Van Kessel and Russell, 1996). The theoretical maximum growth was assumed to be 0.5 g cells g^{-1} CHO for all CHO pools apart from A2 CHO (lactic acid) which has a Y_G of 0.108 g cells g^{-1} lactic acid due to the lower ATP yield per mole of lactic acid fermented (Lanzas et al., 2007). The Y_G of 0.5

g cells g^{-1} CHO is higher than previous versions of the CNCPS as protozal growth and predation are included mechanistically in this model. Russell et al. (2009) describe the model using a closed system where an initial rumen CHO pool is digested or passed until the pool is exhausted. However, in an animal, pools would be replenished during meals and the process would be continuous. Further, when feeding a TMR, a range of CHO sources would be consumed, with varying kd, meaning bacteria would be growing at varying rates and would be partitioning energy differently. Integrating the model structure described by Russell et al. (2009) into the framework described in Chapter 3 allowed for microbial growth to be predicted in a continuous, steady state system with the spectrum of CHO sources and kd represented for any given diet.

An example of how the model of Russell et al. (2009) was integrated into the current model to estimate bacterial growth on fiber CHO is in Figure 4.1. Definitions of the abbreviations used in Figure 4.1 are in Tables 4.1, 4.2 and 4.3. Briefly, B3 slow CHO R and B3 fast CHO represent the pools of slowly and rapidly digesting NDF in the rumen as described by Raffrenato (2011). The NDF in these pools is degraded by bacteria and used for functions of maintenance and growth as described above. The same general structure is used for NFB fermenting A2, A3, A4, B1 and B2 CHO. A complete list of the bacterial pools and flows, organized by gastrointestinal compartment, are in Tables 4.2 and 4.3. The equations used to calculate the pools and flows are in Tables 4.8 and 4.9.

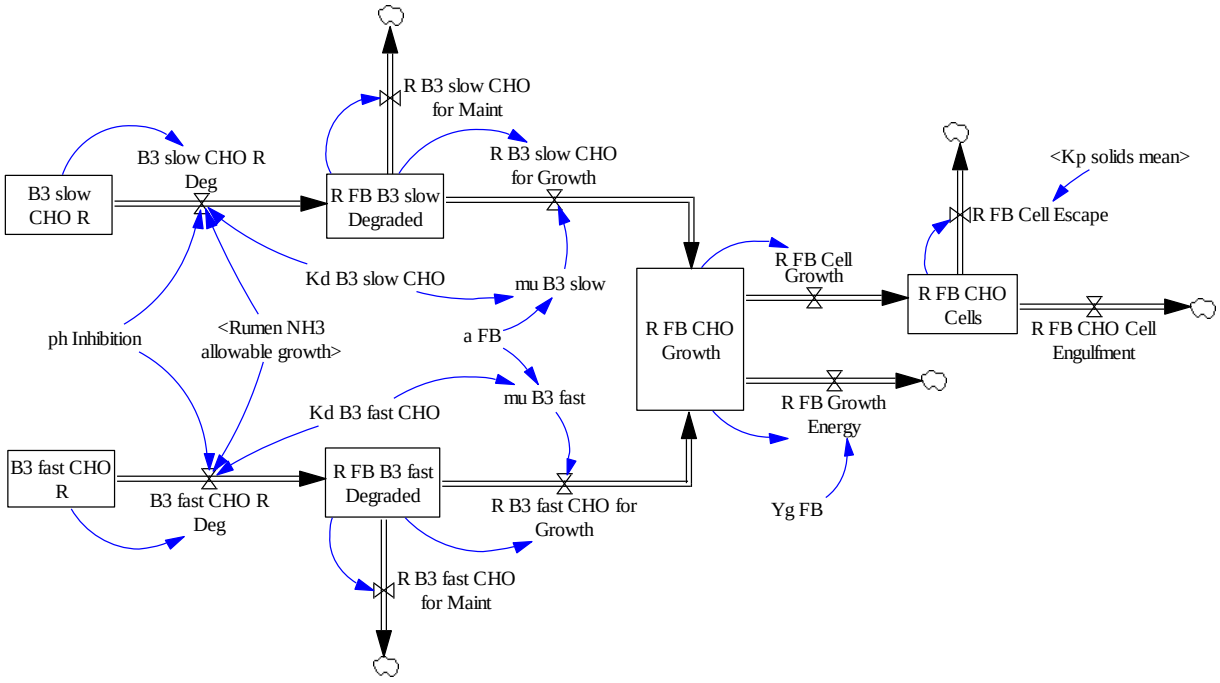

Figure 4.1. Diagrammatic representation of microbial growth from slowly and rapidly degrading NDF using the model of Russell et al. (2009) modified for NDF pool degradation characteristics from Raffrenato (2011).

Important differences between the current model, and the model of Russell et al. (2009) include restriction of bacterial growth due to low rumen N (Rumen NH_3 allowable growth), escape of bacteria from the rumen (R FB Cell Escape and R NFB Cell Escape) and engulfment of bacteria by protozoa (R FB CHO Cell Engulfment and R NFB Cell Engulfment). Russell et al. (2009) ignored N limitation citing the extensive recycling of urea in ruminants. However, there is good consensus in the literature that low rumen N levels impact CHO digestion and microbial growth (Broderick et al., 2008, Broderick, 2003, Lee et al., 2011, Lee et al., 2012, Schwab et al., 2005). The current model adjusts bacterial growth when rumen NH_3 falls below 5.0 mg/dl (Satter and Roffler, 1975) using a 'lookup' adjustment (Figure 4.2A). The lookup structure is used to avoid erratic model behavior and instability that can occur when conditional statements are used

(Sterman, 2000). Different aspects of growth were adjusted for FB and NFB, respectively. Fiber digestion appears more directly affected by low rumen N than the digestion of non-fiber CHO (Hoover, 1986). This is evident through lower apparent total tract NDF digestion in cows fed adequate and restricted protein diets, respectively (Broderick et al., 2008, Broderick, 2003, Lee et al., 2011, Lee et al., 2012). To replicate this behavior in the model, fiber kd was multiplied by the adjustment factor in Figure 4.2A corresponding to the concentration of rumen NH_3-N which reduced the rate of rumen digestion, microbial growth, and increased NDF passage to the lower gastrointestinal compartments (see Figure 4.1). Non-fiber bacteria were assumed to digest CHO at the same rate, but lower their growth efficiency through energy spilling reactions (Van Kessel and Russell, 1996). To replicate this behavior, the proportion of energy used to generate NFB cells was reduced, again using the adjustment in Figure 4.2A. This indirectly lowered Y_G which increased the energy required to grow, effectively spilling energy. The stimulatory effects of peptide utilization on bacterial growth efficiency were also included (Figure 4.2B), similar to previous versions of the CNCPS (Russell et al., 1992). However, rather than expressing yield improvement relative to the ratio of AA to total organic matter, the ratio of NH_3 utilization relative to AA utilization was used (Russell and Sniffen, 1984). Nitrogen uptake by bacteria in the rumen is calculated by multiplying the rate of cell growth by the N content of the cell DM. It is assumed the proportion of pre-formed AA uptake by NFB is relative to availability. Therefore, the ratio of peptide and free AA N (PAA N R) in the rumen to ammonia (NH_3) determines the AA uptake rate of NFB. An important feedback loop exists where N uptake is modulated by reducing cell growth when rumen NH_3 drops below 5.0 mg/dl (Figure 4.2A), thereby reducing demand. In Vensim, a feedback loop is what defines an interaction between two or more variables. The effect of pH was modeled using the lookup adjustment in Russell et al. (2009).

Prediction of pH was calculated using the equations in Fox et al. (2004). Bacterial cells were assumed to disappear from the rumen either through escape or by protozoal engulfment as discussed in Section 4.3.2.

Bacteria passing through into the small intestine were partitioned according to their chemical composition and digested as described in Chapter 3. Bacterial growth in the large intestine uses the same growth kinetics and assumptions as the rumen. The transit time through the large intestine is assumed to be 7 hours (k_p = 14 % h^{-1}) as explained in Chapter 3. The N for microbial growth in the large intestine comes from either urea recycled directly into the intestine or flowing through from the small intestine and includes endogenous gastrointestinal secretions.

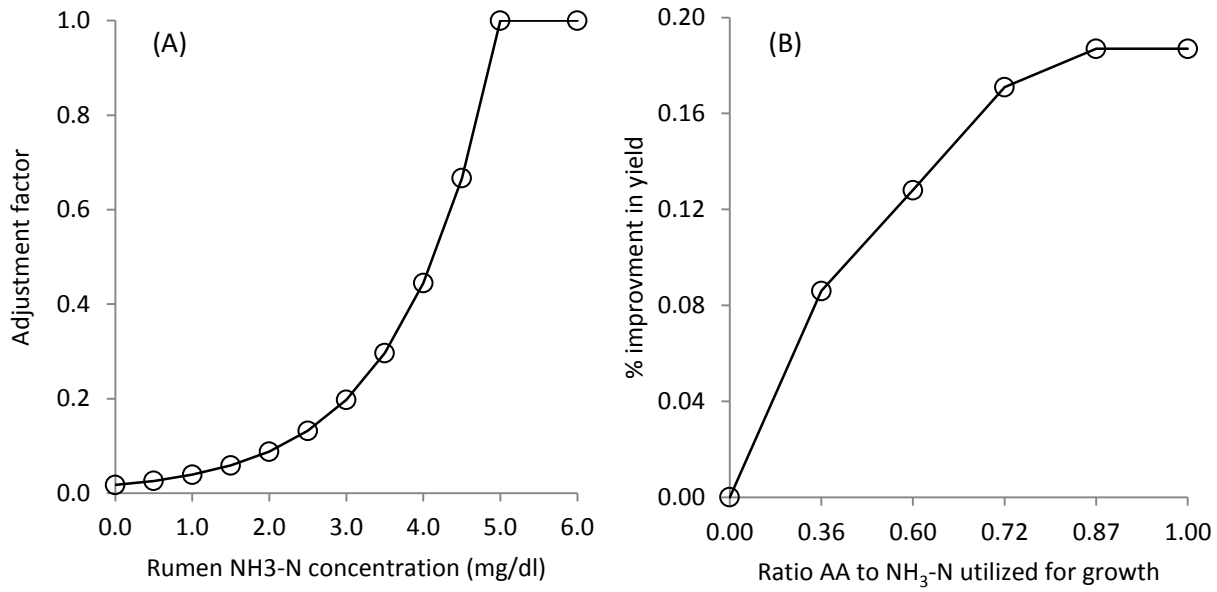

Figure 4.2. Lookup factors used to adjust microbial growth for rumen ammonia (A) and AA N use (B). Microbial cell growth is adjusted when rumen NH_3-N is < 5.0 mg/dl (X axis; A) using the corresponding adjustment factor on the Y axis. Similarly, bacterial growth yield is increased according to the ratio of AA N and NH_3 N (X axis). Growth yield increases from 100% of the expected yield when NH_3-N provides 100% of the growth N to a maximum of 118% of the expected yield when AA N provides >87% of the growth N. Both adjustments are made dynamically during the simulation.

Table 4.1. Model inputs and constants used to calculate bacterial growth and digestion

Input[1]	Units	Description
Yg FB	g cells/g CHO	Theoretical maximum rumen FB yield without maintenance
Yg LI FB	g cells/g CHO	Theoretical maximum LI FB yield without maintenance
Yg NFB	g cells/g CHO	Theoretical maximum rumen FB yield without maintenance
Yg LI NFB	g cells/g CHO	Theoretical maximum LI FB yield without maintenance
a FB	g CHO/hr	FB maintenance coefficient
a NFB	g CHO/hr	NFB maintenance coefficient
Kd A2 CHO*i*	%/hr	Rate of A2 CHO degradation
Kd A3 CHO*i*	%/hr	Rate of A3 CHO degradation
Kd A4 CHO*i*	%/hr	Rate of A4 CHO degradation
Kd B1 CHO*i*	%/hr	Rate of B1 CHO degradation
Kd B2 CHO*i*	%/hr	Rate of B2 CHO degradation
Kd B3 fast CHO*i*	%/hr	Rate of B3 fast CHO degradation
Kd B3 slow CHO*i*	%/hr	Rate of B3 slow CHO degradation
Kp solids mean	%/hr	Mean solids passage rate
LI transit time	%/hr	Transit time through the LI
FB N	% DM	N content of FB cells
FB AA N	% N	Proportion of AA N in FB cell N
FB NA N	% N	Proportion of nucleic acid N in FB cell N
FB CW N	% N	Proportion of cell wall N in FB cell N
FB CHO	% DM	CHO content of FB cells
FB EE	% DM	EE content of FB cells
FB Ash	% DM	Ash content of FB cells
NFB N	% DM	N content of NFB cells
NFB AA N	% N	Proportion of AA N in NFB cell N
NFB NA N	% N	Proportion of nucleic acid N in NFB cell N
NFB CW N	% N	Proportion of cell wall N in NFB cell N
NFB CHO	% DM	CHO content of NFB cells
NFB EE	% DM	EE content of NFB cells
NFB Ash	% DM	Ash content of NFB cells
ID FB AA N	%	Proportion of FB AA N digested in the SI
ID FB NA N	%	Proportion of FB nucleic acid N digested in the SI
ID FB CW N	%	Proportion of FB cell wall N digested in the SI
ID FB CHO	%	Proportion of FB CHO digested in the SI
ID FB EE	%	Proportion of FB EE digested in the SI
ID FB Ash	%	Proportion of FB ash digested in the SI
ID NFB AA N	%	Proportion of NFB AA N digested in the SI
ID NFB NA N	%	Proportion of NFB nucleic acid N digested in the SI
ID NFB CW N	%	Proportion of NFB cell wall N digested in the SI
ID NFB CHO	%	Proportion of NFB CHO digested in the SI
ID NFB EE	%	Proportion of NFB EE digested in the SI
ID NFB Ash	%	Proportion of NFB ash digested in the SI

[1] Subscript *i* refers to the i^{th} feed in the diet.

Table 4.2. Bacterial pools and substrates by gastrointestinal compartment

Compartment	Pool[1]	Units	Description
Rumen	*Fiber bacteria*		
	R FB B3 fast Degraded*i*	g CHO	Degraded B3 fast CHO
	R FB B3 slow Degraded*i*	g CHO	Degraded B3 slow CHO
	R FB B3 fast Maint	g CHO	B3 fast CHO used for maintenance
	R FB B3 slow Maint	g CHO	B3 slow CHO used for maintenance
	R FB CHO Growth	g CHO	Fiber CHO used for growth
	R FB CHO Energy	g CHO	Fiber CHO used to generate energy to grow
	R FB CHO Cells	g CHO	Fiber used for cell growth
	Non-fiber bacteria		
	R NFB A2 Degraded*i*	g CHO	Degraded A2 CHO
	R NFB A3 Degraded*i*	g CHO	Degraded A3 CHO
	R NFB A4 Degraded*i*	g CHO	Degraded A4 CHO
	R NFB B1 Degraded*i*	g CHO	Degraded B1 CHO
	R NFB B2 Degraded*i*	g CHO	Degraded B2 CHO
	R NFB A2 Maint	g CHO	A2 CHO used for maintenance
	R NFB A3 Maint	g CHO	A3 CHO used for maintenance
	R NFB A4 Maint	g CHO	A4 CHO used for maintenance
	R NFB B1 Maint	g CHO	B1 CHO used for maintenance
	R NFB B2 Maint	g CHO	B2 CHO used for maintenance
	R NFB CHO Growth	g CHO	Non-fiber CHO used for growth
	R NFB CHO Energy	g CHO	Non-fiber CHO used to generate energy to grow
	R NFB CHO Cells	g CHO	Non-fiber CHO used for cell growth
Small intestine	*Rumen Fiber bacteria*		
	R FB N SI	g N	FB N in the SI
	R FB CHO SI	g CHO	FB CHO in the SI
	R FB EE SI	g EE	FB EE in the SI
	R FB Ash SI	g Ash	FB ash in the SI
	Rumen non-fiber bacteria		
	R NFB N SI	g N	NFB N in the SI
	R NFB CHO SI	g CHO	NFB CHO in the SI
	R NFB EE SI	g EE	NFB EE in the SI
	R NFB Ash SI	g Ash	NFB ash in the SI
Large intestine	*Rumen Fiber bacteria*		
	R FB AA N LI	g AA N	AA N from rumen FB in the LI
	R FB NA N LI	g NA N	Nucleic acid N from rumen FB in the LI
	R FB CW N LI	g CW N	Cell wall N from rumen FB in the LI
	R FB CHO LI	g CHO	CHO from rumen FB in the LI
	R FB EE LI	g EE	EE from rumen FB in the LI
	R FB Ash LI	g Ash	Ash from rumen FB in the LI

Table 4.2. *(Continued)*

Compartment	Pool[1]	Units	Description
	Rumen non-fiber bacteria		
	R NFB AA N LI	g AA N	AA N from rumen NFB in the LI
	R NFB NA N LI	g NA N	Nucleic acid N from rumen NFB in the LI
	R NFB CW N LI	g CW N	Cell wall N from rumen NFB in the LI
	R NFB CHO LI	g CHO	CHO from rumen NFB in the LI
	R NFB EE LI	g EE	EE from rumen NFB in the LI
	R NFB Ash LI	g Ash	Ash from rumen NFB in the LI
	Large intestine fiber bacteria		
	LI FB B3 fast Degraded*i*	g CHO	Degraded B3 fast CHO degraded in the LI
	LI FB B3 slow Degraded*i*	g CHO	Degraded B3 slow CHO in the LI
	LI FB B3 fast Maint	g CHO	B3 fast CHO used for maintenance by FB in the LI
	LI FB B3 slow Maint	g CHO	B3 slow CHO used for maintenance by FB in the LI
	LI FB CHO Growth	g CHO	Fiber CHO used for growth by FB in the LI
	LI FB CHO Energy	g CHO	Fiber CHO used to generate energy to grow by FB in the LI
	LI FB CHO Cells	g CHO	Fiber used for cell growth by FB in the LI
	Large intestine non-fiber bacteria		
	LI NFB A4 Degraded*i*	g CHO	Degraded A4 CHO in the LI
	LI NFB B1 Degraded*i*	g CHO	Degraded B1 CHO in the LI
	LI NFB B2 Degraded*i*	g CHO	Degraded B3 CHO in the LI
	LI NFB A4 Maint	g CHO	A4 CHO used for maintenance by NFB in the LI
	LI NFB B1 Maint	g CHO	B1 CHO used for maintenance by NFB in the LI
	LI NFB B2 Maint	g CHO	B2 CHO used for maintenance by NFB in the LI
	LI NFB CHO Growth	g CHO	Non-fiber CHO used for growth by NFB in the LI
	LI NFB CHO Energy	g CHO	Non-fiber CHO used to generate energy to grow by NFB in the LI
	LI NFB CHO Cells	g CHO	Non-fiber CHO used for cell growth by NFB in the LI

[1] Subscript *i* refers to the i^{th} feed in the diet.

Table 4.3. Bacteria and bacterial substrate flows by gastrointestinal compartment

Compartment	Flow[1]	Units	Description
Rumen	*Fiber bacteria*		
	B3 fast CHO R Deg*i*	g CHO/hr	Degradation of B3 fast CHO
	B3 slow CHO R Deg*i*	g CHO/hr	Degradation of B3 slow CHO
	R B3 fast CHO for Maint*i*	g CHO/hr	B3 fast CHO being used for maintenance
	R B3 slow CHO for Maint*i*	g CHO/hr	B3 slow CHO being used for maintenance
	R B3 fast CHO for Growth*i*	g CHO/hr	B3 fast CHO being used for growth
	R B3 slow CHO for Growth*i*	g CHO/hr	B3 slow CHO being used for growth
	R FB Growth Energy	g CHO/hr	Fiber CHO being used to generate energy to grow
	R FB Cell Growth	g CHO/hr	Fiber being used for cell growth
	R FB CHO Cell Engulfment	g FB cells/hr	Engulfment of FB cells by PZ
	R FB Cell Escape	g FB cells/hr	Escape of FB cells to the SI
	Non-fiber bacteria		
	A2 CHO R Deg*i*	g CHO/hr	Degradation of A2 CHO
	A3 CHO R Deg*i*	g CHO/hr	Degradation of A3 CHO
	A4 CHO R Deg*i*	g CHO/hr	Degradation of A4 CHO
	B1 CHO R Deg*i*	g CHO/hr	Degradation of B1 CHO
	B2 CHO R Deg*i*	g CHO/hr	Degradation of B2 CHO
	R A2 CHO for Maint*i*	g CHO/hr	A2 CHO being used for maintenance
	R A3 CHO for Maint*i*	g CHO/hr	A3 CHO being used for maintenance
	R A4 CHO for Maint*i*	g CHO/hr	A4 CHO being used for maintenance
	R B1 CHO for Maint*i*	g CHO/hr	B1 CHO being used for maintenance
	R B2 CHO for Maint*i*	g CHO/hr	B2 CHO being used for maintenance
	R A2 CHO for Growth*i*	g CHO/hr	A2 CHO being used for growth
	R A3 CHO for Growth*i*	g CHO/hr	A3 CHO being used for growth
	R A4 CHO for Growth*i*	g CHO/hr	A4 CHO being used for growth
	R B1 CHO for Growth*i*	g CHO/hr	B1 CHO being used for growth
	R B2 CHO for Growth*i*	g CHO/hr	B2 CHO being used for growth
	R NFB Growth Energy	g CHO/hr	Non-fiber CHO being used to generate energy to growth
	R NFB Cell Growth	g CHO/hr	Non-fiber CHO being used for cell growth
	R NFB CHO Cell Engulfment	g NFB cells/hr	Engulfment of NFB cells by PZ
	R NFB Cell Escape	g NFB cells/hr	Escape of NFB cells to the SI
Small intestine	*Fiber bacteria*		
	R FB AA N ID	g AA N/hr	Digestion of FB AA N in the SI
	R FB NA N ID	g NA N/hr	Digestion of FB nucleic acid N in the SI
	R FB CW N ID	g CW N/hr	Digestion of FB cell wall N in the SI
	R FB CHO ID	g CHO/hr	Digestion of FB CHO in the SI
	R FB EE ID	g EE/hr	Digestion of FB EE in the SI
	R FB Ash ID	g Ash/hr	Digestion of FB ash in the SI
	R FB AA N Pass	g AA N/hr	Passage of FB AA N from the SI to the LI
	R FB NA N Pass	g NA N/hr	Passage of FB nucleic acid N from the SI to the LI
	R FB CW N Pass	g CW N/hr	Passage of FB cell wall N from the SI to the LI
	R FB CHO Pass	g CHO/hr	Passage of FB CHO from the SI to the LI

Table 4.3. *(Continued)*

Compartment	Flow[1]	Units	Description
	R FB EE Pass	g EE/hr	Passage of FB EE from the SI to the LI
	R FB Ash Pass	g Ash/hr	Passage of FB ash from the SI to the LI
	Non-fiber bacteria		
	R NFB AA N ID	g AA N/hr	Digestion of NFB AA N in the SI
	R NFB NA N ID	g NA N/hr	Digestion of NFB nucleic acid N in the SI
	R NFB CW N ID	g CW N/hr	Digestion of NFB cell wall N in the SI
	R NFB CHO ID	g CHO/hr	Digestion of NFB CHO in the SI
	R NFB EE ID	g EE/hr	Digestion of NFB EE in the SI
	R NFB Ash ID	g Ash/hr	Digestion of NFB ash in the SI
	R NFB AA N Pass	g AA N/hr	Passage of NFB AA N from the SI to the LI
	R NFB NA N Pass	g NA N/hr	Passage of NFB nucleic acid N from the SI to the LI
	R NFB CW N Pass	g CW N/hr	Passage of NFB cell wall N from the SI to the LI
	R NFB CHO Pass	g CHO/hr	Passage of NFB CHO from the SI to the LI
	R NFB EE Pass	g EE/hr	Passage of NFB EE from the SI to the LI
	R NFB Ash Pass	g Ash/hr	Passage of NFB ash from the SI to the LI
Large intestine	*Rumen fiber bacteria*		
	R FB AA N Out	g AA N/hr	AA N from rumen FB passing out in the feces
	R FB NA N Out	g NA N/hr	Nucleic acid N from rumen FB passing out in the feces
	R FB CW N Out	g CW N/hr	Cell wall N from rumen FB passing out in the feces
	R FB CHO Out	g CHO/hr	CHO from rumen FB passing out in the feces
	R FB EE Out	g EE/hr	EE from rumen FB passing out in the feces
	R FB Ash Out	g Ash/hr	Ash from rumen FB passing out in the feces
	Rumen non-fiber bacteria		
	R NFB AA N Out	g AA N/hr	AA N from rumen NFB passing out in the feces
	R NFB NA N Out	g NA N/hr	Nucleic acid N from rumen NFB passing out in the feces
	R NFB CW N Out	g CW N/hr	Cell wall N from rumen NFB passing out in the feces
	R NFB CHO Out	g CHO/hr	CHO from rumen NFB passing out in the feces
	R NFB EE Out	g EE/hr	EE from rumen NFB passing out in the feces
	R NFB Ash Out	g Ash/hr	Ash from rumen NFB passing out in the feces
	Large intestine fiber bacteria		
	B3 fast CHO LI Deg*i*	g CHO/hr	Degradation of B3 fast CHO
	B3 slow CHO LI Deg*i*	g CHO/hr	Degradation of B3 slow CHO
	LI B3 fast CHO for Maint*i*	g CHO/hr	B3 fast CHO being used for maintenance
	LI B3 slow CHO for Maint*i*	g CHO/hr	B3 slow CHO being used for maintenance
	LI B3 fast CHO for Growth*i*	g CHO/hr	B3 fast CHO being used for growth
	LI B3 slow CHO for Growth*i*	g CHO/hr	B3 slow CHO being used for growth
	LI FB Growth Energy	g CHO/hr	Fiber CHO being used to generate energy to grow
	LI FB Cell Growth	g CHO/hr	Fiber being used for cell growth
	LI FB N Out	g N/hr	N from LI FB passing out in the feces

Table 4.3. *(Continued)*

Compartment	Flow[1]	Units	Description
	LI FB CHO Out	g CHO/hr	CHO from LI FB passing out in the feces
	LI FB EE Out	g EE/hr	EE from LI FB passing out in the feces
	LI FB Ash Out	g Ash/hr	Ash from LI FB passing out in the feces
	Large intestine non-fiber bacteria		
	A4 CHO LI Deg*i*	g CHO/hr	Degradation of A4 CHO
	B1 CHO LI Deg*i*	g CHO/hr	Degradation of B1 CHO
	B2 CHO LI Deg*i*	g CHO/hr	Degradation of B2 CHO
	LI A4 CHO for Maint*i*	g CHO/hr	A4 CHO being used for maintenance
	LI B1 CHO for Maint*i*	g CHO/hr	B1 CHO being used for maintenance
	LI B2 CHO for Maint*i*	g CHO/hr	B2 CHO being used for maintenance
	LI A4 CHO for Growth*i*	g CHO/hr	A4 CHO being used for growth
	LI B1 CHO for Growth*i*	g CHO/hr	B1 CHO being used for growth
	LI B2 CHO for Growth*i*	g CHO/hr	B2 CHO being used for growth
	LI NFB Growth Energy	g CHO/hr	Non-fiber CHO being used to generate energy to growth
	LI NFB Cell Growth	g CHO/hr	Non-fiber CHO being used for cell growth
	LI NFB N Out	g N/hr	N from LI NFB passing out in the feces
	LI NFB CHO Out	g CHO/hr	CHO from LI NFB passing out in the feces
	LI NFB EE Out	g EE/hr	EE from LI NFB passing out in the feces
	LI NFB Ash Out	g Ash/hr	Ash from LI NFB passing out in the feces

[1] Subscript *i* refers to the i^{th} feed in the diet.

4.3.2 Protozoa growth

4.3.2.1 General model structure

Previous versions of the CNCPS have accounted for protozoa by reducing the Y_G of bacteria from 0.5 to 0.4 g cells g^{-1} CHO (Russell et al., 1992). However, in high producing dairy cows protozoa can contribute up to 10% of the microbial N flowing from the rumen and have important effects on the dynamics of N metabolism in the rumen (Firkins et al., 2007, Hristov and Jouany, 2005). To capture these effects, aspects of protozoal growth and metabolism were added to the current model.

Although many types of protozoa exist in the rumen, the most important are the ciliates of which there are two groups: Holotrich protozoa (**HPZ**) and Entodiniomorphid protozoa (**EPZ**)

(Williams and Coleman, 1988). The model considers HPZ and EPZ separately based on their preferred growth substrates. Carbohydrate metabolism follows the same model structure as bacterial growth with some differences which are described below. The model structure was deemed appropriate given protozoa require energy for the same general purposes of maintenance and growth as bacteria and exist in the same environment (Williams and Coleman, 1988). Carbohydrates are assumed to be the dominant source of energy to grow with bacteria providing the major source of AA (Williams and Coleman, 1988).

4.3.2.2 Carbohydrate engulfment

Protozoal growth is calculated separately for each carbohydrate pool. It is assumed that EPZ consume starch (B1), soluble fiber (B2) and NDF (B3 slow, B3 fast and C) and that HPZ consume sugar (A4) (Coleman, 1986, Williams and Coleman, 1988). This is a simplification as both types of protozoa can consume each of these substrates (Coleman, 1986). However, HPZ tend to prefer soluble CHO and contribute little to fiber digestion while EPZ rapidly engulf starch granules and have been shown to also break down cellulose and pectin (Coleman, 1986, Williams and Coleman, 1988).

Protozoa initially engulf material which is then metabolized within the cell (Coleman, 1992). In order for material to be engulfed, it must first be of an appropriate size (Onodera and Henderson, 1980). The rate at which starch digests in the rumen is a function of both physical and chemical characteristics of which particle size is an important component (Offner et al., 2003). It was assumed, on a relative basis, the same physical and chemical characteristics among different feeds would impact the ability of both bacteria and protozoa to digest CHO. Also, kd would provide a reasonable proxy for differentiating engulfment rates among feeds due to

particle size. Therefore, the rate of engulfment for each substrate was determined by adjusting the kd of each CHO source from each feed by a 'capacity restriction'. Coleman (1992) measured a maximum uptake of starch granules of approximately 1.8 g CHO g^{-1} protozoal cells. Engulfment rate was adjusted using a lookup function where kd was multiplied by an adjustment factor according to the ratio of engulfed CHO to protozoal cells (Figure 4.3A). When engulfed CHO exceeded 1.8 g CHO g^{-1} protozoal cells (Coleman, 1992), engulfment rate exponentially declined which provided a feedback loop in the model where engulfment of material was linked to the protozoal cell mass (Figure 4.3A). This same system was used for each of the substrates that could be engulfed. Engulfment rate was also adjusted according to the predicted rumen pH. It is widely reported that excess starch consumption can kill protozoa, and in some cases completely defaunate the rumen (Hristov and Jouany, 2005). It seems more likely this is linked to rumen pH than starch intake *per se* (Dehority, 2005). To model the effect of pH on protozoal growth, the relationship of pH and concentration of protozoa presented by Dehority (2005) was used to derive the adjustment factor in Figure 4.3B. Rumen pH was predicted empirically according to Fox et al. (2004).

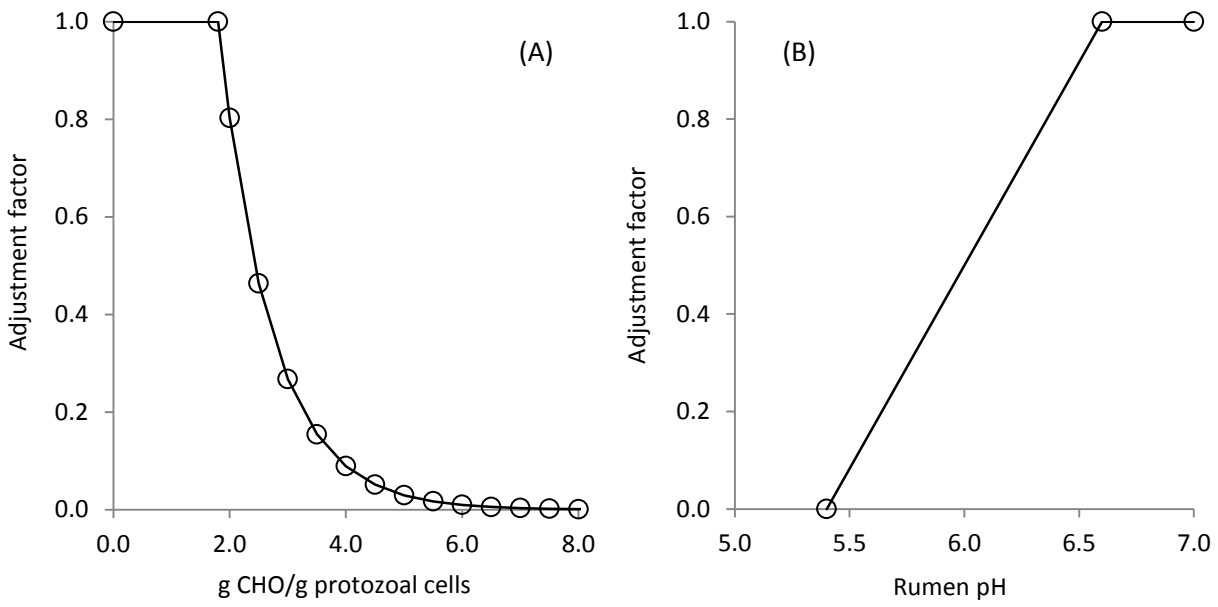

Figure 4.3. Engulfment adjustments for protozoa due to cell capacity (A) and rumen pH (B)

4.3.2.3 Growth and metabolism

Once engulfed, the model assumes material is either metabolized, returned to the rumen pool as protozoa lyse, or escapes through to the small intestine within protozoa as they pass. Breakdown of a substrate within protozoa is relative to the substrate pool size, but occurs slowly (Williams and Coleman, 1988). Slow growth rates and long rumen retention times mean protozoa have higher maintenance requirements and lower growth efficiency relative to bacteria (Hristov and Jouany, 2005). At a macro level, protozoal composition is relatively similar to bacteria (Czerkawski, 1976), and given they exist in the same environment and utilize the same substrates to grow, the ATP yield per unit of digested material should be similar (Stouthamer, 1973). To model this, Y_G is set at 0.5 g cells g^{-1} CHO and '*a'* is set at 0.03 g CHO g^{-1} cells h^{-1}, which is the same as NFB. The kd of each CHO source is again used as a proxy to differentiate digestion rate among engulfed material. Although the particle size of engulfed material will be similar, chemical characteristics that affect kd are assumed to still be present, and different

among substrates and feeds. To utilize the feed library data for protozoa, the digestion rates were multiplied by a reduction factor to account for the slower metabolic rate of protozoa relative to bacteria. The factor used was 0.5 which meant that on average, CHO digestion was approximately 0.25 g CHO g^{-1} protozal cell h^{-1}, similar to reports by Coleman (1992). Reducing the kd also increased the predicted maintenance costs through the equation $m\mu = (kd - a) - a$ (% h^{-1}) which lowered the growth efficiency.

4.3.2.4 Escape and lysis

Disappearance of protozoa from the rumen can occur by either passage or lysis (Ankrah et al., 1990, Hristov and Jouany, 2005). Autolysis is typically reported to be extensive with 66-85% of protozoa recycling within the rumen (Dijkstra et al., 1998). Further, concentrations of protozoa at the duodenum in sheep and goats are typically 20-40% lower than in rumen fluid suggesting protozoa have the ability to avoid passage and remain in the rumen (Hristov and Jouany, 2005). Under these conditions, lysis becomes an important mechanism to control the protozoal pool size in the rumen, as was shown by Dijkstra et al. (1998). Firkins et al. (2007) offers a different viewpoint for high producing dairy cows where rapid rumen turnover and high rates of passage mean a large portion of protozoa simply pass out of the rumen making extensive lysis less important. Under these conditions protozoal pools sizes were lower (4.8-12.7% microbial N), passage rates were similar to feed particles and cell passage was relative to the rumen pool size (Sylvester et al., 2005). To replicate this behavior in the model, protozoa were assumed to pass with the solids passage rate and the flow was assumed to be relative to the pool size. Ankrah et al. (1990) estimated approximately half the disappearance of protozoa in the rumen could be attributed to passage or dilution and half due to lysis meaning the rate of lysis would be similar to the rate of passage. However, these estimates were made in steers fed once a day, which again,

might not reflect the situation in a high producing dairy cow (Firkins et al., 2007). In the current model, disappearance due to lysis was assumed to be half the rate of passage which gave predicted pool sizes in a similar range to those reported by Sylvester et al. (2005).

4.3.2.5 Nitrogen consumption and bacterial predation

Unlike bacteria, protozoa cannot synthesize their own AA and must rely on the consumption of preformed AA for protein synthesis (Williams and Coleman, 1988). Bacteria comprise the single most important AA source, possibly because of their high AA content and consistent supply, although varying amounts of dietary protein are also consumed (Coleman, 1986, Firkins et al., 2007). Compared to CHO consumption, bacterial engulfment is slow where protozoa 'graze' bacteria in a continuous process (Firkins et al., 2007). Engulfed proteins are partially incorporated into protozoal cell proteins and partially released into the rumen medium as either peptides and AA or NH_3 (Walker et al., 2005). *In vitro* studies have shown approximately 50% of engulfed proteins are incorporated into protozoal proteins, while the other 50% are excreted (Hristov and Jouany, 2005). Coleman and Hall (1984) calculated the potential protein synthesis from the uptake of bacteria and free AA and showed, if considered together, bacterial AA would contribute approximately 2/3 to protein synthesis and free AA approximately 1/3. Using these relationships, protozoal N uptake can be calculated as double the requirement for cell growth and bacterial predation can be calculated at 2/3 of this N uptake. It is difficult to find quantitative estimations of AA N release relative to NH_3 in the literature, although protozoa are known to have high deaminase activity (Walker et al., 2005). Therefore, it was assumed that half the N released was in the form of AA N and half as NH_3. The model assumes both NFB and FB are engulfed and follows the hypothesis of Dijkstra et al. (1998) that fibrolytic bacteria are engulfed as a consequence of being attached to fiber particles that are engulfed. Therefore, engulfment of

FB is calculated by multiplying the grams of fiber engulfed by the ratio FB to fiber in the rumen (g FB N g^{-1} fiber), with the assumption being all FB in the rumen are attached. Engulfment of NFB is then calculated as 2/3 the engulfed N – FB engulfment with non-bacterial AA providing the balance of the N consumption.

4.3.2.6 Other growth substrates

The CHO fraction of engulfed bacteria and lysed protozoa were assumed to provide an energy yielding substrate for protozoal growth. Protozoa are known to also engulf other protozoa in the rumen (Williams and Coleman, 1988). For simplicity, only bacterial engulfment was considered in this model, however, lysed protozoa were assumed to be consumed by other protozoa and the CHO used as an energy source to grow. The same general structure was used to calculate protozoal cell yield from engulfed microbial material as other CHO sources. The rate of digestion of microbial CHO was assumed to be 40 % hr^{-1}, similar to sugar (Van Amburgh et al., 2010).

4.3.2.7 Summary of protozoal growth

Figure 4.1 is a diagrammatic representation of EPZ growth on B1 CHO (Starch) used in the model and serves to summarize the relationships described above. In Figure 4.4, protozoa compete for rumen available starch (B1 CHO R) with bacterial degradation (B1 CHO R Deg) and escape of starch to the small intestine (B1 CHO Escape). The rate at which protozoa engulf starch particles is calculated using the rate of starch digestion for each feed (Kd B1 CHO) which is adjusted to ensure engulfment does not exceed EPZ cell capacity (EPZ capacity restriction) and for the effect of rumen pH (pH engulfment adjustment). Substrate engulfment is the first step in supplying energy for protozoa to grow, and if set to 0, will stop protozoal growth and can be

used to simulate the effects of rumen defaunation. Once engulfed, starch is either degraded (EPZ B1 CHO Deg), escapes within the protozoal cells to the small intestine (EPZ B1 Escape), or is released back into the rumen available pool as protozoa lyse (EPZ B1 Engulfed Recycled). The rate of degradation (EPZ Kd B1 CHO) is calculated using the kd for each feed which is adjusted by a factor of 0.5 to represent the slower metabolic rate relative to bacteria (EPZ metabolic rate relative to bacteria). The escape of starch to the small intestine within protozoal cells and the release of starch back to the rumen available pool is calculated by multiplying the rate of cell escape and cell lysis, respectively, by the ratio of engulfed starch to cell mass (Ratio EPZ B1 engulfed to EPZ B1 Cells). Once degraded, the material is either used for maintenance or growth according to the system described for bacteria. The cell mass of protozoa can either escape to the small intestine (EPZ B1 Cell Escape) or lyse (EPZ B1 Cell Lysis). Escape and lysis provide the negative feedback required by the model to control protozal cell mass which allows the simulation to reach steady state. This system is replicated for each growth substrate used by protozoa in the model. A complete list of the protozoal pools and flows, organized by gastrointestinal compartment, are in Tables 4.4 and 4.5. The equations used to calculate the pools and flows are in Tables 4.10 and 4.11.

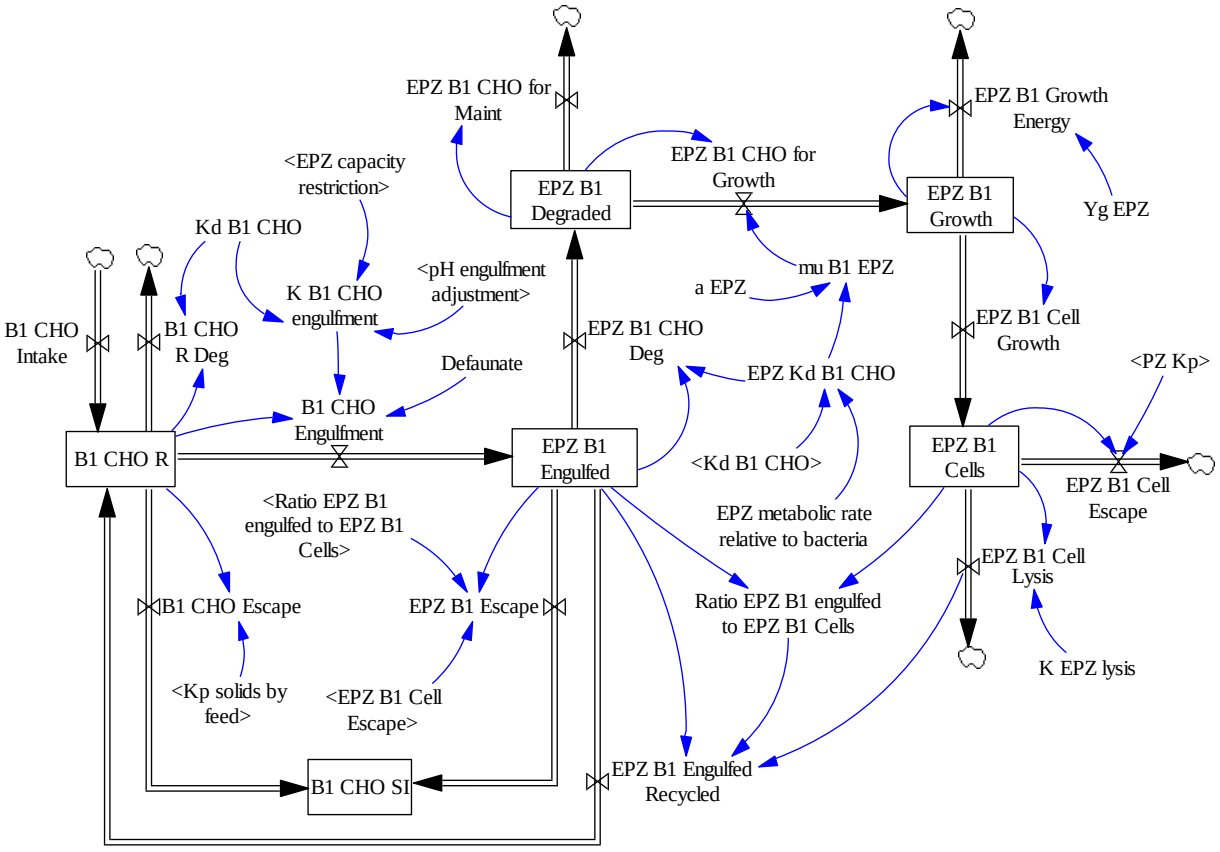

Figure 4.4. Schematic representation of the model used to predict engulfment, recycling, and metabolism of B1 CHO (Starch) in the rumen by Entodiniomorphid protozoa (EPZ).

Table 4.4. Protozoal pools by gastrointestinal compartment.

Compartment	Pool[1]	Units	Description
Rumen	*Entodiniomorphid protozoa*		
	EPZ B1 Engulfed*i*	g CHO	B1 CHO engulfed by EPZ
	EPZ B2 Engulfed*i*	g CHO	B2 CHO engulfed by EPZ
	EPZ B3 fast Engulfed*i*	g CHO	B3 fast CHO engulfed by EPZ
	EPZ B3 slow Engulfed*i*	g CHO	B3 slow CHO engulfed by EPZ
	EPZ C Engulfed*i*	g CHO	C CHO engulfed by EPZ
	EPZ Engulfed M	g CHO	Microbial CHO engulfed by EPZ
	EPZ B1 Degraded*i*	g CHO	B1 CHO degraded by EPZ
	EPZ B2 Degraded*i*	g CHO	B2 CHO degraded by EPZ
	EPZ B3 fast Degraded*i*	g CHO	B3 fast CHO degraded by EPZ
	EPZ B3 slow Degraded*i*	g CHO	B3 slow CHO degraded by EPZ
	EPZ Degraded M	g CHO	Microbial CHO degraded by EPZ
	EPZ B1 Maint	g CHO	B1 CHO used by EPZ for maintenance
	EPZ B2 Maint	g CHO	B2 CHO used by EPZ for maintenance
	EPZ B3 fast Maint	g CHO	B3 fast CHO used by EPZ for maintenance
	EPZ B3 slow Maint	g CHO	B3 slow CHO used by EPZ for maintenance
	EPZ M Maint	g CHO	Microbial CHO used by EPZ for maintenance
	EPZ B1 Growth	g CHO	B1 CHO used by EPZ for growth
	EPZ B2 Growth	g CHO	B2 CHO used by EPZ for growth
	EPZ Fiber Growth	g CHO	Fiber CHO used by EPZ for growth
	EPZ M Growth	g CHO	Microbial CHO used by EPZ for growth
	EPZ B1 Energy*i*	g CHO	B1 CHO used by EPZ to generate energy to grow
	EPZ B2 Energy	g CHO	B2 CHO used by EPZ to generate energy to grow
	EPZ Fiber Energy	g CHO	Fiber CHO used by EPZ to generate energy to grow
	EPZ M Energy	g CHO	Microbial CHO used by EPZ to generate energy to grow
	EPZ B1 Cells	g CHO	B1 CHO used for cell growth
	EPZ B2 Cells	g CHO	B2 CHO used for cell growth
	EPZ Fiber Cells	g CHO	Fiber CHO used for cell growth
	EPZ M Cells	g CHO	Microbial CHO used for cell growth
	Holotrich protozoa		
	HPZ A4 Engulfed*i*	g CHO	A4 CHO engulfed by HPZ
	HPZ Engulfed M	g CHO	Microbial CHO engulfed by HPZ
	HPZ A4 Degraded*i*	g CHO	A4 CHO degraded by HPZ
	HPZ Degraded M	g CHO	Microbial CHO degraded by HPZ
	HPZ A4 Maint	g CHO	A4 CHO used by HPZ for maintenance
	HPZ M Maint	g CHO	Microbial CHO used by HPZ for maintenance
	HPZ A4 Growth	g CHO	A4 CHO used by HPZ for growth
	HPZ M Growth	g CHO	Microbial CHO used by HPZ for growth
	HPZ A4 Energy	g CHO	A4 CHO used by HPZ to generate energy to grow
	HPZ M Energy	g CHO	Microbial CHO used by HPZ to generate energy to grow
	HPZ A4 Cells	g CHO	A4 CHO used by HPZ for cell growth
	HPZ M Cells	g CHO	Microbial CHO used by HPZ for cell growth

Table 4.4. *(Continued)*

Compartment	Pool[1]	Units	Description
Small intestine	*Protozoa*		
	PZ N SI	g N	PZ N in the SI
	PZ CHO SI	g CHO	PZ CHO in the SI
	PZ EE SI	g EE	PZ EE in the SI
	PZ Ash SI	g Ash	PZ ash in the SI
Large intestine	*Protozoa*		
	PZ AA N LI	g AA N	AA N from PZ in the LI
	PZ NA N LI	g NA N	Nucleic acid N from PZ in the LI
	PZ CW N LI	g CW N	Cell wall N from PZ in the LI
	PZ CHO LI	g CHO	CHO from PZ in the LI
	PZ EE LI	g EE	EE from PZ in the LI
	PZ Ash LI	g Ash	Ash from PZ in the LI

[1] Subscript *i* refers to the i^{th} feed in the diet.

Table 4.5. Protozoal flows by process and compartment.

Compartment	Flow[1]	Units	Description
Substrate intake and cycling	*Entodiniomorphid protozoa*		
	B1 CHO Engulfment*i*	g CHO/hr	Engulfment of B1 CHO
	B2 CHO Engulfment*i*	g CHO/hr	Engulfment of B2 CHO
	B3 fast CHO Engulfment*i*	g CHO/hr	Engulfment of B3 fast CHO
	B3 slow CHO Engulfment*i*	g CHO/hr	Engulfment of B3 slow CHO
	C CHO Engulfment*i*	g CHO/hr	Engulfment of C CHO
	EPZ Bacterial CHO Engulfed	g CHO/hr	Engulfment of bacterial CHO
	EPZ Engulfed Lysed PZ CHO	g CHO/hr	Engulfment of lysed PZ CHO
	EPZ B1 Engulfed Recycled*i*	g CHO/hr	Engulfed B1 CHO returning to the rumen pool
	EPZ B2 Engulfed Recycled*i*	g CHO/hr	Engulfed B2 CHO returning to the rumen pool
	EPZ B3 fast Engulfed Recycled*i*	g CHO/hr	Engulfed B3 fast CHO returning to the rumen pool
	EPZ B3 slow Engulfed Recycled*i*	g CHO/hr	Engulfed B3 slow CHO returning to the rumen pool
	EPZ C Engulfed Recycled*i*	g CHO/hr	Engulfed C CHO returning to the rumen pool
	EPZ B1 Escape*i*	g CHO/hr	Engulfed B1 CHO escaping in PZ cells
	EPZ B2 Escape*i*	g CHO/hr	Engulfed B2 CHO escaping in PZ cells
	EPZ B3 fast Escape*i*	g CHO/hr	Engulfed B3 fast CHO escaping in PZ cells
	EPZ B3 slow Escape*i*	g CHO/hr	Engulfed B3 slow CHO escaping in PZ cells
	EPZ C Escape*i*	g CHO/hr	Engulfed C CHO escaping in PZ cells
	Holotrich protozoa		
	A4 CHO Engulfment*i*	g CHO/hr	Engulfment of A4 CHO
	HPZ Bacterial CHO Engulfed	g CHO/hr	Engulfment of bacterial CHO
	HPZ Engulfed Lysed PZ CHO	g CHO/hr	Engulfment of lysed PZ CHO
	HPZ A4 Engulfed Recycled*i*	g CHO/hr	Engulfed A4 CHO retiring to the rumen pool
	HPZ A4 Escape*i*	g CHO/hr	Engulfed A4 CHO escaping in PZ cells
Growth and metabolism	*Entodiniomorphid protozoa*		
	EPZ B1 CHO Deg*i*	g CHO/hr	Degradation of B1 CHO by EPZ
	EPZ B2 CHO Deg*i*	g CHO/hr	Degradation of B2 CHO by EPZ
	EPZ B3 fast CHO Deg*i*	g CHO/hr	Degradation of B3 fast CHO by EPZ
	EPZ B3 slow CHO Deg*i*	g CHO/hr	Degradation of B3 slow CHO by EPZ
	EPZ M Deg	g CHO/hr	Degradation of microbial CHO by EPZ
	EPZ B1 CHO for Maint*i*	g CHO/hr	B1 CHO used by EPZ for maintenance
	EPZ B2 CHO for Maint*i*	g CHO/hr	B2 CHO used by EPZ for maintenance
	EPZ B3 fast CHO for Maint*i*	g CHO/hr	B3 fast CHO used by EPZ for maintenance
	EPZ B3 slow CHO for Maint*i*	g CHO/hr	B3 slow CHO used by EPZ for maintenance
	EPZ M for Maint	g CHO/hr	Microbial CHO used by EPZ for maintenance
	EPZ B1 CHO for Growth*i*	g CHO/hr	B1 CHO used by EPZ for growth
	EPZ B2 CHO for Growth*i*	g CHO/hr	B2 CHO used by EPZ for growth
	EPZ B3 fast CHO for Growth*i*	g CHO/hr	B3 fast CHO used by EPZ for growth
	EPZ B3 slow CHO for Growth*i*	g CHO/hr	B3 slow CHO used by EPZ for growth

Table 4.5. *(Continued)*

Compartment	Flow[1]	Units	Description
	EPZ M for Growth	g CHO/hr	Microbial CHO used by EPZ for growth
	EPZ B1 Growth Energy	g CHO/hr	B1 CHO used by EPZ to generate energy to grow
	EPZ B2 Growth Energy	g CHO/hr	B2 CHO used by EPZ to generate energy to grow
	EPZ Fiber Growth Energy	g CHO/hr	Fiber CHO used by EPZ to generate energy to grow
	EPZ M Growth Energy	g CHO/hr	Microbial CHO used by EPZ to generate energy to grow
	EPZ B1 Cell Growth	g CHO/hr	B1 CHO used for EPZ cell growth
	EPZ B2 Cell Growth	g CHO/hr	B2 CHO used for EPZ cell growth
	EPZ Fiber Cell Growth	g CHO/hr	Fiber CHO used for EPZ cell growth
	EPZ M Cell Growth	g CHO/hr	Microbial CHO used for EPZ cell growth
	EPZ B1 Cell Lysis	g EPZ cells/hr	Lysis of EPZ cells grown with B1 CHO
	EPZ B2 Cell Lysis	g EPZ cells/hr	Lysis of EPZ cells grown with B2 CHO
	EPZ Fiber Cell Lysis	g EPZ cells/hr	Lysis of EPZ cells grown with fiber CHO
	EPZ M Cell Lysis	g EPZ cells/hr	Lysis of EPZ cells grown with microbial CHO
	EPZ B1 Cell Escape	g EPZ cells/hr	Escape of EPZ cells grown with B1 CHO
	EPZ B2 Cell Escape	g EPZ cells/hr	Escape of EPZ cells grown with B2 CHO
	EPZ Fiber Cell Escape	g EPZ cells/hr	Escape of EPZ cells grown with fiber CHO
	EPZ M Cell Escape	g EPZ cells/hr	Escape of EPZ cells grown with microbial CHO
	Holotrich protozoa		
	HPZ A4 CHO Deg*i*	g CHO/hr	Degradation of A4 CHO by HPZ
	HPZ M Deg	g CHO/hr	Degradation of microbial CHO by HPZ
	HPZ A4 CHO for Maint*i*	g CHO/hr	A4 CHO used by HPZ for maintenance
	HPZ M for Maint	g CHO/hr	Microbial CHO used by HPZ for maintenance
	HPZ A4 CHO for Growth*i*	g CHO/hr	A4 CHO used by HPZ for growth
	HPZ M for Growth	g CHO/hr	Microbial CHO used by HPZ for growth
	HPZ A4 Growth Energy	g CHO/hr	A4 CHO used by HPZ to generate energy to grow
	HPZ M Growth Energy	g CHO/hr	Microbial CHO used by HPZ to generate energy to grow
	HPZ A4 Cell Growth	g CHO/hr	A4 CHO used for HPZ cell growth
	HPZ M Cell Growth	g CHO/hr	Microbial CHO used for HPZ cell growth
	HPZ A4 Cell Lysis	g HPZ cells/hr	Lysis of EPZ cells grown with A4 CHO
	HPZ M Cell Lysis	g HPZ cells/hr	Lysis of EPZ cells grown with microbial CHO
	HPZ A4 Cell Escape	g HPZ cells/hr	Escape of EPZ cells grown with A4 CHO
	HPZ M Cell Escape	g HPZ cells/hr	Escape of EPZ cells grown with microbial CHO

Table 4.5. *(Continued)*

Compartment	Flow[1]	Units	Description
Small intestine	*Protozoa*		
	PZ AA N ID	g AA N/hr	Digestion of PZ AA N in the SI
	PZ NA N ID	g NA N/hr	Digestion of PZ nucleic acid N in the SI
	PZ CW N ID	g CW N/hr	Digestion of PZ cell wall N in the SI
	PZ CHO ID	g CHO/hr	Digestion of PZ CHO in the SI
	PZ EE ID	g EE/hr	Digestion of PZ EE in the SI
	PZ Ash ID	g Ash/hr	Digestion of PZ ash in the SI
	PZ AA N Pass	g AA N/hr	Passage of PZ AA N from the SI to the LI
	PZ NA N Pass	g NA N/hr	Passage of PZ nucleic acid N from the SI to the LI
	PZ CW N Pass	g CW N/hr	Passage of PZ cell wall N from the SI to the LI
	PZ CHO Pass	g CHO/hr	Passage of PZ CHO from the SI to the LI
	PZ EE Pass	g EE/hr	Passage of PZ EE from the SI to the LI
	PZ Ash Pass	g Ash/hr	Passage of PZ ash from the SI to the LI
Large intestine	*Protozoa*		
	PZ AA N Out	g AA N/hr	PZ AA N passing out in the feces
	PZ NA N Out	g NA N/hr	PZ nucleic acid N passing out in the feces
	PZ CW N Out	g CW N/hr	PZ cell wall N passing out in the feces
	PZ CHO Out	g CHO/hr	PZ CHO passing out in the feces
	PZ EE Out	g EE/hr	PZ EE passing out in the feces
	PZ Ash Out	g Ash/hr	PZ ash passing out in the feces

[1] Subscript *i* refers to the i^{th} feed in the diet.

4.4 Model behavior

Examples of how predictions of microbial growth behave under different dietary conditions, with and without protozoa, are presented in Figure 4.5 and Table 4.7. Dietary comparisons include high and low levels of forage at high or low levels of intake. The diet makeup, chemical composition and level of intake for each comparison are in Table 4.6. Diets were formulated to provide a 600 kg animal with enough energy and protein to support 45 kg milk at the high level of intake and 20 kg milk/d at the low level of intake. Simulations are run for 300 hours which is the time required for all diets to reach steady state within the rumen submodel.

Table 4.6. Example diets with high and low levels of forage at high and low intakes used to demonstrate the behaviour of microbial growth in the model

	High intake		Low intake	
	Low forage	High forage	Low forage	High forage
DMI (kg/d)	25.0	25.0	15.0	15.0
Diet ingredient (% DM)				
Corn Silage	12.0	43.6	12.0	43.6
Grass Hay	20.0	13.0	20.0	13.0
Alfalfa Hay	10.0	13.0	10.0	13.0
Corn meal	32.0	18.0	32.0	18.0
Soybean Meal	12.0	12.0	12.0	12.0
Soybean Hulls	12.0	0.0	12.0	0.0
Blood meal	0.0	0.4	0.0	0.4
Protected fat	2.0	0.0	2.0	0.0
Forage (% of diet DM)	42.0	70.0	42.0	70.0
Diet composition (% DM)				
CP	15.5	15.5	15.5	15.5
Starch	29.2	29.5	29.2	29.5
NDF	34.3	34.6	34.3	34.6
EE	5.2	3.2	5.2	3.2
Ash	4.7	5.1	4.7	5.1

Predicted rumen pools of FB N and NFB N are reduced by protozoal growth (Figure 4.5). This occurs due to predation and also competition for substrate. Non-fiber bacteria are most affected as they exist in the fluid phase and are more accessible for protozoa to engulf (Dijkstra et al., 1998). Fiber bacteria are also engulfed as a collateral effect of fiber engulfment (Dijkstra et al., 1998). Protozoal pool sizes when intake was high were 4.2% and 9.2% of the microbial N for the low and high forage diets, respectively, and are within the range and follow the same trend reported by Sylvester et al. (2005). Pool sizes on the lower intake diets are higher which is due to lower predicted passage. A positive feedback exists within the model where, as the protozoal cell mass increases, more substrate can be engulfed. This is controlled by lysis, passage and also the ability of protozoa to digest engulfed material. Engulfment is typically more rapid than digestion (Coleman, 1992), which leads to an accumulation of substrate within the cell and restricts further engulfment (Figure 4.3A). Engulfment rates in the examples presented ranged from 0.46 to 0.97 g CHO g^{-1} PZ cells hr^{-1} (Table 4.7) which is comparable to the range reported by Coleman (1992) for fed cells. Likewise, the digestion rate of engulfed material (0.16 – 0.30 g CHO g^{-1} PZ cells hr^{-1}) was comparable to values measured by Coleman (1992). The low cell mass of protozoa on the low forage diet at high intake results in a high ratio of engulfed CHO to protozoal cells (3.55) and restricts further engulfment (Figure 4.3A). The low forage diet has a slightly lower pH which also restricts substrate engulfment. Protozoa can have a stabilizing effect on rumen pH by lowering the available CHO pool (Hristov and Jouany, 2005) and the model estimates lower available CHO in the presence of protozoa (Table 4.7), however, a more mechanistic approach to calculate pH is needed to adequately model this effect. Important differences exist in rumen NH_3-N among the faunated and defaunted simulations. Protozoa make a significant contribution to microbial protein turnover in the rumen which increases peptides,

free AA and NH_3-N (Walker et al., 2005). In situations where rumen N is deficient, the effect of protozoa in the model stimulates bacterial growth and CHO digestion through increasing the rumen N supply, although net microbial flow out of the rumen is still reduced through predation. Predicted microbial turnover ranged from ~10% to 40% which is lower than what is typically reported (Hristov and Jouany, 2005), but this might be expected in high producing animals (Firkins et al., 2007). Overall efficiencies of microbial growth in the faunated simulations ranged from 17.4 to 28.5 g microbial N kg^{-1} RD OM which is similar to the finding of Broderick et al. (2010). Values in the defaunated simulations were higher than what might be expected and demonstrates the importance of including protozoa in the model.

Predictions of protozoal growth were most sensitive to the rates of lysis and passage. Figure 4.6 has examples of predicted microbial pools sizes when lysis or passage are set to 0, or when both lysis and passage are reduced to half the normal model values (passage = solids kp; lysis = 0.5 × passage). Eliminating protozoal passage had the most pronounced effect on the rumen cell N with protozoal N increasing to ~55% of microbial N (Figure 4.6C) which is closer to most literature reports (Hristov and Jouany, 2005). Given many of the studies in the literature were completed on sheep or steers at low levels of intake, protozoal sequestration mechanisms were probably more effective and cell passage very low. It would be possible to implement these mechanisms in the current model by restricting the pool size that was available to pass at low levels of intake. However, for high producing dairy cows predictions are consistent with expected results.

Table 4.7. Predicted rumen parameters and microbial growth efficiency with and without protozoa in diets with high (70%) and low (42%) forage content at high (25 kg/d) and low (15 kg/d) levels of intake.

		High intake		Low intake	
Item[1]	Faunation[2]	Low forage	High forage	Low forage	High forage
Rumen PZ N pool (% microbial N)	F	4.2%	9.2%	10.1%	23.3%
Bacterial CHO digestion (% total)	F	94.1%	87.8%	89.8%	81.0%
PZ CHO digestion (% total)	F	6.0%	12.3%	10.2%	19.0%
Rate of PZ CHO engulfment (g CHO g^{-1} PZ cells hr^{-1})	F	0.97	0.74	0.68	0.46
Rate of PZ CHO digestion (g CHO g^{-1} PZ cells hr^{-1})	F	0.30	0.24	0.21	0.16
Ratio of engulfed CHO to PZ cell mass (g CHO g^{-1} PZ cells)	F	3.55	2.24	2.53	1.54
Capacity engulfment adjustment	F	0.16	0.40	0.32	0.67
pH engulfment adjustment	F	0.84	0.88	0.84	0.88
Microbial N turnover (%)	F	10.1%	20.3%	21.5%	39.3%
Rumen NH_3-N (mg/dl)	F	10.3	11.0	15.8	17.6
	D	8.0	6.9	12.2	10.7
Rumen pdCHO pool size (g)	F	5807	5505	3718	3432
	D	5988	6121	3866	3664
MGE (g microbial N kg^{-1} RD OM)	F	28.5	27.3	19.9	17.4
	D	31.3	33.2	23.8	25.0

[1] Abbreviations include: PZ = protozoa; CHO = carbohydrates; pdCHO = potentially digestible carbohydrates; MGE = microbial growth efficiency; OM = organic matter; RD = rumen digested.

[2] Faunation indicates if the rumen is faunated (F) or defaunated (D).

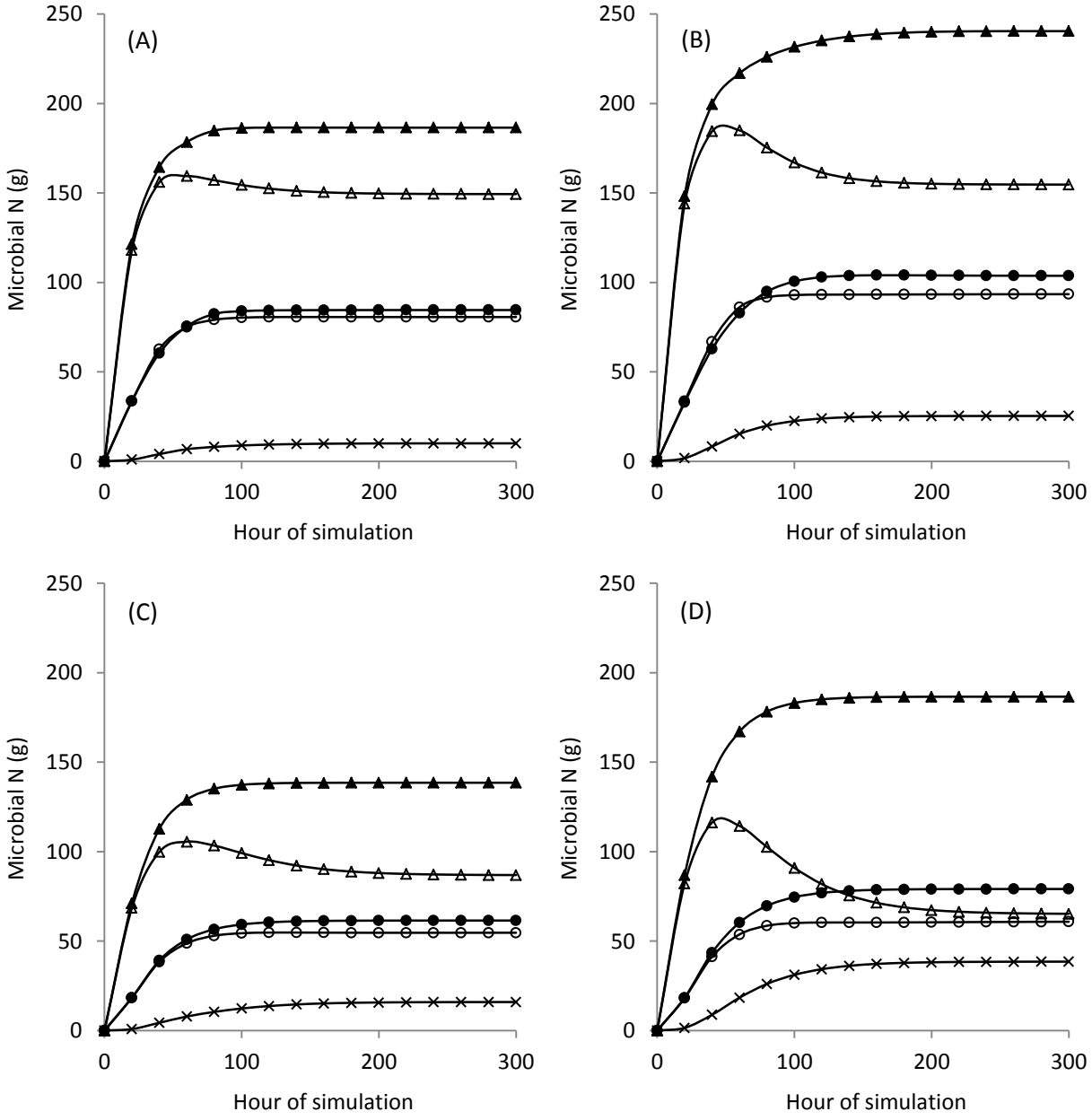

Figure 4.5. Rumen microbial N pools in diet simulations at high intakes with low (A) or high (B) levels of forage or low intakes with low (C) or high (D) levels of forage where the rumen was either faunated or defaunated. Microbial populations in the faunated rumen include: Non-fiber bacteria (Δ), fiber bacteria (○) and protozoa (×). Microbial populations in the defaunated rumen include: Non-fiber bacteria (▲) and fiber bacteria (●).

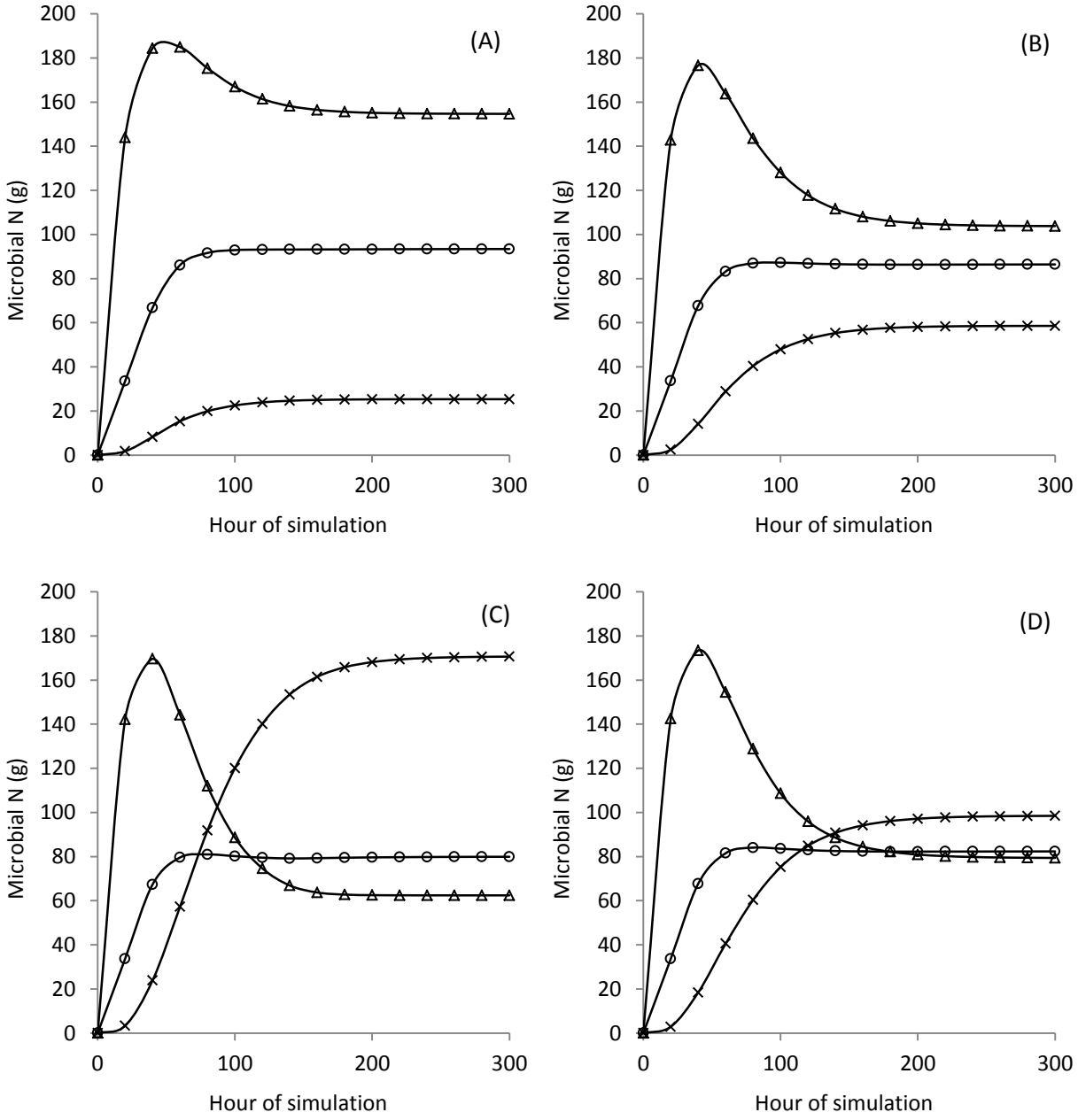

Figure 4.6. Rumen microbial N pools at high intake and high forage (Figure 4.5B) where protozoal lysis and passage are either: normal (A); passage is normal but lysis is 0 (B); lysis is normal but passage is 0 (C); passage and lysis are both half of normal (D). Microbial populations include: Non-fiber bacteria (Δ), fiber bacteria (○) and protozoa (×).

4.5 Implications

Rumen metabolism is a dynamic process with many interacting factors influencing the digestion of nutrients and the supply of microbial protein to the animal. Important additions to this version of the CNCPS include estimations of protozoal growth and a mechanistic large intestine. Protozoa have an important influence on microbial supply to the animal and nutrient cycling within the rumen while the large intestine contributes a varying amount to CHO digestion and is an important component of whole body N metabolism. Construction of this new dynamic version of the CNCPS provides new capability to estimate these interactions and their effects on the rumen environment for application in routine diet formulation.

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4.7 Appendix

Table 4.8. Differential equations used to calculate bacterial pools. The equations follow the general form d/dt $pool_t = flow_t$

Pool[1]	Equation	
Fiber bacteria		
R FB B3 fast Degraded*i*	B3 fast CHO R Deg*i* - R B3 fast CHO for Growth*i* - R B3 fast CHO for Maint*i*	(1.1)
R FB B3 slow Degraded*i*	B3 slow CHO R Deg*i* - R B3 slow CHO for Growth*i* - R B3 slow CHO for Maint*i*	(1.2)
R FB B3 fast Maint	sum(R B3 fast CHO for Maint*i*)	(1.3)
R FB B3 slow Maint	sum(R B3 slow CHO for Maint*i*)	(1.4)
R FB CHO Growth	sum(R B3 slow CHO for Growth*i*) +(sum(R B3 fast CHO for Growth*i*) - R FB Growth Energy - R FB Cell Growth	(1.5)
R FB CHO Energy	R FB Growth Energy	(1.6)
R FB CHO Cells	R FB Cell Growth - R FB Cell Escape - R FB CHO Cell Engulfment	(1.7)
Non-fiber bacteria		
R NFB A2 Degraded*i*	A2 CHO R Deg*i* - R A2 CHO for Growth*i* - R A2 CHO for Maint*i*	(1.8)
R NFB A3 Degraded*i*	(A3 CHO R Deg*i* × 0.5) - R A3 CHO for Growth*i* - R A3 CHO for Maint*i*	(1.9)
R NFB A4 Degraded*i*	A4 CHO R Deg*i* - R A4 CHO for Maint*i* - R A4 CHO for Growth*i*	(1.10)
R NFB B1 Degraded*i*	B1 CHO R Deg*i* - R B1 CHO for Growth*i* - R B1 CHO for Maint*i*	(1.11)
R NFB B2 Degraded*i*	B2 CHO R Deg*i* - R B2 CHO for Growth*i* - R B2 CHO for Maint*i*	(1.12)
R NFB A2 Maint	sum(R A2 CHO for Maint*i*)	(1.13)
R NFB A3 Maint	sum(R A3 CHO for Maint*i*)	(1.14)
R NFB A4 Maint	sum(R A4 CHO for Maint*i*)	(1.15)
R NFB B1 Maint	sum(R B1 CHO for Maint*i*)	(1.16)
R NFB B2 Maint	sum(R B2 CHO for Maint*i*)	(1.17)
R NFB CHO Growth	(sum(R A2 CHO for Growth*i* + R A3 CHO for Growth*i* + R A4 CHO for Growth*i* + R B1 CHO for Growth*i* + R B2 CHO for Growth*i*)) - R NFB Cell Growth - R NFB Growth Energy	(1.18)
R NFB CHO Energy	R NFB Growth Energy	(1.19)
R NFB CHO Cells	R NFB Cell Growth - R NFB Cell Escape - R NFB CHO Cell Engulfment	(1.20)
Rumen Fiber bacteria		
R FB N SI	FB Cell N Escape + FB PAA N Escape - R FB CW N Pass - R FB AA N ID - R FB AA N Pass - R FB CW N ID - R FB NA N ID - R FB NA N Pass	(1.21)
R FB CHO SI	R FB CHO Escape - R FB CHO Ab - R FB CHO Pass	(1.22)
R FB EE SI	R FB EE Escape - R FB EE Ab - R FB EE Pass	(1.23)
R FB Ash SI	R FB Ash Escape - R FB Ash Ab - R FB Ash Pass	(1.24)
Rumen non-fiber bacteria		
R NFB N SI	NFB Cell N Escape + NFB PAA N Escape - R NFB AA N ID - R NFB AA N Pass - R NFB CW N ID - R NFB NA N ID - R NFB NA N Pass - R NFB CW N Pass	(1.25)
R NFB CHO SI	R NFB CHO Escape - R NFB CHO AB - R NFB CHO Pass	(1.26)
R NFB EE SI	R NFB EE Escape - R NFB EE Ab - R NFB EE Pass	(1.27)
R NFB Ash SI	R NFB Ash Escape - R NFB Ash Ab - R NFB Ash Pass	(1.28)

Table 4.8. *(Continued)*

Pool[1]	Equation	
Rumen Fiber bacteria		
R FB AA N LI	R FB AA N Pass - R FB AA N Out	(1.29)
R FB NA N LI	R FB NA N Pass - R FB NA N Out	(1.30)
R FB CW N LI	R FB CW N Pass - R FB CW N Out	(1.31)
R FB CHO LI	R FB CHO Pass - R FB CHO Out	(1.32)
R FB EE LI	R FB EE Pass - R FB EE Out	(1.33)
R FB Ash LI	R FB Ash Pass - R FB Ash Out	(1.34)
Rumen non-fiber bacteria		
R NFB AA N LI	R NFB AA N Pass - R NFB AA N Out	(1.35)
R NFB NA N LI	R NFB NA N Pass - R NFB NA N Out	(1.36)
R NFB CW N LI	R NFB CW N Pass - R NFB CW N Out	(1.37)
R NFB CHO LI	R NFB CHO Pass - R NFB CHO Out	(1.38)
R NFB EE LI	R NFB EE Pass - R NFB EE Out	(1.39)
R NFB Ash LI	R NFB Ash Pass - R NFB Ash Out	(1.40)
Large intestine fiber bacteria		
LI FB B3 fast Degraded*i*	B3 fast CHO LI Deg*i* - LI B3 fast CHO for Growth*i* - LI B3 fast CHO for Maint*i*	(1.41)
LI FB B3 slow Degraded*i*	B3 slow CHO LI Deg*i* - LI B3 slow CHO for Growth*i* - LI B3 slow CHO for Maint*i*	(1.42)
LI FB B3 fast Maint	sum(LI B3 fast CHO for Maint*i*)	(1.43)
LI FB B3 slow Maint	sum(LI B3 slow CHO for Maint*i*)	(1.44)
LI FB CHO Growth	sum(LI B3 fast CHO for Growth*i*) + sum(LI B3 slow CHO for Growth*i*) - LI FB Growth Energy - LI FB Cell Growth	(1.45)
LI FB CHO Energy	LI FB Growth Energy	(1.46)
LI FB CHO Cells	LI FB Cell Growth - LI FB CHO Cells Out	(1.47)
Large intestine non-fiber bacteria		
LI NFB A4 Degraded*i*	A4 CHO LI Deg*i* - LI A4 CHO for Growth*i* - LI A4 CHO for Maint*i*	(1.48)
LI NFB B1 Degraded*i*	B1 CHO LI Deg*i* - LI B1 CHO for Growth*i* - LI B1 CHO for Maint*i*	(1.49)
LI NFB B2 Degraded*i*	B2 CHO LI Deg*i* - LI B2 CHO for Growth*i* - LI B2 CHO for Maint*i*	(1.50)
LI NFB A4 Maint	sum(LI A4 CHO for Maint*i*)	(1.51)
LI NFB B1 Maint	sum(LI B1 CHO for Maint*i*)	(1.52)
LI NFB B2 Maint	sum(LI B2 CHO for Maint*i*)	(1.53)
LI NFB CHO Growth	sum(LI A4 CHO for Growth*i*) + sum(LI B1 CHO for Growth*i*) + sum(LI B2 CHO for Growth*i*) - LI NFB Growth Energy - LI NFB Cell Growth	(1.54)
LI NFB CHO Energy	LI NFB Growth Energy	(1.55)
LI NFB CHO Cells	LI NFB Cell Growth - LI NFB CHO Cells Out	(1.56)

[1] Subscript *i* refers to the i^{th} feed in the diet.

Table 4.9. Equations used to calculate the flows between bacterial pools

Flow[1]	Equation	
Fiber bacteria		
B3 fast CHO R Deg*i*	((B3 fast CHO R*i* × Kd B3 fast CHO*i*) × ph Inhibition) × Rumen NH3 allowable growth	(2.1)
B3 slow CHO R Deg*i*	((B3 slow CHO R*i* × Kd B3 slow CHO*i*) × ph Inhibition) × Rumen NH3 allowable growth)	(2.2)
R B3 fast CHO for Maint*i*	R FB B3 fast Degraded*i*	(2.3)
R B3 slow CHO for Maint*i*	R FB B3 slow Degraded*i*	(2.4)
R B3 fast CHO for Growth*i*	R FB B3 fast Degraded*i* × mu B3 fast*i*	(2.5)
R B3 slow CHO for Growth*i*	R FB B3 slow Degraded*i* × mu B3 slow*i*	(2.6)
R FB Growth Energy	R FB CHO Growth × ((1 / Yg FB) - 1)	(2.7)
R FB Cell Growth	R FB CHO Growth	(2.8)
R FB CHO Cell Engulfment	FB Cell N Engulfed / FB N	(2.9)
R FB Cell Escape	R FB CHO Cells × Kp solids mean	(2.10)
Non-fiber bacteria		
A2 CHO R Deg*i*	A2 CHO R*i* × Kd A2 CHO*i*	(2.11)
A3 CHO R Deg*i*	A3 CHO R*i* × Kd A3 CHO*i*	(2.12)
A4 CHO R Deg*i*	A4 CHO R*i* × Kd A4 CHO*i*	(2.13)
B1 CHO R Deg*i*	B1 CHO R*i* × Kd B1 CHO*i*	(2.14)
B2 CHO R Deg*i*	B2 CHO R*i* × Kd B2 CHO*i*	(2.15)
R A2 CHO for Maint*i*	R NFB A2 Degraded*i*	(2.16)
R A3 CHO for Maint*i*	R NFB A3 Degraded*i*	(2.17)
R A4 CHO for Maint*i*	R NFB A4 Degraded*i*	(2.18)
R B1 CHO for Maint*i*	R NFB B1 Degraded*i*	(2.19)
R B2 CHO for Maint*i*	R NFB B2 Degraded*i*	(2.20)
R A2 CHO for Growth*i*	(R NFB A2 Degraded*i* × mu A2 CHO*i*) × 0.5	(2.21)
R A3 CHO for Growth*i*	R NFB A3 Degraded*i* × mu A3 NFC*i*	(2.22)
R A4 CHO for Growth*i*	R NFB A4 Degraded*i* × mu A4 CHO*i*	(2.23)
R B1 CHO for Growth*i*	R NFB B1 Degraded*i* × mu B1 CHO*i*	(2.24)
R B2 CHO for Growth*i*	R NFB B2 Degraded*i* × mu B2 CHO*i*	(2.25)
R NFB Growth Energy	R NFB CHO Growth × ((1 / Yg NFB) - 1)	(2.26)
R NFB Cell Growth	(R NFB CHO Growth × Rumen NH3 allowable growth) × Peptide effect	(2.27)
R NFB CHO Cell Engulfment	NFB Cell N Engulfed / NFB N	(2.28)
R NFB Cell Escape	R NFB CHO Cells × Kp solids mean	(2.29)
Fiber bacteria		
R FB AA N ID	(R FB N SI × FB AA N) × ID FB AA N	(2.30)
R FB NA N ID	(R FB N SI × FB NA N) × ID FB NA N	(2.31)
R FB CW N ID	(R FB N SI × FB CW N) × ID FB CW N	(2.32)
R FB CHO ID	R FB CHO SI × ID FB CHO	(2.33)
R FB EE ID	R FB EE SI × ID FB EE	(2.34)

Table 4.9. *(Continued)*

Flow[1]	Equation	
R FB Ash ID	R FB Ash SI × ID FB Ash	(2.35)
R FB AA N Pass	(R FB N SI × FB AA N) × (1 - ID FB AA N)	(2.36)
R FB NA N Pass	(R FB N SI × FB NA N) × (1 - ID FB NA N)	(2.37)
R FB CW N Pass	(R FB N SI × FB CW N) × (1 - ID FB CW N)	(2.38)
R FB CHO Pass	R FB CHO SI × (1 - ID FB CHO)	(2.39)
R FB EE Pass	R FB EE SI × (1 - ID FB EE)	(2.40)
R FB Ash Pass	R FB Ash SI × (1 - ID FB Ash)	(2.41)
Non-fiber bacteria		
R NFB AA N ID	(R NFB N SI × NFB AA N) × ID NFB AA N	(2.42)
R NFB NA N ID	(R NFB N SI × NFB NA N) × ID NFB NA N	(2.43)
R NFB CW N ID	(R NFB N SI × NFB CW N) × ID NFB CW N	(2.44)
R NFB CHO ID	R NFB CHO SI × ID NFB CHO	(2.45)
R NFB EE ID	R NFB EE SI × ID NFB EE	(2.46)
R NFB Ash ID	R NFB Ash SI × ID NFB Ash	(2.47)
R NFB AA N Pass	(R NFB N SI × NFB AA N) × (1 - ID NFB AA N)	(2.48)
R NFB NA N Pass	(R NFB N SI × NFB NA N) × (1 - ID NFB NA N)	(2.49)
R NFB CW N Pass	(R NFB N SI × NFB CW N) × (1 - ID NFB CW N)	(2.50)
R NFB CHO Pass	R NFB CHO SI × (1 - ID NFB CHO)	(2.51)
R NFB EE Pass	R NFB EE SI × (1 - ID NFB EE)	(2.52)
R NFB Ash Pass	R NFB Ash SI × (1 - ID NFB Ash)	(2.53)
Rumen fiber bacteria		
R FB AA N Out	R FB AA N LI × LI transit time	(2.54)
R FB NA N Out	R FB NA N LI × LI transit time	(2.55)
R FB CW N Out	R FB CW N LI × LI transit time	(2.56)
R FB CHO Out	R FB CHO LI × LI transit time	(2.57)
R FB EE Out	R FB EE LI × LI transit time	(2.58)
R FB Ash Out	R FB Ash LI × LI transit time	(2.59)
Rumen non-fiber bacteria		
R NFB AA N Out	R NFB AA N LI × LI transit time	(2.60)
R NFB NA N Out	R NFB NA N LI × LI transit time	(2.61)
R NFB CW N Out	R NFB CW N LI × LI transit time	(2.62)
R NFB CHO Out	R NFB CHO LI × LI transit time	(2.63)
R NFB EE Out	R NFB EE LI × LI transit time	(2.64)
R NFB Ash Out	R NFB Ash LI × LI transit time	(2.65)
Large intestine fiber bacteria		
B3 fast CHO LI Deg*i*	B3 fast CHO LI*i* × Kd B3 fast CHO*i*	(2.66)
B3 slow CHO LI Deg*i*	B3 slow CHO LI*i* × Kd B3 slow CHO*i*	(2.67)
LI B3 fast CHO for Maint*i*	LI FB B3 fast Degraded*i*	(2.68)
LI B3 slow CHO for Maint*i*	LI FB B3 slow Degraded*i*	(2.69)

Table 4.9. *(Continued)*

Flow[1]	Equation	
LI B3 fast CHO for Growth*i*	LI FB B3 fast Degraded*i* × mu LI B3 fast*i*	(2.70)
LI B3 slow CHO for Growth*i*	LI FB B3 slow Degraded*i* × mu LI B3 slow*i*	(2.71)
LI FB Growth Energy	LI FB CHO Growth × ((1 / Yg LI FB) - 1)	(2.72)
LI FB Cell Growth	LI FB CHO Growth × LI N availability	(2.73)
LI FB N Out	LI FB Cell N × LI transit time	(2.74)
LI FB CHO Out	LI FB CHO Cells Out × FB CHO	(2.75)
LI FB EE Out	LI FB CHO Cells Out × FB EE	(2.76)
LI FB Ash Out	LI FB CHO Cells Out × FB Ash	(2.77)
Large intestine non-fiber bacteria		
A4 CHO LI Deg*i*	A4 CHO LI*i* × Kd A4 CHO*i*	(2.78)
B1 CHO LI Deg*i*	B1 CHO LI*i* × Kd B1 CHO*i*	(2.79)
B2 CHO LI Deg*i*	B2 CHO LI*i* × Kd B2 CHO*i*	(2.80)
LI A4 CHO for Maint*i*	LI NFB A4 Degraded*i*	(2.81)
LI B1 CHO for Maint*i*	LI NFB B1 Degraded*i*	(2.82)
LI B2 CHO for Maint*i*	LI NFB B2 Degraded*i*	(2.83)
LI A4 CHO for Growth*i*	LI NFB A4 Degraded*i* × mu LI A4*i*	(2.84)
LI B1 CHO for Growth*i*	LI NFB B1 Degraded*i* × mu LI B1*i*	(2.85)
LI B2 CHO for Growth*i*	LI NFB B2 Degraded*i* × mu LI B2*i*	(2.86)
LI NFB Growth Energy	LI NFB CHO Growth × ((1 / Yg LI NFB) - 1)	(2.87)
LI NFB Cell Growth	LI NFB CHO Growth × LI N availability	(2.88)
LI NFB N Out	LI NFB Cell N × LI transit time	(2.89)
LI NFB CHO Out	LI NFB CHO Cells Out × NFB CHO	(2.90)
LI NFB EE Out	LI NFB CHO Cells Out × NFB EE	(2.91)
LI NFB Ash Out	LI NFB CHO Cells Out × NFB Ash	(2.92)

[1] Subscript *i* refers to the i^{th} feed in the diet.

Table 4.10. Differential equations used to calculate protozoal pools. The equations follow the general form $d/dt\ pool_t = flow_t$

Pool[1]	Equation	
Entodiniomorphid protozoa		
EPZ B1 Engulfed*i*	B1 CHO Engulfment*i* - EPZ B1 CHO Deg*i* - EPZ B1 Engulfed Recycled*i* - EPZ B1 Escape*i*	(3.1)
EPZ B2 Engulfed*i*	B2 CHO Engulfment*i* - EPZ B2 CHO Deg*i* - EPZ B2 Engulfed Recycled*i* - EPZ B2 Escape*i*	(3.2)
EPZ B3 fast Engulfed*i*	B3 fast CHO Engulfment*i* - EPZ B3 fast CHO Deg*i* - EPZ B3 fast Engulfed Recycled*i* - EPZ B3 fast Escape*i*	(3.3)
EPZ B3 slow Engulfed*i*	B3 slow CHO Engulfment*i* - EPZ B3 slow CHO Deg*i* - EPZ B3 slow Engulfed Recycled*i* - EPZ B3 slow Escape*i*	(3.4)
EPZ C Engulfed*i*	C CHO Engulfment*i* - EPZ C Engulfed Recycled*i* - EPZ C Escape*i*	(3.5)
EPZ Engulfed M	EPZ Bacterial CHO Engulfed - EPZ M Deg - EPZ Engulfed Lysed PZ CHO	(3.6)
EPZ B1 Degraded*i*	EPZ B1 CHO Deg*i* - EPZ B1 CHO for Growth*i* - EPZ B1 CHO for Maint*i*	(3.7)
EPZ B2 Degraded*i*	EPZ B2 CHO Deg*i* - EPZ B2 CHO for Growth*i* - EPZ B2 CHO for Maint*i*	(3.8)
EPZ B3 fast Degraded*i*	EPZ B3 fast CHO Deg*i* - EPZ B3 fast CHO for Growth*i* - EPZ B3 fast CHO for Maint*i*	(3.9)
EPZ B3 slow Degraded*i*	EPZ B3 slow CHO Deg*i* - EPZ B3 slow CHO for Growth*i* - EPZ B3 slow CHO for Maint*i*	(3.10)
EPZ Degraded M	EPZ M Deg - EPZ M for Growth - EPZ M for Maint	(3.11)
EPZ B1 Maint	sum(EPZ B1 CHO for Maint*i*)	(3.12)
EPZ B2 Maint	sum(EPZ B2 CHO for Maint*i*)	(3.13)
EPZ B3 fast Maint	sum(EPZ B3 fast CHO for Maint*i*)	(3.14)
EPZ B3 slow Maint	sum(EPZ B3 slow CHO for Maint*i*)	(3.15)
EPZ M Maint	EPZ M for Maint	(3.16)
EPZ B1 Growth	sum(EPZ B1 CHO for Growth*i*) - EPZ B1 Growth Energy - EPZ B1 Cell Growth	(3.17)
EPZ B2 Growth	sum(EPZ B2 CHO for Growth*i*) - EPZ B2 Growth Energy - EPZ B2 Cell Growth	(3.18)
EPZ Fiber Growth	sum(EPZ B3 fast CHO for Growth*i*) + sum(EPZ B3 slow CHO for Growth*i*) - EPZ Fiber Growth Energy - EPZ Fiber Cell Growth	(3.19)
EPZ M Growth	EPZ M for Growth - EPZ M Cell Growth - EPZ M Growth Energy	(3.20)
EPZ B1 Energy*i*	EPZ B1 Growth Energy	(3.21)
EPZ B2 Energy	EPZ B2 Growth Energy	(3.22)
EPZ Fiber Energy	EPZ Fiber Growth Energy	(3.23)
EPZ M Energy	EPZ M Growth Energy	(3.24)
EPZ B1 Cells	EPZ B1 Cell Growth - EPZ B1 Cell Lysis - EPZ B1 Cell Escape	(3.25)
EPZ B2 Cells	EPZ B2 Cell Growth - EPZ B2 Cell Escape - EPZ B2 Cell Lysis	(3.26)
EPZ Fiber Cells	EPZ Fiber Cell Growth - EPZ Fiber Cell Escape - EPZ Fiber Cell Lysis	(3.27)
EPZ M Cells	EPZ M Cell Growth - EPZ M Cell Escape - EPZ M Cell Lysis	(3.28)
Holotrich protozoa		
HPZ A4 Engulfedi	A4 CHO Engulfment*i* - HPZ A4 CHO Deg*i* - HPZ A4 Engulfed Recycled*i* - HPZ A4 Escape*i*	(3.29)
HPZ Engulfed M	HPZ Bacterial CHO Engulfed + HPZ Engulfed Lysed PZ CHO - HPZ M Deg	(3.30)
HPZ A4 Degraded*i*	HPZ A4 CHO Deg*i* - HPZ A4 CHO for Growth*i* - HPZ A4 CHO for Maint*i*	(3.31)
HPZ Degraded M	HPZ M Deg - HPZ M for Growth - HPZ M for Maint	(3.32)
HPZ A4 Maint	sum(HPZ A4 CHO for Maint*i*)	(3.33)
HPZ M Maint	HPZ M for Maint	(3.34)

Table 4.10. *(Continued)*

Pool[1]	Equation	
HPZ A4 Growth	sum(HPZ A4 CHO for Growth*i*) - HPZ A4 Growth Energy - HPZ A4 Cell Growth	(3.35)
HPZ M Growth	HPZ M for Growth - HPZ M Cell Growth - HPZ M Growth Energy	(3.36)
HPZ A4 Energy	HPZ A4 Growth Energy	(3.37)
HPZ M Energy	HPZ M Growth Energy	(3.38)
HPZ A4 Cells	HPZ A4 Cell Growth - HPZ A4 Cell Lysis - HPZ A4 Cell Escape	(3.39)
HPZ M Cells	HPZ M Cell Growth - HPZ M Cell Escape - HPZ M Cell Lysis	(3.40)
Protozoa		
PZ N SI	PZ Cell N Escape + PZ PAA N Escape - PZ AA N ID - PZ AA N Pass - PZ CW N ID - PZ CW N Pass - PZ NA N ID - PZ NA N Pass	(3.41)
PZ CHO SI	PZ CHO R Escape - PZ CHO Ab - PZ CHO Pass	(3.42)
PZ EE SI	PZ EE R Escape - PZ EE Ab - PZ EE Pass	(3.43)
PZ Ash SI	PZ Ash R Escape - PZ Ash Ab - PZ Ash Pass	(3.44)
Protozoa		
PZ AA N LI	PZ AA N Pass - PZ AA N Out	(3.45)
PZ NA N LI	PZ NA N Pass - PZ NA N Out	(3.46)
PZ CW N LI	PZ CW N Pass - PZ CW N Out	(3.47)
PZ CHO LI	PZ CHO Pass - PZ CHO Out	(3.48)
PZ EE LI	PZ EE Pass - PZ EE Out	(3.49)
PZ Ash LI	PZ Ash Pass - PZ Ash Out	(3.50)

[1] Subscript *i* refers to the i^{th} feed in the diet.

Table 4.11. Equations used to calculate the flows between protozoal pools

Flow[1]	Equation	
Entodiniomorphid protozoa		
B1 CHO Engulfment*i*	B1 CHO R*i* × K B1 CHO engulfment*i*	(4.1)
B2 CHO Engulfment*i*	B2 CHO R*i* × K B2 CHO engulfment*i*	(4.2)
B3 fast CHO Engulfment*i*	B3 fast CHO R*i* × K engulfment FC EPZ*i*	(4.3)
B3 slow CHO Engulfment*i*	B3 slow CHO R*i* × K engulfment FC EPZ*i*	(4.4)
C CHO Engulfment*i*	C CHO R*i* × K engulfment FC EPZ*i*	(4.5)
EPZ Bacterial CHO Engulfed	(Engulfed bacterial Cells × Bacterial CHO) × Prop EPZ Cell Growth	(4.6)
EPZ Engulfed Lysed PZ CHO	PZ CHO Lysed × Prop EPZ Cell Growth	(4.7)
EPZ B1 Engulfed Recycled*i*	((Ratio EPZ B1 engulfed to EPZ B1 Cells × EPZ B1 Cell Lysis) / sum(EPZ B1 Engulfed*i*)) × EPZ B1 Engulfed*i*	(4.8)
EPZ B2 Engulfed Recycled*i*	((EPZ B2 Cell Lysis × Ratio EPZ B2 Cells to EPZ B2 Engulfed) / sum(EPZ B2 Engulfed*i*)) × EPZ B2 Engulfed*i*	(4.9)
EPZ B3 fast Engulfed Recycled*i*	((EPZ Fiber Cell Lysis × Ratio of EPZ B3 fast engulfed to EPZ fiber Cells) / sum(EPZ B3 fast Engulfed*i*)) × EPZ B3 fast Engulfed*i*) + (EPZ B3 fast Engulfed*i* × EPZ fiber excretion)	(4.10)
EPZ B3 slow Engulfed Recycled*i*	(((EPZ Fiber Cell Lysis × Ratio of EPZ B3 slow engulfed to EPZ fiber Cells) / sum(EPZ B3 slow Engulfed*i*)) × EPZ B3 slow Engulfed*i*) + (EPZ B3 slow Engulfed*i* × EPZ fiber excretion)	(4.11)
EPZ C Engulfed Recycled*i*	(((EPZ Fiber Cell Lysis × Ratio of EPZ C engulfed to EPZ fiber Cells) / sum(EPZ C Engulfed*i*)) × EPZ C Engulfed*i*) + (EPZ C Engulfed*i* × EPZ fiber excretion)	(4.12)
EPZ B1 Escapei	((Ratio EPZ B1 engulfed to EPZ B1 Cells × EPZ B1 Cell Escape) / sum(EPZ B1 Engulfed*i*)) × EPZ B1 Engulfed*i*	(4.13)
EPZ B2 Escape*i*	((EPZ B2 Cell Escape × Ratio EPZ B2 Cells to EPZ B2 Engulfed) / sum(EPZ B2 Engulfed*i*)) × EPZ B2 Engulfed*i*	(4.14)
EPZ B3 fast Escape*i*	((EPZ Fiber Cell Escape × Ratio of EPZ B3 fast engulfed to EPZ fiber Cells) / sum(EPZ B3 fast Engulfed*i*)) × EPZ B3 fast Engulfed*i*	(4.15)
EPZ B3 slow Escape*i*	((EPZ Fiber Cell Escape × Ratio of EPZ B3 slow engulfed to EPZ fiber Cells) / sum(EPZ B3 slow Engulfed*i*)) × EPZ B3 slow Engulfed*i*	(4.16)
EPZ C Escape*i*	((EPZ Fiber Cell Escape × Ratio of EPZ C engulfed to EPZ fiber Cells) / sum(EPZ C Engulfed*i*)) × EPZ C Engulfed*i*	(4.17)
Holotrich protozoa		
A4 CHO Engulfment*i*	A4 CHO R*i* × K A4 CHO engulfment*i*	(4.18)
HPZ Bacterial CHO Engulfed	(Engulfed bacterial Cells × Bacterial CHO) × Prop HPZ Cell Growth	(4.19)
HPZ Engulfed Lysed PZ CHO	PZ CHO Lysed × Prop HPZ Cell Growth	(4.20)
HPZ A4 Engulfed Recycled*i*	((HPZ A4 Cell Lysis × Ratio HPZ A4 Cells to HPZ A4 Engulfed) / sum(HPZ A4 Engulfed*i*)) × HPZ A4 Engulfed*i*	(4.21)
HPZ A4 Escape*i*	((HPZ A4 Cell Escape × Ratio HPZ A4 Cells to HPZ A4 Engulfed) / sum(HPZ A4 Engulfed*i*)) × HPZ A4 Engulfed*i*	(4.22)

Table 4.11. *(Continued)*

Flow[1]	Equation	
Entodiniomorphid protozoa		
EPZ B1 CHO Deg*i*	EPZ B1 Engulfed*i* × EPZ Kd B1 CHO*i*	(4.23)
EPZ B2 CHO Deg*i*	EPZ B2 Engulfed*i* × EPZ Kd B2 CHO*i*	(4.24)
EPZ B3 fast CHO Deg*i*	EPZ B3 fast Engulfed*i* × EPZ Kd B3 fast CHO*i*	(4.25)
EPZ B3 slow CHO Deg*i*	EPZ B3 slow Engulfed*i* × EPZ Kd B3 slow CHO*i*	(4.26)
EPZ M Deg	EPZ Engulfed M × Kd EPZ M CHO	(4.27)
EPZ B1 CHO for Maint*i*	EPZ B1 Degraded*i*	(4.28)
EPZ B2 CHO for Maint*i*	EPZ B2 Degraded*i*	(4.29)
EPZ B3 fast CHO for Maint*i*	EPZ B3 fast Degraded*i*	(4.30)
EPZ B3 slow CHO for Maint*i*	EPZ B3 slow Degraded*i*	(4.31)
EPZ M for Maint	EPZ Degraded M	(4.32)
EPZ B1 CHO for Growth*i*	EPZ B1 Degraded*i* × mu B1 EPZ*i*	(4.33)
EPZ B2 CHO for Growth*i*	EPZ B2 Degraded*i* × mu B2 EPZ*i*	(4.34)
EPZ B3 fast CHO for Growth*i*	EPZ B3 fast Degraded*i* × mu B3 fast EPZ*i*	(4.35)
EPZ B3 slow CHO for Growth*i*	EPZ B3 slow Degraded*i* × mu B3 slow EPZ*i*	(4.36)
EPZ M for Growth	EPZ Degraded M × mu M CHO EPZ	(4.37)
EPZ B1 Growth Energy	EPZ B1 Growth × ((1/Yg EPZ) - 1)	(4.38)
EPZ B2 Growth Energy	EPZ B2 Growth × ((1/Yg EPZ) - 1)	(4.39)
EPZ Fiber Growth Energy	EPZ Fiber Growth × ((1/Yg EPZ) - 1)	(4.40)
EPZ M Growth Energy	EPZ M Growth × ((1/Yg EPZ) - 1)	(4.41)
EPZ B1 Cell Growth	EPZ B1 Growth × PZ NFB N allowable growth	(4.42)
EPZ B2 Cell Growth	EPZ B2 Growth × PZ NFB N allowable growth	(4.43)
EPZ Fiber Cell Growth	EPZ Fiber Growth × PZ NFB N allowable growth	(4.44)
EPZ M Cell Growth	EPZ M Growth	(4.45)
EPZ B1 Cell Lysis	EPZ B1 Cells × K EPZ lysis	(4.46)
EPZ B2 Cell Lysis	EPZ B2 Cells × K EPZ lysis	(4.47)
EPZ Fiber Cell Lysis	EPZ Fiber Cells × K EPZ lysis	(4.48)
EPZ M Cell Lysis	EPZ M Cells × K EPZ lysis	(4.49)
EPZ B1 Cell Escape	EPZ B1 Cells × PZ Kp	(4.50)
EPZ B2 Cell Escape	EPZ B2 Cells × PZ Kp	(4.51)
EPZ Fiber Cell Escape	EPZ Fiber Cells × PZ Kp	(4.52)
EPZ M Cell Escape	EPZ M Cells × PZ Kp	(4.53)
Holotrich protozoa		
HPZ A4 CHO Deg*i*	HPZ A4 Engulfed*i* × HPZ Kd A4 CHO*i*	(4.54)
HPZ M Deg	HPZ Engulfed M × Kd HPZ M CHO	(4.55)
HPZ A4 CHO for Maint*i*	HPZ A4 Degraded*i*	(4.56)
HPZ M for Maint	HPZ Degraded M	(4.57)
HPZ A4 CHO for Growth*i*	HPZ A4 Degraded*i* × mu A4 HPZ*i*	(4.58)
HPZ M for Growth	HPZ Degraded M × mu M CHO HPZ	(4.59)

Table 4.11. *(Continued)*

Flow[1]	Equation	
HPZ A4 Growth Energy	HPZ A4 Growth × ((1/Yg HPZ) - 1)	(4.60)
HPZ M Growth Energy	HPZ M Growth × ((1/Yg HPZ) - 1)	(4.61)
HPZ A4 Cell Growth	HPZ A4 Growth × PZ NFB N allowable growth	(4.62)
HPZ M Cell Growth	HPZ M Growth	(4.63)
HPZ A4 Cell Lysis	HPZ A4 Cells × K HPZ lysis	(4.64)
HPZ M Cell Lysis	HPZ M Cells × K HPZ lysis	(4.65)
HPZ A4 Cell Escape	HPZ A4 Cells × PZ Kp	(4.66)
HPZ M Cell Escape	HPZ M Cells × PZ Kp	(4.67)
Protozoa		
PZ AA N ID	(PZ N SI × PZ AA N) × ID PZ AA N	(4.68)
PZ NA N ID	(PZ N SI × PZ NA N) × ID PZ NA N	(4.69)
PZ CW N ID	(PZ N SI × PZ CW N) × ID PZ CW N	(4.70)
PZ CHO ID	PZ CHO SI × ID PZ CHO	(4.71)
PZ EE ID	PZ EE SI × ID PZ EE	(4.72)
PZ Ash ID	PZ Ash SI × ID PZ Ash	(4.73)
PZ AA N Pass	(PZ N SI × PZ AA N) × (1 - ID PZ AA N)	(4.74)
PZ NA N Pass	(PZ N SI × PZ NA N) × (1 - ID PZ NA N)	(4.75)
PZ CW N Pass	(PZ N SI × PZ CW N) × (1 - ID PZ CW N)	(4.76)
PZ CHO Pass	PZ CHO SI × (1 - ID PZ CHO)	(4.77)
PZ EE Pass	PZ EE SI × (1 - ID PZ EE)	(4.78)
PZ Ash Pass	PZ Ash SI × (1 - ID PZ Ash)	(4.79)
Protozoa		
PZ AA N Out	PZ AA N LI × LI transit time	(4.80)
PZ NA N Out	PZ NA N LI × LI transit time	(4.81)
PZ CW N Out	PZ CW N LI × LI transit time	(4.82)
PZ CHO Out	PZ CHO LI × LI transit time	(4.83)
PZ EE Out	PZ EE LI × LI transit time	(4.84)
PZ Ash Out	PZ Ash LI × LI transit time	(4.85)

[1] Subscript *i* refers to the i^{th} feed in the diet.

CHAPTER 5: A REVISED SYSTEM OF PREDICTING AMINO ACID REQUIREMENTS WITHIN THE UPDATED STRUCTURE OF THE CORNELL NET CARBOHYDRATE AND PROTEIN SYSTEM

5.1 Abstract

Improved predictions of the true and optimum AA supply to dairy cows in ration formulation models like the Cornell Net Carbohydrate and Protein System (CNCPS) would provide an opportunity to balance diets closer to animal requirements and improve nutrient utilization. Predictions of true AA supply in a dynamic version of the CNCPS were refined by modeling endogenous N (EN) transactions along the entire gastrointestinal tract (GIT) including incorporation of EN into microbial N supply. Studies that used isotopic enrichment of N (^{15}N-Leucine) to mark endogenous components were used to develop the model. Predictions were close to measured data at the duodenum, ileum and in the feces. Incorporation of EN into microbial N and the original source of EN at various points in the GIT and in the feces were also accurately predicted. Optimum AA supply was determined using a dataset of published studies that infused AA post-ruminally. A logistic model was used to estimate additional AA requirements above the physiological processes quantified by the model. The optimum AA supply to maximize AA use and minimize wastage was determined where the third derivative of the logistic model was 0. The optimum AA supply differed among AA but requirements for Met (5.7% EAA) and Lys (15.1 % EAA) were similar to other recommendations. A loglogistic relationship was observed when the efficiency of AA use was regressed against AA supply relative to ME but no relationship was found when AA supply was expressed relative to MP. This suggests considering AA supply relative to energy could improve predictions of AA utilization.

5.2 Introduction

An improved understanding of both, the true, and optimum supply of AA to a dairy cow can provide an opportunity to balance AA closer to animal requirements and reduce total protein feeding while still maintaining high levels of production (Haque et al., 2012). This strategy can also reduce feed costs and lower the environmental impact of dairy production (Higgs et al., 2012). Amino acids flowing to the duodenum encompass three major fractions: Un-degraded feed, microbial and endogenous AA (Lapierre et al., 2006). Combined, these fractions represent the gross AA supply, potentially available to the animal. However, the endogenous fraction, and its contribution to the microbial pool make establishing the net AA supply complex (Ouellet et al., 2002). The contribution of endogenous N to the microbial pool and un-degraded dietary pool represent a recycling of previously absorbed AA that cannot be considered new supply (Lapierre et al., 2006). Currently, the prediction of AA supply in the Cornell Net Carbohydrate and Protein System (**CNCPS**) is the sum of AA from feed and bacteria that escape the rumen and are digested in the small intestine and does not consider endogenous AA or protozoa (O'Connor et al., 1993). Incorporating both endogenous AA and protozoa into the CNCPS would refine and possibly improve predictions of the true supply of AA to the animal.

Requirements in the CNCPS are calculated individually for different physiological processes and divided by a transfer coefficient (efficiency of use) to give total AA requirements (O'Connor et al., 1993). Previous versions of the CNCPS have assumed the protein requirements for maintenance are the sum of scurf, urinary protein and metabolic fecal N (Fox et al., 2004). Metabolic fecal nitrogen (**MFN**) is typically estimated using regression techniques with past versions of the NRC and CNCPS using the estimates of Swanson (1977). Fox et al., (2004) suggested these calculations may have shortcomings due to the contribution of microbial

nitrogen from hind gut fermentation to total fecal nitrogen. The regression techniques used would consider microbial N as endogenous N (**EN**). Hence, the N or AA requirement for maintenance estimated by the model using these predictions might be over-estimated. The assumption used when considering MFN in the maintenance requirement of an animal is that for the protein to be a cost, it needs to be excreted. However, considerably more EN is secreted into the rumen of dairy cows than escapes in the free form or incorporated in bacteria (Marini et al., 2008, Ouellet et al., 2010a, Ouellet et al., 2002). This means the balance has to be degraded in the rumen and the N absorbed as ammonia. Once degraded, essential AA are lost to the animal and can only be replaced by the diet or rumen microorganisms appearing in the duodenum. Therefore, it makes sense to consider all protein secreted in to the gastrointestinal tract (**GIT**) which is not recovered in the small intestine a maintenance cost, not just what appears in the feces.

The objectives of this study were to replace current predictions of MFN with estimations of EN transactions through the whole GIT in the dynamic version of the CNCPS described in Chapters 3 and 4. In doing this, the true supply of AA to the small intestine from all sources can be refined and the shortcomings of the current predictions improved. A second objective was to evaluate the efficiency of transfer of AA to milk and maintenance using the predicted net supply and requirements of the new model. Interactions between protein and energy play an important role in determining how an animal will utilize absorbed AA and it has been recommended they be considered together (Hanigan et al., 1998, Lobley, 2007). These interactions were investigated in determining the optimum AA requirements for this version of the model.

5.3 Materials and methods

5.3.1 Modeling endogenous AA losses in the gut

Predictions of EN losses into the GIT were modeled mechanistically to capture the various transactions along the GIT and between microbial pools. Gross EN to the forestomach and intestines were estimated according to Ouellet et al. (2010a) and Ouellet et al. (2002) which were subsequently partitioned into individual components (Table 5.1) using estimates reported in Egan et al. (1984). The studies by Ouellet and co-workers directly measured EN using ^{15}N-Leucine in cows with multiple cannulas. Using this technique, different precursor pools are available to represent the site of EN production and have different levels of isotopic enrichment. In dairy cows, the enrichment of milk probably gives a good representation of tissues that are rapidly turning over like the pancreas and secretions while the intestinal mucosa is known to directly contribute to EN through desquamation (Ouellet et al., 2002). Values from the mucosa precursor pool were used to estimate microbial enrichment as EN contributions to the rumen would largely be from desquamation (Egan et al., 1984). Free EN at the duodenum was assumed to be best represented by the 'combined' precursor pool (Ouellet et al., 2010a) due to the contribution of pancreatic secretions, bile and secretions into the abomasum. Data using a 'combined' precursor pool are not presented in Ouellet et al. (2002). Therefore, the relative difference between the 'combined' and 'mucosa' precursor pools (combined = 60% of mucosa) presented in Ouellet et al. (2010a) were used to calculate a combined value for the data in Ouellet et al. (2002). Endogenous secretions early in the small intestine were assumed to be largely recovered. Therefore, EN measured at the ileum and in the feces would predominantly be from sloughed keratinized cells with poor digestibility and would be best represented by the mucosa precursor pool. Endogenous contributions are reasonably consistent among diets when expressed relative to DMI or OMI (Marini et al., 2008, Ouellet et al., 2010a, Ouellet et al., 2002, Tamminga et al.,

1995). Thus, the model expresses each component as g EN per kg DMI. Quantitative estimates of fluxes to and from the various pools in the model were estimated by setting the kinetic parameters and digestibility coefficients in the model to align predictions at various points in the gut to measured data (Ouellet et al., 2010a, Ouellet et al., 2002). A summary of the model inputs used to estimate the EN transactions are in Table 5.1.

Endogenous N in the rumen has three potential fates: 1) It is degraded to ammonia; 2) escapes the rumen; 3) or is incorporated into microbial protein. Degradation and passage are estimated using the kinetic relationships described in Chapter 3 where free EN is assumed to flow in the liquid phase. Incorporation into microbial protein is estimated using two derivations of the microbial model described in Chapter 4. The first derivation (Figure 5.1) is used to predict total microbial enrichment of ^{15}N and includes the transfer of labelled NH_3 within the rumen. The second (Figure 5.2) predicts the enrichment of ^{15}N from only peptides and AA and excludes any transfer from NH_3. The studies of Ouellet exclude the transfer of ^{15}N from recycled urea, but it is still possible for $^{15}NH_3$ to be produced in the rumen by bacteria and protozoa and incorporated into microbial protein. The model assumes if EN is degraded to NH_3, the AA are lost to the animal, and are only recoverable if incorporated into microbial protein intact. Therefore, the first model estimates total ^{15}N enrichment of microbial protein, including transfer from NH_3 (Figure 5.1), and is used to set the kinetics and digestibility coefficients relative to the measured data, while the second model is used to estimate true EN AA uptake by the microbes and subsequent endogenous AA recovery.

Table 5.1. Endogenous contributions and digestion coefficients used to predict endogenous AA requirements and supply in the models outlined in Figures 5.1 and 5.2.

Endogenous component	Secretion (g N/kg DMI)	Kd (%/hr)[2]	ID (%)[3]
Saliva	0.9	150	5
Rumen sloughed cells	4.3	150	5
Omasum/abomasum sloughed cells	0.3	0.0	70
Omasum/abomasum secretions	0.2	0.0	70
Pancreatic secretions	0.4	0.0	70
Bile	0.1	0.0	70
Small intestine sloughed cells[1]	0.7	75	50
Small intestine secretions[1]	0.7	75	50
Large intestine sloughed cells	0.3	150	N/A

[1] Includes secretions past the pancreatic and bile duct and prior to the terminal ileum

[2] Rate of microbial degradation in either the rumen or large intestine

[3] Digestion in the small intestine

Transactions in the first model (Figure 5.1) begin with labeled EN (LEN) that is degraded (LEN to R) and enters the peptide and free AA (**PAA**) pool in the rumen (LEN PAA R). From there, the LEN can escape (LEN PAA Escape), be degraded to NH_3 (LEN PAA Deg) or be taken up by non-fiber bacteria (LEN PAA Uptake NFB) or protozoa (LEN PAA Engulfment). Protozoa either incorporate the LEN (PZ LEN Engulfed Incorporated), excrete it as PAA (PZ LEN Engulfed excreted as PAA), or excrete it as NH_3 (PZ LEN Engulfed excreted as NH_3). Labelled PZ can escape the rumen (PZ Cell LEN Escape) or lyse (PZ Cell LEN Lysis). Protozoal excretion of PAA, NH_3 and lysis has the effect of transferring EN through numerous rumen N pools and also allows FB to be enriched through the labeled NH_3 pool (NH_3 LEN R) which can also escape (FB Cell LEN Escape). Enrichment of microbial protein through the NH_3 pool is not considered available for recovery as AA given the AA itself has been degraded. Therefore, these same transactions are considered in Figure 5.2 excluding the transfer through NH_3.

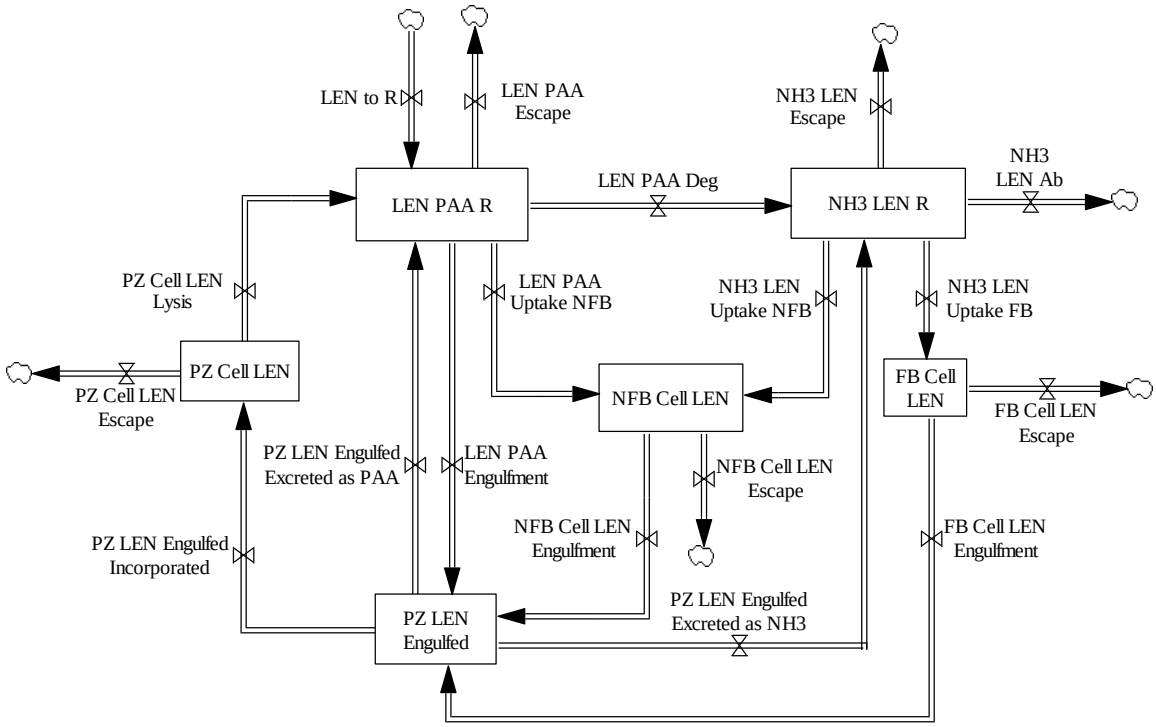

Figure 5.1. Schematic representation of the model used to predict the incorporation of labelled endogenous N (LEN) into rumen microorganisms

Transactions in the second model (Figure 5.2) again begin with EN that is degraded to PAA (EPAA) entering the rumen PAA pool (EPAA R). Once in the EPAA R pool, it can either escape in the liquid phase, be degraded to NH_3 or be taken up by NFB (EPAA Uptake) or PZ (EPAA Engulfed). Any EPAA converted to NH_3 cannot be recovered as EPAA and is eliminated from the model (EPAA NH3). Endogenous PAA taken up by NFB can either escape or be engulfed by PZ (NFB EPAA Cell Engulfed). Protozoa cause some recycling of EPAA through the EPAA R pool. Finally, protozoal and NFB N of endogenous origin escaping to the small intestine (PZ Cell EPAA Escape and NFB Cell EPAA Escape, respectively) have the potential to be recovered in the small intestine as AA from microbial protein.

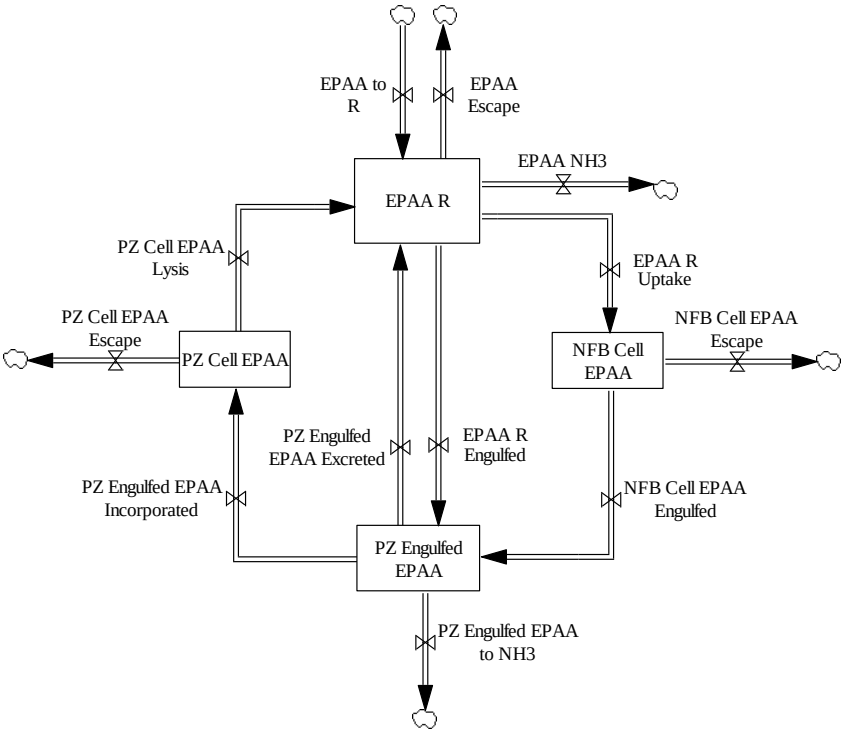

Figure 5.2. Schematic representation of the model used to predict the incorporation of endogenous peptides and AA (EPAA) into rumen microorganisms

Each individual source of EN can be tracked within the model, as either free EN, or incorporated in microbial protein, from the initial transfer into the gut, to its final fate. An AA profile is applied to each component using the profiles in Table 5.2. Microbial AA of endogenous origin are not considered new supply and are subtracted off digested microbial AA using the profile of the original source. Endogenous AA in microbial protein are assumed to be evenly distributed through the cell N and digestion is relative to the digestion of total microbial N. Free EN can be recovered if it is digested in the small intestine otherwise the AA are considered lost. Losses occur from degradation and absorption as NH_3 in the rumen and large intestine, or excretion in the feces. The total cost of endogenous AA can be calculated as total entry into the gut less recovery in the small intestine.

Table 5.2. Profiles of essential AA (EAA; % EAA N), EAA N (% AA N) and AA N (% total N) for endogenous N components predicted by the model. The proportion of AA N not accounted for as EAA N represents the contribution of non-essential AA to endogenous secretions.

Endogenous component	Met	Lys	Arg	Thr	Leu	Ile	Val	His	Phe	Trp	EAA N	AA N
Saliva[1]	1.0%	12.4%	24.7%	13.2%	13.2%	6.5%	12.9%	8.7%	4.6%	2.8%	48.9%	80.0%
Rumen sloughed cells[2]	2.5%	18.5%	29.2%	6.7%	12.8%	6.3%	8.5%	8.5%	4.8%	2.2%	56.1%	79.0%
Omasum/abomasum sloughed cells[2]	2.5%	18.5%	29.2%	6.7%	12.8%	6.3%	8.5%	8.5%	4.8%	2.2%	56.1%	79.0%
Omasum/abomasum secretions[3]	1.9%	19.4%	21.9%	10.6%	7.1%	6.9%	10.1%	13.5%	5.5%	3.0%	52.2%	64.9%
Pancreatic secretions[4]	2.0%	16.2%	18.0%	10.6%	13.0%	7.7%	12.4%	12.6%	5.0%	2.6%	51.3%	94.3%
Bile[5]	2.6%	13.3%	13.4%	9.8%	13.4%	7.4%	13.3%	18.9%	4.7%	3.0%	7.2%	51.5%
Small intestine sloughed cells[6]	1.9%	14.3%	23.7%	13.2%	9.8%	7.5%	11.8%	9.5%	5.4%	3.0%	39.2%	72.9%
Small intestine secretions[6]	1.9%	14.3%	23.7%	13.2%	9.8%	7.5%	11.8%	9.5%	5.4%	3.0%	39.2%	72.9%
Large intestine sloughed cells[2]	2.5%	18.5%	29.2%	6.7%	12.8%	6.3%	8.5%	8.5%	4.8%	2.2%	56.1%	79.0%

[1] Salivary protein (Yisehak et al., 2012)

[2] Rumen epithelia (Larsen et al., 2000)

[3] Abomasal isolates (Ørskov et al., 1986)

[4] Pancreatic juice from Hamza (1976) reported by Larsen et al. (2000)

[5] Cow bile (Larsen et al., 2000)

[6] Ileal endogenous AA (Jansman et al., 2002)

5.3.2 Estimating total AA requirements

Amino acid requirements estimated in the CNCPS (**AAR**) include milk, growth, reserves, pregnancy, scurf, metabolic urinary losses and endogenous losses in the gut. Endogenous losses in this model are calculated as previously described with the other requirements according to Fox et al. (2004). Amino acids used for other processes not accounted for by the model (**AAO**) can be calculated by taking the difference between predicted AA supply (**AAS**) and AAR. The term often used to describe AAO is 'efficiency of use' which can vary depending on AA supply relative to other nutrients and the physiological state of the animal (Doepel et al., 2004, Hanigan et al., 1998). In order to balance a ration in a manner where individual EAA supply is not excessive, but also not limiting, estimates of the optimum level of AAO relative to AAR are required. In this model, the approach used to generate these estimates was similar to the study of Doepel et al. (2004). Briefly, a dataset was constructed of studies that infused AA into the abomasum, duodenum, or intravenously (Table 5.3). Infusion studies were used so that the addition of AA above the basal diet was known and limited the reliance on model predictions (Doepel et al., 2004). The final dataset included 41 publications, 51 experiments and 218 treatment means. Descriptive statistics for the dataset are in Table 5.4. Information reported in the publications was entered in model. Often, limited information was presented on the chemical composition of the dietary components. In this situation, the reported information was used, and uncertain values were predicted using an extension of the method described in Chapter 2. 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. As described previously, infused AA

were assumed to be 100% available to the animal (Doepel et al., 2004). Once compiled, each treatment was evaluated through the model to estimate AAS and AAR for each of the 10 EAA. A logistic model with three parameters was used to fit the data which was previously shown to give the most appropriate fit (Doepel et al., 2004). The selected model has the form

$$y = \frac{\theta_1}{1+ \theta_2 e^{\theta_3(x)}} \quad [1]$$

where *y* is the AAR (g/d), *x* is the predicted AAS (g/d), $\theta_{1\text{-}3}$ are the model parameters used to described the sigmoidal shape of the curve. The optimum supply of AA was considered to be the point on the curve where the rate of change in the ratio of AAR:AAS was the most rapid, or, in other words, the rate at which cows were changing the way they managed additional AAS was most rapid (Figures 5.4 and 5.5). This can be calculated when the third derivative of the logistic model is zero. The third derivative has the form

$$\frac{d^3y}{dx^3} = -\theta_1\theta_2{\theta_3}^3 e^{\theta_3(x)} \frac{1-4\theta_2 e^{\theta_3(x)}+ {\theta_2}^2 e^{2\theta_3(x)}}{(1+\theta_2 e^{\theta_3(x)})^4} \quad [2]$$

and the zero point of interest is calculated using the equation

$$x = \frac{1}{\theta_3}\log\left(\frac{2-\sqrt{3}}{\theta_2}\right) \quad [3]$$

where *x* is considered the optimum AAS for the dataset used. By substituting *x* into equation [1], and dividing *y* (AAR) by *x* (AAS) the optimum ratio of AAR to AAS can be calculated, and

therefore, the optimum level of additional AA for other functions not considered by the model. When balancing a ration, the total required supply (g AA/d) can be calculated by dividing AAR by the optimum ratio of AAR to AAS. The same calculations were also performed for MP.

The relationship between ratio of AAR and AAS and AA supply relative to other nutrients (g AA/ Mcal ME and g AA/ 100g MP) was also investigated. A loglogistic model with three parameters was used to fit this relationship with the form

$$y = \theta_1 - \log\left(1 + \theta_2 e^{-\theta_3(x)}\right) \quad [4]$$

where *y* is the ratio of AAR to AAS, *x* is AA supply expressed relative to Mcals of ME or 100g MP and $\theta_{1\text{-}3}$ are the model parameters used to describe the shape of the curve. The optimum supply of a given EAA relative to ME or MP can then be found by rearranging formula [4] and solving for *x* using the AAR:AAS (*y*) previously calculated.

$$x = \frac{-1}{\theta_3} log\left(\frac{e^{\theta_1 - y} - 1}{\theta_2}\right) \quad [5]$$

Given the information presented by studies published in the literature is typically limited compared to the inputs required by the CNCPS, a large number of assumptions have to made. To limit the influence of potential input errors, points were weighted on the likelihood of being an outlier. The scheme used was the Tukey Biweight and was implemented according to Motulsky and Christopoulos (2004). Data analysis was performed using the non-linear modelling function in SAS (2010).

Table 5.3. Studies included in the dataset used to estimate additional AA requirements

Studies included in the data set	
(Aldrich et al., 1993)	(Köning et al., 1984)
(Bruckental et al., 1991)	(Lapierre et al., 2009)
(Cant et al., 1991)	(Lynch et al., 1991)
(Choung and Chamberlain, 1992a)	(Mackle et al., 1999a)
(Choung and Chamberlain, 1992b)	(Mackle et al., 1999b)
(Choung and Chamberlain, 1993)	(Metcalf et al., 1996)
(Choung and Chamberlain, 1995a)	(Pisulewski et al., 1996)
(Choung and Chamberlain, 1995b)	(Raggio et al., 2006)
(Choung and Chamberlain, 1995c)	(Relling and Reynolds, 2008)
(Clark et al., 1977)	(Rius et al., 2010)
(Cohick et al., 1986)	(Robinson et al., 2000)
(Doepel and Lapierre, 2010)	(Rogers et al., 1984)
(Doepel and Lapierre, 2011)	(Schwab et al., 1976)
(Griinari et al., 1997)	(Schwab et al., 1992a)
(Guinard and Rulquin, 1994)	(Schwab et al., 1992b)
(Guinard and Rulquin, 1995)	(Seymour et al., 1990)
(Guinard et al., 1994)	(Vanhatalo et al., 1999)
(Huhtanen et al., 1997)	(Varvikko et al., 1999)
(Kim et al., 1999)	(Vicini et al., 1988)
(Kim et al., 2000)	(Weekes et al., 2006)
(King et al., 1991)	

Table 5.4. Descriptive statistics of the dataset used to estimate AA requirements

	Mean	SD	Min	Max
DMI (kg/d)	18.0	3.1	11.0	27.6
DIM (d)	107	51	28	240
Body weight (kg)	551	55	487	733
Milk yield (kg/d)	26.3	5.85	10.7	40.0
Milk fat (%)	3.98	2.65	2.37	41.90
Milk true protein (%)	2.88	0.20	2.38	3.52
Fat yield (kg/d)	1.01	0.51	0.53	8.09
Milk true protein yield (kg/d)	0.76	0.16	0.32	1.11

5.4 Results and Discussion

5.4.3 Endogenous N flows

The mechanistic framework developed in Chapters 3 and 4 enabled EN to be modeled in all parts of the GIT including the microbial transactions in the rumen and large intestine. Model estimates compared to measurements taken from multi-cannulated animals in the studies of Ouellet and coworkers are in Table 5.5. Model predicted flows of EN at the duodenum were similar to measured values. The greatest difference was observed in the prediction of microbial EN in the 'Inoc' and 'Formic' treatments (Ouellet et al., 2010a). The model assumes microbes do not differentiate between the original source of N in the rumen with uptake being based on the relative availability of each source (Marini et al., 2008). Silages fed in the 'Inoc' and 'Formic' treatments had higher levels of soluble protein than the hay treatment (Martineau et al., 2007) which increased the availability of feed N in the rumen relative to EN and resulted in lower predicted microbial uptake of EN. The rate of CHO digestion in the rumen also impacts predictions of EN uptake through its effect on microbial growth (see Chapter 4). Therefore, more accurate estimates of CHO digestion kinetics could improve model predictions. Although differences in EN secretion into the foregut among dietary treatments has been observed (Ouellet

et al., 2010a), the mechanism of action is still unclear (Larsen et al., 2000). Therefore, expressing EN secretion relative to DMI seemed appropriate until the factors involved are better understood. Further down the GIT, estimates were similar to measured values at the terminal ileum and in the feces (Table 5.5).

Total EN transactions through each compartment in the model for the 'Hay' treatment in Ouellet et al. (2010a) are summarized in Figure 5.3. These data were generated using the model in Figure 5.2 where EN transfers through the NH_3 pool were excluded. The 'Hay' treatment was chosen given the close agreement between model and measured values. Total EN secretions into the gut were 135.4 g/d of which 46.4 g/d was recovered as either free EN in the duodenum or incorporated in microbial protein. The balance (89.0 g/d) was considered lost by the animal and part of the maintenance requirements for protein. Of the 89.0 g/d lost, 31.8 g/d appeared in the feces and 57.2 g/d was degraded in the GIT to NH_3. The total estimated requirement (89.0 g/d) when expressed relative to DMI is 5.1 g EN/ kg DMI which, surprisingly, is similar to current model estimates of MFN for the same diet (5.0 g MFN/kg DMI).

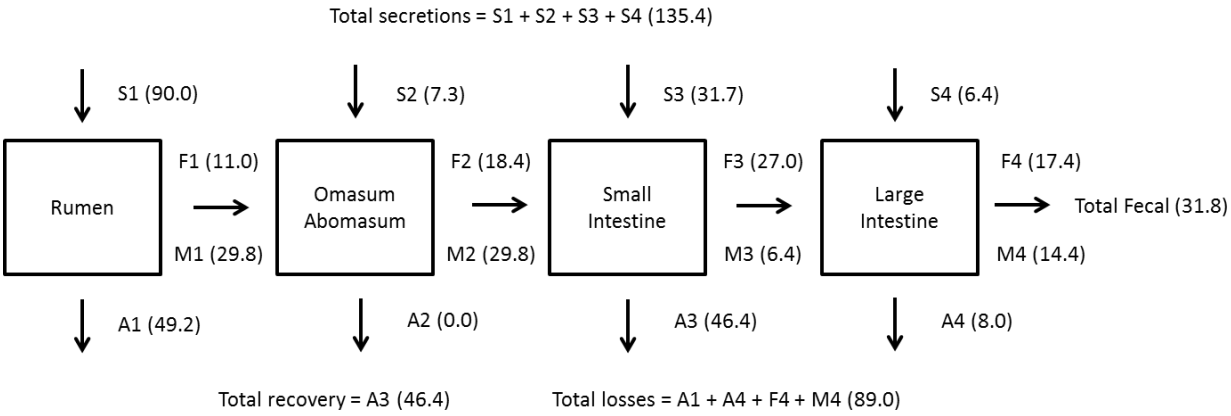

Figure 5.3. Model predicted endogenous transactions (g endogenous N/d) by compartment for the hay treatment presented in Ouellet et al. (2010a). S1-S4 are the endogenous secretions into the gut; F1-F4 are the flows of free endogenous N; M1-M4 are the flow of endogenous N in bacteria; A1-A4 is the endogenous N absorption at different sites. Recovery is only possible in the small intestine (A3) where the N can be absorbed as AA.

Table 5.5. Measured and model predicted endogenous flows along the gut (g EN/kg DMI)

	HF[1]		LF		Hay		Formic		Inoc		Average	
Endogenous flow	Study	Model	Study	Model	Study	Model	Study	Model	Study	Model	Study	Model
Total Duodenum	3.4	3.8	3.7	3.6	4.9	4.8	4.3	4.1	4.7	4.1	4.2	4.1
Microbial	2.0	2.3	2.3	2.1	3.3	3.3	3.1	2.6	3.4	2.5	2.8	2.6
Free[2]	1.3	1.5	1.4	1.5	1.6	1.5	1.2	1.5	1.3	1.5	1.4	1.5
Total Ileum		2.0		2.0	2.1	2.3	2.4	2.2	2.9	2.1	2.5	2.2
Secreted in the forestomach[3]		1.3		1.3	1.3	1.6	1.8	1.5	1.8	1.5	1.6	1.5
Secreted in the intestine		0.7		0.7	0.8	0.7	0.6	0.7	1.1	0.7	0.8	0.7
Fecal	1.8	2.0	2.0	1.9	2.4	2.3	2.1	2.1	2.5	2.1	2.1	2.1
Secreted in the forestomach	1.4	1.3	1.3	1.3	1.8	1.6	1.5	1.5	1.7	1.5	1.6	1.4
Secreted in the intestine[4]	0.4	0.7	0.6	0.6	0.6	0.7	0.6	0.7	0.8	0.7	0.6	0.7

[1] HF and LF are from Ouellet et al. (2002); Hay, Formic and Inoc are from Ouellet et al. (2010b)

[2] Estimated using the combined precursor pool. All other data represent the mucosa precursor pool

[3] Includes pancreatic secretions and bile

[4] Includes contributions from the large intestine

5.4.4 Amino acid requirements

Requirements for each individual EAA in the CNCPS are predicted for processes that are quantified by the model (maintenance, lactation, pregnancy, growth) and subsequently divided by the efficiency of transfer to that process to give the total AA requirement (Fox et al., 2004, O'Connor et al., 1993). The efficiency of transfer could also be thought of as the additional requirement for each AA relative to the requirements quantified by the model. Such processes include oxidation across the gut or in other tissues, anaplerotic requirements, synthesis of non-essential AA, gluconeogenesis etc. (Lapierre et al., 2005, Lapierre et al., 2006, Lemosquet et al., 2010, Lobley, 2007). The apparent efficiency of AA use for any given diet can be calculated by dividing model predicted AAR by AAS, which can be variable, and typically decreases as AAS increases relative to AAR and also energy (Hanigan et al., 1998). This decrease in apparent efficiency of AA use represents AA being increasingly used for purposes other than those quantified or described by the model. If the utilization of each AA for every process in metabolism could be adequately quantified, the term 'efficiency of use' would become obsolete as it would be 100% (there would be no additional requirement above model predictions). The ability of cows to direct AA to other uses demonstrates the interactions among different nutrients and is an example of the metabolic flexibility that allows productivity to be maintained across a wide range of nutrient inputs and supply (Lobley, 2007). The pertinent question for ration balancing is: what level of additional AA supply is required above the predicted requirements for milk protein synthesis and body protein requirements to maximize productivity and minimize AA wastage? The answer to this question is going to differ among models as supply and requirements are calculated in different ways. For example, changing the maintenance requirements from using MFN as in previous version of the CNCPS to estimating AA loss

through the GIT using isotopic enrichment techniques considers 9 different sources of EN, each with a different AA profile (Table 5.2), and so it would be expected that AA requirements among models would be different.

The optimum supply of EAA in this study was defined where the rate of change in which additional AA supply was being used for other purposes was most rapid. This point was defined by Doepel et al. (2004) as the required AA supply and is equivalent to the break-point in the segmented linear model used in the NRC (2001). Previous versions of the CNCPS have treated different physiological functions separately with the original values coming from a range of sources outlined in O'Connor et al. (1993). Lapierre et al. (2007) suggested using a single factor to calculate total AA requirement for maintenance and milk production makes more biological sense as it is difficult to localize the large number of processes that are encompassed in AAO. Recommendations for v6.1 of the CNCPS were presented by Lapierre et al. (2007) and have been implemented in the most recent update of the model v6.5 (Van Amburgh et al., 2013). Model parameters and the fit summary for the logistic model used to make the calculations in this study are described in Table 5.6. The variation explained by the logistic model was similar to Doepel et al. (2004). Examples of model fit and optimum supply for Met and Lys are in Figures 5.4 and 5.5. The optimum ratio of model predicted AAR to AAS for each AA and MP are in Table 5.6. As explained, it is difficult to compare the ratio of AAR:AAS among studies due to the different way models calculate AAR. However, it is possible to compare the optimum AAS expressed as % EAA and also in g/d relative to the study of Doepel et al. (2004) given the similarities in the datasets. The required supply and balance of EAA in the current study compared with Doepel et al. (2004) are remarkably similar despite the differences in the models

used to estimate supply. The largest differences were for the BCAA, which are lower in this study, and Met, which is higher. The reason for these differences is unclear but could be due to variation in the AA profiles of feeds and different estimates of microbial protein supply.

Table 5.6. Model parameters, RMSE, R^2 and model outcomes for the logistic model fit between predicted AA requirement and supply

	Model parameters							
AA	θ_1	θ_2	θ_3	RMSE	R^2	AAR:AAS[1]	g/d[2]	% EAA
Arg	66.72	3.17	-0.03	3.31	0.79	0.55	96.4	10.2%
His	39.22	2.77	-0.05	2.47	0.76	0.70	43.9	4.5%
Ile	79.32	3.93	-0.03	4.85	0.74	0.61	102.7	10.8%
Leu	135.12	2.81	-0.01	8.52	0.72	0.67	158.3	17.1%
Lys	114.87	3.21	-0.02	7.33	0.72	0.62	145.1	15.1%
Met	39.23	2.49	-0.04	2.40	0.73	0.53	58.2	5.7%
Phe	69.30	3.52	-0.02	4.23	0.74	0.53	103.4	10.7%
Thr	69.54	3.50	-0.02	4.23	0.74	0.53	102.9	10.7%
Trp	20.74	4.42	-0.10	1.04	0.81	0.58	28.1	2.9%
Val	93.80	2.99	-0.02	6.10	0.68	0.62	118.8	12.4%
MP[3]	1625.35	3.67	-0.002	93.35	0.76	0.73	1751.8	N/A

[1] Optimum ratio of predicted AA requirement (AAR) and supply (AAS)

[2] Optimum AA supply for the dataset used

[3] MP = Metabolizable protein

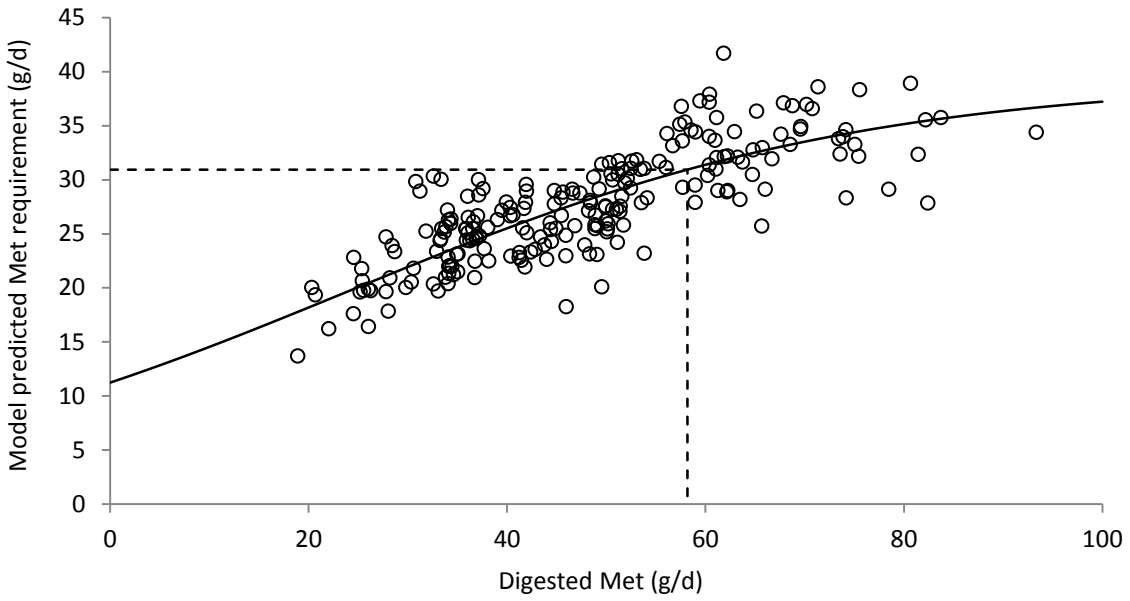

Figure 5.4. Logistic fit of model predicted Met requirement and Met supply. The dashed line represents the optimum ratio of Met requirement and Met supply

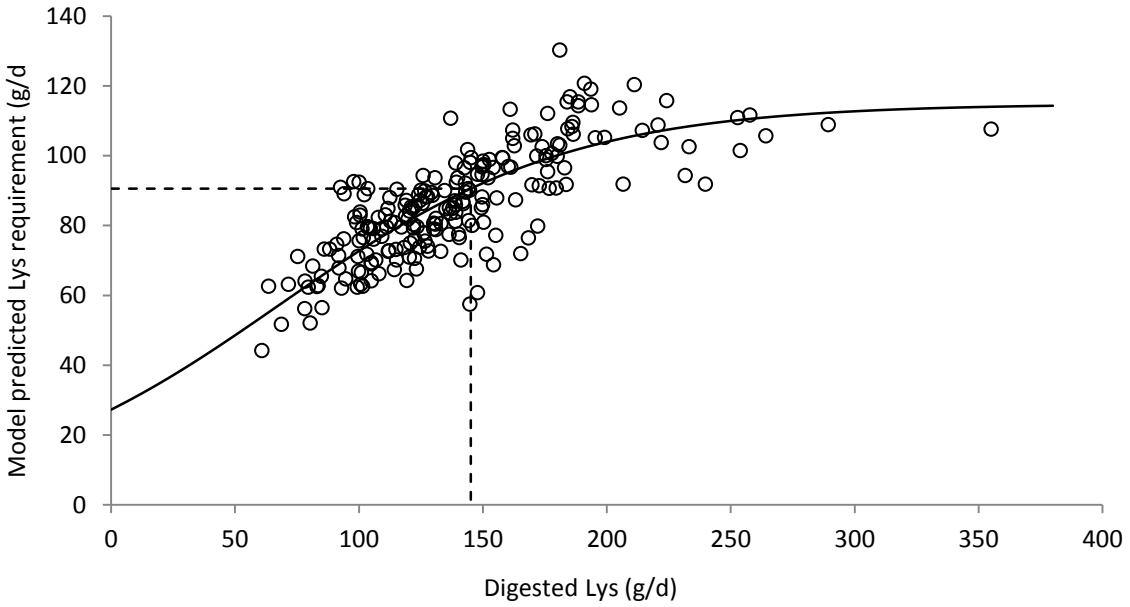

Figure 5.5. Logistic fit of model predicted Lys requirement and Lys supply. The dashed line represents the optimum ratio of Lys requirement and Lys supply

5.4.5 Interactions between amino acid supply and energy

The impact of energy supply on the utilization of AA was investigated by regressing the ratio of AAR and AAS against AA supply relative to total ME and total MP supply. No relationship was found when AA were expressed relative to MP, but a loglogistic relationship was observed when expressed relative to ME. The optimum supply of each EAA relative to ME was determined by using the optimum ratio of AAR to AAS calculated in the previous analysis and solving for *x* using the loglogistic model Eq. 5. Examples of the loglogistic fit and optimum supply relative to ME for Met and Lys are in Figures 5.6 and 5.7, respectively. The model parameters, summary of fit and optimum AA supply relative to ME for all 10 EAA are in Table 5.7. Typically, recommendations for AA balancing are made relative to total MP supply. This approach has been successful in establishing Met and Lys requirements from dose response studies (NRC, 2001, Rulquin et al., 1993, Schwab, 1996). The studies used to estimate these requirements are unique in that they isolate the response to single AA while holding all other variables constant. The data used in this study were different in that 81% of the treatments simultaneously infused greater than 1 AA with the average number of AA infused >8. Interestingly, the optimum supply of Met and Lys estimated in this study was 15.1% and 5.7% of EAA, respectively, which is similar to results found in other studies that used different approaches (Rulquin et al., 1993, Schwab, 1996, Schwab et al., 1992b). However, under these circumstances, no relationship was observed between the 'efficiency' of AA use when AA supply was expressed relative to MP supply but a strong relationship was observed when AA were expressed relative to ME supply which is in agreement the findings of Van Straalen et al. (1994). These data suggests when balancing rations it might be more appropriate to consider AA supply relative to ME which is the approach used in swine (NRC, 2012). Establishing requirements for monogastrics is less complicated than in ruminants as the true AA supply is

more easily determined (Lapierre et al., 2006). Interestingly, the predicted Lys requirement for a lactating sow in the NRC (2012) model is 2.72 g Lys/Mcal ME which is similar to the 3.03 g Lys/Mcal ME calculated in this study for dairy cows. Likewise, the recommended ratios for each EAA and Lys are similar in the dairy cow and sow with the exception of Met and His (Table 5.7). These data suggest, as improvements are made to the predictions of true AA supply in dairy cows, consideration of the approach used to balance AA in other species where AA supply is more easily determined could provide opportunities to improve productivity and the efficiency of nutrient use.

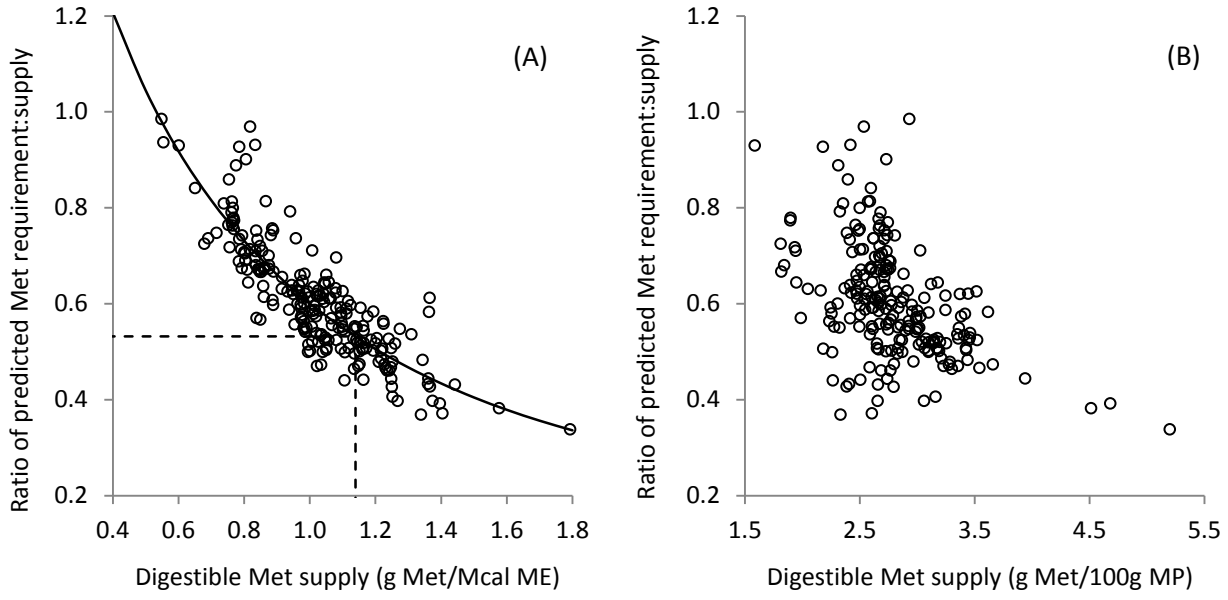

Figure 5.6. Relationship between model predicted Met requirement:supply and Met supply relative to ME (A) or MP (B). The dashed line in (A) represents the Met supply at the optimum ratio of model predicted Met requirement and supply. No significant relationship was determined in (B).

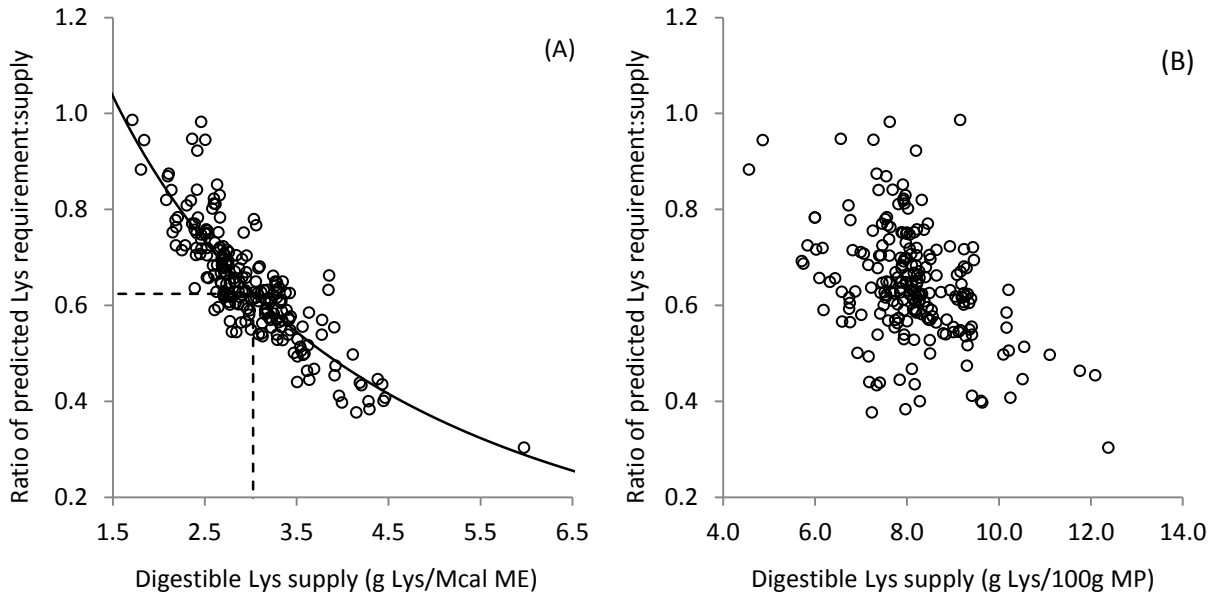

Figure 5.7. Relationship between model predicted Lys requirement:supply and Lys supply relative to ME (A) or MP (B). The dashed line in (A) represents the Lys supply at the optimum ratio of model predicted Lys requirement and supply. No significant relationship was determined in (B).

Table 5.7. Model parameters and fit summary for the loglogistic relationship between AA requirement and supply as well as optimum supply of each EAA relative to ME and relative to Lys.

	Model parameters							
AA	θ_1	θ_2	θ_3	R^2	RMSE	g AA/ Mcal ME	Lys:AA Dairy[1]	Lys:AA Swine[2]
Arg	0.14	-0.88	0.47	0.80	0.05	2.04	1.49	1.85
His	0.19	-1.01	1.01	0.79	0.07	0.91	3.33	2.50
Ile	-0.53	-0.87	0.12	0.71	0.06	2.16	1.40	1.78
Leu	-0.27	-0.90	0.11	0.79	0.06	3.42	0.89	0.89
Lys	0.02	-0.89	0.23	0.73	0.06	3.03	1.00	1.00
Met	0.16	-0.97	1.01	0.75	0.06	1.14	2.66	3.71
Phe	0.09	-0.81	0.39	0.72	0.05	2.15	1.40	1.82
Thr	-0.53	-0.84	0.12	0.71	0.05	2.14	1.41	1.49
Trp	-0.21	-0.81	0.67	0.68	0.05	0.59	5.16	5.33
Val	-0.09	-0.88	0.22	0.75	0.06	2.48	1.22	1.15

[1] Optimum Lys:EAA ratio for the data set used

[2] Optimum Lys:EAA ratio for a lactating sow (NRC, 2012)

5.5 Conclusions

Predictions of endogenous N transactions along the entire GIT have been incorporated into a dynamic version of the CNCPS. This has replaced metabolic fecal N used in previous versions of the CNCPS in estimating AA requirements for maintenance. Model predictions for endogenous transactions along the GIT are close to measured data and have refined the predictions of true AA supply to the animal. Additional AA and MP requirements above the physiological processes quantified by the CNCPS were also estimated. The optimum supply of Met and Lys relative to total EAA were similar to other studies. A loglogistic relationship was observed when the efficiency of AA use was regressed against AA supply relative to ME suggesting expressing AA supply relative to energy could improve predictions of AA utilization. Recommendations for each EAA are given in g AA / Mcal ME and also in a ratio with Lys and are similar to the requirements of swine, suggesting that post-absorptive metabolism could be similar once the supply of EAA is better understood.

5.6 References

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CHAPTER 6: A DYNAMIC VERSION OF THE CORNELL NET CARBOHYDRATE AND PROTEIN SYSTEM: PREDICTING NITROGEN AND AMINO ACID SUPPLY

6.1 Abstract

Balancing the amino acid supply in dairy cow diets has received increased attention in an effort to improve animal productivity, increase N utilization and reduce feed costs. Ration balancing tools like the Cornell Net Carbohydrate and Protein System (CNCPS) and National Research Council model (NRC) allow for consideration of AA supply in the field. In this study, the ability of a new, dynamic version of the CNCPS to predict N and AA flows from the rumen was evaluated using literature studies that reported N flows (n = 16) and AA flows (n = 11) from sampling at the omasum. The adequacy of model predictions for each parameter were assessed using numerous statistics including concordance correlation coefficients (CCC), squared coefficient of determination based on a mean study effect (R^2_{MP}) and linear regression parameters. Model predicted flows of microbial N (MN) were close to measured values and were predicted accurately (Slope = 0.94) and precisely (R^2_{MP} = 0.88; CCC = 0.93). Rumen undegraded feed (RUN), which would include endogenous secretions, was predicted precisely (R^2_{MP} = 0.82; CCC = 0.90), but some prediction bias was observed (Slope = 0.83). Overall, total non-ammonia N (NAN) was predicted with a high level of accuracy and precision (R^2_{MP} = 0.93; CCC = 0.96) and with little bias (Slope = 0.94) indicating the model could accurately predict, and partition, the N flowing from the rumen. Compared to measured data, AA flows were over-predicted which was unexpected given the close agreement with the predicted flows of MN, RUN and NAN. Predictions of Leu, Arg and Thr were most accurate (Slope = 0.86, 0.82, 0.85, respectively; R^2_{MP} = 0.84, 0.79, 0.77, respectively) while predictions of Lys and Ile were least

accurate (Slope = 0.69 and 0.68, respectively; R^2_{MP} = 0.58 and 0.75, respectively). Discrepancies were observed between reported AA flows and AA flows that could be calculated from the reported N flows. It is possible that sample preservation or other factors could have reduced the recovery of certain AA during analysis and the reported AA flows from omasal flow studies are under-estimated.

6.2 Introduction

In non-ruminant nutrition, protein supply is considered in its individual components with a specific focus on essential and conditionally essential AA (NRC, 2012). Compared to a ruminant, predicting AA supply in a non-ruminant is simple, as the intake of digestible protein also represents the supply. In ruminants, the extensive degradation of dietary protein by rumen microorganisms and synthesis of microbial protein alters the supply to the animal and makes predicting the true AA supply challenging. Despite the challenges, AA balancing in dairy cows has received a lot attention in an effort to improve animal productivity and reduce feed costs. Ration formulations systems such as the CNCPS (Fox et al., 2004, Tylutki et al., 2008, Van Amburgh et al., 2013) and the NRC (2001) are important tools that allow nutritionists to consider AA supply in the field, without which, the concept of balancing ruminant diets for AA would be essentially theoretical.

The original system for calculating AA supply in the CNCPS was described by O'Connor et al. (1993) and has been used in all subsequent versions of the model (Fox et al., 2004, Tylutki et al., 2008, Van Amburgh et al., 2013). Published evaluations have shown the model can predict the supply of microbial and dietary protein reasonably well (Offner and Sauvant, 2004, Pacheco

et al., 2012), but the prediction of individual AA at the duodenum can be biased (Pacheco et al., 2012). A new, dynamic version of the CNCPS was constructed that included N components that have been previously omitted from the model including rumen protozoa (Chapter 4) and endogenous N secretions along the entire gastrointestinal tract (**GIT**) (Chapter 5). These components were included within the dynamic framework described in Chapter 3 which includes a new system of calculating post-ruminal N digestion based on an *in vitro* estimate of indigestible protein developed by Ross (2013). The objective of this study was to evaluate the ability of the new version of the CNCPS to predict N and AA flows out of the rumen.

6.3 Materials and methods

6.3.1 Calculation of nitrogen and amino acid flows

The system used to calculate N supply from feed, rumen microorganism and endogenous sources has been described in Chapters 3, 4, and 5, respectively. The N components arriving at the duodenum are described in Table 6.1. The sum of the individual components in Table 6.1 give the total non-ammonia N (**NAN**) arriving at the duodenum and is equivalent to the sample that would be measured *in vivo* from a duodenal cannula. Endogenous components secreted post-ruminally can be removed from the calculation to give an estimate of the N that would be measured using omasal sampling.

Amino acid flows (g/d) to the omasum or duodenum are estimated by partitioning the N from each component (Table 6.1) into AA N (% total N), then into N from each individual AA and dividing by the concentration of N in the AA (% molar mass) to give grams of AA. The different N fractions within a feed (A2, B1, B2 and C; Table 6.1) are pooled and considered as a single

flow when calculating AA supply while the individual microbial and endogenous components are considered separately. The calculation is described as follows:

$$AA_{ki} = \frac{((N\ flow_i \times AA\ N_i) \times AA\ N_{ki}}{N\ conc_k} \quad [1]$$

where:

AA_{ki} is the k^{th} AA (g/d) from the i^{th} N component (g/d)

N $flow_i$ is the flow of the i^{th} N component (g/d)

AA N_i is the proportion of AA N in the i^{th} N component (% total N)

AA N_{ki} is the proportion of N from the k^{th} AA in the AA N of the i^{th} N component (% AA N)

N $conc_k$ is the N concentration in the k^{th} AA (% molar mass)

The total AA flow can then be calculated by summing the individual AA flows:

$$AA_k = \sum_{i=1}^{n} AA_{ki} \quad [2]$$

where:

AA_k is the total supply of the k^{th} AA (g/d)

AA_{ki} is the k^{th} AA (g) from the i^{th} N component (g/d)

6.3.2 Calculation of nitrogen and amino acid digestion

Digestion of feed N in the small intestine is estimated using either the system described by Sniffen et al. (1992) or the system described in Chapter 3 that uses the *in vitro* estimate of indigestible N developed by Ross (2013). To summarize, if an estimate from the assay of Ross

(2013) is available, the fractions of feed N escaping the rumen are pooled and the digestibility is calculated as follows:

$$Intestinal\ digestibility_i = 1 - \left(\frac{Indigestible\ N_i}{A2\ N_i + B1\ N_i + B2\ N_i + C\ N_i + PAA\ N_i}\right) \quad [3]$$

where:

i represents the ith feed in the diet

Indigestible N is estimated using the assay of Ross (2013)

A2 N, B1 N, B2 N, C N and PAA N represent model predicted N escape for each fraction, including peptides and free AA.

The total predicted non-ammonia N flow from each feed is then multiplied by the intestinal digestibility value calculated in Eq. [3] to estimate N digestion and ignores the previously used detergent approach for fractionation. If the *in vitro* indigestible N estimate is not available the system of Sniffen et al. (1992) is used where static digestibility coefficients from the CNCPS feed library are applied to each N fraction to estimate digestion. This is summarized by the following equation:

$$N\ digested_i = \sum_{j=1}^{n} Nflow_{ij} \times ID_{ij} \quad [4]$$

where:

$N\ digested_i$ is the total N digested for the i^{th} feed

$N\ flow_{ij}$ is flow of N from the j^{th} N fraction of the i^{th} feed

ID_{ij} is the intestinal digestion coefficient for the j^{th} N fraction of the i^{th} feed

Microbial N is partitioned into cell wall N, which is considered completely indigestible, nucleic acid N and AA N which are considered completely digestible, respectively (Chapter 3). Endogenous N components are digested according to the digestion coefficients in Chapter 5. A summary of the N components digested in the small intestine are in Table 6.2.

Amino acid digestion is calculated the same way as in Eq. [1], but rather than using the total N flow, digested N is used:

$$AA_{ki} = \frac{((N\ Digested_i \times AA\ N_i) \times AA\ N_{ki}}{N\ conc_k} \quad [5]$$

where:

AA_{ki} is the k^{th} AA (g/d) from the i^{th} N component (g/d)

N $Digested_i$ is the digested N from the i^{th} N component (g/d)

AA N_i is the proportion of AA N in the i^{th} component of digested N (% total N)

AA N_{ki} is the proportion of N from the k^{th} AA in the AA N of the i^{th} component of digested N (% AA N)

N $conc_k$ is the N concentration in the k^{th} AA (% molar mass)

Table 6.1. Nitrogen components arriving in the small intestine

Duodenal nitrogen flows[1]	Flow	Description[2]
Feed		
	A2 N Escape*i*	Escape of A2 N from the rumen
	B1 N Escape*i*	Escape of B1 N from the rumen
	B2 N Escape*i*	Escape of B2 N from the rumen
	C N Escape*i*	Escape of C N from the rumen
	Feed PAA N Escape*i*	Escape of PAA originating from feed
Microbial		
	FB Cell N Escape	Escape of FB cell N from the rumen
	NFB Cell N Escape	Escape of NFB cell N from the rumen
	PZ Cell N Escape	Escape of PZ cell N from the rumen
	FB PAA N Escape	Escape of PAA originating from FB
	NFB PAA N Escape	Escape of PAA originating from NFB
	PZ PAA N Escape	Escape of PAA originating from PZ
Endogenous		
	End N OA Flow*j*	Escape of endogenous N from the rumen
	End PAA N Escape*j*	Escape of PAA originating from endogenous secretions

[1] Subscript *i* represents the *i*th feed in the diet; subscript *j* represents the *j*th endogenous secretion

[2] A2 N = Soluble non-ammonia N; B1 = insoluble N; B2 = fiber bound N; C = unavailable N (acid detergent insoluble N); FB = fiber bacteria; NFB = non-fiber bacteria; PZ = protozoa; PAA = peptides and free AA.

Table 6.2. Nitrogen components digested in the small intestine

Intestinal nitrogen digestion[1]	Flow	Description[2]
Feed		
	A2 N ID*i*	Digestion of A2 N in the SI
	B1 N ID*i*	Digestion of B1 N in the SI
	B2 N ID*i*	Digestion of B2 N in the SI
	C N ID*i*	Digestion of C N in the SI
	Feed PAA N ID*i*	Digestion of PAA originating from feed in the SI
Microbial		
	R FB AA N ID	Digestion of FB AA N in the SI
	R FB NA N ID	Digestion of FB nucleic acid N in the SI
	R FB CW N ID	Digestion of FB cell wall N in the SI
	R NFB AA N ID	Digestion of NFB AA N in the SI
	R NFB NA N ID	Digestion of NFB nucleic acid N in the SI
	R NFB CW N ID	Digestion of NFB cell wall N in the SI
	PZ AA N ID	Digestion of PZ AA N in the SI
	PZ NA N ID	Digestion of PZ nucleic acid N in the SI
	PZ CW N ID	Digestion of PZ cell wall N in the SI
Endogenous		
	End N ID*j*	Digestion of endogenous N in the SI

[1] Subscript *i* represents the *i*th feed in the diet; subscript *j* represents the *j*th endogenous secretion

[2] A2 N = Soluble non-ammonia N; B1 = insoluble N; B2 = fiber bound N; C = unavailable N (acid detergent insoluble N); FB = fiber bacteria; NFB = non-fiber bacteria; PZ = protozoa; PAA = peptides and free AA; SI = small intestine.

6.3.3 Evaluation dataset

A database was compiled from published studies that measured microbial N (**MN**), rumen undegraded feed N (which would include endogenous N; **RUN**), total non-ammonia N (**NAN**) (16 publications; 61 treatment means) and AA (11 publications; 43 treatment means) flows at the omasum (Table 6.3). Information reported in the study on animal characteristics, their environment and diets were entered in model. 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 predicted using an extension of the method described in Chapter 2. 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.

Table 6.3. Omasal sampling studies used to evaluate model N flows and AA flows

Study	Amino acid flows reported
Ahvenjärvi et al. (1999)	
Ahvenjärvi et al. (2002)	x
Ahvenjärvi et al. (2006)	
Brito et al. (2006)	x
Brito et al. (2007a)	x
Brito et al. (2007b)	x
Brito et al. (2009)	x
Broderick and Reynal (2009)	x
Choi et al. (2002)	
Korhonen et al. (2002b)	x
Colmenero and Broderick (2006)	
Owens et al. (2008a)	
Owens et al. (2008b)	
Reynal and Broderick (2003)	x
Reynal and Broderick (2005)	x
Vanhatalo et al. (2009)	x

6.3.4 Statistical analysis

A mixed model using the restricted maximum likelihood model (**REML**) procedure of SAS (2010) was used to analyze the data using the model:

$$Y_{ij} = (\beta_0 + b_{0i}) + \beta_1 X_{ij} + \varepsilon_{ij}$$

where:

Y_{ij} is the expected outcome for the dependent variable Y observed at repetition j of the continuous variable X in study i,

β_0 is the overall intercept across all studies,

b_{0i} is the random effect of study i,

β_1 is the overall slope of Y on X across all studies,

X_{ij} is the data associated with repetition j of the continuous variable X in study i, and

ε_{ij} is random variation

The variance components in the model adhered to the following assumptions: $b_{0i} \sim N(0,\sigma^2_0)$, $b_{1i} \sim N(0,\sigma^2_1)$, and $\varepsilon_{ij} \sim N(0,\sigma^2_\varepsilon)$. The squared sample correlation coefficients reported were based on either the BLUP ($\mathbf{R^2_{BLUP}}$) or model predictions using a mean study effect ($\mathbf{R^2_{MP}}$). The random effect of study in the mixed model analysis typically accounts for a high proportion of variation and is important in ensuring parameter estimates are not biased (St-Pierre, 2001). However, the large portion of variation explained by the study effect result in high R^2_{BLUP} values. In practice, R^2_{BLUP} can be misleading as random farm-to-farm variation cannot be accounted for given that no measured values exist to compare model predictions to. Consequently, R^2_{MP} values were also presented which use an average study effect across the whole data set and give a better indication of the amount of variation the model may explain in the practical situation. Further information on mixed model methodology can be found in a review by St-Pierre (2001).

Additional model adequacy statistics were calculated to give further insight into the accuracy, precision, and sources of error in the model (Tedeschi, 2006). Root mean square prediction

errors (RMSPE) were used to indicate accuracy. A decomposition of the MSPE was also performed to give an estimation of the error due to central tendency (mean bias), regression (systematic bias), and random variation (Bibby and Toutenburg, 1977). Concordance correlation coefficients (**CCC**) were used to simultaneously account for accuracy and precision. Concordance correlation coefficients can vary from 0 to 1, with a value of 1 indicating that no deviation from the Y = X line has occurred. Further description of these statistics is provided by Tedeschi (2006).

6.4 Results

6.4.5 Nitrogen flows

Model predicted N flows estimated by the model were similar to measured values for MN, RUN and NAN (Figures 6.1, 6.2 and 6.3, respectively). Microbial N and NAN were predicted with a high level of accuracy and precision (CCC = 0.96 and 0.93, respectively) and with little bias (Table 6.4). Predictions of RUN were accurate (CCC = 0.90) but some bias was observed (19% systematic bias and 6% mean bias). The random effect of study explained the majority of the variation in NAN and MN while most of the variation in RUN was residual error.

6.4.6 Amino acid flows

Relative to the reported data, the model over-predicted AA flows for all the EAA. The over-prediction was greatest for Ile and Lys (Figure 6.4C and E) and least for Arg, Leu and Thr (Figure 6.4A, D and H). The random effect of study accounted for greater than half the variation for all EAA other than Ile and Leu and R^2_{BLUP} ranged from 0.86 – 0.94 (Table 6.4). The variation explained using a mean study effect (R^2_{MP}) was lower, and varied among AA. Methionine and

Phe were the most variable and Leu and Arg were the least variable (Table 6.4). The bias associated with predictions was mostly mean and random bias apart from His and Phe which were higher in systematic and random bias.

Calculation of Lys flow using the reported omasal MN flow (Figure 6.2) and typical bacterial AA composition (Clark et al., 1992) was higher than the total reported Lys flow in many studies (Figure 6.5). In this case, if apparent RUN Lys was back calculated from total reported Lys flow and calculated microbial Lys flow, the RUN Lys was negative, which is impossible. Using these calculations, the maximum contribution of Lys from RUN for any diet was 30% while the microbial contribution ranged from 70% - 129% of the measured Lys flow (Figure 6.5).

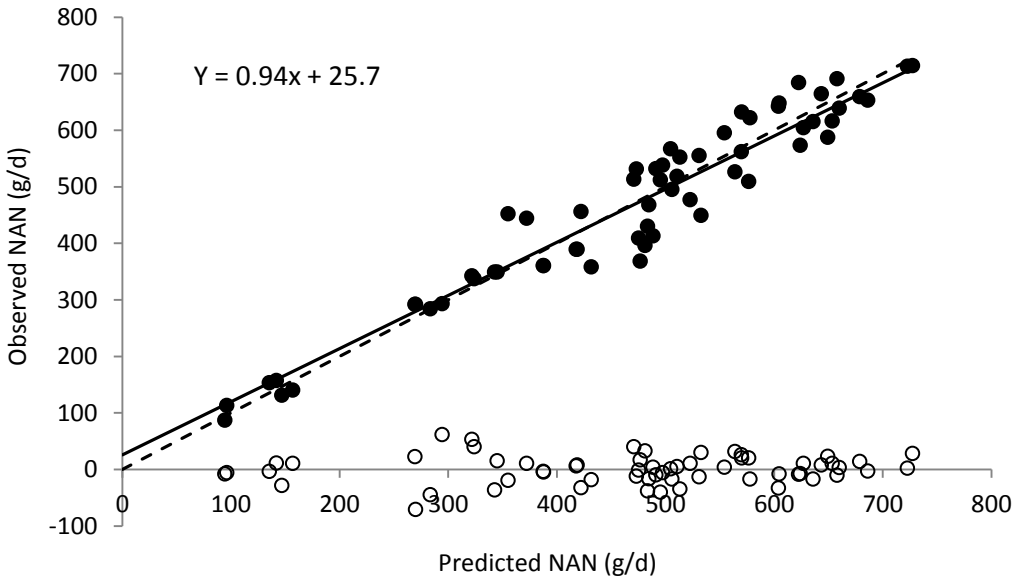


Figure 6.1 Predicted and observed non-ammonia N (NAN) flows at the omasum (●) and residual error (○) from the mixed model regression analysis. The solid line (—) represents the linear regression and the dashed line (- - -) is the unity line. Regression statistics are in Table 6.4.

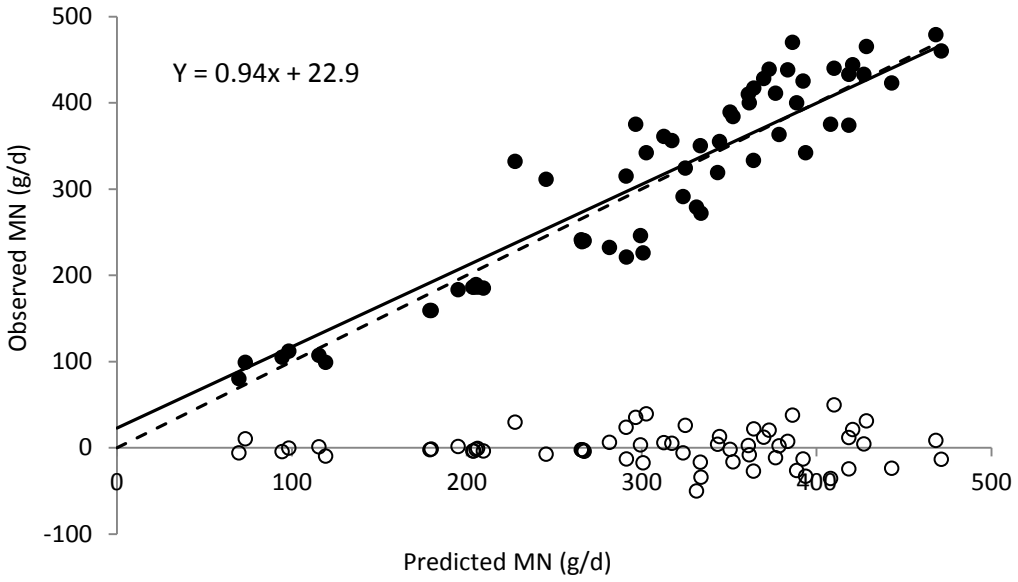


Figure 6.2. Predicted and observed microbial N (MN) flows at the omasum (●) and residual error (○) from the mixed model regression analysis. The solid line (—) represents the linear regression and the dashed line (- - -) is the unity line. Regression statistics are in Table 6.4

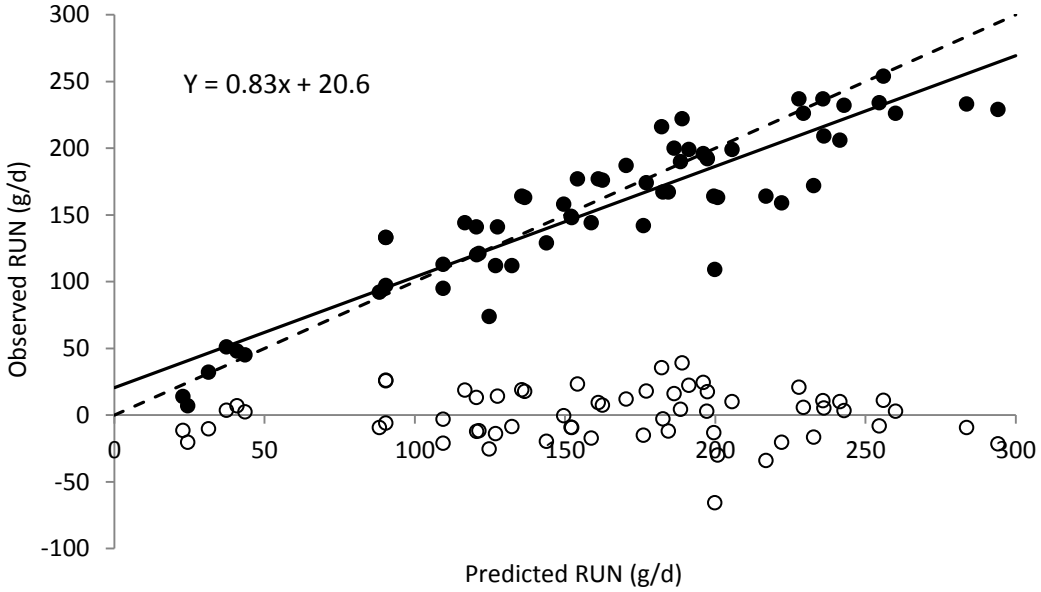
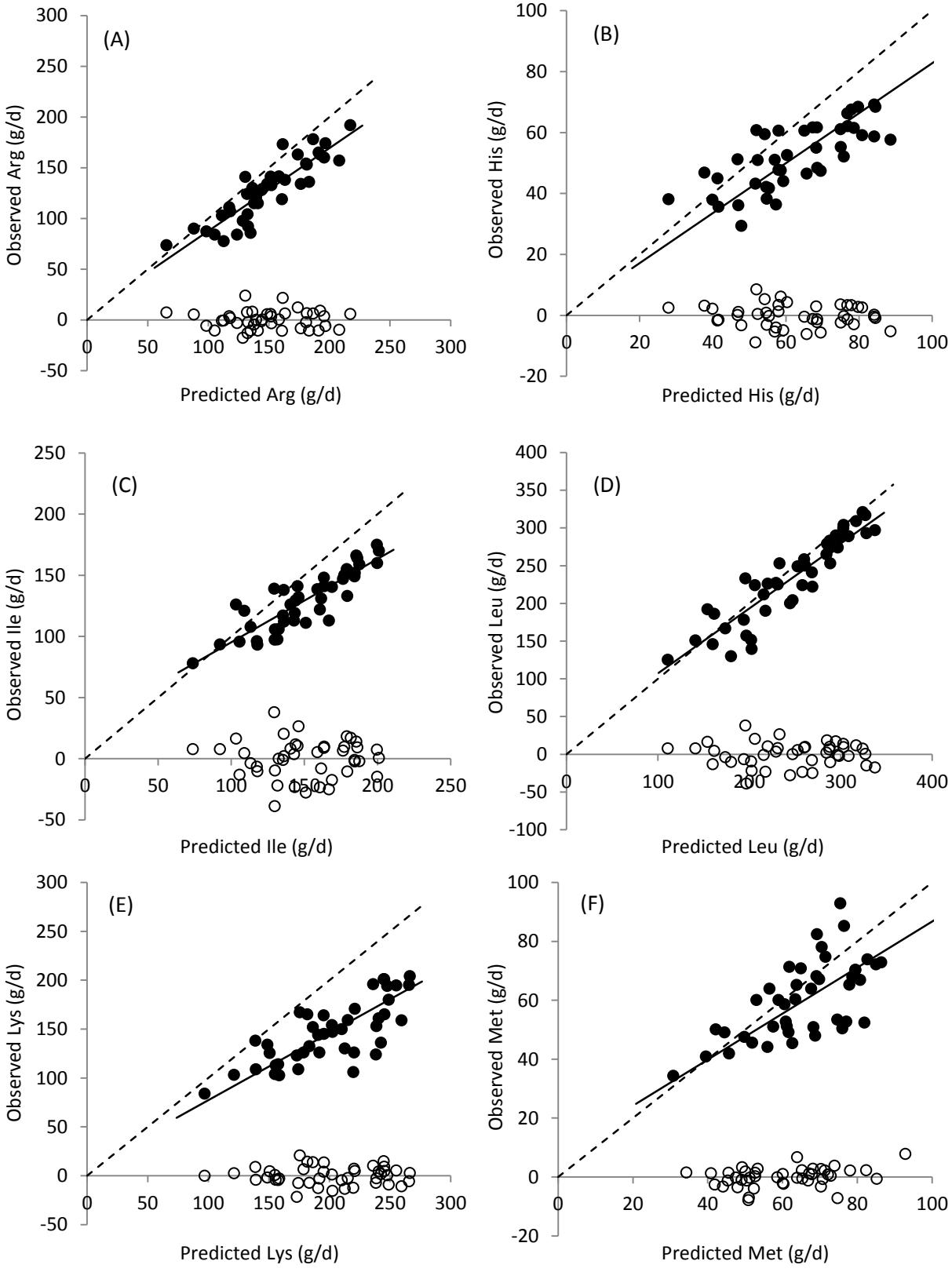


Figure 6.3. Predicted and observed rumen un-degraded and endogenous N flows (RUN) at the omasum (●) and residual error (○) from the mixed model regression analysis. The solid line (—) represents the linear regression and the dashed line (- - -) is the unity line. Regression statistics are in Table 6.4.


(A)
Observed Arg (g/d)
Predicted Arg (g/d)
(B)
Observed His (g/d)
Predicted His (g/d)
(C)
Observed Ile (g/d)
Predicted Ile (g/d)
(D)
Observed Leu (g/d)
Predicted Leu (g/d)
(E)
Observed Lys (g/d)
Predicted Lys (g/d)
(F)
Observed Met (g/d)
Predicted Met (g/d)

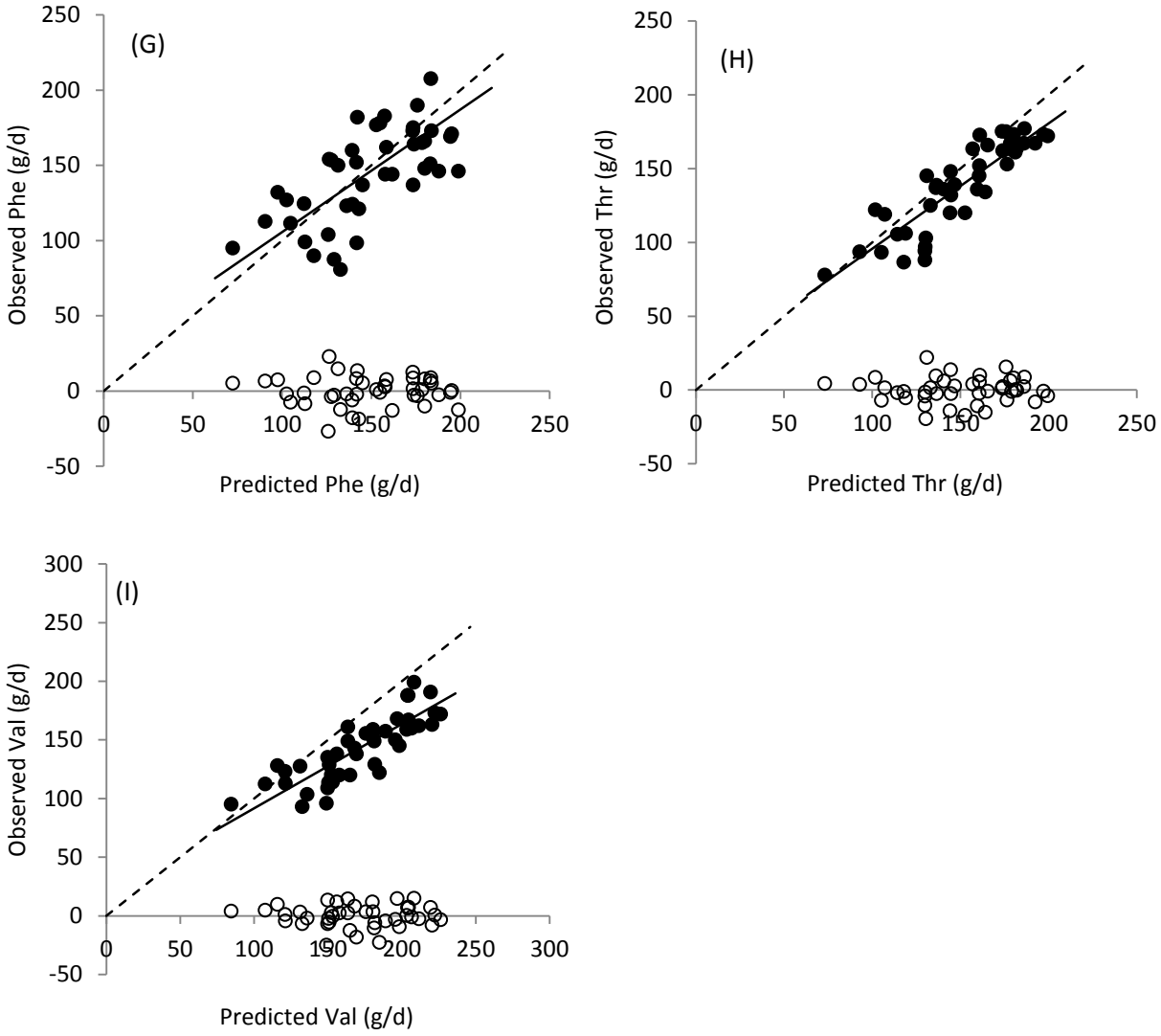


Figure 6.4. Predicted and observed essential AA flows at the omasum (●) and residual error (○) from the mixed model regression analysis. The solid line (—) represents the linear regression and the dashed line (- - -) is the unity line. Regression statistics are in Table 6.4.

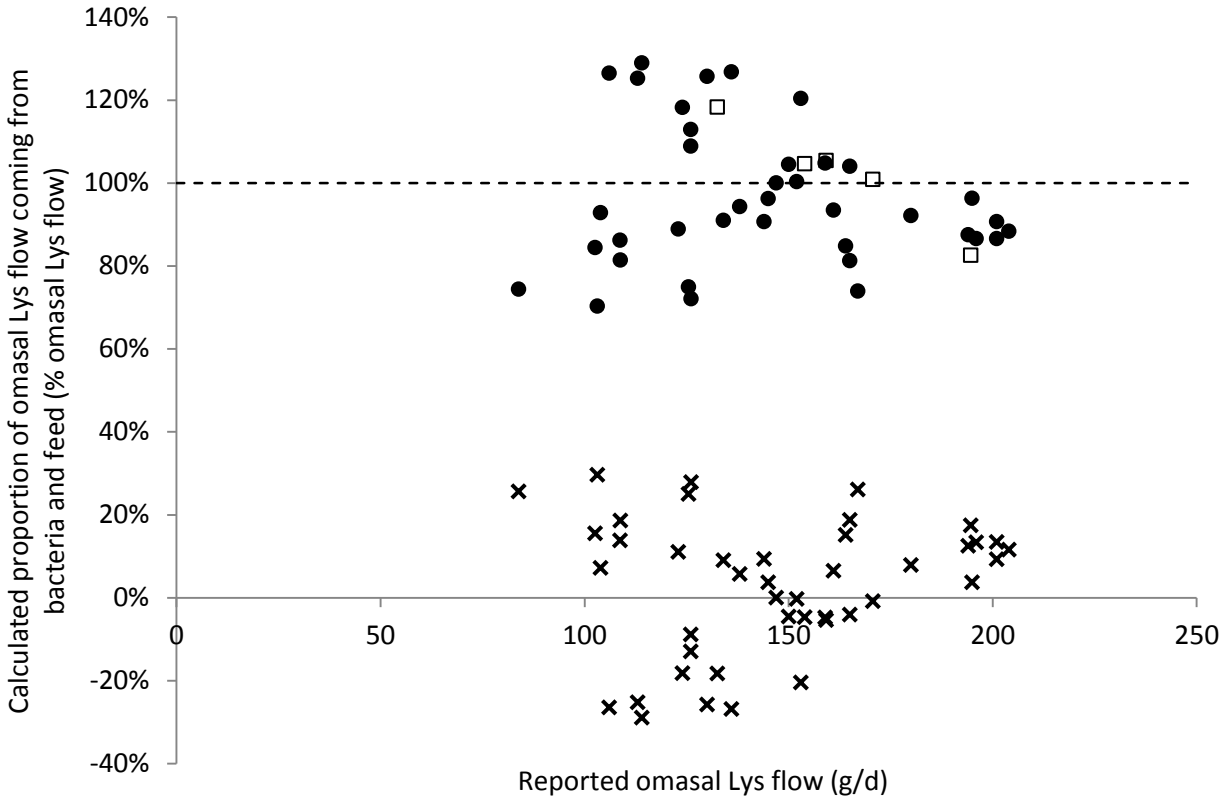


Figure 6.5. The proportion of calculated bacterial Lys flow from microbial N flows estimated using ^{15}N (●) or purine derivatives (□) compared with feed (×) relative to reported total Lys flows at the omasum. Bacterial Lys was calculated from the measured microbial N flows at the omausm and the chemical composition reported in Clark et al. (1992); 67% AA N (% total cell N); 11.2% Lys N (% AA N); Lys N (19.2 % molar mass). Feed Lys was calculated as the difference between total reported Lys and calculated bacterial Lys. The dashed line (- - -) represents 100% of the reported Lys flow. Values greater than 100% mean the calculated bacterial Lys was greater than the total measured Lys from all sources.

Table 6.4. Model adequacy statistics for the prediction of nitrogen components and essential AA from the Cornell Net Carbohydrate and Protein System version 7 (CNCPS) relative to values measured at the omasum

						Variance component[5] (%)				MSPE Partitioned[8] (%)		
Omasal component (g/d)	R^2_{BLUP}[2]	R^2_{MP}[3]	RMSE[4]	Slope	Intercept	Study	Residual	CCC[6]	RMSPE[7]	U^M	U^S	U^R
Non-ammonia N	0.98	0.93	27.9	0.94	25.7	63.5	36.5	0.96	43.8	0%	2%	98%
Microbial N	0.97	0.88	22.2	0.94	22.9	74.8	25.2	0.93	40.2	1%	1%	98%
RUN[1]	0.90	0.82	20.6	0.83	20.6	32.4	67.6	0.90	28.3	6%	19%	75%
Arg	0.92	0.79	10.0	0.82	5.9	54.1	45.9	0.73	26.1	65%	6%	29%
His	0.91	0.61	4.25	0.82	0.8	74.9	25.1	0.65	25.7	3%	17%	80%
Ile	0.86	0.75	10.0	0.68	27.1	40.3	59.7	0.65	26.3	64%	15%	21%
Leu	0.92	0.84	17.5	0.86	21.2	45.4	54.6	0.89	26.7	24%	5%	71%
Lys	0.92	0.58	10.4	0.69	8.4	78.7	21.3	0.36	60.9	80%	9%	11%
Met	0.94	0.42	3.67	0.78	8.6	88.3	11.7	0.60	12.0	20%	6%	74%
Phe	0.90	0.44	10.8	0.82	8.6	81.4	18.6	0.65	25.7	3%	17%	80%
Thr	0.92	0.77	9.7	0.85	10.9	57.1	42.9	0.81	18.8	39%	5%	56%
Val	0.88	0.69	10.6	0.72	19.8	58.4	41.6	0.56	34.8	70%	11%	19%

[1] RUN = Rumen undegraded and endogenous N

[2] R^2_{BLUP} = squared sample correlation coefficient based on BLUP.

[3] R^2_{MP} = squared sample correlation coefficient based on model-predicted estimates.

[4] RMSE = Root mean square error.

[5] Percentage of variance related to the effect of study and random variation.

[6] Concordance correlation coefficient.

[7] RMSPE = Root mean square prediction error.

[8] MSPE = Mean square prediction error partitioned to: U^M= mean bias; U^S = systematic bias; U^R = random variation. $U^M + U^S + U^R = 100$

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6.5 Discussion

The model described here, and in previous chapters, represents an implementation of recent advancements that have been made in the understanding of N availability to the animal, including improvements in the characterization of feed chemistry (Chapter 2), quantification of endogenous N flows (Ouellet et al., 2010, Ouellet et al., 2002), estimates of N availability in the small intestine (Ross, 2013) and changes to estimates of microbial growth to include protozoa (Chapter 4). The broad goal of these updates has been to improve the models ability to predict N flows out of the rumen, to the small intestine, and the availability of AA to the animal. Validating the changes to the model against animal data is an important step in establishing the efficacy of the model updates (Tedeschi, 2006). The data used to evaluate the model was sourced from studies that measured N flows at the omasum. The omasal sampling technique described by Huhtanen et al. (1997) has advantages over sampling in other compartments (abomasum or duodenum) that include less contamination with endogenous material and less invasive surgery that can affect the performance and lifespan of the cows used. All studies in the current dataset 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 like Cr_2O_3 that are often used in studies that have sampled at the duodenum (Firkins et al., 2007, Huhtanen et al., 2010). Microbial N flows were estimated using either ^{15}N (n = 11) or purine bases (n = 5). Previous model evaluations (Pacheco et al., 2012) and the NRC (2001) have used data from studies that measured N and AA flows at the duodenum. Although a larger dataset is available if duodenal sampling is considered (40 studies; 154 treatments; Pacheco et al., 2012), we chose to restrict this dataset to studies that sampled at the omasum to limit endogenous contamination and marker bias.

Previous evaluations of the CNCPS have found predictions of microbial flows to the duodenum to be accurate and to compare favorably to other available models (Offner and Sauvant, 2004, Pacheco et al., 2012). Although deemed accurate, both evaluations reported regression slopes <1 (0.70 and 0.91, respectively) suggesting prediction bias. Incorporation of protozoa into the dynamic structure of the current model (Chapter 4) represents a considerable change in the system used to estimate microbial growth in the CNCPS. Compared to omasal sampling data, predictions of microbial N flows were more accurate and had less bias than previous versions of the model (slope = 0.94; Figure 6.2; Table 6.4). Predictions were also closer to measured data than the NRC (2001) which was shown to under-predict microbial N flows (slope = 1.26), particularly when observed flows were high (Broderick et al., 2010). Measured microbial growth efficiency (g MN/ kg OM truly digested in the rumen) in the study of Broderick et al. (2010) was within the expected range and similar to other studies (Clark et al., 1992) suggesting the observed flows were reasonable. Therefore, predictions of microbial growth in this version of the CNCPS appear to have improved.

Prediction of RUN was more variable than MN and tended to be over-predicted when RUN flows were high (Figure 6.3). What is generally reported as feed N will typically also include endogenous secretions as feed N is calculated as the difference between total NAN and MN (Broderick et al., 2010). Any error in the prediction of MN or NAN will be pooled in the estimates of RUN and, therefore, more variability might be expected. Also, the predictions of RUN rely on library values to estimate the rate of N digestion of the various N fractions which can vary within, and among feeds (Broderick, 1987, NRC, 2001). Estimating digestion rates of feed N *in vitro* is challenging due to contamination with microbial protein (Broderick, 1987).

However, relying on library values is no doubt one of the major limitations to improving predictions of AA supply in ration formulation models. Although some bias was observed in this version of the CNCPS, the slope and intercept were closer to unity than observed for the NRC (2001) by Broderick et al. (2010). Less bias was observed in RUN for version 6 of the CNCPS using duodenal data (Slope = 0.94; Intercept = 24.6 g N; Pacheco et al., 2012). However, more endogenous N would be expected in the dataset of Pacheco et al. (2012) which would reduce the apparent over-prediction observed at the omasum in this study. The CNCPS v6 (Fox et al., 2004, Tylutki et al., 2008) does not include predictions of endogenous N, therefore, the apparent accuracy of version 6 of the model compared to duodenal measurements suggests an over-prediction of undegraded dietary protein flow out of the rumen.

In this analysis, total NAN was predicted accurately, precisely and with little bias (Table 6.4). The relationship was similar to the NRC (2001) which was also able to accurately predict total NAN flowing from the rumen (Broderick et al., 2010). These data represent an improvement from the evaluation of Pacheco et al. (2012) which can probably be attributed to the improvement in the prediction of microbial yield. However, some caution is necessary when comparing the studies due to the differences in the datasets used to complete the evaluation.

Amino acid flows were over-predicted by the model relative to measured omasal flows for the AA considered in this study. This was unexpected given the close agreement between N flows from the model and measured data. The variation in AA flows differed among AA with the greatest variation seen in His, Lys, Met and Phe (Table 6.4). Given the model calculates AA flow by applying an AA profile to the predicted N flow (Eq. 1), and the N flows appeared to be

accurate, three possibilities can be formulated to explain the bias: 1) the AA profiles of the N constituents comprising the omasal N flow do not adequately represent what is truly flowing at the omasum; 2) there is analytical error associated with measuring AA which differs among AA; and 3) the omasal N flows reported by the studies are biased, and given the agreement between the model and the measured data, the model is also biased. Certainly, Met analysis is technically challenging and requires an additional pre-oxidation step before acid hydrolysis of the sample (Allred and MacDonald, 1988). However, all studies used in the current dataset reported using the correct procedure for Met analysis, and similar variance was observed in the prediction of other AA that do not require this step (Lys, His, Phe). The AA profiles of feeds used by the model were updated in an earlier study (Chapter 2) using a contemporary dataset and it is unlikely the possible variance in these profiles could cause an over-prediction of the magnitude observed. For example, dietary Lys in the study of Reynal and Broderick (2005) was reported to be approximately 4.5% AA among the treatments reported which was similar to model predictions (data not shown). If the predicted Lys content was over-predicted by 2% points (6.5% AA), at the highest levels of RUN flow (~250 g/d), this would represent a difference of ~20 g Lys/d which is less than half the difference observed (~70 g/d) at high levels of Lys flow (Figure 6.4E). It is also unclear why the predictions of certain AA (Arg, Leu, Thr) had less variation and bias than others given the N flows used to make the calculations were the same. These findings are consistent with the study of Pacheco et al. (2012) who also reported differences in slopes among AA for version 6 of the CNCPS. Interestingly, the directional differences in the regression slopes of the AA reported in Pacheco et al. (2012) were similar among the models evaluated (i.e. Arg ~0.6; Leu ~0.9; Ile ~0.6), with the exception of the NRC. The factorial equations used in the NRC (2001) were derived using measured duodenal flow

from a dataset similar to the evaluation set used in Pacheco et al. (2012), therefore, it is not surprising NRC predictions were close to the duodenal data. Variation has been observed in the profile of AA in whole feeds and residues after exposing feeds to fermentation (Edmunds et al., 2013, Erasmus et al., 1994). However, it is unclear what portion of this is due to the challenges of correcting for microbial contamination of samples after rumen exposure. Stern et al. (1983) calculated differential rates of AA digestion and showed Lys, Ile, His and Arg were the four most rapidly degraded AA in corn gluten meal and AA degradation differed among AA. This could partially explain the difference in slopes among AA, but again, the magnitude of the differences observed could not explain the observed bias in the AA flows.

Continuing with the investigation of predicted Lys flow in the current study, we calculated the likely Lys flow using the observed microbial N flow measured at the omasum and the composition of bacteria reported in Clark et al. (1992). The Lys flows calculated using the reported microbial N flow were, in many cases, greater than the total Lys flow measured at the omasum, which is obviously impossible (Figure 6.5). The bacterial composition reported by Clark et al. (1992) is consistent with other literature reports (Czerkawski, 1976, Korhonen et al., 2002a, Storm and Ørskov, 1983, Volden et al., 1999) and it seems unlikely that differences in microbial composition would be responsible for the observed inconsistency in the data suggesting another source of error. Possibilities may include: 1) the measured microbial N flows were over-estimated or 2) there was error associated with the AA analysis of the omasal digesta. Given that the microbial N flows appear consistent with typical values of microbial protein synthesis (Broderick et al., 2010) error associated with the AA analysis seems more likely. Treatment of samples with formaldehyde, which is commonly used to stop bacterial cells from

lysing (Isaacson et al., 1975) has been shown to lower the recovery of Lys, His, Tyr, Cys and Glu (Gruber and Mellon, 1968) and also interact with Arg, Thr and other AA (Barry, 1976) and could explain the some of the inconsistencies observed between the data and the model predictions, although not all studies reported the use of formaldehyde. Another explanation could be that Maillard reactions are occurring between Lys and readily available carbohydrates, especially since Lys is the EAA with greatest bias (Van Soest, 1994). Maillard reactions typically require heat, and all studies reported freeze drying the samples used for analysis, however, reactions can also be chemically induced (Gerrard et al., 2002, 2003), and is well documented problem in the pharmaceutical industry (Wu et al., 2011). When examining the contribution of predicted EAA N flows relative to observed NAN flows, the average contribution was 39%, which is similar to the concentration of EAA N in bacteria (Volden et al., 1999) and also similar to EAA N in feeds when expressed on a whole feed basis. This suggests the predicted contribution of EAA N to the total NAN flow is reasonable and suggests the measured AA flows, based on incomplete recovery of EAA as analyzed could be underestimated. Further investigation into the efficacy of current procedures of AA analysis on digesta samples is warranted and would aid in the interpretation of data used to validate prediction models.

6.6 Conclusions

A new version of the CNCPS was evaluated for its ability to predict nitrogen and AA flows from the rumen. Data were evaluated using a dataset from literature values that measured N and AA flows at the omasum. Model predictions were close to measured data for microbial, feed and total non-ammonia N flows at the omasum but over-predicted the flow of essential AA. Discrepancies were observed between reported AA flows and AA flows that could be calculated

from the reported N flows. It is possible sample preservation or other factors could have reduced the recovery of certain AA during analysis and the reported AA flows are under-estimated.

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CHAPTER 7: BALANCING DAIRY CATTLE DIETS FOR METHIONINE OR ALL ESSENTIAL AMINO ACIDS REALATIVE TO ENERGY AT NEGATIVE AND ADEQUATE LEVELS OF RUMEN NITROGEN

7.1 Abstract

Improving the ability of ration balancing systems to predict the AA supply and requirement in lactating dairy cattle provides an opportunity to improve animal productivity, reduce feeds costs and improve N utilization. Updates have been made to the Cornell Net Carbohydrate and Protein System (CNCPS) which now includes estimations of rumen protozoa, endogenous N secretions and a new system for calculating post-ruminal N digestion. The goal of this study was to evaluate the ability of the updated model to balance diets of high producing dairy below or close to requirements for both rumen N and EAA and evaluate the impact on N utilization. To do this, sixty-four high producing dairy cows were randomly assigned to 1 of 4 treatments. The treatments were 1) limited in Met, MP and rumen N (Base); 2) adequate in Met but limited MP and rumen N (Base+M); 3) adequate in Met and rumen N, but limited MP (Base+MU); 4) adequate in MP, rumen N and balanced for all EAA (Positive). Dietary CP was 13.5, 13.6, 14.6 and 15.6 % DM for the Base, Base+M, Base+MU and Positive treatments, respectively. No differences were observed in DMI or milk yield (24.1 - 24.8 and 40.0 - 41.8 kg/d, respectively). Energy corrected milk, fat and true protein yield were greater (3.3, 0.09 and 0.11 kg/d, respectively; $P < 0.001$) in cows fed the Positive compared to the Base treatment. True protein concentration in milk was higher ($P < 0.001$) and milk fat tended to be higher ($P < 0.10$) in cows fed the Positive and Base+MU treatments than cows fed the Base and Base+M treatments. Using the updated Cornell Net Carbohydrate and Protein System to evaluate the diets and environment, cattle fed the Base, Base+M and Base+MU treatments were predicted to have a negative MP

balance (-231, -310 and -142 g/d, respectively), while cattle fed the Positive treatment consumed 33 g MP/d excess to requirements. Bacterial growth was predicted to be depressed by 16% and 17% for the Base and Base+M treatments, respectively, due to the predicted rumen N balance which corresponded with lower (P <0.05) apparent total tract NDF digestion. The study demonstrates high levels of milk production can be achieved when diets are formulated on a N basis, ignoring CP and focusing on rumen N balance and EAA, even when crude protein is <14 % DM provided adequate AA are supplied to the small intestine. Further, this study demonstrates that N utilization can be improved and the environmental impact of dairy production reduced through more precise predictions of N and AA requirements and predicted supply.

7.2 Introduction

Ration formulation systems continue to evolve as new information becomes available and the understanding of biological systems improves. The accurate prediction of AA requirement and supply in dairy cattle has been of particular interest in an attempt to improve animal performance, reduce feed costs and increase N utilization (Lapierre et al., 2006). Recommendations for dietary Lys and Met supply are well established (NRC, 2001, Rulquin et al., 1993, Schwab, 1996) and numerous studies have demonstrated improvements in animal productivity when the balance of Lys and Met is improved (Armentano et al., 1997, Chen et al., 2011, Noftsger and St-Pierre, 2003). In addition to Lys and Met, the potential for other EAA to limit milk production has been investigated including the branched chain AA, Arg and His (Appuhamy et al., 2011, Haque et al., 2012, Haque et al., 2013, Lee et al., 2012a, Lee et al., 2012b). Increases to milk, milk protein, and also DMI have been observed when His was added to diets predicted to be His deficient (Lee et al., 2012a, Lee et al., 2012b), but mixed results have

been observed when adding BCAA and Arg (Appuhamy et al., 2011, Haque et al., 2012, Haque et al., 2013). The interactions and inter-conversion between protein and energy could impact expected responses from additional AA supply, particularly BCAA which are extensively oxidized and act as precursors for the synthesis of other required metabolites (Lemosquet et al., 2010, Lobley, 2007). Further, provision of additional energy can reduce the oxidation of BCAA in the mammary gland and demonstrates the ability of the animal to adjust metabolism according to the profile of nutrients provided (Raggio et al., 2006). Given the interactions between protein and energy it has been suggested they be considered together in ration formulation systems, rather than as separate entities (Lobley, 2007).

The repeatability of a response from AA balancing may also be influenced by the ability of ration formulation systems to accurately estimate true AA deficiencies. Pacheco et al. (2012) conducted an evaluation of four commercially available ration balancing programs to predict EAA supply and concluded that, while predictions were generally accurate, all programs, including the CNCPS, had areas where significant improvements could be achieved. A new, dynamic version of the Cornell Net Carbohydrate and Protein System (**CNCPS)** has been constructed that includes rumen protozoa (Chapter 4) and endogenous N secretions along the entire gastro-intestinal tract which have not been directly included in previous versions of the CNCPS (Chapter 5). The model also includes a new system for calculating post-ruminal N digestion based on an *in vitro* estimate of indigestible protein developed by Ross (2013). Research efforts have been focused on improving the capability of the CNCPS to precisely estimate N and AA availability to the animal to allow for the formulation of rations that more closely match animal requirements. An evaluation of the model showed predictions were close to

measured data for microbial, feed and total non-ammonia N flows at the omasum (Chapter 6). New optimum requirements for each EAA relative to metabolizable energy (ME) supply have also been established (Chapter 5) and appear to explain more variation in AA utilization than current recommendations expressed relative to MP supply.

The objectives of this study were 1) to use the new model to balance the diets of high producing dairy cattle for Met or all EAA using the requirements established in Chapter 5 and, 2) to test the models sensitivity in predicting rumen N supply. Our hypothesis was that milk production will be maximized by providing adequate rumen N and a balanced supply of all EAA relative to energy.

7.3 Materials and methods

7.3.1 Animals and diets

The experiment was conducted at the Cornell Teaching and Research Facility (Harford, NY) from May – August 2013. All procedures carried out in the study were approved by the Cornell University Institutional Animal Care and Use Committee. Sixty-four lactating Holstein dairy cattle [16 primiparous and 48 multiparous; 100 ± 31 DIM at the beginning of the study; 624 ± 68 kg BW; 3.0 ± 0.2 BCS (1-5 scale)] were randomly assigned to one of four treatments. Treatment assignment was balanced for parity, energy corrected milk and DIM. Cattle were housed in individual tiestalls and fed a TMR once daily at approximately 0900 h with a 10% target refusal rate. All cows were treated with rBST (Posilac) on a 14 d cycle according to label (Elanco Animal Health, Greenfield, IN). The experiment proceeded in three phases. Phase 1 was a 7 day adjustment period to allow cows to become accustomed to the housing conditions in the tie-stall

barn. Phase 2 was a 14 day reference period where all cattle were fed the same diet and data were collected to be used as a covariate in the statistical model. Phase 3 was the experimental period where cattle were fed treatment diets which lasted 100 days. The intended treatments were 1) balanced (assuming 45 kg ECM) for ME, MP, MP Lys and rumen N but limited in MP Met (Base); 2) balanced for ME, MP, MP Lys, rumen N and balanced for MP Met with supplemental Met (Base+M); balanced for ME, MP, MP Lys, MP Met with excess rumen N through supplementing urea (Base+MU); balanced to be adequate in all EAA and excess rumen N (Positive). Due to large changes in the chemical composition of the corn silage being fed through the experiment (Table 7.2), diets ended up lower in total N than expected. Accordingly, the resulting treatments can be described as 1) balanced for ME (assuming 45 kg ECM), but limited in MP and rumen N (Base); 2) balanced for ME and MP Met but limited in MP and rumen N (Base+M); 3) balanced for ME, MP Met, with adequate rumen N, but limited in MP (Base+MU); 4) balanced for ME, MP, all EAA and adequate in rumen N (Positive).

7.3.2 Sample collection and analysis

Body weight and body condition score (1-5 scale) were measured weekly. Cows were milked two times per day at 0900 and 2000 h and milk weights were recorded at each milking. Milk samples were collected on two days each week (4 consecutive milkings). Samples were placed in tubes containing 2-bromo-2-nitropropane-1,3-diol and analyzed for fat, true protein, lactose, and MUN (Dairy One, Ithaca NY) using fourier transform infrared spectroscopy (Milkoscan 6000; Foss Electric, Hillerød, Denmark). Milk component yield was calculated using the milk weight and composition of each individual milking during sampling and summed to give the daily yield.

Dry matter intake was measured daily for each animal. Samples of TMR and ORTS for each diet were sampled twice each week, composited, and analyzed using near infrared reflectance spectroscopy (**NIR**) for the chemical components presented in Table 7.1 (Cumberland Valley Analytical Services, Maugansville, MD). The dry matter content of each TMR was measured weekly by drying at 100°C in a forced air oven. Forage samples were taken weekly and analyzed by wet chemistry for the chemical components presented in Table 7.1 (Cumberland Valley Analytical Services, Maugansville, MD). Corn silage dry matter was measured 5 d per wk using a Koster Moisture Tester. Individual ingredients in the grain mix were sourced from the provider (CNY Feed Inc., Jordan, NY) three times during the experiment and analyzed by wet chemistry for the same components as the forages. Subsamples of all ingredients were taken and dried at 60°C, ground to 2 mm using a Wiley Mill and analyzed for AA concentration and indigestible N. For the analysis of AA, sample aliquots (2 mg N) were hydrolyzed at 110ºC for 21-hr in a block heater (Gehrke et al., 1985) with 5-ml 6 M HCl after flushing with N_2 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 water, evaporated again, which was repeated two more times to remove the acid and dissolved in 2 ml sample buffer for analysis. Additional aliquots (2 mg N) were preoxidized with 1 ml performic acid (4.5 ml 88% formic acid, 0.5 ml 30% hydrogen peroxide, 25 mg phenol) for 16 h on ice prior to acid hydrolysis for analysis of Met and Cys. Amino acids were separated on a lithium cation exchange column using a three-buffer step gradient and column temperature gradient. Detection was at 560 nm following ninhydrin post column derivation on an HPLC System Gold with 32 Karat software (Beckman-Coulter, Inc., Fullerton, CA). Standards (250 nM/ml) for Asp, Thr, Ser, Glu, Gly, Ala, Val, Met, Ile, Leu, Tyr, Phe, NH3, Lys, His, Arg and Cys (125 nM/ml)

were prepared by diluting a purchased stock (Amino acid standard H, #20088; Pierce Chemical; Rockford, IL) with the sample buffer. Internal standards (250 nM/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 N) were hydrolyzed using 1.2 g $Ba(OH)_2$ at 110ºC 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. Concentrate feeds were also analyzed for indigestible N using the *in vitro* procedure described by Ross (2013).

Blood samples (10 ml) were collected from every cow, once each week (1100 h), by venipuncture of the coccygeal vein into heparinized Vacutainers (Becton Dickinson, Rutherford, NJ), immediately placed on ice then centrifuged (1,500 × g for 15 min at 4°C) to obtain plasma and frozen at −20°C before analysis. Samples were analyzed for plasma urea N (**PUN**) using an enzymatic colorimetric assay based on a commercial kit (No. 640; Sigma Chemical Co., St. Louis, MO). Three times during the study (wk 2 of the covariate period; wk 5 and 10 of the experimental period), an additional blood sample was taken and analyzed for plasma AA. Equal volumes (0.65 ml) of plasma and ice-cold sulfosalicylic acid (10%) containing the internal standard norleucine (250 nM) were mixed, vortexed extensively and refrigerated on ice for 12 h with occasional vortexing. Samples were then centrifuged (15,800 × g for 30 min at 4°C) and 1 ml of supernatant was lyophilized, reconstituted in 0.5 ml of 3N LiOH, filtered through a 0.2 µm

filter and frozen at –20°C until analysis. Analysis was by an automated ion-exchange chromatography system as described above.

Sampling of feces was conducted by taking spot fecal samples (~500 g / cow) eight times over a 3 d period (d1 = 1100, 1700 and 2300 h; d2 = 0500, 1400 and 2000 h; d3 = 0200 and 0800), three times during the experiment (wk 2 of the covariate period; wk 5 and 10 of the experimental period) and frozen (-20°C). Samples were subsequently thawed, composited by cow (8 samples / cow) and blended to ensure uniformity. An aliquot (1000 g) was dried at 60°C in a forced air oven for 96 h and ground to 1 mm in a Wiley Mill. Samples of TMR and ORTS were also collected for 2 d beginning the day prior to the first fecal sampling. The TMR samples were taken at the time of feed delivery, composited within treatment, and three aliquots per treatment were frozen (–20°C). Individual ORTS samples for each cow, each day (2 d), were collected and stored frozen at –20°C. Samples were subsequently thawed and dried at 60°C in a forced air oven and ground to 1 mm in a Wiley Mill. The dry matter content of both the TMR and ORTS were measured and used to estimate DMI for each cow during the collection period. The ground fecal, TMR and ORTS samples were analyzed for aNDFom and $uNDF_{240}$ (Cumberland Valley Analytical Services, Maugansville, MD) and were used to estimate total tract NDF digestion as described by Huhtanen et al. (1994).

7.3.3 Statistical analysis

Data were analyzed using a restricted maximum likelihood model in SAS (2010). The model is described as follows:

$$Y_{ijklm} = \mu + c_i + T_j + D_k + TD_{jk} + P_l + X_i + V_i + W_m + \varepsilon_{ijklm}$$

where Y_{ijklm} is the dependent variable, μ is the overall mean, c_i is the random effect of the i^{th} cow, T_j is the effect of the j^{th} treatment, D_k is the k^{th} day, TD_{jk} is the interaction between the j^{th} treatment and k^{th} day, P_l is the l^{th} parity, X_i is the mean covariate measure for the i^{th} cow, V is the variation in the mean covariate measure for the i^{th} cow, W_m is the blocking effect of the m^{th} period of weather (m = 1, 2, 3) and ε_{ijklm} is the residual error. The effect of weather was added to the model to account for a period of hot humid conditions during the experiment. Three periods were defined: m=1 30 d period of moderate temperatures; m=2 33 d period of hot, humid conditions where the mean of the minimum and maximum temperature for a 24 h period was > 18°C; m=3 34 d of moderate temperatures. For the analysis of PUN, the term D_k referred to the k^{th} week rather than day as blood was sampled 1 d each wk. The terms D_k, TD_{jk}, V_i and H_m were not included in the model used to analyze total tract NDF digestibility or plasma AA as these parameters were only sampled 3 times per cow (1 covariate measure and 2 experimental measures). The means presented for data other than CNCPS model outputs are least squares means. Significant differences among means ($P < 0.05$) were calculated using a Student's *t*-test and are indicated by different subscripts. Values presented for CNCPS outputs are raw means.

Table 7.1. Ingredients and chemical composition of experimental diets

Ingredient, % DM	Base[1]	Base+M	Base+MU	Positive
Corn Silage	46.98	46.49	46.75	46.13
Grass Hay	8.53	8.53	8.42	8.46
Corn grain ground fine	15.73	15.84	15.66	15.12
Corn gluten feed	8.69	8.75	8.66	7.07
Soybean meal	6.21	6.25	6.18	7.89
Soyhulls	2.07	2.08	2.06	2.10
SoyPLUS[2]	2.07	2.08	2.06	4.11
Molasses Dried	2.07	2.08	2.06	1.20
NutraCor[3]	1.90	1.92	1.90	1.64
Urea	0.08	0.08	0.52	0.12
AjiPro-L[4]	0.10	0.10	0.09	0.00
Smartamine M[5]	0.00	0.08	0.08	0.09
Blood meal[6]	1.66	1.67	1.65	2.18
Minerals and vitamins[7]	3.92	4.05	3.91	3.88
Chemical components[8], % DM				
CP	13.5	13.6	14.6	15.6
SP, % CP	38.8	38.6	38.8	37.8
Ammonia, % SP	7.5	7.5	7.9	7.4
ADICP, % CP	8.6	8.6	8.5	8.3
NDICP, % CP	12.1	12.1	11.9	12.0
Acetic acid	1.1	1.1	1.1	1.1
Propionic acid	0.1	0.0	0.0	0.0
Lactic acid	2.5	2.5	2.5	2.5
WSC	4.7	4.7	4.6	4.4
Starch	31.9	31.9	31.5	30.9
Soluble fiber	4.5	4.5	4.4	4.5
ADF	16.6	16.5	16.4	16.5
NDF	29.7	29.6	29.3	29.3
Lignin, % NDF	10.2	10.2	10.1	10.3
$uNDF_{240}$, % NDF	21.5	21.4	21.2	21.5
Ash	7.3	7.4	7.3	7.3
EE	4.7	4.7	4.6	4.4

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

[2] SoyPLUS (West Central Cooperative, Ralston, IA) rumen protected soybean meal

[3] NutraCor (Energy Feeds International, San Leandro, CA) rumen protected fat

[4] AjiPro-L (Ajinomoto Heartland Inc, Chicago, IL) rumen protected Lys (L-Lys 40% DM)

[5] Smartamine M (Adisseo USA Inc, Alpharetta, GA) rumen protected Met (60% MP Met)

[6] Blood meal (Perdue AgriBusiness)

[7] Contained on a DM basis: 19.2 % sodium bicarbonate, 2.4 % Magnesium oxide, 38.3 % ground limestone, 7.2 % sodium chloride, 1.4 % vitamin E, 12.0 % potassium sulfate, 16.8 % potassium carbonate and 2.7 % mineral and vitamin premix (calcium 0.75%, magnesium 9.54%, sulfur 19.25 %, iodine 330 ppm, cobalt 501 ppm, iron 0.1 ppm, zinc 25,709 ppm, manganese 22,306, selenium 214 ppm, vitamin A 3,702 KIU/kg, vitamin D 923 KIU/kg, vitamin E 12,490 IU/kg; Mercer Milling Company, Liverpool, NY 13088)

[8] Values represent model formulations based on measured chemical components from individual ingredients. Chemical components are expressed as % DM unless stated. SP = soluble protein; ADICP = CP insoluble in acid detergent; NDICP = CP insoluble in neutral detergent; WSC = water soluble carbohydrates; uNDF240 = undigested NDF after 240 hours of in vitro fermentation; EE = ether extract.

Table 7.2. Chemical composition of corn silage for each week of the experiment

	Week of experiment															
Chemical component[1]	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Mean	SD
CP	8.0	7.3	6.7	7.7	7.1	7.2	7.4	7.3	7.2	7.4	7.3	6.4	6.6	7.3	7.2	0.42
SP, % CP	55.5	66.4	57.6	62.5	58.5	61.7	59.0	61.0	62.9	65.1	61.1	53.4	57.7	63.7	60.4	3.68
Ammonia, % SP	15.7	18.5	17.0	13.5	15.7	14.4	13.6	15.0	16.3	16.2	16.7	15.4	16.6	17.0	15.8	1.39
ADICP, % CP	10.4	9.4	10.1	9.3	9.5	10.2	8.9	8.9	8.9	9.3	8.0	11.7	11.1	10.6	9.7	1.00
NDICP, % CP	12.9	9.2	10.3	10.0	9.5	11.3	10.7	10.2	9.7	10.7	9.9	12.8	11.3	10.7	10.7	1.12
Acetic acid	3.1	3.5	3.3	1.8	2.3	3.1	2.0	1.8	1.5	2.3	2.7	2.7	1.8	1.8	2.4	0.65
Propionic acid	0.5	0.2	0.1	0.0	0.0	0.2	0.1	0.1	0.1	0.0	0.1	0.2	0.0	0.0	0.1	0.13
Lactic acid	1.7	5.5	5.6	5.2	5.7	5.2	5.8	6.3	6.7	6.1	4.7	4.2	5.6	7.0	5.4	1.29
Total VFA	5.3	9.2	9.0	7.0	8.0	8.5	7.9	8.2	8.3	8.4	7.5	7.1	7.4	8.8	7.9	1.0
WSC	0.9	0.9	1.0	1.0	0.9	0.9	0.9	0.9	0.9	1.1	0.8	0.7	0.9	1.3	0.9	0.14
Starch	36.1	36.3	37.8	35.3	39.2	37.7	40.1	39.9	39.7	38.0	40.9	41.5	41.2	36.4	38.6	2.06
Soluble fiber	4.1	2.8	2.0	4.3	3.0	2.1	4.1	3.7	3.2	2.8	3.8	2.0	4.3	4.1	3.3	0.87
ADF	23.6	21.5	21.7	23.9	20.7	21.8	18.9	19.5	20.1	21.9	19.8	22.4	19.7	21.4	21.2	1.51
NDF	39.1	37.5	37.4	38.5	36.1	37.5	33.0	33.8	34.5	36.5	34.0	36.8	34.2	36.4	36.1	1.90
Lignin, % NDF	7.6	7.3	6.7	7.2	7.5	8.2	8.6	8.7	8.8	7.4	7.8	7.7	7.3	6.6	7.7	0.68
$NDFD_{24}$, % NDF	53.5	51.5	54.0	48.9	52.7	53.0	53.3	53.5	54.6	53.8	54.8	53.9	52.5	51.4	53.0	1.5
$uNDF_{240}$, % NDF	31.2	27.4	26.4	26.9	27.3	22.5	22.9	22.5	22.0	25.8	27.8	23.6	26.5	24.6	25.5	2.65
Ash	3.1	2.7	2.7	2.8	2.5	2.6	2.9	2.7	2.6	2.6	2.5	2.3	2.6	3.1	2.7	0.23
EE	3.5	3.4	3.4	3.4	3.2	3.5	3.6	3.6	3.6	3.1	3.2	3.2	2.8	2.5	3.3	0.32

[1] Chemical components are expressed as % DM unless stated. SP = soluble protein; ADICP = CP insoluble in acid detergent; NDICP = CP insoluble in neutral detergent; WSC = water soluble carbohydrates; $NDFD_{24}$ = digested NDF after 24 hours of in vitro fermentation; $uNDF_{240}$ = undigested NDF after 240 hours of in vitro fermentation; EE = ether extract

Table 7.3. Chemical composition of dry grass hay and major concentrate ingredients

Chemical component[1]	Dry grass hay	Corn grain ground fine	Soyhulls	Corn gluten feed	Soyplus	Soybean meal	Blood meal
CP	7.9	8.6	11.2	19.1	47.6	52.8	98.3
SP, % CP	22.2	10.2	16.1	34.0	7.1	15.7	62.7
Ammonia, % SP	0.0	0.0	0.0	0.0	0.0	0.0	0.0
ADICP, % CP	15.9	6.3	11.8	14.5	3.2	0.9	0.7
NDICP, % CP	31.5	9.3	34.7	19.4	18.5	1.3	0.8
Indigestible N, % N[2]	N/D	20.0	22.8	26.0	9.1	7.2	1.7
WSC	7.5	2.8	2.7	6.0	12.8	13.1	0.2
Starch	2.0	74.7	1.7	17.7	2.6	3.2	0.0
Soluble fiber	8.4	0.7	4.8	12.0	1.3	13.8	0.0
ADF	41.7	4.3	49.7	11.0	8.9	4.8	0.0
NDF	67.6	8.6	72.4	37.1	22.5	8.0	0.0
Lignin, % NDF	10.7	22.3	3.8	9.7	7.2	11.9	0.0
$uNDF_{240}$, % NDF	35.2	19.7	6.7	16.4	14.7	24.1	0.0
Ash	4.9	1.3	5.4	5.8	6.9	7.7	1.3
EE	1.8	3.3	1.9	2.4	6.4	1.6	0.1

[1] Chemical components expressed as % DM unless stated. SP = soluble protein; ADICP = CP insoluble in acid detergent; NDICP = CP insoluble in neutral detergent; WSC = water soluble carbohydrates; $uNDF_{240}$ = undigested NDF after 240 hours of in vitro fermentation; EE = ether extract

[2] Measured according to Ross (2013); N/D = not determined

Table 7.4. Amino acid composition of dietary ingredients

AA, g/100g AA	Corn Silage	Dry grass hay	Corn grain ground fine	Soyhulls	Corn gluten feed	Soyplus	Soybean meal	Blood meal
EAA								
Arg	1.8	5.1	4.3	4.9	3.9	7.0	7.2	3.7
His	1.4	1.4	2.1	2.0	2.6	2.2	2.3	5.9
Ile	4.2	4.0	3.0	3.8	3.0	3.7	3.6	0.5
Leu	10.9	7.9	11.9	6.5	9.3	7.8	7.5	12.7
Lys	3.0	4.6	2.6	6.3	2.1	4.7	5.5	7.6
Met	6.9	8.3	7.3	5.1	5.6	4.3	4.5	3.7
Phe	3.9	4.6	4.3	3.5	3.5	4.9	4.8	7.6
Thr	4.8	5.2	4.1	4.0	4.5	4.4	4.3	4.8
Trp	0.6	1.3	0.9	1.5	0.7	1.5	1.5	2.0
Val	5.6	5.3	4.3	4.6	4.4	4.1	3.8	6.5
NEAA								
Ala	10.4	6.8	7.5	4.9	7.4	4.6	4.4	9.0
Asp	6.1	9.3	4.7	7.9	4.8	9.0	8.9	6.9
Cys	5.9	7.1	6.1	7.9	8.3	3.2	4.1	2.1
Glu	15.2	10.7	17.8	11.4	17.6	19.7	19.2	10.6
Gly	4.4	5.7	3.5	9.2	4.6	4.3	4.2	4.4
Pro	7.6	5.1	7.8	5.2	9.5	5.2	5.1	3.5
Ser	4.8	5.0	5.0	6.8	5.0	5.6	5.5	5.7
Tyr	2.3	2.4	2.8	4.4	3.2	3.6	3.5	2.7
EAA, % AA	43.2	47.7	44.8	42.2	39.7	44.7	45.0	55.0
NEAA, % AA	56.8	52.3	55.2	57.8	60.3	55.3	55.0	45.0
AA N, % total N	57.1	60.0	74.0	71.8	62.3	74.0	77.1	75.4

7.4 Results

7.4.4 Animal performance

No differences were observed in DMI or milk yield. Energy corrected milk yield was higher ($P < 0.001$) in cattle fed the Positive treatment compared other treatments (Table 7.5). No differences were observed in fat or true protein in cows fed the Base, Base+M or Base+MU treatments, but cattle fed the Positive treatment produced more true protein than the Base treatment and more fat than the Base and Base+M treatments ($P < 0.05$). True protein concentration in milk was higher ($P < 0.001$) and milk fat tended to be higher ($P < 0.10$) in cattle fed the positive and Base+MU treatments than cows fed the Base and Base+M treatments. Lactose %, body weight and BSC were similar among treatments (Table 7.5).

Table 7.5. Effects of treatment diets on milk production, intake, body weight and body condition scores.

	Base[1]	Base+M	Base+MU	Positive	SEM	*P*-value
Intake and milk production, kg/d						
Dry matter intake	24.1	24.5	24.8	24.7	0.48	0.717
Energy correct milk yield[2]	38.5[a]	39.3[a]	40.0[a]	41.8[b]	0.67	0.005
Milk yield	40.0	40.6	40.7	41.8	0.68	0.288
True protein yield	1.13[a]	1.18[ab]	1.18[ab]	1.22[b]	0.019	0.009
Fat yield	1.30[a]	1.28[a]	1.34[ab]	1.41[b]	0.038	0.047
Lactose yield	1.93	1.94	1.95	2.00	0.036	0.344
Milk composition, %						
True protein	2.88[a]	2.93[ab]	2.96[b]	2.98[b]	0.023	0.009
Fat	3.31	3.20	3.34	3.51	0.088	0.078
Lactose	4.84	4.85	4.85	4.86	0.010	0.799
Body weight and condition						
Body weight, kg/d	625	631	633	623	5.2	0.430
Body weight change, kg/wk	1.40	1.45	2.14	1.98	0.455	0.515
BCS, 1-5 scale	3.06	3.09	3.07	3.08	0.021	0.713

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

[2] Estimated according to Tyrrell and Reid (1965)

7.4.5 Nitrogen utilization

Nitrogen intake was similar among cow fed the base and Base M treatments but was higher for cows fed the Base MU and positive treatments (~60 g/d and ~90 g/d, respectively) which corresponded with higher levels of dietary CP (Table 7.1). Milk urea N and PUN in cows fed the Base and Base M treatments were similar and were lower ($P < 0.001$) than the Base MU and positive treatments (Table 7.6). Milk urea N was slightly higher than PUN but both measures were in the same general range. Productive N was higher in cows fed the positive treatment due to the higher milk protein yield (Table 7.5). Predictions of fecal and urinary N increased as dietary N intake increased. Urinary N was ~60 g higher in cows fed the positive treatment

compared to the Base treatment and fecal N was ~20 g higher which corresponded with lower N use efficiency. Cows fed the Base and Base M treatments had the highest N use efficiency (0.37 and 0.38, respectively) and, based on predicted N excretion, partitioned 1.65 and 1.70 more N to productive uses than urine (Table 7.6). Total NDF and potentially digestible (**pd**) NDF intake were not different among treatments although indigestible fiber tended to be higher for cows fed the Base treatment (Table 7.7). Apparent total tract NDF and pd NDF digestion was higher ($P < 0.05$) in cows fed the Base MU and positive treatments indicating the higher N intake improved rumen N balance.

Table 7.6. Nitrogen intake, utilization and excretion for each treatment

	Base[1]	Base+M	Base+MU	Positive	SEM	*P*-value
N intake, mg/dl	521.6[a]	532.1[a]	581.9[b]	615.1[c]	13.20	< 0.001
MUN, mg/dl	6.9[a]	7.3[a]	9.1[b]	10.4[c]	0.30	< 0.001
PUN[2], mg/dl	5.9[a]	5.7[a]	8.5[b]	8.7[b]	0.54	< 0.001
Productive N[3], g/d	192.3[a]	198.9[ab]	198.6[ab]	205.8[b]	3.87	0.025
Fecal N[4], g/d	213.8[a]	217.3[a]	228.0[b]	234.5[b]	4.77	< 0.001
Urinary N[4], g/d	129.4[a]	129.8[a]	169.5[b]	189.3[c]	8.99	< 0.001
Productive N:Urinary N	1.65[a]	1.70[a]	1.29[b]	1.13[c]	0.108	< 0.001
Productive N:Intake N	0.37[a]	0.38[a]	0.35[b]	0.34[b]	0.010	< 0.001

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

[2] PUN = plasma urea N.

[3] Productive N = N used for milk, growth, pregnancy and reserves (Fox et al., 2004)

[4] Predicted using the equations of Higgs et al. (2012)

Table 7.7. Fiber intake and apparent total tract digestion for each treatment

	Base[1]	Base+M	Base+MU	Positive	SEM	*P*-value
Intake, kg/d						
NDF	8.19	7.99	7.80	7.69	0.222	0.295
pd NDF[2]	5.89	5.86	5.68	5.58	0.161	0.367
$uNDF_{240}$[3]	2.30	2.13	2.12	2.11	0.061	0.052
Apparent digestion, %						
NDF	40.8[ab]	40.5[b]	42.9[a]	42.9[a]	0.008	< 0.05
pd NDF	56.7[ab]	55.2[b]	59.0[a]	59.2[a]	0.011	< 0.05

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

[2] pd NDF = potentially digestible NDF

[3] $uNDF_{240}$ = undigested NDF after a 240 hour in vitro fermentation

7.4.6 Amino acid balance

Predicted AA supply expressed relative to ME for each treatment is in Table 7.8. Compared to the ideal supply calculated in Chapter 5, the Base treatment was low in Arg, Ile, Lys, Met and Val. The Base+M treatment was similar to the Base treatment but with adequate Met (1.13 g Met/mcal ME). All AA were adequate in cattle fed the Positive treatment other than Ile which was 0.16 g/mcal ME lower than the ideal supply.

Table 7.8. Predicted AA supply for each treatment compared with the ideal supply (g digested AA/Mcal ME)

AA	Ideal[1]	Base[2]	Base+M	Base+MU	Positive	SEM
Arg	2.04	1.85	1.86	1.96	2.15	0.006
His	0.91	1.01	1.01	1.05	1.19	0.003
Ile	2.16	1.83	1.83	1.94	2.00	0.005
Leu	3.42	3.64	3.65	3.81	4.15	0.012
Lys	3.03	2.83	2.82	2.98	3.09	0.007
Met	1.14	0.93	1.13	1.17	1.25	0.003
Phe	2.15	2.12	2.12	2.22	2.42	0.006
Thr	2.14	2.16	2.16	2.27	2.43	0.007
Trp	0.59	0.60	0.60	0.63	0.69	0.002
Val	2.48	2.33	2.33	2.45	2.62	0.007
Lys:Met	2.66	3.04	2.51	2.54	2.47	0.002

[1] Based on calculations in Chapter 5

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

Treatment differences ($P < 0.05$) in plasma AA concentrations were observed in Gln, Gly, Ser, Arg and Met (Table 7.9). Methionine concentration was lower in the Base treatment compared with the other treatments and corresponded to the dietary supplementation of Met (Table 7.1). Arginine increased as protein supply increased and reflected the Arg supply relative to ME (Table 7.8). Essential AA in the plasma were higher in the Positive treatment but similar among the other treatments, including cows fed the Base+MU treatment, despite the higher predicted AA supply. Non-essential AA were not affected by treatment, however, 3-Methylhistidine was lower ($P < 0.05$) in cows fed the Positive treatment.

Table 7.9. Plasma AA concentration (g/100 g AA) for each experimental treatment

	Base	Base+M	Base+MU	Positive	SEM	*P*-value
Non-essential						
Ala	8.92	8.55	9.16	7.89	0.411	0.102
Asn	4.08	3.99	3.94	3.37	0.553	0.751
Asp	0.91	0.92	0.72	0.84	0.105	0.437
Cit	6.32	6.96	7.31	7.32	0.345	0.107
Cys	1.80	1.94	1.92	1.85	0.078	0.526
Gln	6.33[a]	6.29[a]	8.04[b]	7.64[b]	0.478	0.011
Glu	6.54	6.45	6.62	6.29	0.301	0.846
Gly	9.24[a]	11.19[b]	8.87[a]	9.10[a]	0.608	0.020
Orn	1.66	1.88	1.69	1.95	0.097	0.066
Pro	4.10	3.63	3.98	4.11	0.271	0.515
Ser	3.71[a]	3.65[a]	3.10[b]	3.08[b]	0.144	0.001
Tyr	3.76	3.60	3.56	3.39	0.138	0.249
Essential						
Arg	4.25[a]	4.37[a]	4.74[ab]	5.09[b]	0.208	0.012
His	3.32	3.47	3.12	3.37	0.166	0.440
Ile	4.45	4.07	4.19	4.22	0.162	0.368
Leu	5.86	5.29	5.19	5.67	0.215	0.067
Lys	4.43	4.24	4.19	4.62	0.170	0.200
Met	1.54[a]	2.19[b]	2.24[b]	2.14[b]	0.088	< 0.001
Phe	2.90	2.61	2.82	2.70	0.136	0.393
Thr	4.89	4.63	4.48	4.57	0.292	0.745
Trp	1.94	1.75	1.72	1.93	0.104	0.266
Val	9.05	8.30	8.39	8.88	0.344	0.284
3-Methylhistidine	0.46[a]	0.38[ab]	0.42[a]	0.31[b]	0.041	0.046
NEAA[2]	104.8	103.1	105.6	102.5	3.51	0.895
EAA[2]	87.0[a]	86.9[a]	85.1[a]	99.8[b]	3.38	0.005
Total AA[2]	204.1[a]	211.6[a]	207.0[a]	230.7[b]	6.12	0.007

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

[2] Expressed as µg/ml

7.4.7 Model predictions

The data presented in Table 7.10 for model predictions are raw means and are not adjusted for the reference period. The cattle consumed approximately 63 mcals ME/d for each of the treatments which provided enough energy to support 42.1 – 46.1 kg milk/d, close to the target of 45 kg ECM/d. Predicted MP supply ranged from 2323 - 2784 g/d for cows fed the Base and

Positive treatments, respectively. Cows fed the Base, Base+M and Base+MU treatments were predicted to have a negative MP balance, while cows fed the Positive treatment consumed 33 g MP/d excess to requirements. Model predicted rumen NH_3 concentration (mg/dl) ranged from 5.1 in the Base and Base+M treatments to 7.8 and 7.5 in the Base+MU and Positive treatments (Table 7.10). From the rumen submodel of the CNCPS, bacterial growth was predicted to be depressed 16 and 17% for the Base and Base+M treatments, respectively due to the low level of rumen NH_3. When considering predicted Lys and Met balance in g/d, Lys was predicted to be negative for all treatments while Met was negative for the Base and Base+M treatments (-15.6 g and -6.9 g, respectively), but close to requirement for the Base+MU and Positive treatments. The apparent efficiency of MP use varied (72%- 83%) but was close to the optimum efficiency calculated in Chapter 5 (73%) in cows fed the Positive treatment.

Table 7.10. Selected outputs from the new version of the Cornell Net Carbohydrate and Protein System.

	Base[1]	Base+M	Base+MU	Positive	SEM
DMI[2], kg/d	23.9	24.8	24.7	24.4	0.12
Actual Milk[2], kg/d	38.0	40.9	38.8	40.9	0.21
ME Supply, Mcals ME/d	61.2	63.2	63.2	62.9	0.28
ME Required, Mcals ME/d	56.3	57.4	57.6	59.6	0.23
ME Balance, Mcals ME/d	4.9	5.8	5.6	3.3	0.26
MP Supply, g/d	2323.0	2418.8	2527.9	2783.9	15.16
MP Required[3], g/d	1864.4	1991.8	1948.7	2008.1	8.24
MP Required at 73% efficiency[4], g/d	2554.0	2728.4	2669.5	2750.9	11.28
Apparent efficiency[5], %	80%	82%	77%	72%	0.32%
MP Balance, g/d	-230.9	-309.7	-141.6	33.0	10.01
MP RUP, g/d	1118.5	1183.4	1180.0	1465.6	8.88
MP Microbial, g/d	1204.5	1235.4	1347.9	1318.3	6.90
MP Microbial, %	51.9%	51.1%	53.4%	47.5%	0.09%
ME allowable milk	42.1	46.1	43.6	44.7	0.29
MP allowable milk	33.9	34.8	36.7	41.5	0.30
ME MP average	38.2	40.8	40.6	44.7	0.28
ME MP first limiting	34.3	35.4	37.6	42.5	0.28
Met supply, g/d	57.1	71.3	74.4	79.1	0.47
Lys supply, g/d	173.4	178.7	188.6	194.9	0.99
Met balance, g/d	-15.6	-6.9	-1.8	0.0	0.34
Lys balance, g/d	-18.3	-27.0	-12.5	-13.3	0.68
Rumen NH_3, mg/dl	5.1	5.1	7.8	7.5	0.07
Bacterial growth depression, %	16%	17%	4%	2%	0.36%

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

[2] Unadjusted means across the entire experiment

[3] MP required represents gross model predicted requirements for MP without accounting for the efficiency of use

[4] MP required at 73% efficiency of use (Chapter 5)

[5] Apparent efficiency of use = MP Required/MP supply

7.5 Discussion

The goal of this study was to use newly developed tools to balance dairy cow diets precisely to requirements for rumen N and to test the concept of balancing essential AA to an ideal profile relative to ME supply (Chapter 5). Due to considerable changes in corn silage composition as the bunk was fed (Table 7.2), diets ended up lower in protein, higher in non-fiber carbohydrates (Starch ~31.5 % DM) and lower in NDF (29.5 % DM) than anticipated. The variable nature of forage composition is a major challenge when attempting to precision feed dairy cows which was evident in this study. While it would have been preferable if the dietary carbohydrate profile was higher in NDF and lower in starch, the lower than expected protein levels tested the models ability to predict rumen N supply and AA balance at a very low intake level. The profile and supply of AA differed by treatment as intended (Table 7.8). Cows fed the Base treatment were predicted to be limited in Arg, Ile, Lys, Met and Val while cows fed the Positive treatment were predicted to be only slightly limited in Ile. Our intention was for cows fed the Base and Base+M treatments to be provided negative and adequate levels of Met, respectively, when Lys was adequate. However, Lys supply was predicted to be below the ideal supply for both treatments while Met supply was as intended (Table 7.8). The predicted Lys:Met ratio was lower than the ideal ratio (2.66) estimated in Chapter 5 indicating that Met supply was at, or excess to requirement relative to Lys for the Base+M, Base+MU and Positive treatments.

When AA balance has been altered in dairy cattle in research and field settings, a variety of responses have been demonstrated. In the study of Chen et al. (2011) an increase in ECM was observed when supplemental Met was provided, but no difference in milk volume was detected. In contrast, Lee et al. (2012b), observed a milk volume response when cows were supplemented

with Met and Lys, or Met, Lys and His, but milk components among the treatments were similar. Other studies have reported changes in both components and volume (Appuhamy et al., 2011, Haque et al., 2012, Noftsger and St-Pierre, 2003). Mepham (1982) classified EAA in 2 groups based on different patterns of mammary utilization where, for group 1 AA (Met, Phe, Tyr and Trp), there was apparent stoichiometric transfer to milk protein while group 2 AA (Ile, Lys, Leu and Val) were generally taken up in excess of milk protein secretion. Different types of responses (volume or components) have been observed among group 1 and 2 AA which can, in-part, be explained by the different ways in which the AA are metabolized (Lapierre et al., 2012). The group 2 AA, taken up in excess, can elicit a milk volume response with the excess carbon used to generate ATP, NEAA and also lactose (Maxin et al., 2013) while the uptake of group 1 AA reflects the output in milk protein and additional uptake is directly linked to an increase in milk protein yield, which can occur independently to an increase in the uptake of group 2 AA (Lemosquet et al., 2010). Cows in the current study produced similar milk volumes among treatments but milk components increased when the dietary AA were closer to the ideal balance (Table 7.8) resulting in higher ECM in cows fed the Positive treatment ($P < 0.01$) and a numerical increase among the Base, Base+M and Base+MU treatments as AA were added. Supplemental Met was the only difference in AA supply between the Base and Base+M treatments, which according to the plasma Met concentration, had been delivered (Table 7.9). Cows did not respond to the increased Met supply as observed in other studies (Chen et al., 2011, Noftsger and St-Pierre, 2003) which might have been due to a limitation of other EAA (Table 7.8). An extended period (33 d) of hot humid weather (mean daytime temperatures = 27.5°C; mean nighttime temperatures = 17°C) was experienced during the study and the barn the cows were housed in was poorly ventilated. Heat stress has been shown to change the metabolism of

lactating cows with plasma NEFA shown to decrease and PUN increase, indicating higher levels of AA oxidation (Wheelock et al., 2010). The effect of the heat and humidity in the current study may have further reduced the availability of circulating AA and impacted the response to supplemental Met. Interestingly, the concentration of EAA and total AA in plasma were not changed from the addition of urea (Base+MU), despite a predicted increase in microbial protein supply (Table 7.10), although differences in some AA were observed (Gln, Ser, Arg). The concentration of arterial EAA has been shown to decrease when urea is given to cows fed diets adequate in rumen N, possibly due to increased hepatic catabolism to provide an N group for the synthesis of urea (Lapierre et al., 2004). It is possible that an increase in AA supply from the Base+MU treatment was offset by an increase in hepatic removal to provide N for the urea cycle resulting in no true increase in AA supply (Reynolds, 1992). Cows fed the positive treatment had increased concentrations of EAA and total AA in plasma ($P < 0.01$) which corresponded with an increase in predicted supply of both group 1 and 2 AA (Table 7.8). Although not significant (P=0.29), milk volume was 1.0-1.8 kg higher in cows fed the Positive treatment, which, when considered together with the changes in milk components, indicates the increase in ECM was due to a combination of both volume and composition.

The low dietary protein concentration in the Base and Base+M treatments (~13.5 % CP) resulted in low PUN (5.7-5.9 mg/dl) and caused a reduction in apparent total tract NDF digestion indicating rumen N supply was limited (Table 7.7). Similar affects have been observed in other studies that fed comparable levels of protein (Colmenero and Broderick, 2006, Lee et al., 2012b). Lee et al. (2012b) also reported a reduction in DMI at low levels of protein which was not observed in the current study. One of the goals of this study was to use new strategies to more

precisely predict AA supply which included using a new assay to estimate the indigestible protein fraction of feeds (Ross, 2013). In addition to the supplemental Met and Lys, AA sources were selected according to the assay of Ross (2013) that had low levels of indigestible N, and high model predicted rumen N escape (Table 7.3). Lee et al. (2012b) suggested the depression in DMI they observed was due to a limitation in AA supply, not rumen N. Data in this experiment partially support this hypothesis, although no increase in DMI was observed when AA supply was increased. Despite being low in CP, the Base and Base+M treatments were not predicted to be severely limited in AA supply (Table 7.8) which probably allowed the cows to maintain DMI and milk production. Nitrogen utilization of cows fed the Base+M treatment was 38% which is higher than typically observed, particularly in mid-lactation cows at high production (Huhtanen and Hristov, 2009). In the Base and Base+M treatments, 1.7 times more N was being partitioned to milk than was being excreted in the urine and demonstrates the potential to reduce the environmental impact of dairy production if cows are fed precisely to requirements.

Cows were able to produce more milk than the model predicted MP supply would support when fed the Base, Base+M and Base+MU treatments. The efficiency factor used to estimate total MP requirements differs among models and depends on the requirements for MP accounted for by the model (Chapter 5). Previous versions of the CNCPS have used 0.67 (Fox et al., 2004) which is the same as the NRC (2001). The current model uses a factor 0.73 which was calculated in Chapter 5 and is higher than previous version of the CNCPS due to an increase in the maintenance requirements accounted for by the model through the inclusion of endogenous losses along the entire gut. Despite this, the apparent efficiency of MP use by cows in the current study ranged from 0.72 – 0.82 for the positive and Base+M treatments, respectively. This might

be due to inaccurate predictions of MP supply, although the efficiency of MP use has been shown to vary depending on the MP supply relative to other nutrients (Metcalf et al., 2008). Metcalf et al. (2008) points out, ration balancing models are not typically designed to be response models. Rather, they are designed to predict nutrient requirements at an optimum level. Therefore, although cows were able to utilize MP with a predicted efficiency of 0.82 when fed the Base+M treatment, it is likely performance would have improved if they were closer to the model predicted requirement using an efficiency factor of 0.73. Predicted bacterial growth depression due to low rumen N in the Base and Base+M treatments corresponded with the reduction in observed total tract NDF digestion (Table 7.7). The model also predicted rumen N supply in the Base+MU and Positive treatments was adequate and no further response would be expected if additional dietary N was supplied. Colmenero and Broderick (2006) measured an increase in NDF digestion when dietary CP was increased from 13.5 – 15 % DM but saw no benefit beyond 15 % which agrees with the findings of this study and suggests the model is sensitive to rumen N supply.

7.6 Conclusions

High levels of milk can be produced when diets are formulated to be adequate in rumen N and EAA supply, even when total dietary CP is low (<14 % DM). Model predictions appeared sensitive to rumen N supply and an increase in ECM was observed when diets were balanced for all EAA relative to dietary energy supply. New laboratory techniques allowed for the selection of high quality ingredients that were predicted to supply high levels of digestible AA to the small intestine and made it possible to formulate diets low in CP that were close to requirements. The

study demonstrates N utilization can be improved in high producing cows and the environmental impact of dairy production reduced through precision feeding of N and AA.

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CHAPTER 8: THE EFFECT OF STARCH-, FIBER-, OR SUGAR-BASED SUPPLEMENTS ON NITROGEN UTILIZATION IN GRAZING DAIRY COWS

8.1 Abstract

Nitrogen utilization in grazing cows is often low due to high concentrations of rapidly soluble and degradable protein in the pasture-based diet. Broadly, opportunities to improve N utilization lie in either reducing the amount of N consumed by the animal, or incorporating more N into milk protein. The goal of this study was to compare the relative importance of dietary N intake and productive N output for improving N utilization in grazing cows fed either starch-, fiber- or sugar-based supplements. Also, the Cornell Net Carbohydrate and Protein System v6.1 (CNCPS) was evaluated as a tool to assess cow performance and improve N utilization in pasture-based systems. Eighty-five cows were randomly assigned to one of five treatments at parturition (17 cows per treatment). Treatments consisted of a pasture only control (P) and pasture with a starch- (St and StN), fiber- (FbN) or a sugar (Sg)-based supplement. The StN and FbN treatments contained additional dietary N. Diets were formulated using the CNCPS to supply similar levels of dietary metabolizable energy, but differing levels of dietary N and metabolizable protein. Nitrogen utilization ranged from 22 to 26 % across the five groups. Cows fed the St treatment had the lowest levels of milk urea N, blood urea N and urinary N excretion and had the highest productive N output (149 g/d). Cows fed the FbN treatment had similar productive N output (137 g/d) and consumed ~100 g/d more dietary N than the St treatment resulting in greater urinary N excretion. Although milk protein yield was moderately greater in the St treatment, quantitatively the difference in N intake (100 g/d) had the greatest effect on N utilization and suggests that controlling dietary N intake should be the first priority when attempting to improve N utilization

in grazing cows. There was no effect of supplementing pasture fed cattle with sugar on production or N utilization under the conditions of this experiment. Predictions of metabolizable energy and protein availability for milk yield from the CNCPS were similar to actual milk yield for all treatments. Model predicted N utilization and excretion reflected the trends observed in the measured data and suggests the CNCPS can be a useful tool for formulating and evaluating diets to improve N utilization in pasture-based systems.

8.2 Introduction

Globally, there is increased pressure to reduce the environmental impact of dairying. Nitrogen management is of particular concern in pasture-based systems due to its impact on water quality in aquifers, rivers and lakes (Ledgard et al., 1999). Improving N utilization in pastoral systems presents a unique set of challenges given the requirement for high levels of pasture consumption for low-cost production, the large demands of N by temperate grasses, and the resultant soluble and rapidly degradable nature of pasture protein (Kolver, 2003).

Nitrogen-use-efficiency can be broadly defined as the proportion of productive N output from the cow (N accretion, milk N or N retained by the conceptus), relative to total N consumed (Calsamiglia et al., 2010). An increase in milk protein yield would increase productive N output and improve N utilization for a given N intake. Any N not secreted in milk, or accreted into tissue, will be lost in either feces or urine (Lapierre and Lobley, 2001). Fecal N is relatively fixed (Marini and Van Amburgh, 2003); the greatest opportunity to improve N utilization, therefore, is in either reducing urinary N output, or increasing productive N output (Broderick, 2003, Marini and Van Amburgh, 2003). In pasture-based systems, previous efforts to improve N capture have

focused on improving energy supply to the rumen, with the objective of incorporating more ammonia into microbial protein and, thereby, increasing the AA flow to the small intestine (Kolver et al., 1998a, Miller et al., 2001, Moorby et al., 2006, Sairanen et al., 2005). Although important, this assumes the milk yield of the cow is limited by AA supply, which when corrected, should increase milk protein synthesis and secretion. Milk protein synthesis appears more closely linked to milk yield, physiological state, and overall nutritional status than simple substrate availability (Cant et al., 2003, Hanigan et al., 2001). Therefore, in a situation where MP is adequate, increasing AA supply will increase hepatic removal, and shift the majority of the associated N back to the urea pool with no real benefit to overall N utilization (Lapierre et al., 2005).

Changing the ratio of starch or sugar to NDF has previously been reported to alter ruminal VFA profiles (Bauman et al., 1971, Beckman and Weiss, 2005), with subsequent effects on milk composition (Beckman and Weiss, 2005, Broderick, 2003). Changing dietary MP and N intake simultaneously tests the effect of substrate supply, dietary N dilution and their comparative importance in improving N utilization, for a given N intake, compared with increasing productive N output. The Cornell Net Carbohydrate and Protein System v6.1 (**CNCPS**) was previously used to evaluate grazing cows by Kolver et al. (1998b), who reported that the model could realistically predict ME and MP supply and subsequent milk production. Recent changes have been made to improve CNCPS predictions, including a re-characterization of various pool constituents, degradation rates, passage rate assignments (Van Amburgh et al., 2010, Van Amburgh et al., 2007) and predictions of N excretion (Higgs et al., 2012). The aim of the current study is to investigate the opportunity to improve productive N output in grazing cows using

starch-, fiber-, and sugar-based supplements formulated to supply balanced ME, with differing MP and N intakes. A second goal was to simulate the experimental conditions in the CNCPS and assess its usefulness as a tool to model N utilization under grazing conditions.

8.3 Materials and methods

Experimental work was conducted at the DairyNZ Lye Farm, Hamilton, New Zealand (37 47' S, 175 19' E) during July and August 2010. Prior approval for animal use was attained from the Ruakura Animal Ethics Committee, Hamilton, New Zealand.

8.3.1 Experimental Design and Treatments

Eighty five dairy cows (53 Friesian and 32 Friesian × Jersey, respectively; 69 Multiparous and 16 Primiparous, respectively) due to calve over a 21-d period were randomly assigned to one of five treatments at parturition (n = 17); treatments were balanced for milk production (mean of the first 100 DIM from the previous lactation for multiparous cows; 17.7 ± 0.7 kg milk/cow per d; mean ± SD), pre-calving BW (549 ± 29 kg), BCS (4.5 ± 0.3; 10-point scale: Roche et al., 2004), and age (4.5 ± 0.2 yr).

Dietary treatments consisted of a pasture only control (**P**) and pasture with starch (**St** and **StN**), fiber (**FbN**) or sugar (**Sg**)-based supplements. The StN and FbN treatments were formulated to supply equal dietary N and MP, while the St and Sg treatments had no additional N. A small amount of soybean meal was added to the StN treatment to make it equivalent to the FbN treatment on a true protein (TP) basis. Corn grain was used as the starch source, wheat-middlings as the fiber source and molasses as the sugar source. Supplements were formulated

using the CNCPS v6.1 (Tylutki et al., 2008, Van Amburgh et al., 2010) and fed to support a target of 30 kg of milk. Chemical composition and DMI for each treatment are presented in Table 8.1. All supplements were offered in pellet form except the Sg treatment, which was in liquid form and fed at a lower rate to prevent adverse health effects. The assumptions used when formulating the supplements were that cows in early lactation (~ 40 DIM), of similar BW, offered between 30 and 40 kg DM/ d of ryegrass-based pasture would consume approximately 15 kg/DM/d (Dalley et al., 1999) and would substitute approximately 0.5 kg of pasture DM/kg of concentrate DM fed (Bargo et al., 2003). Supplements were introduced gradually over a 3 d period and offered in two equal portions at a.m. and p.m. milking. The Sg treatment was provided orally in a diluted bolus (3:1 molasses:water) after each milking. All cows calved in an 18 d period and started their allocated treatment immediately after parturition. The experiment concluded on the same day for all cows meaning the experimental period ranged from 6.5 to 9 wk depending on the calving date.

Table 8.1. Feed intake and chemical composition of experimental diets.

	Diet[1]				
Item[2]	P	St	StN	FbN	Sg
Intake			kg/day		
DMI	11.7	13.8	13.9	15.1	12.6
Ingredient			% of DM		
Pasture	100.0	72.2	78.4	68.7	86.7
Corn meal	0.0	25.0	16.7	0.0	0.0
Wheat Middlings	0.0	2.8	2.2	29.8	0.0
Soybean Meal (48%)	0.0	0.0	1.8	0.0	0.0
Fat	0.0	0.0	0.0	1.1	0.0
Urea	0.0	0.0	0.9	0.4	0.0
Molasses	0.0	0.0	0.0	0.0	13.3
Chemical composition					
CP	28.1	23.0	27.3	25.0	25.0
SP (% CP)	46.0	41.4	42.9	45.7	53.0
ADICP (% CP)	7.9	6.5	6.8	6.6	7.2
NDICP (% CP)	33.3	26.9	28.1	26.8	29.3
NDF	39.5	32.6	34.0	38.5	34.3
ADF	22.0	17.5	18.5	19.1	19.1
Lignin (% NDF)	5.7	8.1	7.2	6.9	13.8
EE	4.9	4.6	4.6	5.7	4.6
Starch	0.3	16.5	11.3	7.1	0.3
Sugar	12.8	10.2	10.9	10.4	21.8
Ash	8.0	6.4	6.8	6.9	8.6

[1] P = Pasture only; St = pasture with a starch-based supplement; StN = pasture with a starch-based supplement and additional N; FbN = pasture with a fiber-based supplement and additional N; Sg = pasture with a sugar-based supplement.

[2] SP = Soluble protein; ADICP = Acid detergent insoluble CP; NDICP = Neutral detergent insoluble CP; EE = Ether extract.

8.3.2 Grazing Management

Cows rotationally grazed 37 hectares permanently subdivided into 1 hectare paddocks (defined grazing area) as one group (n = 85). Each paddock was further subdivided using a temporary electric fence to establish grazing conditions that encouraged pasture to be harvested to a post-grazing residual mass of 1,500-1600 kg DM/ha. This has been reported to balance the dual goals of achieving high DMI while maximizing pasture production and quality for future grazing events (Hoogendoorn et al., 1992, Lee et al., 2008). Cows in early lactation have increasing DMI; therefore, pasture allowance was continually reassessed to maintain the target residual pasture mass. Pasture allowance was 29 ± 5 kg DM/cow per d for the last 3 weeks of the study. Pre- and post-grazing compressed sward heights for the same period were 22.9 ± 2.3 and 10.6 ± 1.2 cm, respectively, and pre- and post-grazing pasture yield was 3243 ± 261 and 1681 ± 233 kg DM/ha, respectively. Measurements were made using a Rising Plate Meter installed with an electronic counter (Farmworks, Palmerston North, New Zealand). Cows had access to a fresh allocation of pasture twice daily and only returned to the same area when a minimum of two leaves had appeared on the majority (> 66%) of perennial ryegrass (*Lolium perenne* L.) tillers. The pasture offered consisted of 90.2 (± 2.8)% perennial ryegrass leaf, 2.5 (± 1.4)% perennial ryegrass stem, 1.5 (± 2.2)% white clover (*Trifolium repens*), 0.6 (± 0.7)% weeds (*Sisymbrium officinale, Achillea millefolium, Taraxacum officinale, Ranunculus sardous*), and 5.2 (± 1.8)% dead material, on a DM basis.

8.3.3 Pasture Measurements

Representative samples of pasture were collected daily by clipping pasture to grazing height from paddocks due to be grazed. Samples were bulked on a weekly basis for the duration of the

experiment, and duplicate samples were dried for 48 h at either 100^{O}C, for DM analysis, or 60^{O}C for analysis of nutrient composition. Samples dried at 60^{O}C were subsequently ground to pass through a 1.0 mm sieve (Christy Lab Mill, Suffolk, UK) and analyzed by wet chemistry for the nutrients required to evaluate the diets in the CNCPS (Tylutki et al., 2008 ; DairyOne, Ithaca, NY).

8.3.4 Animal Measurements

8.3.4.1 DMI

Mean group pasture DMI was calculated as the product of the difference between the pre- and post-grazing pasture mass and area grazed daily (Roche et al., 1996). Supplement offered and refused was measured at each milking. Estimations of individual cow pasture DMI were obtained using the n-alkane technique outlined by Kennedy et al. (2003). Briefly, each cow was dosed twice daily (at milking) with a capsule containing 356 mg of n-dotriacontane (C32; i.e. 712 mg C32/cow per d) for a 10-d period on weeks 6 and 7 of the experiment. Fecal grab samples were collected twice daily from each cow (after milking) during the last 5 d of the 10 d period. The fecal samples from each cow for the 5 d period were bulked and stored at –17^{O}C awaiting alkane analysis. During the same 5 d period, pasture samples were plucked to grazing height, following close observation of the grazing animal, to represent pasture grazed. The n-alkane concentration (C25-C36) in pasture, supplement and feces were determined using gas chromatography (Mayes et al., 1986). The ratio of pasture C33 (tritriacontane) to dosed C32 (n-dotriacontane) was used to estimate pasture DMI. Estimates of daily pasture DMI were calculated as follows:

$$\text{Daily pasture intake (kg DM/cow)} = \frac{F_i/F_j \times (D_j + I_s \times S_j) - I_s \times S_i}{P_i - (P_j \times F_i/F_j)}$$

where F_i, S_i and P_i are the concentrations (mg/kg of DM) of the natural odd-chain n-alkane (C33) in feces, supplement and pasture, respectively, F_j, S_j and P_j are the concentrations (mg/kg of DM) of the dosed even-chain n-alkane (C32) in feces, supplements and pasture, respectively, and D_j and I_S are the dose rate (mg/ d) of the even-chain n-alkane (C32) and supplement intake, respectively.

8.3.4.2 Milk and BW

Individual milk yields were recorded daily (GEA, Oelde, Germany). Fat, TP, and lactose concentrations in milk were determined by a Milkoscan FT120 (Foss Electric, Hillerød, Denmark) on a composite from a.m. and p.m. samples collected once (two consecutive days) each week for the duration of the experiment. Milk composition data were verified by reference techniques for a sub-set of milk samples (milk fat: Röese-Gottlieb method; IDF, 1987; CP: Kjeldahl techniques; Barbano et al., 1991). Body weight and BCS were measured weekly following the a.m. milking; BCS was assessed on a 10-point scale, where 1 is emaciated and 10 is obese (Roche et al., 2004). These scores can be converted to the 5-point scale of Wildman et al. (1982) using the regression equation generated by Roche et al. (2004; 5-point BCS = 1.5 + 0.32 10-point BCS).

8.3.4.3 Blood

Two 10 mL evacuated blood tubes containing either a sodium heparin pellet (158 IU sodium heparin) or EDTA (0.117 mL of 15% K_3EDTA) to prevent coagulation were collected from each cow by coccygeal venipuncture prior to treatment allocation and weekly thereafter. Plasma was separated (1,120 *g*, 10 min, 4^{O}C) and frozen at -20 ºC prior to analysis. Plasma from the EDTA tubes was analyzed for NH_3 concentration (mmol/L), based on the enzymatic kinetic assay described by Da Fonseca-Wollheim (1973). Plasma from the sodium heparin tubes were analyzed for NEFA, BHBA, glucose and urea. Determination of NEFA (mmol/L; colorimetric method using a commercial kit: WAKO, Osaka, Japan), BHBA (mmol/L; BHBA dehydrogenase assay based on formation of acetoacetate and NADH after addition of NAD), glucose (mmol/L; hexokinase method based on formation of NADPH), and urea (mmol/L; urease hydrolysis method) were performed on a Hitachi Modular P800 analyzer (Roche, Basel, Switzerland) at 30^{O}C by Gribbles Veterinary Pathology Ltd., Hamilton, New Zealand. The inter- and intra-assay CV was < 2% for all assays.

8.3.4.4 Urine

Mid-stream urine samples were collected once each week during voluntary urination of cows immediately prior to the morning milking. After collection, samples were divided into 50 mL aliquots for the analysis of creatinine, urea, uric acid, allantoin, urea and total N. The aliquot's used for the analysis of urea and total N were reduced to pH ≤ 2 using approximately 3 mL of 6 mol/L hydrochloric acid and frozen at -20 ºC prior to analysis. Creatinine, uric acid (mmol/L; enzymatic colorimetric assay) and urea (mmol/L; kinetic UV assay) were analyzed using commercial kits (Roche Diagnostic NZ Ltd., Auckland, New Zealand) by Gribbles Veterinary

Pathology Ltd., Hamilton, New Zealand. Allantoin was analyzed on a spectrophotometer using a colorimetric assay (Young and Conway, 1942) and total N was analyzed using the Leco total combustion method (Institute of Food, Nutrition and Human Health, Massey University, New Zealand).

8.3.5 CNCPS Inputs

Data used in the CNCPS represented the mean of a 5 d period in wk 7 of the study, coinciding with the n-alkane DMI estimation. Dietary inputs, including DMI, feed ingredients, and the chemical composition of ration are in Table 8.1. Animal inputs, including milk production, initial BCS, and BW change are in Table 8.4. Other inputs, including stage of lactation, breed and parity are consistent with the previous description in this section. The contribution of tissue mobilization to predictions of ME and MP milk (Table 8.4) were estimated from BW change. The chemical composition (fat:protein) of mobilized body reserves changes depending on the BCS of the animal (Fox et al., 1999). To account for this, the composition of reserves mobilized was calculated using the BW change and initial BCS from Table 8.4 and equations in Fox et al. (2004). Briefly, initial BW and BCS were used to calculate a reference BW at BCS 3 (1 – 5 scale). Mobilized fat and protein were then estimated using the reference BW and the change from initial to final BW (Fox et al., 2004). Change in BW was preferred to BCS as an estimate of tissue mobilization due to the difficulty in ascertaining small changes in BCS over one time period (Ferguson et al., 1994). It was assumed that when MP supply was excess to requirements, protein mobilized from tissue was used as an energy source and contributed to ME supply.

8.3.6 Statistical Analysis

Data are expressed as means of the last three weeks of the study and were analyzed using a restricted maximum likelihood model (REML) in GenStat 13.2 (VSN International, 2010). The model included the fixed effects of calving group (three groups to account for calving date), age (primiparous and multiparous), week of study, treatment, and the interaction of calving group and week. The effects of calving group, parity and week of study were included to account for non-treatment variation and are not considered important in explaining treatment effects. Cow was included as a random effect. Treatment effects were considered significant at $P < 0.05$. The LSD for the error degrees of freedom was approximately 2 × the SE of the difference (**SED**).

8.4 Results

8.4.7 Animal Observations

The type of supplement fed to cattle on treatment affected milk yield ($P < 0.01$), yield of TP ($P < 0.001$) and lactose ($P < 0.01$), but not milk fat (Table 2). Cows fed the St treatment had the highest milk and TP yields ($P < 0.01$) followed by the FbN and StN treatments. Cows fed the Sg treatment had similar milk and milk components to cows fed the P control. Concentrations of fat, TP and lactose were affected ($P < 0.01$) by treatment (Table 8.2). Milk fat concentration was lower in cows fed the St supplement than cows in the other four groups, which did not differ from each other. True protein concentration was greater ($P < 0.01$) in the St treatment compared with the P and Sg treatments, but similar to the StN and FbN treatments. Milk urea N concentration was lower in cows fed the St treatment ($P < 0.001$) compared with all other treatments.

Table 8.2. Effects of supplementing different carbohydrate types to grazing dairy cows in early lactation on milk yield and milk composition.

	Diet[1]						
Item	P	St	StN	FbN	Sg	SED[2]	*P*-value[3]
Yield (kg/d)							
Milk	23.1	27.7	25.5	26.2	23.6	1.34	0.005
Fat	1.03	1.07	1.11	1.16	1.06	0.061	0.326
TP	0.74	0.95	0.85	0.87	0.73	0.038	<0.001
Lactose	1.13	1.38	1.25	1.28	1.14	0.063	0.001
Milk composition (%)							
Fat	4.44	3.88	4.39	4.41	4.57	0.209	0.016
TP	3.20	3.43	3.34	3.34	3.11	0.075	0.001
Lactose	4.89	4.99	4.91	4.92	4.85	0.037	0.010
MUN (mmol/L)	7.24	5.10	7.09	6.40	6.60	0.228	<0.001

[1] P = Pasture only; St = pasture with a starch-based supplement; StN = pasture with a starch-based supplement and additional N; FbN = pasture with a fiber-based supplement and additional N; Sg = pasture with a sugar-based supplement.

[2] SED = Standard error of the difference.

[3] Refers to the overall treatment effect. The least significant difference for this study is 2 × SED. Therefore, individual treatment means were considered significantly different when they differed by > 2 × SED.

Table 8.3. Effects of supplementing different carbohydrate types to grazing dairy cows in early lactation on parameters of N and energy metabolism.

	Diet[1]						
Item[2]	P	St	StN	FbN	Sg	SED[3]	*P*-value[4]
Blood parameters (mmol/L)							
BUN	7.05	5.10	6.72	6.07	6.60	0.227	<0.001
Glucose	4.05	4.20	4.12	4.34	3.97	0.093	0.002
NEFA	0.54	0.54	0.52	0.63	0.65	0.052	0.036
BHBA[5]	0.62 (-0.21)	0.38 (-0.42)	0.48 (-0.32)	0.42 (-0.37)	0.65 (-0.19)	-0.041	<0.001
Urine parameters[5]							
PD:Creatinine	2.97 (0.47)	2.86 (0.46)	2.49 (0.40)	3.09 (0.49)	2.49 (0.40)	-0.045	0.141
N:Creatinine	0.23 (-0.65)	0.16 (-0.79)	0.19 (-0.71)	0.21 (-0.68)	0.20 (-0.70)	-0.039	0.004
Urea:Creatinine	78.44 (1.89)	54.63 (1.74)	75.40 (1.88)	73.16 (1.86)	69.27 (1.84)	-0.025	<0.001

[1] P = Pasture only; St = pasture with a starch-based supplement; StN = pasture with a starch-based supplement and additional N; FbN = pasture with a fiber-based supplement and additional N; Sg = pasture with a sugar-based supplement.

[2] PD = Purine derivatives (allantoin + uric acid)

[3] SED = Standard error of the difference.

[4] Refers to the overall treatment effect. The least significant difference for this study is 2 × SED. Therefore, individual treatment means were considered significantly different when they differed by > 2 × SED.

[5] Data were log transformed for statistical analysis. Numbers outside the brackets are back-transformed values and numbers inside the brackets are log transformed. The SED corresponds to the transformed values.

Cows fed the St treatment had more than 1 mmol/L less urea in blood than the other treatments, which was consistent with the ratios of urinary urea to creatinine and urinary N to creatinine (Table 8.3). There were no differences in the ratio of purine derivatives (**PD**) to creatinine. Blood concentrations of BHBA were elevated in control and Sg cows, but similar among the other treatments. Blood NEFA concentrations were greater ($P < 0.05$) in cows fed the Sg and FbN treatments, but similar among the other treatments.

8.4.8 CNCPS Predictions

Predictions from the CNCPS are in Table 8.4. Metabolizable energy and MP intake was similar among the St and FbN treatments and Sg and StN treatments, respectively, but predicted MP allowable milk was considerably higher than ME allowable milk. Cows fed the StN and FbN treatments were similar in total N intake (~ 600 g N/d), which was approximately 100 g greater than the St and Sg treatments and 70 g greater than the P control. When tissue mobilization was included into the model, predicted ME and MP allowable milk were balanced and similar to actual milk production.

Predicted urinary N excretion followed the trends evident in ratios of urinary N and urinary urea to creatinine (Table 8.3). The ratios of productive N:urinary N, productive N:intake N and milk TP:MP supply were all greater in cows fed the St treatment, but similar among the other treatments.

Table 8.4. CNCPS inputs and predictions for the effect of supplementing different carbohydrate types on N use parameters.

	Diet[1]				
Item	P	St	StN	FbN	Sg
Milk actual (kg/d)	23	28	26	26	24
ME Milk predicted (kg/d)[2]	22	27	27	28	26
MP Milk predicted (kg/d)[2]	23	25	26	27	24
Diet allowable ME milk (kg/d)	7	15	14	15	10
Diet allowable MP milk (kg/d)	23	24	26	27	24
Initial BW (kg)	441	467	442	448	477
Final BW (kg)	417	449	422	427	453
Initial BCS[3]	3.8	4.1	3.8	4.0	4.1
Final BCS	3.7	4.0	3.7	4.0	4.0
N intake (g/d)	527	507	607	604	504
Productive N (g/d)	116	149	134	137	115
Fecal N (g/d)	155	163	182	187	155
Urine N (g/d)	303	230	331	320	279
N Balance (g/d)	-48	-34	-39	-40	-45
MP intake (g/d)	1548	1697	1776	1889	1547
MP Bacteria (% MP intake)	34%	42%	38%	38%	40%
ME intake (MJ/d)	123	154	151	159	136
Productive N:Urine N	0.38	0.65	0.40	0.43	0.41
Productive N:Intake N	0.22	0.29	0.22	0.23	0.23
Milk TP:MP Supply	0.48	0.56	0.48	0.46	0.47

[1] P = Pasture only; St = pasture with a starch-based supplement; StN = pasture with a starch-based supplement and additional N; FbN = pasture with a fiber-based supplement and additional N; Sg = pasture with a sugar-based supplement.

[2] Includes contributions from body reserves.

[3] Measured on a 1-10 scale (Roche et al., 2004)

8.5 Discussion

Efficiency of N utilization in dairy cows is typically low, averaging around 25%, but can range from 15% to 40% (Calsamiglia et al., 2010). Trends evaluated over a wide range of dietary and management conditions indicate that dietary CP concentration is the most important factor influencing the efficiency of N use (Huhtanen and Hristov, 2009). Efficiencies as high as 43% (Frank and Swensson, 2002) and 37% (Noftsger and St-Pierre, 2003) have been reported in the literature in TMR-fed cows and as high as 38% in cows fed ryegrass-based diets (Moorby et al., 2006). The observed N efficiencies for cows in the current study ranged from 22% to 29% (Table 8.4), which, according to Calsamiglia et al. (2010), would be classified as ranging from low to moderately high, respectively. Cows with the highest N-use efficiency in the current study (St) still wasted 10% more N than cows in the study of Moorby et al. (2006). The major difference between the two studies was the CP content of the pasture, which was 28.1% in the current study (Table 8.1) compared with approximately 10% in the study of Moorby et al. (2006). Attempts have been made to improve the retention of dietary N by synchronizing the supply of energy and protein in the rumen, both, through supplementation (Kolver et al., 1998a), and also through feeding pasture cultivars bred to have higher sugar content (Edwards et al., 2007). Effects have generally been transient, with no real improvement in N utilization suggesting N intake is a more important factor in improving N utilization (Edwards et al., 2007, Henning et al., 1993, Kim et al., 1999, Kolver et al., 1998a).

Pasture CP in the current study was higher than anticipated (Table 8.1); this resulted in predicted MP allowable milk being approximately 10 kg higher than ME allowable milk (Table 8.4). Unfortunately, the StN treatment was less palatable than the other treatments and this

resulted in lower than formulated ME intake. Despite this, N intake and predicted MP allowable milk were similar among cows fed the St and Sg treatments and StN and FbN treatments (Table 8.4) allowing the comparison of both dietary N dilution, and the effect of carbohydrate type. Compared with the P control, cows fed the Sg treatment had a lower ratio of urinary urea:creatinine and MUN; however, urinary N:creatinine and BUN were similar. This suggests a small N dilution effect consistent with the reduction in dietary N intake (Table 8.4), but no improvements in the overall efficiency of N use. In contrast, cows fed the St treatment consumed the same amount of dietary N as the Sg treatment, but had a 0.06 unit greater N-use-efficiency (Table 8.4). Cows fed the St treatment consumed more ME than the Sg treatment. However, cows fed the Sg treatment also consumed more ME than the P control and there was no difference between these two treatments. Supplementing with sucrose has previously been reported to drop rumen pH and decrease the rate of NDF digestion (Chamberlain et al., 1993, Huhtanen and Khalili, 1991). In the experiment of Huhtanen and Khalili (1991), cows were fed 1 kg DM/d of sucrose which reduced the rate of NDF digestion by 1.5 %/hr. Pasture NDF in the current study was calculated to digest at a rate of 7 %/hr (Van Amburgh et al., 2003). Reducing this digestion rate from 7 to 5.5 %/hr in the CNCPS reduced the ME allowable milk from 26 to 24 kg and probably explains the lack of response in the Sg treatment. Pasture in the current study was 12.8 % sugar and the administration of molasses increased the sugar content in the diet of the Sg cows to 21.8% (Table 8.1). There was no difference in the ratio of PD:creatinine suggesting total microbial growth was not changed by treatment (Valadares et al., 1999), although there was a tendency for PD:creatinine to be lower in Sg and StN cows. Therefore, supplementing molasses to cows consuming high quality spring pasture using the method

employed in the current experiment (1.5 kg/d split in two feeds at milking) has no production benefit.

The effect of supplementing a fermentable fiber source was also investigated (FbN) and compared with supplementing starch (StN and St). Cows fed the FbN treatment had lower BUN and MUN concentrations than cows fed the StN treatment; however, there were no differences in N excretion (Table 8.3) or predicted N utilization (Table 8.4). Numerically, cows fed the St treatment produced more milk protein than cows fed the FbN treatment. Rius et al. (2010) reported increased milk protein synthesis with the addition of post-ruminal starch, a result, most likely, of increased concentrations of insulin and IGF-1 (Griinari et al., 1997, Mackle et al., 1999, Rius et al., 2010). In the current study, differences in N excretion among treatments can be largely explained by differences in N intake (Table 8.4) with only subtle differences in productive N when ME intake was similar. When comparing the St and FbN treatments, approximately 90% of the difference in N excretion can be attributed to a reduction in N intake, whereas 10% can be attributed to higher productive N output. These findings are in agreement with the conclusion of Huhtanen and Hristov (2009) that reducing N intake is the most important factor in reducing N losses from dairy operations.

Predictions from the CNCPS suggest that cows were consuming adequate dietary MP at the given level of milk, but dietary ME was limited; this is similar to the results of Kolver et al. (1998b). A major difficulty when conducting grazing studies is accurately estimating DMI (Bargo et al., 2003). Intakes reported in this study (Table 8.4) are based on the n-alkane technique described by Kennedy et al. (2003). The dosing and sampling period for this assay

coincided with a period of prolonged wet weather, which reduced pasture utilization and probably explains the low pasture DMI (Holmes et al., 2002). However, pasture intake could also have been underestimated if the recovery of dosed C32 n-alkane was lower than expected (Kennedy et al. 2003). Blood NEFA increased sharply and BW decreased sharply over this period (data not presented), which is consistent with the ME deficit predicted by the CNCPS. The model predicted cows fed the Sg treatment and P control to be most limited in dietary ME intake compared with milk produced, which is consistent with higher concentrations of BHBA (Table 8.3). Mobilization of tissue accounted for 36% of the ME requirement for cows fed the P control which is comparable to levels reported from cows in the first three weeks of lactation, but are high for cows in the fifth week of lactation (Pedernera et al., 2008; Komaragiri and Erdman, 1997). Including the recorded change in BW into the CNCPS aligned predicted ME and MP milk closely with actual milk for all treatments (Table 8.4) and is consistent with previous evaluations of the CNCPS under grazing conditions (Kolver et al., 1998b). Predictions of N excretion reflect measured N excretion (Table 8.3). Cows fed the St treatment were predicted to excrete 70 g/d less urinary N than the P control and approximately 100 g/d less than the StN and FbN treatments, respectively. Although 70 g/d may seem inconsequential, in a herd of 1,000 cows this reduction is equivalent to 1,000 kg less urea excreted/wk and, if sustained, represents a considerable reduction in N loss to the environment. Similar effects could be achieved if CP levels in pasture were reduced (Moorby et al., 2006). Given only one data point was modeled per treatment, care must be taken when interpreting these results. However, model predictions were consistent with the recorded data and suggest the CNCPS can be successfully used as a tool to formulate diets to improve N utilization in grazing cows.

8.6 Conclusions

Nitrogen utilization can be improved by including high energy, low protein supplements into the diets of grazing dairy cows. Reducing dietary N intake appears to be the most important factor in improving N utilization when ME intake is the same. However, subtle improvements in milk protein output can be achieved by feeding starch, compared with fiber- or sugar-based supplements. Feeding additional sugar to cows fed high quality spring pasture had no real benefit in the current study. Predictions from the CNCPS were consistent with measured data and predicted ME and MP allowable milk were close to measured milk production when estimations of tissue mobilization were included. Predictions of N utilization also reflected the measured data, indicating that the CNCPS is a useful tool in formulating diets to reduce N losses to the environment.

8.7 Acknowledgements

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CHAPTER 9: SUMMARY

Since the original publications of the CNCPS in the early 90's, work has been ongoing to improve the models capability to predict nutrient supply and requirements of dairy cattle with a focus of field application. This dissertation describes a shift from the original structure of the model that calculates statically, to a dynamic structure that calculates over time. Table 9.1 has a summary of the major updates to the CNCPS since version 6.0 (Tylutki et al., 2008) that have resulted in v6.1, v6.5 and v7.0. Contributions from this dissertation that have been implemented into v6.5 of the model include updates to the chemistry and AA profiles of feeds in the feed library and re-structuring of the protein pools (Chapter 2; Table 9.1). Updates that have resulted in v7.0 are described in detail through this dissertation and the major changes are listed in Table 9.1. Data from the experiment described in Chapter 7 were simulated in v6.5 and v7.0 of the CNCPS and serve to demonstrate differences in model predictions between the two versions (Table 9.2).

Predicted ME supply is slightly higher in v7.0 (~1.0 Mcal/d) which is partially due to the incorporation of NDF passage rates from the NorFor system (Chapter 3) which have resulted in slower NDF passage and higher levels of predicted NDF digestion in the rumen. Version 6.5 predicts higher levels of MP supply for all treatments. This is most pronounced in the Base and Base+M treatments which is largely due to higher levels of predicted microbial growth. Rumen N balance is predicted to be adequate in all treatments in v6.5. In contrast, v7.0 predicts the Base and Base+M treatments to be ~15% below requirement which is reflected in the predictions of microbial MP supply (Table 9.2) and is consistent with the lower observed NDF digestion in these treatments (Table 7.7). Differences in net protein requirements are due to the different

ways in which metabolic losses in the GIT are calculated. Version 7.0 mechanistically estimates endogenous losses along the entire GIT (Chapter 5; Table 9.1), while v6.5 uses an empirical estimate of metabolic fecal N (Fox et al., 2004). Although net protein requirements are different, MP requirements are similar as each version of the model uses a different efficiency of use to estimate MP from net protein (v6.5 = 67%; v7.0 = 73%). Because of the similar predicted MP requirement, and higher predicted MP supply in v6.5, MP allowable milk was closer to actual milk for the low protein diets (Base, Base+M and Base+MU) but was over-predicted for the positive treatment, while v7.0 predicted cows were limited in MP for the low protein treatments, but was adequate for the positive treatment. Predictions of Met balance were similar among model versions; however, Lys balance was considerably lower in v6.5 than v7.0, despite predicted MP supply being higher. Amino acid balance appeared to more closely reflect animal performance for v7.0 of the CNCPS, while total MP supply was closer in v6.5 for the low protein treatments. Rigorous evaluations are a critical component of model development process. Further evaluations over a wide range of situations will further establish the relative performance of v7.0 of the CNCPS compared with v6.5 and other models used in the global dairy industry and demonstrate the usefulness of the model as an on-farm ration balancing tool.

Table 9.1. Major developments in the CNCPS after the description of version 6.0 by Tylutki et al. (2008) resulting in v6.1, v6.5 and v7.0

v6.1	v6.5	v7.0
• Re-organization of passage rate assignments so soluble protein fractions flow with the liquid passage rate (Van Amburgh et al., 2007) • Reduction the digestion rates of A and B1 protein fractions to be more consistent with literature reports (Van Amburgh et al., 2007) • Reduction in the digestion rates of sugars to better reflect gas production data (Van Amburgh et al., 2007)	• Updated feed chemistry in the feed library (Chapter 2) • Updated pool structure for the protein fractions in the model where the A pool, previously defined as non-protein N, was changed to ammonia and is now defined as the A1 pool (Chapter 2) • Updated AA profiles of feeds in the feed library (Chapter 2) • Combined efficiency of AA use for milk production and maintenance (Lapierre et al., 2007) • Capability to use $uNDF_{240}$ rather than lignin × 2.4 to characterize unavailable fiber (Raffrenato, 2011)	• New dynamic structure for the entire gastro-intestinal model (Chapter 3) • Expansion of the post-rumen model to include a separate large and small intestine (Chapter 3) • Development of a mechanistic large intestine (Chapter 3) • Inclusion of protozoa in the microbial sub-model (Chapter 4) • New system to mechanistically estimate N recycling (Chapter 3) • Capability to model different meal patterns (Chapter 3) • Capability to estimate N digestibility using an in vitro estimate of indigestible N (Ross, 2013) • Inclusion of endogenous N transactions along the gastro-intestine tract (Chapter 5) • Revised efficiencies of AA use (Chapter 5) • Expansion of potentially digestible NDF from 1 to 2 pools (Raffrenato, 2011) and the implementation of new passage rates for NDF from (NorFor, 2011)

Table 9.2. Comparison of model predictions for v6.5 and v7.0 of the CNCPS using the dietary treatments from Chapter 7

	Base[1]		Base+M		Base+MU		Positive	
	v7.0	v6.5	v7.0	v6.5	v7.0	v6.5	v7.0	v6.5
DMI[2], kg/d	23.9		24.8		24.7		24.4	
Actual milk[2], kg/d	38.0		40.9		38.8		40.9	
ME supply, Mcals ME/d	61.2	60.0	63.2	62.0	63.2	61.7	62.9	61.0
ME required, Mcals ME/d	56.3	56.3	57.4	57.4	57.6	57.6	59.6	59.6
ME balance, Mcals ME/d	4.9	3.7	5.8	4.6	5.6	4.1	3.3	1.4
MP supply, g/d	2323	2527.2	2418.8	2635.6	2527.9	2613.7	2783.9	2828.0
Net protein required[3], g/d	1864.4	1929.1	1991.8	2054.8	1948.7	1999.7	2008.1	2049.4
MP required[4], g/d	2554.0	2515.5	2728.4	2691.1	2669.5	2611.8	2750.9	2691.2
MP balance, g/d	-230.9	11.7	-309.7	-55.5	-141.6	1.9	33	136.9
MP RUP, g/d	1118.5	1197.8	1183.4	1267.6	1180	1258.4	1465.6	1516.9
MP microbial, g/d	1204.5	1329.4	1235.4	1368.1	1347.9	1355.3	1318.3	1311.1
MP microbial, %	51.9%	52.8%	51.1%	52.1%	53.4%	52.1%	47.5%	46.7%
ME allowable milk	42.1	42.0	46.1	46.3	43.6	43.4	44.7	43.9
MP allowable milk	33.9	38.3	34.8	39.6	36.7	38.9	41.5	44.2
ME MP average	38.2	40.1	40.8	43.0	40.6	41.2	44.7	44.1
ME MP first limiting	34.3	38.0	35.4	39.5	37.6	38.8	42.5	42.1
Met supply, g/d	57.1	72.3	71.3	86.9	74.4	86.1	79.1	89.8
Lys supply, g/d	173.4	195.6	178.7	202.3	188.6	200.4	194.9	200.8
Met balance, g/d	-15.6	-13.4	-6.9	-4.1	-1.8	-0.7	0	-5.2
Lys balance, g/d	-18.3	-45.4	-27	-53.6	-12.5	-49.0	-13.3	-51.4
Rumen NH_3 balance, % required	84%	106%	83%	106%	96%	116%	98%	113%

[1] Base = balanced (using v7.0) for ME (assuming 45 kg ECM), but limited in MP and rumen N; Base+M = balanced for ME and MP Met but limited in MP and rumen N; Base+MU = balanced for ME, MP Met, with adequate rumen N, but limited in MP; Positive = balanced for ME, MP, all EAA and adequate rumen N

[2] Unadjusted means across the entire experiment

[3] Net protein required without accounting for the efficiency of use

[4] Metabolizable protein requirement including the efficiency of use of 73% for v7.0 (Chapter 5)

9.1 References

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