MODELS TO PREDICT RUMINAL CARBOHYDRATE AND NITROGEN SUPPLY AND NITROGEN EXCRETION IN CATTLE

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MODELS TO PREDICT RUMINAL CARBOHYDRATE AND NITROGEN SUPPLY AND NITROGEN EXCRETION IN CATTLE

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To mitigate the negative environmental impact of farming, it is important that diets are formulated to accurately match requirements. For that, an adequate characterization of feed composition and its variability is crucial. The original Cornell Net Carbohydrate and Protein (CNCPS) feed carbohydrate and protein fractionation schemes were evaluated and modified to improve predictions of the rumen degradable protein (RDP), rumen undegradable protein (RUP) and microbial protein supply. For carbohydrates, a new expanded scheme was developed; the CA1 is volatile fatty acids (VFA), CA2 is lactic acid, CA3 is other organic acids, CA4 is sugars, CB1 is starch, CB2 is soluble fiber, CB3 is available neutral detergent fiber (NDF), and CC is unavailable NDF. The expanded scheme accounted for more variation in changes in silage quality and non-fiber carbohydrate composition.

The CNCPS and National Research Council (NRC) protein schemes were evaluated using Monte Carlo techniques. Both schemes shared similar limitations including (1) the range of RDP and RUP was over-predicted; (2) the methods used to estimate degradation rates had low accuracy and repeatability, and (3) the assumptions underlying the kinetic models were too restrictive to mimic ruminal digestion. The CNCPS protein scheme was revised and alternative schemes were developed. Predictions of RDP and RUP were improved by assigning rates obtained with the inhibitory in vitro system to a combined insoluble protein B fraction, or by redefining

A and B1 fractions as the non amino-N and amino-N in the soluble fraction, respectively.

Urea recycled to the rumen may represent an important source of N for microbes. A dynamic mechanistic model was developed to be used as a component of ration formulation models to predict N recycling to the GIT and urinary urea N. Recycling processes were modeled as positive feedbacks, while renal excretion was modeled as a negative feedback. Both processes were assumed to be regulated by N intake. Model simulations suggested that accurately accounting for urea recycled to the rumen reduces degradable nitrogen needed in the diet, and the use of the NRC 1985 empirical equation to predict urea recycling to the rumen may greatly underestimate recycling in lactating dairy cows.

BIOGRAPHICAL SKETCH

Cristina Lanzas was born on December 31, 1977 at Barcelona, Spain. After completing secondary school studies in Vic, in 1995, she enrolled in the Veterinary College of the Universitat Autònoma de Barcelona. In 2000, she completed the degree of Veterinary Medicine with Highest Honors. During 2001 to 2003, she completed a Master's degree program in Animal Science under the guidance of Dr. Alice Pell, at Cornell University. During her Master's program, she became interested in nutritional modeling, which led her to enrolled in a Ph.D degree program in 2003 under the supervision of Dr. Danny Fox with a major in Animal Science and minors in Animal Nutrition and Environmental Engineering . After completion of her Ph.D program, she will undertake a postdoctoral position involving modeling infectious and production diseases in the Department of Population Medicine and Diagnostic Sciences at the College of Veterinary Medicine, Cornell University.

To my grandfather Pedro,
with whom this journey began
and will not see the wonderful
outcomes

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LIST OF ABBREVIATIONS

AA	Amino acid
AAN	Amino nitrogen
AD	Acid detergent
ADICP	Acid detergent insoluble crude protein
aNDR	Neutral detergent fiber assayed with amylase and without sodium sulfite
BUN	Blood urea nitrogen concentration
CHO	Carbohydrates
CNCPS	Cornell Net Carbohydrate and Protein System
CPM	Cornell-Penn-Miner Net Carbohydrate and Protein System
DM	Dry matter
EAA	Essential amino acid
EE	Ether extract
FC	Fiber carbohydrates
GFR	Glomerular filtration rate
HMCG	High moisture corn grain
IIV	Inhibitory in vitro system
MCP	Microbial crude protein
ME	Metabolizable energy
MP	Metabolizable protein
NAAN	Non amino nitrogen
ND	Neutral detergent
NDF	Neutral detergent fiber
NDICP	Neutral detergent insoluble crude protein

NFC	Non-fiber carbohydrates
NPN	Non-protein nitrogen
NRC	National Research Council
PDF	Probability distribution function
RAN	Rumen ammonia nitrogen concentration
RDP	Rumen degradable protein
RMSE	Root mean standard error
RMSPE	Root mean square prediction error
RUP	Rumen undegradable protein
SRC	Standard regression coefficients
TCA	Tricholoroacetic acid
VFA	Volatile fatty acids
Y_g	Maximum rumen microbial growth yield

INTRODUCTION

Over the last decade, public concern about issues related to the impact of animal agriculture on the environment has grown. Currently, the agenda for agriculture policies in developed countries incorporates a variety of issues including the role of agriculture in environmental pollution, food safety, excretion of hormonal and antibiotic residues and pathogens to the environment, and animal welfare (Powers, 2003). For the years to come, assuring more efficient production and a safe and nutritious food supply while maintaining profitability will remain a great challenge. More systematic quantitative approaches are needed to cope with the increasing complexity that naturally arises as the number of factors involved in decision making increases.

The Latin verb *simulare* means to mimic. The purpose of a simulation model is to mimic real systems so that their behavior can be studied. Models are valuables tools in both research and field applications. They integrate knowledge in a readily usable way, providing predictions and guidance. In research, a hypothesis, which is nothing but a mental model, can be expressed in mathematical and formal terms to provide a quantitative description and mechanistic understanding of a biological system (Thornley, 2000). When creating models, areas where knowledge is lacking can be highlighted, and *ad hoc* experimentation can be reduced (Thornley, 2000).

Nutritional models help on farm decision-making by predicting animal performance and nutrient excretion and assessing diet adequacy under a wide range of management and feeding situations. Because beef and dairy farming are significant contributors to environmental nitrogen (N) pollution in the developed world, environmental legislation requires farms to quantify and adjust N budgets (NRC,

2003). Thus to mitigate the negative environmental impact, it is important that diets are formulated that meet, but do not exceed N requirements of rumen microbes and amino acids (AA) requirements of the animals. At present, some aspects of current nutritional models require further improvements, in particular predictions of (i) dietary supply of rumen degradable protein (RDP) and rumen undegradable protein (RUP), (ii) extent of ruminal N recycling, (iii) N requirements of rumen microorganisms, and (iv) microbial protein supply (Schwab, et al., 2005).

The objectives of this Ph.D. thesis were (i) to develop and evaluate feed carbohydrate and protein fractionation schemes to improve predictions of dietary supply of RDP and RUP and microbial protein supply, and (ii) conceptualize and develop a dynamic model of N fluxes in dairy cows that characterizes the role of N excretion and recycling on N efficiency. The overall objective was to improve the usefulness of nutritional models to accurately balance diets for N. The literature review covers aspects of feed chemistry, N metabolism, and dynamic systems theory that are the basis for the principles and assumptions of the subsequent chapters.

CHAPTER 1

LITERATURE REVIEW: FEED CARBOHYDRATE AND PROTEIN SYSTEMS AND NITROGEN RECYCLING IN RUMINANTS

1.1. Feed carbohydrate and protein fractionation systems

A key aspect of nutritional models is the description and characterization of feed composition and its variability. The level of aggregation in describing feeds is the result of a compromise among quality and availability of inputs, sensitivity and risk of use of the model, and model objectives.

1. 1. 1. Feed carbohydrates

Carbohydrates (CHO) consist of monosaccharide sugars in chains of varying lengths and have the general chemical formula $C_n(H_2O)_n$. They represent the largest component of rations for ruminants. The biochemical description of the CHO most commonly found in feedstuffs is presented in Table 1.1. Starch, fructans, and galactans are storage reserve compounds. Sucrose can be stored in feeds such as sugar beets, but its main function in plants is transport (Van Soest, 1994). Starch is the predominant reserve CHO and is stored in seeds, as well as in leaves and stems of tropical grasses and legumes (Van Soest, 1994). Fructosans are stored in leaves and stems of temperate grasses, and galactans are found in legume seeds (Van Soest, 1994). Pectin, hemicellulose and cellulose are components of the plant cell wall.

Table 1.1. Common carbohydrates found in feedstuffs (Van Soest, 1994).

Carbohydrate		Simple sugar component	Linkage
Monosaccharides	Glucose		
	Galactose		
	Fructose		
Disaccharides	Lactose	Glucose, galactose	β 1-4
	Sucrose	Glucose, fructose	β, α, 1-2
	Cellobiose	Glucose	β 1-4
	Maltose	Glucose	α 1-4
Oligo and Polisaccharides	Dextrin	Glucose	α 1-4, α 1-6
	Fructans	Fructose	β 2-6, β 2-1
	Galactans	Galactose	α 1-6
	Starch	Glucose	α 1-4, α 1-6
	Cellulose	Glucose	β 1-4
	Pectin	Arabinose, galactose	α 1-4
	Hemicellulose	Arabinose, xylose, galactose, glucuronic acids	

For ruminants, if the goal of a nutritional model is to predict animal responses to varying nutrient supply, a CHO scheme should group CHO based on differences in their supply of energy-yielding compounds, and their effect on microbial protein production. Based on these criteria, the most meaningful and simple partition of CHO is between fiber (FC) and non-fiber (NFC). Insoluble dietary fiber is defined as the slowly digestible or indigestible organic matter of feeds that occupies space in the gastrointestinal tract of animals (Mertens, 1997). Differences in the amount and the chemical properties of fiber in a diet can affect animal performance. High levels of fiber in the diet reduce ration digestibility and restrict intake due to their fill effect of fiber (Mertens, 1997). The lower level of digestible energy intake results in reductions in milk production. Conversely, with low levels of fiber in the diet, adverse effects on rumen fermentation can occur and may lead to rumen acidosis. Therefore due to the

importance of balancing diets for fiber content, laboratory methods have been developed that allow determination of fiber in feeds.

The neutral detergent fiber procedure (NDF) is the most widely accepted method for determining fiber content in feedstuffs. Van Soest and Wine (1967) observed that feeds could be divided into a readily available soluble fraction and a fibrous residue that was incompletely digested. They developed the NDF method to match the nutritional definition of fiber (Van Soest and Wine, 1967). A large number of modifications of the method exists. The NDF method approved by the Association of Official Analytical Chemists International (Mertens, 2002) uses sodium sulfite to remove proteinaceous material from the insoluble fiber and amylase to reduce starch contamination,. The NDF method isolates components other than the fibrous CHO (hemicelluloses and celluloses). It also recovers tannin-protein complexes, protein, ash, silica and lignin (Van Soest, et al., 1991). Therefore, NDF assayed with amylase and sodium sulfite and corrected for residual nitrogen (NDICP) and ash is the most accurate way to estimate FC in commercial laboratories.

The NDF values in model feed libraries represent averages determined over a span of many years. A current problem with these values is the lack of consistency in the methods and corrections used to determine them. Particularly in models where NFC is calculated by difference, methods of feed analysis and subsequent corrections affect estimates of both the FC and NFC fractions, and therefore the impact of a given feed on model predictions of digestibility and animal performance.

Rate and extent of degradation of plant cell wall varies with forage species, and maturity (Van Soest, 1994). Lignin, waxes, and the cuticle of the epidermis interfere with microbial degradation of fiber polysacarids by acting as a physical barrier (Wilson and Mertens, 1995). In addition, plant anatomy and cell type influences fiber digestibility (Akin, 1989).

Until recently, the NFC fraction has been treated as a fairly homogenous, highly digestible fraction. In the most recent Nutrient Requirements of Dairy Cattle (NRC, 2001), NFC is assumed to be 98 % truly digestible, and is modified by an adjustment factor based on processing of feed. However, studies indicate that manipulating dietary NFC influences ruminal fermentation, total tract digestion, animal performance, milk composition, and animal health (Hall, 2002). Although the Dairy NRC (2001) only provided recommendations for a maximum concentration of NFC in the diet (~ 32 to 42 % of the diet DM), it acknowledged that the optimal concentration of NFC depends on several factors including type of NFC components, interactions between NFC and both the fiber and protein fractions, processing effects, dry matter intake, and the physiological state of the animal. The interaction of these factors was well illustrated in a study by Heldt et al (1999), which determined the effect of the interaction between different NFC sources and the RDP level in the diet on rumen fermentation in steers. At low RDP levels (0.031 % BW/d), all types of supplemented NFC (starch, glucose, fructose and sucrose) depressed NDF digestibility. At high RDP levels (0.122 % BW/d), supplemented NFC enhanced NDF digestibility compared to the control (unsupplemented). Sugars had a greater effect than starch, and within sugars, monosaccharides had a greater effect than disaccharides. At low levels of RDP, N is the first limiting nutrient, and thus the competition for N between microbes that utilize NFC and FC may become the dominant interaction. As ruminal N level increases, the competition may be overcome and the enhancement of microbial growth through the provision of growth factors such as branched chain volatile fatty acids from microbial turnover may become more evident. Non-fiber CHO and FC interact through different mechanisms. Khaili and Huhtanen (1991) reported a depression of NDF digestibility when sucrose was supplemented at 16 % of the ration (1 kg sucrose). The depression was reversed by

adding buffers (0.25 kg/d of sodium bicarbonate) to the diet. The rates of NDF digestion were decreased by sucrose supplementation, but rates of passage were not affected by neither sucrose or buffer supplementations (Huhtanen and Khalili, 1991). In addition, some ruminal bacteria produced bacteriocins, which may also play a role in depressing fiber fermentation at neutral pH (Piwonka and Firkins, 1996, Rychlik and Russell, 2002).

Carbohydrates also differ in their ability to support microbial growth (Hall and Herejk, 2001, Strobel and Russell, 1986) because of differences in rates of fermentation, predominant fermentative pathways, and allocation of energy between reserves and growth, among other factors. Based on the fermentation products reported by Strobel and Russell (1986) and assuming a maximum yield of microbial mass of 25 g per mmol of ATP, starch is the NFC that is calculated to support the highest level of microbial growth yield, while xylan and pectin supported the lowest yield (Table 1. 2). Overall, pentoses support less microbial growth than hexoses. At pH below 6, microbial protein synthesis was depressed for all the tested soluble CHO (Strobel and Russell, 1986); but , fermentation was depressed only for cellobiose and pectin. Several factors contribute to reduce protein synthesis at low pH, including depression of CHO utilization, switch to low energy lactate-yielding pathways, and energy spilling (Russell, 1998, Van Kessel and Russell, 1996).

Table 1. 2. Production of fermentation acids and methane and prediction of microbial yield when pure carbohydrates (CHO) are digested at neutral pH *in vitro*.

	Fermentation products[1] (mmol/mmol carbohydrate used)								
	Ace-tate	Prop-ionate	Buty-rate	CH_4	Lac-tate	Total	ATP yield[2]	Y_{ATP}[1,3]	Max Y_g[4]
Starch	0.66	0.38	0.10	0.35	0.12	1.61	2.06	14.8	51.4
Sucrose	0.51	0.23	0.12	0.21	0.40	1.47	1.82	16.8	45.6
Cellobiose	0.66	0.28	0.09	0.22	0.24	1.48	1.86	16.6	46.4
Xylan	0.67	0.30	0.04	0.13	0.00	1.13	1.44	15.2	36.0
Pectin	1.16	0.15	0.02	0.09	0.00	1.43	1.68	12.8	42.0

[1] As reported by Strobel and Russell (1986) at neutral pH for a 10 hour incubation.

[2] ATP yield is the amount of ATP produced (mmol ATP) per 100 g CHO fermented. The following mol ATP/mol of end-product were assumed: 2 for acetate, 3 for propionate, 3 for butyrate, 2 for CH_4, 2 for lactate (Isaacson, et al., 1975).

[3] Y_{ATP} is defined as the mg of microbial dry matter produced per mmol ATP.

[4] Y_g is maximum microbial growth yield (g microbial dry matter/100 g CHO), calculated as ATP yield × Max Y_{ATP}. The maximum Y_{ATP} is assumed to be 25 (Isaacson, et al., 1975).

Within NFC, the simplest carbohydrates (mono-, di-, and oligosaccharides) are grouped as sugars, but little research has been done to determine the nutritional equivalence of the compounds included in the sugar fraction for ruminants. *In vitro* studies have shown differences between sugars. *Streptococcus bovis* grew more slowly on lactose than on glucose (Bond, et al., 1998). Galactose derived from lactose was diverted through the tagatose pathway, which resulted in a lower growth (Bond, et al., 1998). Differences in fermentation rates also have been reported for glucose, fructose, and arabinose (Molina, 2002). *In vivo* studies have been less conclusive than *in vitro* studies. Feeding lactose increased proportions of ruminal butyrate, and decreased acetate and branched chain VFA production (DeFrain, et al., 2004), but studies have

failed to show differences in performance between animals receiving supplemental lactose or other sugars such as sucrose (Maiga, et al., 1995).

Non-fiber CHO compounds that are not digested by mammalian enzymes are included in the soluble fiber fraction. These compounds are pectic substances, β-glucans, fructans, and gums (Van Soest, 1994). Despite being classified together, they have different fermentation characteristics. Overall, they are readily digested by microbes (Biggs and Hancock, 1998, Engstrom, et al., 1992, Hatfield and Weimer, 1995). The main product of pectin fermentation is acetate (Table 2), and pectin utilization is depressed at low pH (Strobel and Russell, 1986). Fructans have a VFA profile similar to sugars and can yield lactic acid (Marounek, et al., 1988).

1.1.2. Feed proteins

Feeds contain a wide array of both non amino and amino N-containing components (Figure 1.1). An appropriate criterion for classifying N containing compounds is their ability to supply both microbial and animal N requirements. The N requirements of rumen microorganisms are met by ammonia, amino acids, and peptides. The N requirements of the animal are met with amino acids, and therefore the quantity and quality (profile) of dietary amino acids are important variables to consider. The best way to describe the nutritive value of N compounds in relation to the previous criterion is to describe them according to their ruminal degradation characteristics (NRC, 2001).

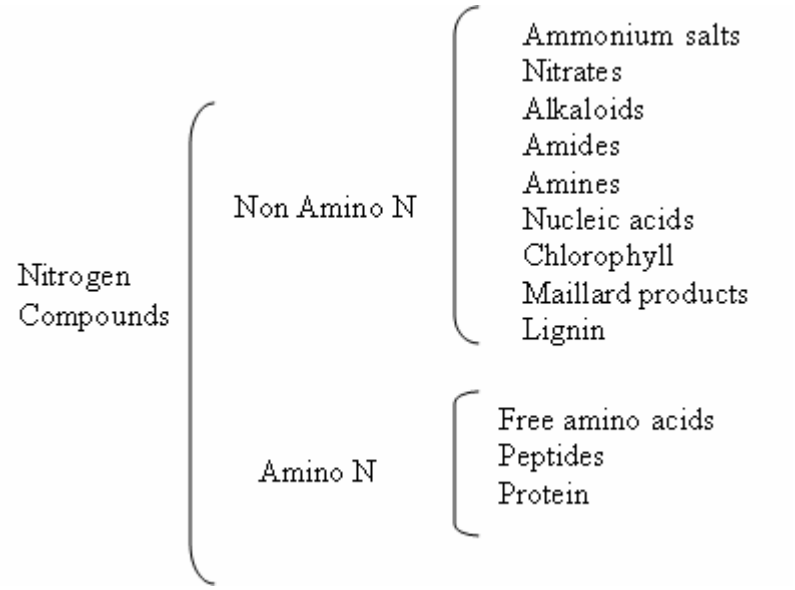


Figure 1. 1. Nitrogen containing components in feeds (Reid, 1994).

The two most common methods used to fractionate N are the *in situ* techniques and the use of solvents. Both methods are discussed in relation to the above criteria in the next section.

1. 1. 2. 1. In situ based fractionation

Fractionations based on *in situ* methods have been the most widely adopted in feed evaluation systems (NRC, 2001) and nutritional models (Dijkstra, et al., 1992, Lescoat and Sauvant, 1995). In the *in situ* method, feed samples are incubated in the rumen inside nylon or Dacron polyester bags. Bags are removed at differing times after commencement of ruminal incubation. Three N fractions are measured (NRC, 2001): an A fraction, which is generally measured as the percentage of N that escapes from the bag during an initial soaking in water, a B fraction, which is the portion of the N associated with particle sizes greater than the pore size of the bag that are susceptible to degradation, and C fraction, which is the percentage of the original N

remaining in the bag at a defined endpoint of incubation. Limitations of the *in situ* method have led researchers to question its usefulness in describing N inputs for balancing N supply with microbial and animal requirements (Schwab, et al., 2005). These limitations include:

(1) The A fraction is assumed to be completely degraded in the rumen (i.e., all RDP), implying that no soluble protein can escape from the rumen, and making no distinction in the N composition of the fraction. However, recent *in vivo* studies showed that some soluble N escapes the rumen as non-ammonia non-microbial N (63-85 g/kg) (Choi, et al., 2002a, Volden, et al., 2002). The A fraction contains variable amounts of NPN, rapidly solubilized protein, and protein in small particles that migrate from nylon bags depending on the feed. The rate of degradation for the small particle fraction may not differ from the rate for the B fraction (Gierus, et al., 2005).

(2) Microbial contamination of the residues results in under prediction of the rates of degradation of the B fraction, especially for high-fiber low-protein feeds (Noziere and Michalet-Doreau, 2000). For high-fiber low-protein feeds, N degradability can be under estimated up to 30 % (Noziere and Michalet-Doreau, 2000). None of the decontamination techniques (i.e. washing, stomaching) removes microbial contamination completely (Noziere and Michalet-Doreau, 2000).

(3) Another issue that arises is that CP degradation may not be equivalent to amino acid degradation. Crude protein degradability tended to be higher compared with total amino acid degradability because the A fraction contains both non amino N as well as amino N (Susmel, et al., 1989, Weisbjerg, et al., 1996). Furthermore, degradabilities differ among individual amino acids; For concentrates, arginine, cysteine, and glutamic acid had a higher effective degradability, and valine, isoleucine, and threonine had a lower effective degradability than average degradability for total amino acids (Hvelplund, et al., 1992). For some feeds, effective degradabilities of

methionine were also lower than the total amino acid treatment (Hvelplund, et al., 1992).

1. 1.2. 2. Solubility based fractionation

The N scheme used in the Cornell Net Carbohydrate and Protein System (CNCPS) fractionates N into five fractions based on solubility; the A fraction is NPN and is analyzed using a protein precipitating agent, the B fraction is true protein and C is unavailable protein (Van Soest, et al., 1981b). The B fraction is further sub-divided into three fractions with different digestion rates (B1, B2, and B3). The B1 fraction is the true protein soluble in borate phosphate buffer, and it is assumed to have very rapid digestion rates (1-4/h). The B3 fraction is insoluble in neutral detergent but is soluble in acid detergent, and it is assumed to represent slowly digestible protein (0.0006-0.0055/h). The C fraction is insoluble in acid detergent solution. The B2 fraction is calculated by difference and is assumed to have rates close to passage rates (0.03-0.16/h). This system of protein fractionation for the CNCPS was first described 25 years ago (Van Soest, et al., 1981b). Some limititations of the system have become apparent through research and field use of the CNCPS.

One of the main problems identified is that there are several disconnects present in the development of the scheme. The assigned digestion rates for the CNCPS protein B fractions in the CNCPS were based on the number of pools and rates identified by a curve-peeling technique using data based on protein *in vitro* solubility when incubated with a protease from *Streptomyces griseus* (Pichard, 1977). Pichard (1977) found that NDICP was highly correlated with the slowly solubilized fraction obtained with the enzyme technique. Subsequently, the rate for the slowly solubilized fraction was assigned to the NDICP (corrected for ADICP) fraction. However, the pool size of the fractions obtained by curve peeling of the enzymatic data do not

always match the pool size of the chemical fractions (Table 1.3), and therefore rates for chemical and enzymatic fractions are not equivalent.

Table 1. 3. Nitrogen fractions based on chemical and enzymatic techniques (Licitra, et al., 1999).

		Chemical data	Enzymatic data[5]	
		Pool size (% N)	Pool size (% N)	Rates (/h)
Alfalfa hay	A + B1[1]	40.1	48.5	--
	B2[2]	57.5	28.9	0.19
	B3[3]	1.5	21.7	0.02
	C[4]	0.9	0.9	0
Blood meal	A + B1[1]	4	1.8	--
	B2[2]	53.9	38.8	0.12
	B3[3]	42.1	63	0.02
	C[4]	0	0	0
Corn gluten meal	A + B1[1]	3.5	2.8	--
	B2[2]	94.5	30.9	0.07
	B3[3]	0.7	65	0.01
	C[4]	1.3	1.3	0
Soybean meal	A + B1[1]	15.5	23.9	--
	B2[2]	75.1	63.4	0.17
	B3[3]	4.5	10.3	0.001
	C[4]	4.9	2.8	0

[1] Chemical fraction is N soluble in buffer solution

[2] Chemical fraction is the N insoluble in buffer solution minus N insoluble in neutral detergent solution (NDIN)

[3] Chemical fraction is NDIN minus N insoluble in acid detergent solution (ADIN)

[4] Chemical fraction is ADIN

[5] The proteolytic enzyme was a protease from *Streptomyces griseus* with a concentration of 0.33 units/mL

In addition, recent studies in which the kinetics of NDICP disappearance has been determined indicated that the digestion rates for the NDICP are considerably higher than are the rates found for the most slowly degraded enzymatic fraction (Coblentz, et al., 1999, Juarez, 1998, McBeth, et al., 2003, Rossi, et al., 1997). With the curve peeling approach, the bias in estimating the slow components is propagated into the estimation of the faster components (Jacquez, 1985), and thus uncertainty in the estimates of the slowest pool transfer to the other identified rates and pool sizes. Inflections in the curves of the natural log of the solubilized N were assumed to be indicative of different first-order pools (Shipley and Clark, 1972). However, inflections in the solubilization curve may also be attributed to other reasons, such as presence of second order kinetics, in which the rate of solubilization is not only a function of the characteristics of the substrate, but also of the enzymatic concentration. End-product accumulation and the decline of the enzymatic activity over time as the proteolytic enzymes degrade themselves results in deviations of the first-order (Krishnamoorthy, et al., 1983). Under these conditions, the pools and rates may be methodological artifacts representative, rather than reflecting intrinsic characteristics of the feed (Mertens, 1993).

The assumption behind the use of N solubility in detergent solutions to fractionate N is that the N associated with NDF is cell wall-bound protein, mostly extensins covalently linked to hemicelluloses. The N insoluble in ADICP is N associated with lignin and Maillard reactions. Sodium sulfite is omitted when analyzing for the NDICP fraction since it is considered that the cleavage of the disulfide bonds by the sodium sulfite is not biologically possible. However, when Pichard (1977) determined the amount of N bound to the cell wall in silages, the differences between the determination with and without Na_2SO_3 were smaller among

silages than hays. Most of the N removed by Na_2SO_3 had been removed during the fermentation process (Pichard, 1977).

There are two types of unavailable N: in forages (lignin-bound N and tannin-protein complexes) and that which is induced by heating and drying. The CNCPS assumes ADICP is indigestible protein completely indigestible, based on the observation that there was a good relationship between ADICP and indigestible N for heat-damaged silages, hays, and dehydrated alfalfa (Goering, et al., 1972). However, additional ADICP produced by heating was partially digested in steamed treated alfalfa (Broderick, et al., 1993), distiller's grains (Nakamura, et al., 1994, Van Soest, 1989), and plant proteins (Hussein, et al., 1995, Nakamura, et al., 1994, Schroeder, et al., 1995), while feeds with a high content of tannins had negative ADICP digestibilites, as the components in the ADICP were binding protein (Waters, et al., 1992). These disparities in behavior reflect the lack of uniformity of the ADICP fraction.

Because peptides and amino acids (AA) may stimulate microbial growth on NFC more than ammonia (VanKessel and Russell, 1996), the distinction between the fraction containing non-amino N and amino-N is important. The CNCPS uses precipitant agents (i.e. trichloroacetic acid, tungstic acid) to partition A and B1 fractions (Sniffen et al., 1992). However, methods based on protein precipitation are not widely available commercially and the factors affecting peptide recoveries have not been fully investigated. It seems that factors other than peptide length affect their precipitation (Hedqvist, 2004).

1.2. Rumen protein digestion

Ruminal N metabolism is a highly complex process that includes multiple steps, including protein hydrolysis, peptide degradation, amino acid deamination, and various pathways of carbon metabolism. Overall microbial N metabolism is highly

related to microbial carbohydrate and energy metabolism (Figure 1.2; (Cotta and Russell, 1996)).

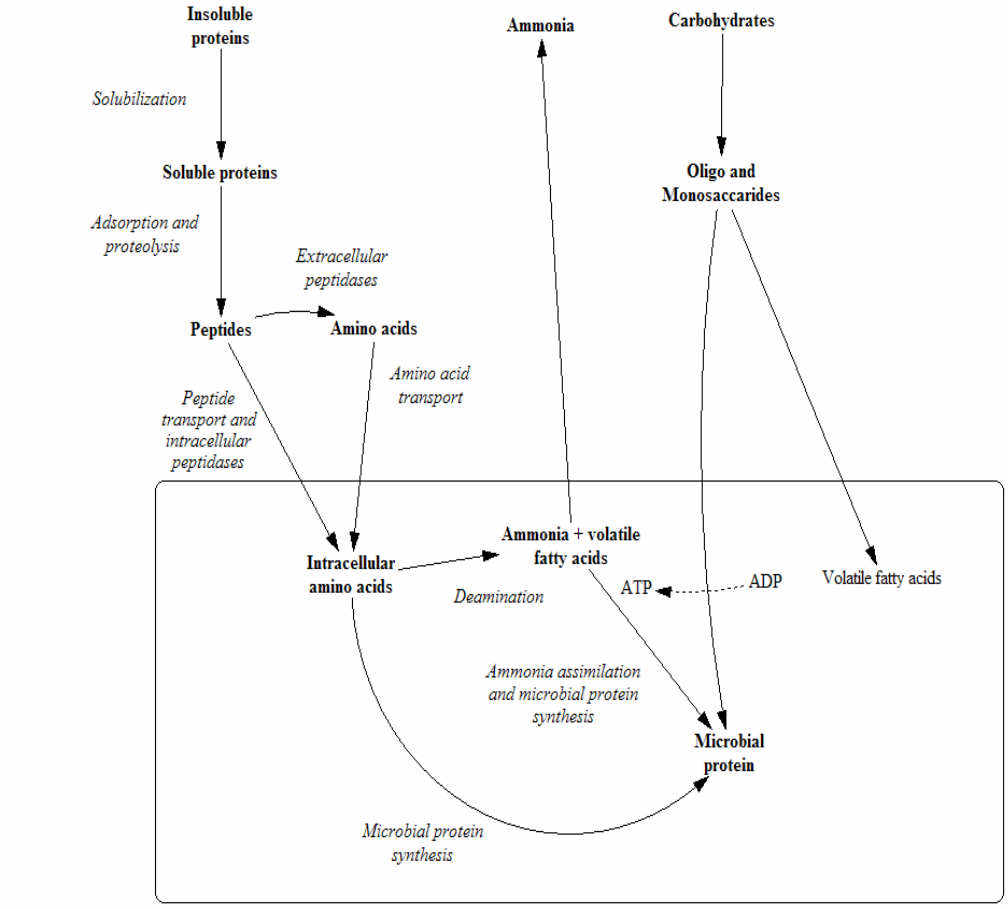


Figure 1. 2. Ruminal nitrogen metabolism pathways, adapted from Russell et al. (1989).

Proteolytic activity is predominantly associated with feed particles, mainly with the small-particle phase (Brock, et al., 1982). Proteolytic activity of the bacteria is of more significance than protozoal or fungal activity (Cotta and Russell, 1996). Proteases are mostly located on the cell surface of bacteria, and thus adsorption of the protein to bacteria is a prerequisite for proteolysis (Broderick, et al., 1989). Among

bacteria, amylolytic bacteria are considered the predominant proteolytic bacteria; *Prevotella spp., Butyrivibrio fibrisolvens, and Streptococcus bovis* are the major organisms involved in protein breakdown because of the high number in the rumen (Cotta and Russell, 1996).

Mixed ruminal protozoa have greater capacity to degrade insoluble, particulate protein than soluble proteins; they engulf and digest chloroplasts (Cotta and Russell, 1996).

The level and type of proteolytic activity in the rumen is highly variable (Falconer and Wallace, 1998). In addition, diet influences rate of proteolytic activity. Feeding highly fermentable diets is associated with an increase in proteolytic activity due to an elevation in the total microbial population (Siddons and Paradine, 1981). High levels of proteolytic activities associated with fresh forage diets have been attributed to an increase in proteolytic activity (Cotta and Russell, 1996). Despite variability in proteolytic activity, no relationships between proteolytic activity and *in situ* rates of protein degradation has been reported (Siddons and Paradine, 1981). Possible reasons for this are, (1) enzymes others than proteases may limit the rate of degradation when the protein is embedded in a matrix, (2) proteolytic activity is in excess, and (3) lack of sensitivity of the *in situ* technique. Chemical and physical characteristics of feeds largely determine rate and extent of protein degradation (Stern, et al., 1994). The effect of protein structure is more evident for soluble proteins. Degradation rates were roughly in proportion to the number of disulfide bonds (Broderick, et al., 1989). Heat treatment, which decreases rumen protein degradability, resulted in a decrease in the percentage of α-helixes and an increase in the percentage of β-sheets (Yu, 2005).

Proteolysis has been proposed to be the main rate-limiting step in ruminal protein degradation (Broderick, et al., 1989). However, *in vivo* experiments showed

(Chen, et al., 1987) that with some diets, accumulation of peptides took place after feeding. Peptidases are cell associated, therefore, so peptide transport and extracellular peptidase activity is not easy to differentiate (Russell, et al., 1989). Following bacterial uptake of small peptides and free AA, there are five distinct intracellular events: (1) cleavage of peptides to free AA, (2) utilization of free AA for protein synthesis, (3) catabolism of free AA to ammonia and carbon skeletons (deamination), (4) utilization of ammonia for re-synthesis of AA, and (5) diffusion of ammonia out of the cell (Figure 1. 2) (NRC, 2001).

1.2.1 *In vitro* methodology

In vitro methods have been extensively used to mimic ruminal digestion and to estimate digestion rates of both feed carbohydrates and proteins. Determining *in vitro* protein digestion presents both methodological challenges. Measuring disappearance of feed proteins is complicated by microbial contamination, while ammonia release is under estimated due to the simultaneous uptake of ammonia for microbial growth. Approaches used to circumvent these problems include (1) the use of inhibitors of microbial protein metabolism, (2) corrections for microbial contamination, and (3) and the use of cell-free enzymes.

1.2.1.1. *In vitro* system with inhibitors

Broderick (1987) used chloramphenicol and hydrazine sulfate to fully recover the products of proteolysis. Chloramphenicol inhibits protein synthesis by blocking formation of amino acyl-tRNA, while hydrazine sulfate inhibits amino acid deamination and NH_3 incorporation (Broderick, 1987). The use of inhibitors did not depress proteolytic activity in short-term incubations (< 4 hours) as judged by the estimates of protein degradation rates obtained (Broderick, 1987), but microbial growth was affected in longer incubations (24 hours) (Siddons, et al., 1982). Although it is possible that the use of short term incubations biases the protein degradation rates

towards the more rapidly degradable protein, the method has proved to be sensitive enough to predict genetic variation for protein degradability in forages (Broderick, et al., 2004a). However, the system may be subject to end-product inhibition, particularly for rapidly degraded proteins. Additionally, the accuracy is reduced for either feeds such as silages, with high levels of ammonia and free amino acids, and for those containing very slowly degraded proteins (Broderick and Cochran, 2000).

1.2.1.2. Corrections for microbial contamination

Ruminal inoculum combined with labeled ammonia (^{15}N) or amino acids (^{14}C) can be used to quantify microbial uptake of protein breakdown products (Atasoglu, et al., 2004, Atasoglu, et al., 2001, Hristov and Broderick, 1994). An indirected way to correct for microbial metabolism was developed by Raab et al. (1983). They determined simultaneously gas production and ammonia release and developed linear regressions between the gas produced and ammonia released. They extrapolated the amount of ammonia which would be released when no fermentable CHO were available. Deviations from linearity were found when a large amount of starch was added to high protein feeds or very low protein content feeds. With high protein feeds, a variable amount of peptides and amino acids were incorporated directly into microbial protein without undergoing deamination, while with low protein content feeds and energy excess conditions, energy spilling occurs, and gas production is disconnected from microbial growth. A different approach was taken by Klopfenstein and colleagues (Haugen, et al., 2006, Mass, et al., 1999). They assumed that treatment with neutral detergent removed microbial contamination and all N removed by the neutral detergent solution was of microbial origin, and therefore the primary fraction of rumen escapable protein was the neutral detergent insoluble crude protein (NDICP) (Mass, et al., 1999). For the forages tested, the assumption seemed reasonable, since estimates calculated using total N corrected for microbial contamination did not differ

from those calculated using NDICP (Mass, et al., 1999). However, the method is not suitable for protein concentrates because in most cases the NDICP represents a small percentage of the total N, and N other than NDICP escapes from the rumen.

1. 3. 1. 3. Cell-free enzymes

Another way of avoiding the problem of microbial contamination is the use of cell-free enzymes. Techniques based on commercial proteases have been extensively studied because there is no need for cannulated animals and they are easier to standardize. However, given the complexity of ruminal protein metabolism and the factors that influence it, it seems unlikely that a single commercial protease would be able to mimic ruminal digestion of protein by microbes. Theoretically, a complex mixture of commercial proteases with activities similar to those found in the rumen/ or microbial-cell preparations could be adequate to mimic rumen proteolysis (Kohn and Allen, 1995, Luchini, et al., 1996). Luchini et al. (1996) tested a mixture of commercial enzymes (trypsin, carbohypeptidase B, chymotrypsin, and carboxypeptidase A). The mixture could not detect differences in digestion rates because of heat damage and did not mimic the digestion rates obtained with strained ruminal fluid.

1. 2. 2. Kinetics of protein digestion

Concepts of classic enzymatic kinetics have been widely applied in modeling digestion in the ruminant. Despite the occurrence of complicated reaction pathways, kinetics of protein digestion generally show simple decay curves with apparent first-order behavior. In a first order rate reaction, at any given moment, a constant fraction (k) of the substrate (S) present undergoes conversion to product over time (t);

$$\frac{dS}{dt} = -kS \qquad [1.1]$$

Graphical procedures can be used to determine the order of a reaction from experimental data (Segel, 1976). The most widely used approach involves plotting transformed time series data and examining the plot for linearity. Another useful plot is called the "phase plot". In a phase plot, the net rate of change of a state variable (i.e. velocity of substrate depletion) is plotted against the state variable itself (i.e. substrate) (Edelstein-Keshet, 1988). Figure 1.3 shows the typical decay curve for a first-order behavior (Panel A). For a first-order reaction, the phase plot (Panel B) and the log transformed plot (Panel C) are linear.

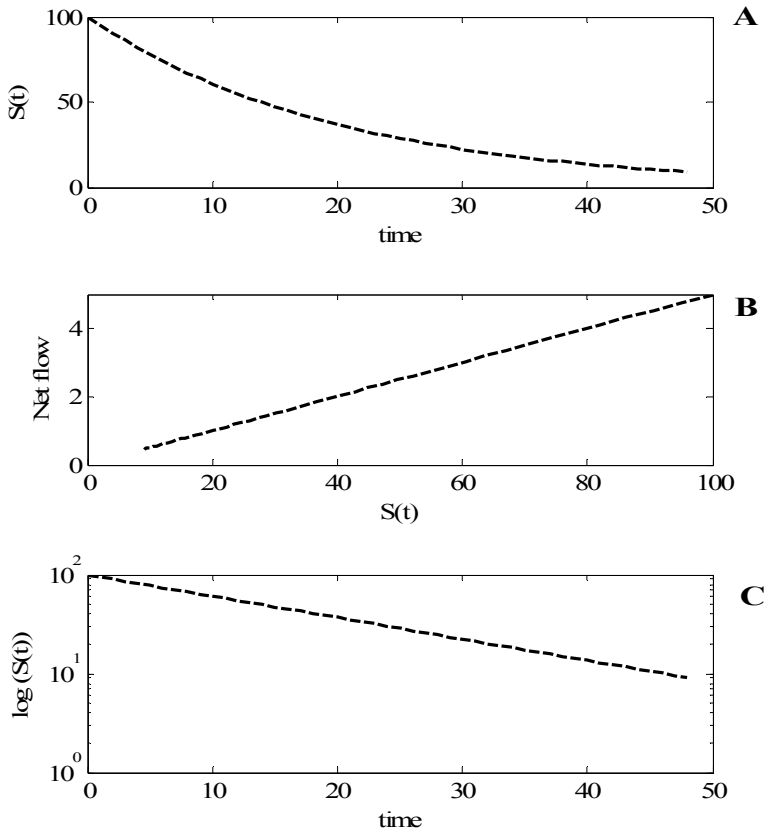


Figure 1.3. Decay curve (Panel A), phase plot (Panel B) and the log transformed plot (Panel C) for first-order kinetics.

The well known Michaelis-Menten plot is an example of a phase plot (Figure 1. 4). Its hyperbolic shape reflects the characteristic that distinguishes enzymatic catalyzed reactions from simple chemical reactions; the dependency of the order of the reaction on substrate concentration (Cornish-Bowden and Wharton, 1988). At a very low substrate level, the velocity of the reaction is essentially linear (first-order); at very high substrate levels, the velocity is essentially independent of the substrate level (zero-order); at intermediate substrate concentrations, velocity follows neither first-order nor zero-order kinetics. The Michaelis-Menten equation ($V = \frac{V \max[S]}{km + [S]}$) is a rather empirical expression describing the plot, in which Vmax represents the maximum velocity that is reached when all the available enzyme is occupied, and km represents the substrate concentration at which the velocity of the reaction is half the maximum velocity.

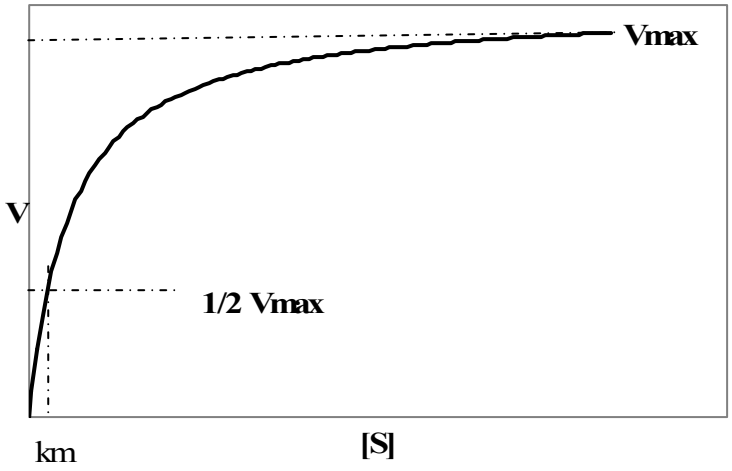


Figure 1. 4. Michaelis-Menten plot

In describing protein digestion as a first-order process, it is assumed that the reaction is substrate limiting, and therefore enzymes/microbes are in excess and that the overall rate of the reaction reflects the rate-limiting step, generally that of proteolysis. In addition, it is assumed that the rate limiting step of the reaction is linked to intrinsic characteristics of feeds and thus the fractional rate is treated as a property of feeds (Mertens, 1993). Nevertheless, it has been shown that more complex reaction mechanisms can give rise to simple decay curves, and thus the interpretation of a simple exponential behavior is more complicated (Srividhya and Schnell, 2006). Bandstra and Tratnyek (2005) demonstrated that the aggregate behavior of multiple reactions of different orders produced a behavior indistinguishable from first-order kinetics. Therefore, in choosing the appropriate kinetic model, emphasis should be placed not only in the empirical modeling of the data, but in theoretical considerations.

1.3. Dynamics of nitrogen cycling

1.3.1 Principles of control and regulation

Animals are biological systems characterized by high complexity and high control. Most biological systems are more than the sums of their parts[1]; they function by virtue of controlled interactions or regulations between their parts (Kalmus, 1966). Two levels of regulation, homeostatic and homeorhetic, take place in animals. Homeostatic regulations smooth nutrient and metabolic flows to maintain a constant internal environment, while homeorhetic regulations controls metabolism in support of the predominant physiological process (Bauman and Currie, 1980). Both homeostatic and homeorhetic regulations involve feedback mechanisms whereby some function of the output of a system is passed to the input. Two types of feedbacks exist; positive and negative feedbacks. Negative feedbacks cause the influence of a disturbance to a

[1] Because biological systems are nonlinear systems. In contrast, the behavior of a linear system is the sum, or superposition, of its components.

regulator to be minimized, so that the system maintains, within limits, a constant output (Milhorn, 1966). Positive feedback leads to continually increasing output after an initial disturbance, and gives the system the ability to access new equilibria (Milhorn, 1966). Positive feedbacks play a key role in regulation of growth and morphogenesis, and reproduction (i.e. onset of puberty or ovulation), while most of the regulation of the endocrine system is mediated through negative feedbacks (e.g. glucose metabolism). Components of the feedback loop are related by causal links (i.e. insulin increases glucose uptake) and each causal link has a polarity. If the dependent variable has the same directionality as the independent variable, the polarity is positive. When the independent variable increases, the dependent variable decreases or vice versa, the polarity is negative. The polarity of the complete loop is the product of the polarities of the causal links of the loop. Formally, the loop polarity is defined as the sign of the open loop gain of the feedback (Eq. 1. 3) (Richardson, 1995). The gain of a feedback refers to the strength of the signal return by the loop. The open loop gain is the partial derivative or the feedback effect of a small change in a variable as it returns to itself. The open loop gain is calculated by the chain rule from the gains of the individual links of a loop (Richardson, 1995).

$$\text{Open loop gain} = \frac{\partial x_1^O}{\partial x_1^I} = \left(\frac{\partial x_1^O}{\partial x_n}\right) \times \left(\frac{\partial x_n}{\partial x_{n-1}}\right) \times \ldots \times \left(\frac{\partial x_2}{\partial x_1^I}\right) \qquad [1.2]$$

$$\text{Loop polarity} = SGN\left(\frac{\partial x_1^O}{\partial x_1^I}\right) = SGN\left[\left(\frac{\partial x_1^O}{\partial x_n}\right) \times \left(\frac{\partial x_n}{\partial x_{n-1}}\right) \times \ldots \times \left(\frac{\partial x_2}{\partial x_1^I}\right) \right] \qquad [1.3]$$

,where SGN is a sign function, returning +1 if its argument is positive and -1 if the argument is negative.

Compartmental models are described by a system of differential equations, in which each compartment is represented by a single differential equation, as demonstrated below.

$$\frac{dx}{dt} = ax + by$$

$$\frac{dy}{dt} = cx + dy$$

can be written in matrix format as, $\dot{x} = Ax$, where $A = \begin{pmatrix} a & b \\ c & d \end{pmatrix}$ and $x = \begin{pmatrix} x \\ y \end{pmatrix}$.

The eigenvalues (λ) of the matrix A indicate the qualitative behaviors the system is capable of (Figure 1. 5). Eigenvalue analyses have been widely used to analyze model behavior and provide qualitative solutions in linear models (Edelstein-Keshet, 1988), and more recently in nonlinear models (Edelstein-Keshet, 1988) and loop dominance analysis (Kampmann and Oliva, 2006, Oliva, 2004).

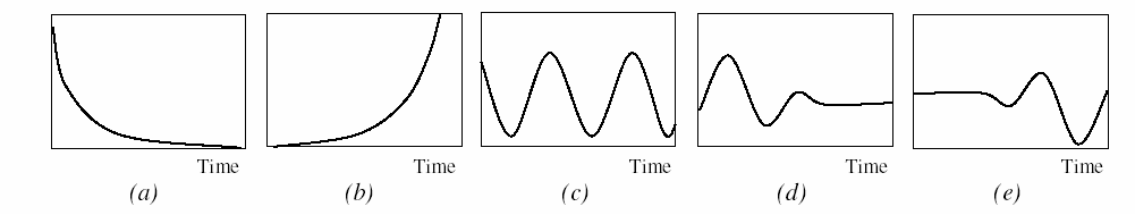


Figure 1. 5. Model behaviors when the eigenvalues are (a) real negative, (b) real positive (c) complex conjugate pair with zero real parts, (d) complex conjugate with negative real parts, and (e) complex conjugate with positive real parts.

Most complex behaviors evolve from the interactions between various feedback loops in the system (Sterman, 2000). The most influential structure in determining some segments of the dynamics of a system is called loop dominance

(Richardson, 1995). For analyzing loop dominance, the eigenvalues of the gain matrix are calculated. The gain matrix (G) is the matrix containing the slopes of the relationship between the net rate of the state variables and the state variables themselves (Kampmann and Oliva, 2006).

$$G = \begin{pmatrix} \frac{\partial \dot{x}_1}{\partial x} & \cdot & \frac{\partial \dot{x}_1}{\partial x_n} \\ \cdot & \cdot & \cdot \\ \frac{\partial \dot{x}_n}{\partial x_1} & \cdot & \frac{\partial \dot{x}_n}{\partial x_n} \end{pmatrix}$$

1.3. 2. Nitrogen recycling

The need to decrease the N content of diets has renewed interest in the mechanisms of N recycling in ruminants and the potential for manipulating N recycling in order to improve its transformation into anabolic products. Recycling of N takes place at different levels and scales (Egan, et al., 1986). At the body level, continual synthesis and breakdown of body protein takes place. At the rumen level, as much as 50 % of the microbial mass is turned over before N passes to the lower gut (Wells and Russell, 1996). Part of the urea in the body is transferred back to the gastrointestinal tract in order to provide N substrate for microbial synthesis. Both protein turnover and intra-ruminal recycling are mostly perceived as sources of inefficiency because they decrease the amount of dietary N transformed into anabolic form. Nevertheless, these recycling mechanisms are beneficial to they animal system by providing plasticity and flexibility, and thus the ability to adapt and respond to a number of physiological and environmental challenges (Lobley, 2003, Stone, et al., 1996).

While metabolites such as glucose are tightly controlled, dynamics of other metabolites, such as urea, are mostly dominated by the presence of different

compartments with different turnover and transfer rates, and the presence of time delays (Sauvant, 1994). Despite this, a remarkable level of regulation of urea metabolism is achieved when low protein diets are fed to ruminants which allows the animal to salvage needed N. As a general trend, the amount of urea recycled back to the gastrointestinal tract (GIT) increases with higher N intakes, but the percentage of synthesized urea that re-enters the gastrointestinal tract decreases as the amount of N fed increases (Figure 1.6).

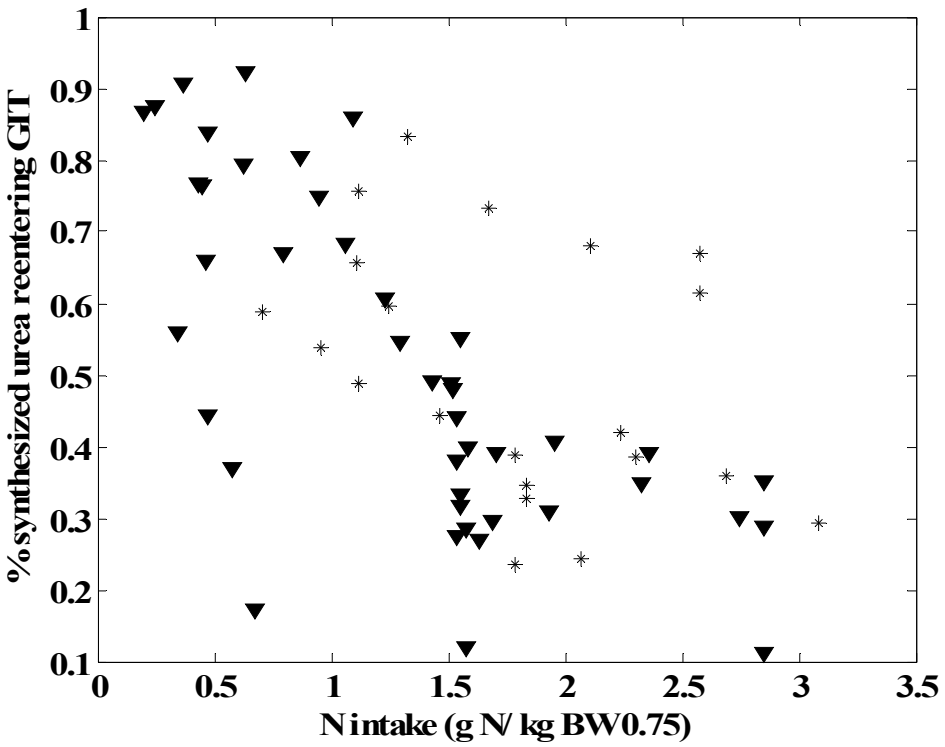


Figure 1.6. Percentage of urea synthesized that reenters the gastrointestinal tract (GIT) in relation to N intake for sheep (▼) and growing cattle (*). Data from Allen and Miller (1976), Bunting et al (1989), Hettiarachchi (1999), Kennedy (1980), Kennedy et al (1981), Marini and Van Amburgh (2003), Marini et al (2004a), Nolan and Leng (1972), Nolan and Stachiw (1979), Norton et al (1982), Obara et al (1993, 1994).

From a feedback perspective, urea metabolism can be represented by the interaction of two main feedbacks (Figure 1.7). Recycling mechanisms are positive feedbacks. In essence, an increase in urea pool size increases the amount of N that is recycled back to the GIT, which in turn increases the N returned to the body urea pool size. Renal excretion is the main negative feedback that counterbalances the "build-up" of N. When the urea pool increases, excretion increases, decreasing the urea pool.

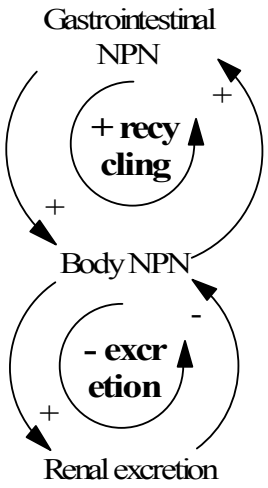


Figure 1.7. Schematic representation of the main feedbacks included in urea (NPN) metabolism. Arrows represent causal links between variables. The positive sign at the arrowheads indicates that both variables have the same directionality, while the negative sign indicates that as one of the variable increases, the dependent variable decreases or vice versa. Positive and negative feedback loops are represented by positive and negative signs within the semi-circle arrow.

Table 1.4 summarizes the equations of a simple N compartmental model that includes the feedbacks represented in Figure 1.7. Degraded CHO are used by microbes with an efficiency Y. Protein degrades to ammonia, which is taken up by the microbes or absorbed through the rumen wall.

Table 1.4. List of the equations for a four-compartment model of nitrogen transactions (Carbohydrates (CHO) and protein (PROT) digested in the gastrointestinal tract (GIT), non-protein nitrogen (NPN) for urea metabolism (GIT and body)).

Mathematical statement	Description
Differential equations	
$\frac{dCHO_{GIT}}{dt} = CHO\,\text{int} - CHO\,\text{deg} - CHOpas$	Carbohydrates pool, g
$\frac{dPROT_{GIT}}{dt} = PROT\,\text{int} - PROT\,\text{deg} - PROTpas$	Protein pool, g
$\frac{dNPN_{GIT}}{dt} = PROT\,\text{deg} + NPNrec - NPNabs - NPNup$	GIT non-protein N pool, g
$\frac{dNPN_{BODY}}{dt} = NPNabs - NPNrec - NPNexc$	Body non-protein N pool, g
Flows	
CHOint= DMintake × CHO	CHO intake, g/d
CHOdeg= $CHO_{GIT} \times kd_{CHO}$	Degraded CHO, g/d
CHOpas= CHO_{GIT} / MRT_{GIT}	Passage CHO, g/d
PROTint= DMintake × PROT	PROT intake, g/d
PROTdeg= $PROT_{GIT} \times kd_{PROT}$	Degraded PROT, g/d
PROTpas= $PROT_{GIT} / MRT_{GIT}$	Passage PROT, g/d
NPNrec= $NPN_{BODY} \times krec$	Recycled NPN, g/d
NPNabs= $NPN_{GIT} \times kabs$	Absorbed NPN, g/d
NPNup=CHOdeg × Ymic × Nmic	Uptake of NPN by microbes, g/d
NPNexc= $NPN_{BODY} \times kexc$	Excreted NPN, g/d
Constants	
$kd_{CHO} = 2.4$	Fractional rate of CHO degradation, d^{-1}
$MRT_{GIT} = 1.6$	Mean retention time, d
$kd_{PROT} = 2.4$	Fractional rate of PROT degradation, d^{-1}
krec = 3.2	Fractional rate of NPN recycling, d^{-1}
kabs = 12	Fractional rate of NPN absorption, d^{-1}
Ymic = 0.35	Microbial yield by unit of degraded CHO, g/g
Nmic= 0.1	Microbial nitrogen content, g/g
kexc= 2.6	Fractional rate of NPN excretion, d^{-}

The model in Table 1.4 assumes that gastrointestinal NPN pool size determines the amount of NPN excreted or recycled. Therefore, the NPN flows are represented as linear functions of body NPN pool size with constant transfer rates (Table 1.4.). For a model with this structure, the resultant open-loop gains are the same independently of the initial values used to determine the gain (Milhorn, 1966). Opening the recycling feedback in the NPN_{GIT} pool (NPN_{GIT} : NPNabs: NPN_{BODY}:NPNrec:NPN_{GIT}), the open gain of the recycling loop is 38.4. For the renal excretion feedback, the open gain is -2.6. The strength of the loops remains constant, and thus a re-partition of the flows between GIT and kidney as displayed in Figure 1.6 can not occur, which suggests that factors other than urea pool size mediate the process.

Mazanov and Nolan (1976) developed first-order linear models of N metabolism for sheep. They concluded that dynamics of N metabolism in sheep were adequately described by constant first-order kinetics. However, the body N pool and flows such as N body losses and recycling were not well represented, and the data were limited to mature sheep fed forage diets. The authors did acknowledge that variable-coefficient models would be more appropriate in representing N transactions.

1. 3. 3. Renal urea excretion

Clearance of a substance from the body is defined as the volume of distribution that is completely cleared per unit of time (Koeppen and Stanton, 1997). The volume of distribution of the urea is the total body water since urea is rapidly distributed throughout this water pool (Visek, 1968). Urea is freely filtered at the glomerulus and partly reabsorbed at the collective tube and renal pelvis (Cirio and Boivin, 1990). Therefore, renal urea clearance can be described as a function of the glomerular filtration rate (GFR, L/d) and its partial reabsorption at the tubular level (cr, coefficient of reabsorption) (Koeppen and Stanton, 1997), with the following equation,

$$\text{Renal clearance (L/d)} = (1 - cr) \times \text{GFR} \quad [1.4]$$

The renal urea excretion (g/d) then can be then calculated as renal urea clearance (L/d) times blood urea concentration (g/L).

The first step in the formation of urine is the production of an ultrafiltrate in the plasma by the glomerulus. The concentrations of non-protein solutes are similar in the plasma and in the ultrafiltrate (Koeppen and Stanton, 1997). Glomerular filtration rate can be determined by the clearances of inulin or creatinine, because these compounds are not subject to reabsorption or active excretion after their filtration (Koeppen and Stanton, 1997). The renal responses that have been described with the feeding of low protein diets include decreased renal plasma flow and GFR (Cirio and Boivin, 1990, Tebot, et al., 2002). However, over a wider range of N intakes, GFR was not significantly related to N intakes (Delaquis and Block, 1995a, Delaquis and Block, 1995b, Maltz and Silanikove, 1996, Marini, et al., 2004a, Marini and Van Amburgh, 2003, Thornton, 1970, Valadares, et al., 1999). Glomerular filtration rate and renal plasma flow are normally held within a narrow range by a process called autoregulation (Koeppen and Stanton, 1997). Two mechanisms are responsible for this autoregulation: one that responds to changes in arterial pressure (myogenic mechanism), and one that responds to changes in the flow rate of tubular fluid (tubuloglomerular feedback) (Koeppen and Stanton, 1997).

Urea reabsorption is mediated through facilitated and active transporters (Sands, 2003). For growing animals, the coefficient of reabsorption (estimated from the ratio between creatinine and urea clearance) had a negative linear relationship with N intake (expressed as percentage of $BW^{0.75}$) (Figure 1.8). Regulated expression of urea transporters is important to deal with varying protein intake (Bagnasco, 2005). Responses to low protein diets include upregulation and increased expression of urea transporters (Bagnasco, 2005).

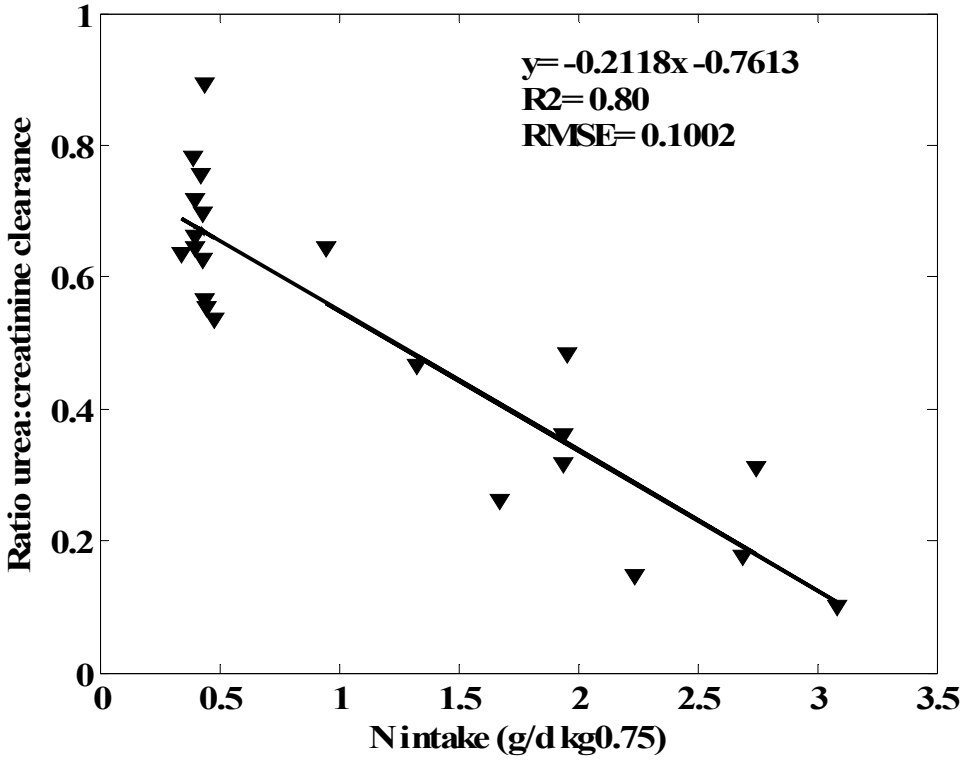


Figure 1.8. Relationship between N intake and the ratio of urea:creatinine clearance for growing animals (N= 22). Data from Boldizarova et al. (1999), Marini and Van Amburgh (2003), Marini et al., (2004a), and Thornton (1970).

1.3.4. Gastrointestinal urea recycling.

Urea is recycled back to the GIT through all sections of the gut wall and saliva (Lapierre and Lobley, 2001). Saliva and gut wall entry are controlled by different mechanisms. The saliva urea entry depends on the saliva flow, which in turns depends on the chewing activity of the animal (Beauchemin, 1991).

Urea entry through the GIT wall is not a simple function of body urea pool size (Egan, et al., 1986). Earlier studies in which urea was infused intravenously and then changes in rumen ammonia concentration (RAN) were measured, found that RAN

increased linearly with increases in blood urea concentrations (BUN), but RAN reached a plateau at approximately BUN of 0.08-0.10 g N/L (Thornton, 1970, Vercoe, 1969). Data from growing sheep and cattle are summarized in Figure 1.10. Rumen wall urea clearance (L/(d×kg$^{0.75}$) had a negative linear relationship with BUN (y= 4.70-18.06×BUN, R^2=0.48, RMSE=1.28) and with RAN (y= 3.87-12.73×RAN, R^2=0.36, RMSE= 1.42) concentrations. Although no clear trend was found between N intake and rumen wall clearance (Figure 1.9), studies have reported increased GIT clearance as levels of dietary N were lowered (Marini and Van Amburgh, 2003).

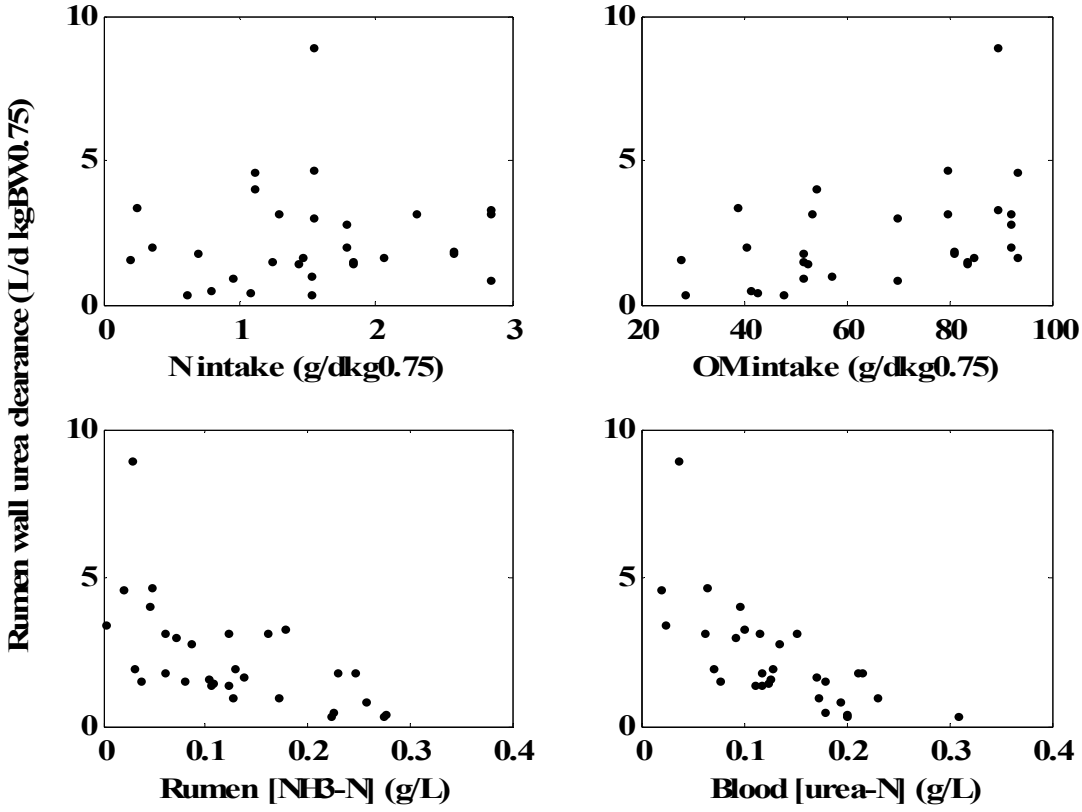


Figure 1.9. Relationships between rumen wall urea clearance and N intake, OM intake, rumen ammonia, and blood urea concentrations for growing ruminants (Hettiarachchi, et al., 1999, Kennedy, 1980, Kennedy, et al., 1981, Norton, et al., 1982, Obara, et al., 1994).

Organic matter intake also had a positive linear relationship with rumen wall urea clearance (Figure 1.9, y= 0.035 × OM intake, R^2= 0.19, RMSE= 1.6). Organic matter fermentability may increase rumen urea transfer through multiple mechanisms (Figure 1.10). The volatile fatty acids produced during fermentation may have a direct effect on the permeability of the rumen wall to urea. Feeding highly fermentable diets is related to increased number and size of rumen wall papillae, and therefore, greater surface area, and to an increase in the surface area of the epithelial capillary network (Remond, et al., 1996). Highly fermentable diets may affect rumen wall clearance indirectly by decreasing RAN; Volatile fatty acids also facilitate ammonia absorption (Bodeker, et al., 1992), because ammonia assimilation is facilitated as the high affinity ammonia assimilation system, which permits ammonia uptake at very low RAN concentrations, and requires ATP (Nolan, 1993).

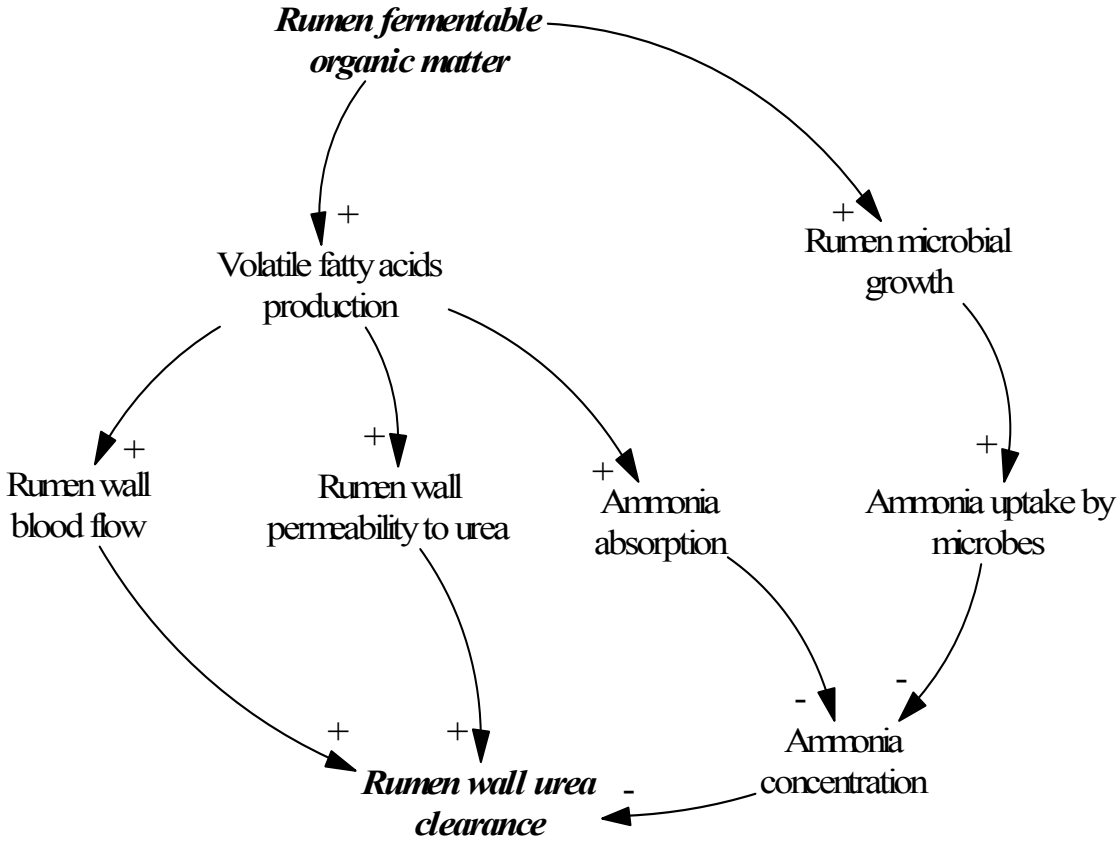


Figure 1.10. Some of the possible pathways through which fermentable organic matter can increase urea transfer. Arrows represent causal links between variables. The positive sign at the arrowheads indicates that both variables have the same directionality in response, while the negative sign indicates that as one of the variables increases, the dependent variable decreases or vice versa.

1. 3. 5. Efficiency of use of recycled nitrogen

In lactating dairy cows, endogenous urea contributed 37.5 % to the bacterial N reaching the duodenum when fed a high-grain diet, and 12.7 % when fed a high-forage diet (Al-Dehneh, et al., 1997). Efficiency of N cycling depends on several factors including:

(1) The residence time of N in the system; urea-N molecules can be recycled to the gut multiple times, and therefore increasing the probability of microbial capture (Lapierre and Lobley, 2001).

(2) The amount and availability of required microbial nutrients nutritional other than ammonia, including amino-N. Although mixed ruminal bacteria have no absolute amino acid requirements (Virtanen, 1966), it is well established that providing amino-N can stimulate microbial growth (Russell, et al., 1989). *In vitro* batch fermentations have shown that the uptake and incorporation of amino-N to microbial cells was linearly related to the relative availability of amino-N (Atasoglu, et al., 1999) . Supplementation of true protein as RDP has either increased (Hume, 1970) or not changed microbial yields (Rooke, et al., 1987). Van Kessel and Russell (1996) demonstrated that peptides and amino acids had little impact on the yield of CHO limited, ammonia-excess cultures, but they improved the growth rate and yield under excess-energy conditions. Amino-N helps to match anabolic and catabolic rates, decreasing the loss of ATP in energy-spilling reactions (Russell, 1993, VanKessel and Russell, 1996). While the incorporation of amino-N is linearly related to its availability, the amount and the type of the responses to supplemented amino-N are not well defined.

(3) The proportion of N attributed to cycling.

(4) Spatial compartmentalization of the rumen. While the urea recycled through saliva may be well mixed at the rumen level, urea transfer through rumen wall may be preferentially used by bacteria attached to the wall (Egan, et al., 1986).

1. 3. 6. Amino acids as a source of urea

Amino acids that are not used for protein synthesis are available as substrates for urea synthesis. Amino acid catabolism includes the catabolism of AA for synthesis of non-protein compounds (e.g. transmethylation reactions, glucose synthesis) and the removal of AA in excess of the animal needs (Bequette, 2003). With the exception of branched chain amino acids, the liver is the main site of amino acid catabolism. A large proportion of the AA presented to the liver are re-circulated AA, and thus the liver catabolizes non-utilized AA, maintaining the blood AA concentrations within certain finites (Lobley, 2003). Estimates of whole body amino acid oxidation for dairy cows have been obtained by using infusions of L[1-^{13}C]leucine (Lapierre, et al., 2002, Lapierre, et al., 2005) (Table 1. 5). Leucine oxidation was found to have a negative linear relationship to leucine used in synthesis for milk protein, while the use for non-milk protein synthesis was fairly constant (55.7 ± 4.05).

Table 1.5. Whole body leucine kinetics determined using constant infusions of L[1-13C] leucine for lactating dairy cows (Lapierre, et al., 2002, Lapierre, et al., 2005).

	Lapierre et al. 2005		Lapierre et al. 2002	
	6 weeks lactation	25 weeks lactation	High MP[2]	Low MP
Production and nutrient supply				
DMI intake, kg/d	25.4	25	17.8	18.1
N intake g/d	670	671	465	472
Milk kg/d	45.5	35.4	NR	NR
Milk protein yield, kg/d	1.43	1.22	NR	NR
Leucine kinetics, mmol/h				
Whole body ILR[1]	114.5	112.9	105.3	84.9
Oxidation	15.9	17.7	22.6	18.7
Synthesis	98.6	95.2	82.7	71.2
Milk protein output	44.3	38	22.3	20.3
Non-milk protein synthesis	54.3	57.2	60.4	50.9

[1] IRL: Irreversible loss rate, [2] MP: Metabolizable protein, NR: not reported.

This literature review indicates much information is available that can be incorporated into nutritional models to improve accuracy of formulating diets for ruminants. Therefore the objectives of this Ph.D. thesis were to utilize published data to: (1) develop and evaluate feed carbohydrate and protein fractionation schemes to improve predictions of dietary supply of RDP and RUP and microbial protein supply, and (2) conceptualize and develop a dynamic model of N fluxes in dairy cows that characterizes the role of N excretion and recycling on N efficiency. The overall objective was to improve the usefulness of nutritional models to accurately balance diets for N.

CHAPTER 2

A REVISED CNCPS FEED CARBOHYDRATE FRACTIONATION SCHEME FOR FORMULATING RATIONS FOR RUMINANTS[2]

2. 1. Abstract

Balancing ruminant diets for appropriate levels and types of dietary carbohydrates (CHO) is necessary to maximize production while assuring the health of the animals. Several feed fractions (i,e, volatile fatty acids (VFA), lactate, sugars, starch) are now being measured in some commercial feed laboratories and this information may assist in better formulating diets. A CHO fractionation scheme based on ruminal degradation characteristics needed for nutritional models is described and its impact on predictions with the Cornell Net Carbohydrate and Protein System (CNCPS) is assessed. Dietary CHO are divided into eight fractions; the CA1 is volatile fatty acids (VFA), CA2 is lactic acid, CA3 is other organic acids, CA4 is sugars, CB1 is starch, CB2 is soluble fiber, CB3 is available neutral detergent fiber (NDF), and CC is unavailable NDF. A Monte Carlo analysis was conducted with an example lactating dairy cow ration to compare the original CNCPS CHO scheme (CA = sugars and organic acids, CB1 = starch and soluble fiber, CB2 = available NDF, CC = unavailable NDF) with the developed CHO scheme. A database was used to obtain distributions and correlations of the feed inputs used in the schemes for the ingredients of the ration (corn and grass silages, high moisture corn, soybean meal, and distillers' grains). The CHO fractions varied in a decreasing order as VFAs, soluble fiber, lactic

[2] Lanzas, C., C. J. Sniffen, S. Seo, L. O. Tedeschi, and D. G. Fox. 2006. A revised CNCPS feed carbohydrate fractionation scheme for formulating rations for ruminants. Anim. Feed Sci. Technol. In Press.

acid, sugar, NDF, starch, and total non-fiber carbohydrates (NFC). Use of the expanded scheme in the CNCPS decreased the microbial CP production, which was sensitive (standard regression coefficient in parenthesis) to corn silage starch (0.55), grass silage NDF rate (0.46), high moisture corn grain starch rate (0.44), and corn silage NDF rate (0.33). Predicted ruminal NFC digestibility remained similar. The expanded CHO scheme provides a more appropriate feed description to account for variation in changes in silage quality and diet NFC composition. However, to fully account for differences in feed CHO utilization, further improvements in the methodology used to estimate the fractions and their corresponding degradation rates, inclusion of dietary factors in dry matter intake predictions, and prediction of ruminal VFA production and pH are necessary.

2. 2. Introduction

Carbohydrates (CHO) are the largest component of rations for lactating dairy cows, and can be partitioned into fiber (FC) and non-fiber carbohydrates (NFC). Fiber CHO (i.e., hemicelluloses and celluloses) is the slowly digestible fraction of feeds that occupies space in the gastrointestinal tract and fiber CHO associated with lignin resists digestion and therefore does not contribute energy to the animal (Mertens, 1997). Carbohydrates soluble in neutral detergent (ND) solution include organic acids, monosaccharides, oligosaccharides, fructans, pectic substances, β-glucans and starch (Hall, 2003). Balancing for an appropriate level and type of NFC is a major challenge in ruminant ration formulation. Feeds vary widely in their amount and composition of NFC, and CHO fractions in NFC differ in rate and extent of fermentation, products of fermentation, and contribution to microbial CP production (Hall and Herejk, 2001, Nocek and Tamminga, 1991), and therefore to animal performance. For example, lactating dairy cows fed diets with by-product feeds high in soluble fiber and sugars had decreased milk protein and increased milk fat yields (Leiva, et al., 2000,

Mansfield, et al., 1994) and lower N efficiency for milk production (Broderick and Radloff, 2004) than those fed high starch diets. Ruminants fed high starch diets that have increased metabolizable energy (ME) tend to have increased microbial amino acid (AA) supply (Oba and Allen, 2003), but are more predisposed to suffer from ruminal acidosis.

The Cornell Net Carbohydrate and Protein System (CNCPS) (Fox, et al., 2004) accounts for effects of variation in feed CHO fractions in predicted feed ME supply, rumen N, and AA balances when developing diets to meet cattle nutrient requirements. Its current feed CHO fractionation scheme divides NFC into two aggregated fractions; an A fraction, which includes organic acids and sugars and a B1 fraction, which includes soluble fiber and starch (Sniffen, et al., 1992). Several limitations of this scheme have become apparent because these fractions are not precisely defined or analyzed (Alderman, 2001, Offner and Sauvant, 2004, Pitt, et al., 1996). It does not account for all of the variability observed in NFC digestibility when various processing treatments are applied (Offner and Sauvant, 2004) . In addition, the description and ruminal digestibility of the fraction containing starch and soluble fiber were highlighted as an area that needed further improvement to accurately predict ruminal VFA production and pH (Pitt, et al., 1996).

Our objectives are to describe a feed CHO fractionation scheme that classifies CHO based on ruminal degradation characteristics and available analytical methods, to evaluate its impact on CNCPS model behavior and sensitivity, and to discuss its application in ruminant ration formulation.

2. 3. Material and methods

2.3.1 Feed carbohydrate fractionation schemes

2. 3. 1. 1. Original carbohydrate fractionation scheme

In the original CNCPS CHO fractionation scheme (Sniffen, et al., 1992), total carbohydrate content in the j^{th} feedstuff is estimated by difference;

$$CHO_j = 1000 - CP_j - EE_j - Ash_j \quad \text{(g/kg DM)} \quad [2.1]$$

Where: Ash_j is the mineral content of the j^{th} feed, g/kg DM; CP_j is the crude protein content of the j^{th} feed, g/kg DM ; and EE_j is the ether extract content of the j^{th} feed, g/kg DM .

Carbohydrates are divided into FC and NFC, with FC defined as NDF. Within FC, the indigestible fiber fraction (CC) is computed as;

$$CC_j = (NDF_j \times Lignin_j \times 2.4) / 1000 \quad \text{(g/kg DM)} \quad [2.2]$$

Where: $Lignin_j$ is the lignin(sa) content of the j^{th} feed, g/kg NDF; NDF_j is the NDF assayed with amylase and without sodium sulfite (aNDR) content of the j^{th} feed, g/kg DM;

The available FC (CB2) is computed as;

$$CB2_j = NDF_j - (NDICP_j \times CP_j)/1000 - CC_j \quad \text{(g/kg DM)} \quad [2.3]$$

Where: CC_j is the indigestible carbohydrate content of the j^{th} feed, g/kg DM; CP_j is the CP content of the j^{th} feed, g/kg DM; NDF_j is the aNDR content of the j^{th} feed, g/kg DM; and $NDICP_j$ is the ND insoluble CP content of the j^{th} feed, g/kg CP.

Non-fiber carbohydrates are calculated by difference;

$$NFC_j = CHO_j - CB2_j - CC_j \quad \text{(g/kg DM)} \quad [2.4]$$

The NFC is divided into fractions CB1 and CA. The CB1 fraction represents soluble fiber and starch, with its degradation rates ranging from 0.05 to 0.50/h. Tabular values were provided for the soluble fiber (Sniffen, et al., 1992). The CA fraction represents the rapidly fermented (1-3/h) water soluble CHO fraction, and is calculated by difference;

$$CB1_j = CB1NFC_j \times NFC_j \times 1000 \quad \text{(g/kg DM)} \quad [2.5]$$

$$CA_j = NFC_j - CB1_j \quad \text{(g/kg DM)} \quad [2.6]$$

Where: CA_j is the sugar content of the j^{th} feed, g/kg DM; $CB1_j$ is the starch and soluble fiber content of the j^{th} feed, g/kg DM; $CB1NFC_j$ is the starch and soluble fiber content of the j^{th} feed, g/kg NFC, and NFC_j is the non-fiber carbohydrate content of the j^{th} feed, g/kg DM.

The Cornell-Penn-Miner (CPM) dairy implementation of the CNCPS model (Boston, et al., 2000) divided the NFC CA and CB1 fractions. The CA fraction was separated into a silage acids fraction (CPM CA1, containing VFA and lactic acid) with degradation rates of 0/h and a sugar fraction (CPM CA2) with degradation rates of 1-3/h. The CB1 fraction was divided into starch (CPM CB1) and soluble fiber fractions (CPM CB2, containing soluble fiber and organic acids). The CPM CB1 and CPM CB2 have identical degradation rates (0.05 to 0.50/ h).

2.3.1.2. New expanded carbohydrate fractionation scheme

Based on ruminal degradation characteristics and available analytical methods, a new scheme, which further disaggregates the original CNCPS and CPM schemes, was developed. Table 1 lists the equations of the new expanded carbohydrate scheme.

Table 2.1. List of the equations for the expanded carbohydrate fractions (g/kg DM)

Fraction	Description	Equation
CHO	Total carbohydrates	$1000- CP_j- EE_j- Ash_j$
CC	Indigestible fiber	$(NDF_j \times Lignin_j \times 2.4)/1000$
CB3	Digestible fiber	$NDF_j – (NDICP_j \times CP_j)/1000 - CC_j$
NFC	Non fiber carbohydrates	$CHO_j- CB3_j-CC_j$
CA1	Volatile fatty acids	$Acetic_j + Propionic_j + Butyric_j + Isobutyric_j$
CA2	Lactic acid	$Lactic_j$
CA3	Organic acids	$Organics_j$
CA4	Sugars	$Sugar_j$
CB1	Starch	$Starch_j$
CB2	Soluble fiber	$NFC_j – CA1_j – CA2_j- CA3_j- CA4_j- CB1_j$

In the expanded CHO fractionation scheme, CHO and CC fractions are calculated as described in equations 2.1 and 2.2. The available FC (CB2, eq. 2.3) was renamed from CB2 to CB3, since the CB1 (eq. 2.5) is divided into starch (CB1) and soluble fiber (CB2). Similar to equations 2.3 and 2.4, available NDF and NFC are computed as;

$$CB3_j = NDF_j - (NDICP_j \times CP_j)/1000 - CC_j \quad \text{(g/kg DM)} \quad [2.7]$$

$$NFC_j = CHO_j - CB3_j - CC_j \quad \text{(g/kg DM)} \quad [2.8]$$

The CA (eq. 2.6) is divided into 4 fractions; volatile fatty acids (VFA) (CA1), lactic acid (CA2), other organic acids (CA3), and sugars (CA4). Although organic acids (CA1, CA2, and CA3) are not carbohydrates, they are included in the carbohydrate fractions because they are judged to be more closely related to carbohydrates than to fat or protein. Fraction CA1 represents VFA;

$$CA1_j = Acetic_j + Propionic_j + Butyric_j + Isobutyric_j \quad \text{(g/kg DM)} \quad [2.9]$$

Where: $Acetic_j$ is the acetic acid content of the j^{th} feed, g/kg DM ; $Propionic_j$ is the propionic content of the j^{th} feed, g/kg DM ; $Butyric_j$ is the butyric acid content of the j^{th} feed, g/kg DM; $Isobutyric_j$ is the isobutyric acid content of the j^{th} feed, g/kg DM.

The VFA can represent up to 60 g/kg of DM of the silages (McDonald, et al., 1991). Volatile fatty acids, which are end-products of fermentation, are not sources of energy for rumen microorganisms. Therefore, their ruminal degradation rates and maximum rumen microbial growth yield (Y_g) are 0.

The fraction CA2 represents lactic acid;

$$CA2_j = Lactic_j \quad \text{(g/kg DM)} \quad [2.10]$$

In fermented feeds, lactic acid is the predominant organic acid, which can be present at 50-150 g/kg DM (McDonald, et al., 1991). In addition to ensiled feeds, lactic acid may be also present in molasses (Table 2. 2) from degradation of invert

sugar, but also includes malic, citric, fumaric and oxalic acids (Amin, 1980). Lactic acid is mainly converted to acetate and propionate in the rumen, with no direct contribution to glucose flux in the animal (Gill, et al., 1986). Based on gas production measurements, the ruminal degradation rate of lactic acid was measured to be 0.07 /h (Molina, 2002). The CNCPS uses a theoretical Y_g of 50 g of microbial cells for 100 g of CHO fermented, or 0.55 mole of hexose fermented (Isaacson, et al., 1975), which assumes approximately 3.63 moles of ATP per mole of hexose, and an ATP yield of 25 g of cells per mole. However, lactic acid supplies less ATP per mole than CHO. For lactic acid, the Y_g was set to 10.8 g cells for 100 g of lactic acid because it was assumed that, on average, 0.65 mole/mole of lactic acid is fermented via the acrylate pathway, which provides 0.33 mole of ATP per mole of lactate and the remaining is fermented mainly through the succinate-propionate pathway, which yields 0.5 mole of ATP per mole of lactate (Counotte, et al., 1981). The Y_g is then decreased by 20 % to account for protozoa predation (Russell, et al., 1992).

The fraction CA3 represents organic acids other than lactic acid;

$$CA3_j = \text{Other Organics}_j \qquad \text{(g/kg DM)} \qquad [2.11]$$

Organic acids other than lactic and VFA are almost undetectable in silages (McDonald, et al., 1991), but in fresh forages, citric, malic, and aconitic acids can comprise more than 100 g/kg of the forage DM (Dijkshoorn, 1973). Acetate is the primary fermentation product from organic acids (Russell and Van Soest, 1984). Based on gas production measurements, the ruminal degradation rate for organic acids was set to 0.05 /h (Molina, 2002), less ATP per mole than CHO and lactic acid. For the CA3 fraction, the Y_g was set to 3.5 g cells for 100 g of organic acids based on the average yields for malic acid (Dimroth and Schink, 1998) and citric acid (Gottschalk, 1986).

Table 2.2. Carbohydrate fractions measured from the expanded scheme in selected feeds and their corresponding degradation rates

	Fractions[a] (g/kg DM)								Degradation rates (/h)			
	CA1[b]	CA2[c]	CA3[d]	CA4	CB1	CB2	CB3	CC	CA4	CB1	CB2	CB3
Energy rich feeds												
Barley grain, steam-rolled	0	0	0	24	523	61	186	58	0.40	0.35	0.30	0.05
Barley grain, ground	0	0	0	24	523	61	186	58	0.40	0.30	0.30	0.05
Beet pulp, dry	0	0	0	133	30	267	259	91	0.40	0.20	0.40	0.08
Citrus pulp, dry	0	0	0	269	12	344	188	56	0.40	0.30	0.30	0.09
Corn grain, cracked	0	0	0	15	748	8	79	5	0.40	0.10	0.20	0.03
Corn grain, ground fine	0	0	0	15	748	8	79	5	0.40	0.15	0.20	0.06
Corn grain, flaked	0	0	0	16	756	8	76	4	0.40	0.25	0.20	0.06
High moisture corn grain, ground	6	17	0	17	714	14	80	5	0.20	0.30	0.20	0.06
Molasses, beet	0	40	55	700	0	0	0	0	0.40	0.30	0.30	0.05
Sorghum grain, ground coarse	0	0	0	24	564	24	205	34	0.40	0.05	0.20	0.03
Soy hulls	0	0	0	7	10	156	616	32	0.40	0.30	0.08	0.08
Cottonseed, whole	0	0	0	23	2	25	350	310	0.40	0.30	0.30	0.06
Forages												
Alfalfa hay	0	0	30	105	18	200	275	151	0.40	0.30	0.35	0.08
Alfalfa silage	16	48	0	31	15	197	303	206	0.20	0.30	0.35	0.06
Corn silage (processed, 250 g/kg DM)	30	54	0	4	309	4	390	108	0.20	0.40	0.30	0.04

Table 2.2 (Continued)

	Fractions[a] (g/kg DM)								Degradation rates (/h)			
	CA1[b]	CA2[c]	CA3[d]	CA4	CB1	CB2	CB3	CC	CA4	CB1	CB2	CB3
Corn silage (unprocessed, 250 g/kgDM)	30	54	0	4	281	32	395	97	0.20	0.40	0.30	0.04
Corn silage (processed, 350 g/kg DM)	26	46	0	8	309	12	395	97	0.20	0.32	0.30	0.04
Corn silage (unprocessed, 350 g/kgDM)	26	46	0	8	309	12	395	97	0.20	0.25	0.30	0.04
Grass pasture	0	0	40	77	4	82	483	92	0.40	0.30	0.30	0.05
Grass silage	22	46	0	48	23	88	466	106	0.20	0.30	0.30	0.06
Legume pasture	0	0	80	156	6	82	213	97	0.40	0.30	0.35	0.08
Protein rich feeds												
Distillers' grains	0	0	0	34	122	103	187	111	0.40	0.17	0.30	0.07
Soybean meal, solvent	0	0	0	109	22	141	80	6	0.40	0.25	0.30	0.06

[a] CA1 = acetic, propionic and butyric acids, CA2 = lactic acid, CA3 = other organic acids, CA4 = sugars, CB1 = starch, CB2 = soluble fiber, CB3 = available neutral detergent fiber (NDF), CC = unavailable NDF (lignin(sa)× 2.4)

[b] Degradation rate for CA1 is 0/h

[c] Degradation rate for CA2 is 0.07/h

[d] Degradation rate for CA3 is 0.05/h

The fraction CA4 includes monosaccharides, disaccharides, and oligosaccharides;

$$CA4_j = Sugars_j \quad (g/kg\ DM) \quad [2.12]$$

The predominant sugars in feeds are glucose, fructose and sucrose (Knudsen, 1997, Van Soest, 1994). Sucrose is the most common sugar, is the principal means of transport in plants and can be stored as a reserve in feeds such as sugar beets (Van Soest, 1994). In legume seeds, raffinose and stachyose represent an important proportion of sugars (Knudsen, 1997). Sugars produce similar amounts of propionate and higher levels of butyrate than starch and, at low pH, produce more lactate than starch (Strobel and Russell, 1986). Using gas production measurements, Molina (2002) reported fermentation rates of 0.40/h for glucose and 0.16/h for arabinose when fermented with a fiber source. As five carbon sugars support less microbial growth than hexoses (Strobel and Russell, 1986), and based on the composition of the sugar fraction in feeds and their ability to support microbial growth, degradation rates for feeds containing mainly sucrose were set at 0.40/h for the sugar fraction (Molina, 2002), but for milk derived products the assigned degradation rate for sugars is 0.30/h as lactose support less microbial growth than sucrose (Bond, et al., 1998, McCormick, et al., 2001). For silages, with the exception of immature corn silages, the sugar fraction does not contain unfermented sugars, in favor of arabinose and other simple sugars derived from the hydrolysis of the side chains of pectin and hemicelluloses (Dewar, et al., 1963, Jones, et al., 1992). Thus, a rate of 0.20/h, closer to the arabinose fermentation rate was assigned to the sugar fraction of silages.

The fraction CB1 represents starch;

$$CB1_j = Starch_j \quad (g/kg\ DM) \quad [2.13]$$

Starch degradability varies depending on the particle size, grain type, processing effect and preservation method (Offner, et al., 2003). Ruminal degradation

rates of starch are feed specific, with values that range from 0.03/h for bird resistant sorghum to 0.40/h for wheat (Table 2.2).

Soluble fiber (CB2) is calculated by difference as;

$$CB2_j = NFC_j - CA1_j - CA2_j - CA3_j - CA4_j - CB1_j \quad (g/kg\ DM) \qquad [2.14]$$

The CB2 fraction which includes β-glucans and pectic substances are defined as dietary fiber because they are not digested by mammalian enzymes. Fermentation of soluble fiber is depressed at low pH and the main VFA produced from its fermentation is acetic acid (Strobel and Russell, 1986). Pectic substances occur in high concentration in by-product feeds such as citrus pulp, beet pulp and soybean hulls, as well as in the cell walls of legume forages (Van Soest, 1994). They ferment quickly, with ruminal degradation rates that range from 0.20 to 0.40/h with the exception of soybean hulls (0.08 /h) (Hall, et al., 1998, Hatfield and Weimer, 1995). β-glucans are present in barley and oat grains at 40-120 g /kg DM and are degraded at similar rates to starch (Engstrom, et al., 1992).

2.3.2. Variability of feed carbohydrate fractions and sensitivity analysis

The expanded CHO fraction scheme was evaluated by completing a sensitivity analysis of the expected variation in feed composition and degradation rates. The sensitivity analysis was conducted using a sample lactating cow diet and expected variation in carbohydrates and their digestion rates. The simulated animal was a lactating dairy cow (650 kg BW and 43 kg milk/day) fed 7.5 kg DM high moisture corn grain (HMCG), 7 kg grass silage, 6 kg corn silage, 3 kg soybean meal, 1 kg distillers grains, 1.1 kg whole cottonseed and a mineral-vitamin mixture. The ration provide 330 g aNDF/kg DM, 410 g NFC/kg DM, 173 g CP/kg DM, and 11.09 MJ/kg DM.

Monte Carlo techniques were used in the sensitivity analysis. In a Monte Carlo analysis, model inputs are described as probability density functions from which

samples are drawn to drive the model and derive probabilities of possible model solutions (Law and Kelton, 2000). The Monte Carlo analysis was done with @Risk version 4.5 (Palisade Corp., Newfield, NY, USA) in a spreadsheet version of the CNCPS (Fox, et al., 2004). In order to describe feed composition as distributions, a database provided by a commercial laboratory (Dairy One, Ithaca, NY, USA) was used. All feeds were analyzed by 'wet' chemistry. For starch analysis, a pre-extraction for sugar was completed and a glucose oxidase-peroxidase assay combined with a peroxide-detecting probe (YSI Incorporated, Yellow Springs, OH, USA) was used. For sugars, a water extraction method was used (Hall, et al., 1999). Feed composition data were fit to a normal distribution. When feed inputs were not statistically normal, the distribution with the best fit to the data was assigned (Table 2.3). Goodness of fit was assessed with several statistics (Chi-squared, Kolmogorov-Smirnov and Anderson-Darling statistical tests) and graphical methods (distribution function differences plots and probability plots) (Law and Kelton, 2000). Minimum and maximum values in the database were used to truncate distributions and a correlation matrix was incorporated to account for correlation among inputs within feeds when sampling (Table 2. 4). For degradation rates, a normal distribution with a SD proportional to their mean was used to account for variability in the rates estimates increases as the mean value increases (Weiss, 1994).

Several sampling techniques that are suitable for Monte Carlo simulation are available (McKay, et al., 1979). The sampling technique chosen for drawing samples from the distribution was the Latin Hypercube, in which the probability density function is divided into intervals of equal probability and from each interval a sample is randomly taken (McKay, et al., 1979). Sampling is forced to represent values at each interval. Ten thousand samples for simulation were completed. For each

sampling, the same random numbers were used to simulate the model with the original and expanded CHO schemes.

The sensitivity analyses are in table 2.6. Model predictions for metabolizable protein (MP) from bacteria, and ruminal NFC digestibility were assessed using the original and expanded CHO fractionation schemes. To assess the impact of feed variability on the model outputs with the two schemes, Bonferroni confidence intervals were computed for the mean and SD of the simulated outputs (Banks, et al., 2004). In addition, a stepwise regression analysis was used to assess the strength of the relationship between specific inputs and outputs. Standard regression coefficients (SRC) were used to rank the inputs and provide a measure of importance based on the effect of moving each variable away from its expected value by a fixed fraction of its SD while retaining all other variables at their expected values (Helton and Davis, 2002).

Table 2.3. Means, coefficients of variation (CV), minimum, maximum and distribution of the feed composition (g/kg DM) for the feeds used in the sensitivity analysis.

	N	Mean	CV	Minimum	Maximum	Distribution [1]
Corn silage						
Ash	6292	44	25.8	12	196	Normal (44, 11)
CP	8908	85	12.4	43	192	Loglogistic (21, 62, 11.3)
NDICP	6018	14	23.9	5	58	Loglogistic (3, 11, 6.1)
EE	6189	33	12.4	13	53	Normal (33, 4)
aNDF	9678	441	13.4	281	743	Normal (441, 59)
Lignin(sa)	6257	35	18.4	9	97	Loglogistic (3, 32, 9.3)
Starch	6353	308	25.4	3	499	Weibull (8.9, 613)
Sugar	6045	41	46.3	1	191	PearsonV (13.6, 747)
Acetic acid	440	23	63.1	0	78	Beta general (1.7, 5.2)
Propionic acid	440	4	130.0	0	31	Beta general (0.4, 4.4)
Butyric acid	440	1	254.7	0	19	Exponential (0.7)
Isobutyric acid	440	6	111.0	1	7	Lognormal (0.6, 0.6)
Lactic acid	440	50	41.3	0	101	Normal (50, 21)

Table 2.3 (Continued)

	N	Mean	CV	Minimum	Maximum	Distribution[1]
Grass silage						
Ash	895	96	27.7	36	226	Loglogistic (14, 77, 5.7)
CP	1385	144	26.7	24	292	Beta general (7.7, 11.7)
NDICP	680	33	27.0	12	78	Lognormal (35, 9)
EE	726	37	25.7	9	103	Normal (37, 10)
aNDF	1384	584	11.9	397	818	Normal (584, 69)
Lignin(sa)	728	69	24.5	19	174	Logistic (68, 9)
Starch	681	24	62.9	1	104	Weibull (1.6, 28)
Sugar	689	28	39.4	8	192	Lognormal (105, 28)
Acetic acid	34	22	74.9	0	63	Loglogistic (-5, 22, 2.6)
Propionic acid	34	2	128.5	0	8	Exponential (2)
Butyric acid	34	4	132.1	0	19	Exponential (4)
Isobutyric acid	34	1	112.0	0	5	Exponential (1)
Lactic acid	34	47	56.8	1	111	Loglogistic (-131, 176, 11.6)
High moisture corn grain						
Ash	1613	17	12.9	11	32	Loglogistic (5, 11, 9.8)
CP	2166	97	10.7	67	149	PearsonV (53.5, 3874)
NDICP	1575	8	23.4	2	19	Logistic (8, 1)

Table 2.3 (Continued)

	N	Mean	CV	Minimum	Maximum	Distribution[1]
EE	1618	44	15.2	21	105	Loglogistic (13, 31, 8.7)
aNDF	2153	101	20.6	51	272	PearsonV (17.3, 1157)
Lignin(sa)	1576	10	23.9	2	25	Logistic (10, 1)
Starch	1602	706	4.3	543	774	Logistic (708, 15)
Sugar	45	22	65.0			Normal (22, 14)
Acetic acid	94	3	113.0			Exponential (3)
Propionic acid	94	0.4	200.0			Exponential (0.4)
Butyric acid	94	0.1	278.0			Exponential (0.1)
Isobutyric acid	94	0.1	300.0			Exponential (0.1)
Lactic acid	94	11	84.0			Normal (11, 9)
Distillers' grains						
Ash	83	63	17.9	32	96	Normal (63, 11)
CP	354	314	7.6	236	406	Normal (314, 24)
NDICP	1427	310	30.6			Normal (310, 95)
EE	286	135	18.0	36	190	Weibull (9.7, 209)
aNDF	284	338	9.5	245	424	Loglogistic(-387, 723, 36.9)
Lignin(sa)	370	57	38.6			Normal (57, 22)
Starch	188	45	51.7	4	229	Loglogistic (-12, 54, 5.7)
Sugar	162	53	41.6	4	138	Loglogistic (-25, 75, 7.1)

Table 2.3 (Continued)

	N	Mean	CV	Minimum	Maximum	Distribution[1]
Soybean meal						
Ash	298	73	30.1			Normal (73, 22)
CP	681	510	6.2	372	569	Logistic (510, 17)
NDICP	124	54	62.4			Normal (54, 34)
EE	322	36	104.4	3	220	PearsonV (1.9, 33)
aNDF	306	123	30.4	70	333	Loglogistic (15, 100, 6.3)
Lignin(sa)	253	14	64.3			Normal (14, 9)
Starch	186	19	60.0			Normal (19, 11)
Sugar	158	135	19.2			Normal (135, 26)
Whole cottonseed						
Ash	99	43	11.9	32	60	Normal (43, 5)
CP	320	241	18.1	114	375	Loglogistic (-163, 401, 16.4)
NDICP	63	24	25.3	17	58	Loglogistic (14, 9, 3.6)
EE	184	225	22.5	122	361	Loglogistic (92, 124, 4.5)
aNDF	311	508	19.8	247	803	Logistic (508, 57)
Lignin(sa)	95	154	24.0	52	250	Normal (154, 37)
Starch	36	11	52.7	1	23	Loglogistic (-1, 11, 2.7)
Sugar	39	59	29.0	34	105	Normal (59, 17)

Table 2.3 (Continued)

[1] The parameters necessary to characterize the distribution are indicated between brackets: a α parameter indicates shape of the distribution, a β parameter indicates scale (e.g. σ for the normal distribution), a γ parameter indicates location (e.g. μ for the normal distribution). The distributions are beta general (α_1, α_2), exponential (β), logistic (α, β), loglogistic (γ, α, β), lognormal (μ, σ), normal (μ, σ), PearsonV (α, β), and Weibull (α, β). When maximum and minimum values are not indicated, the original database was not available to fit the distributions. A normal or exponential (for volatile fatty acids) distribution was assumed.

Table 2.4. Correlation matrix (Spearman correlations) of the feed fractions for the feeds used in the sensitivity analysis (P<0.05) [Blanks indicate no significant (i.e. P>0.05) correlations].

Corn silage													
	Ash	CP	NDICP	EE	aNDF	Lignin(sa)	Starch	Sugar	Acetic	Propionic	Butyric	Iso butyric	Lactic
Ash	1	0.38	0.38	-0.21	0.50	0.47	-0.61	0.14	0.29		0.24		0.24
CP		1	0.45		0.22	0.21	-0.40	0.16					0.21
NDICP			1		0.47	0.49	-0.49	0.24				0.27	-0.18
EE				1	-0.36	-0.22	0.31	-0.38	0.30	0.27			
aNDF					1	0.64	-0.92	0.15	0.17				0.12
Lignin(sa)						1	-0.66	0.16					
Starch							1	-0.27	-0.24				-0.25
Sugar								1	-0.39	-0.28			
Acetic									1	0.65			0.10
Propionic										1			-0.21
Butyric											1		
Isobutyric												1	-0.28
Lactic													1

Table 2.4. (Continued)

Grass silage													
	Ash	CP	NDICP	EE	aNDF	Lignin(sa)	Starch	Sugar	Acetic	Propionic	Butyric	Isobutyric	Lactic
Ash	1	0.59	0.25	0.36	-0.37	-0.28	-0.55	-0.27	0.49		0.49	0.42	0.45
CP		1	0.41	0.75	-0.8	-0.44	-0.19						0.48
NDICP			1		-0.21	0.15							
EE				1	-0.67	-0.58	-0.10		0.48				0.79
aNDF					1	0.44	-0.20	-0.32					
Lignin(sa)						1		-0.32					-0.58
Starch							1	0.43	-0.68				-0.59
Sugar								1	-0.49				-0.52
Acetic									1	0.60			
Propionic										1			
Butyric											1	0.74	
Isobutyric												1	
Lactic													1

Table 2.4. (Continued)

High moisture corn grain								
	Ash	CP	NDICP	EE	aNDF	Lignin(sa)	Starch	Sugar
Ash	1	0.35	0.24	0.31	0.27	-0.10	-0.57	
CP		1	0.24	0.52	0.10	-0.21	-0.32	
NDICP			1	0.27	0.30	0.33	-0.13	
EE				1	0.16	-0.29	-0.44	
aNDF					1	0.21	-0.46	
Lignin(sa)						1		
Starch							1	
Sugar								1
Distillers' Grains								
	Ash	CP	NDICP	EE	aNDF	Lignin(sa)	Starch	Sugar
Ash	1	0.30			0.23			
CP		1		0.26			-0.43	-0.16
NDICP			1					
EE				1	0.13		-0.14	
aNDF					1		-0.14	
Lignin(sa)						1		
Starch							1	
Sugar								1

Table 2.4. (Continued)

Soybean meal

	Ash	CP	NDICP	EE	aNDF	Lignin(sa)	Starch	Sugar
Ash	1							
CP		1		0.53	0.52			
NDICP			1					
EE				1	0.44			
aNDF					1			
Lignin(sa)						1		
Starch							1	
Sugar								1

Whole cottonseed

	Ash	CP	NDICP	EE	aNDF	Lignin(sa)	Starch	Sugar
Ash	1	0.52		0.68	0.58		-0.39	0.62
CP		1		0.57	0.61	-0.29		0.45
NDICP			1					
EE				1	0.69	-0.51	-0.44	0.65
aNDF					1	0.41		-0.66
Lignin(sa)						1		-0.55
Starch							1	
Sugar								1

2. 4. Results and discussion

2.4.1. Feed carbohydrate fractionation schemes and analytical methods

Table 2. 2 lists average CHO fractions for common feedstuffs. Volatile fatty acids and lactic acid values for silages are currently available from fermentation profiles offered by commercial laboratories. Dry matter content of the silages was a poor predictor of total VFA content (Figure 2.1). The amount of DM in silage was negatively, and exponentially, related to the amount of fermentation end products during ensiling (Figure 2.1). Lactic acid content was positively, and linearly, related to the amount of EE of grass silages (Lactic (g/kg DM) = 18.9 EE (g/kg DM) – 66.1, R^2= 0.58, RMSE= 18.2) and legume silages (Lactic (g/kg DM) = 28.1 EE (g/kg DM) – 30.2, R^2= 0.46, RMSE= 20.2). Both EE and lactic acid increased with the extent of fermentation. For corn silages, both VFA's and lactic acid were poorly related with other feed fractions (Table 2.4) and DM (Figure 2.1).

Overall, the correlations among feed inputs were low or moderate (i.e., $r < 0.70$), (Table 2. 4), which prevents use of more common feed analyses, such as NDF assays to predict fractions that are less commonly assayed, such as sugar contents. The components with the highest correlation were the starch and aNDF contents of corn silage, which were strongly linearly related (Starch (g/kg DM) = 845.4 – 12.1 NDF (g/kg DM), R^2= 0.84, RMSE= 31.1) due to the increase of grain content with plant maturity.

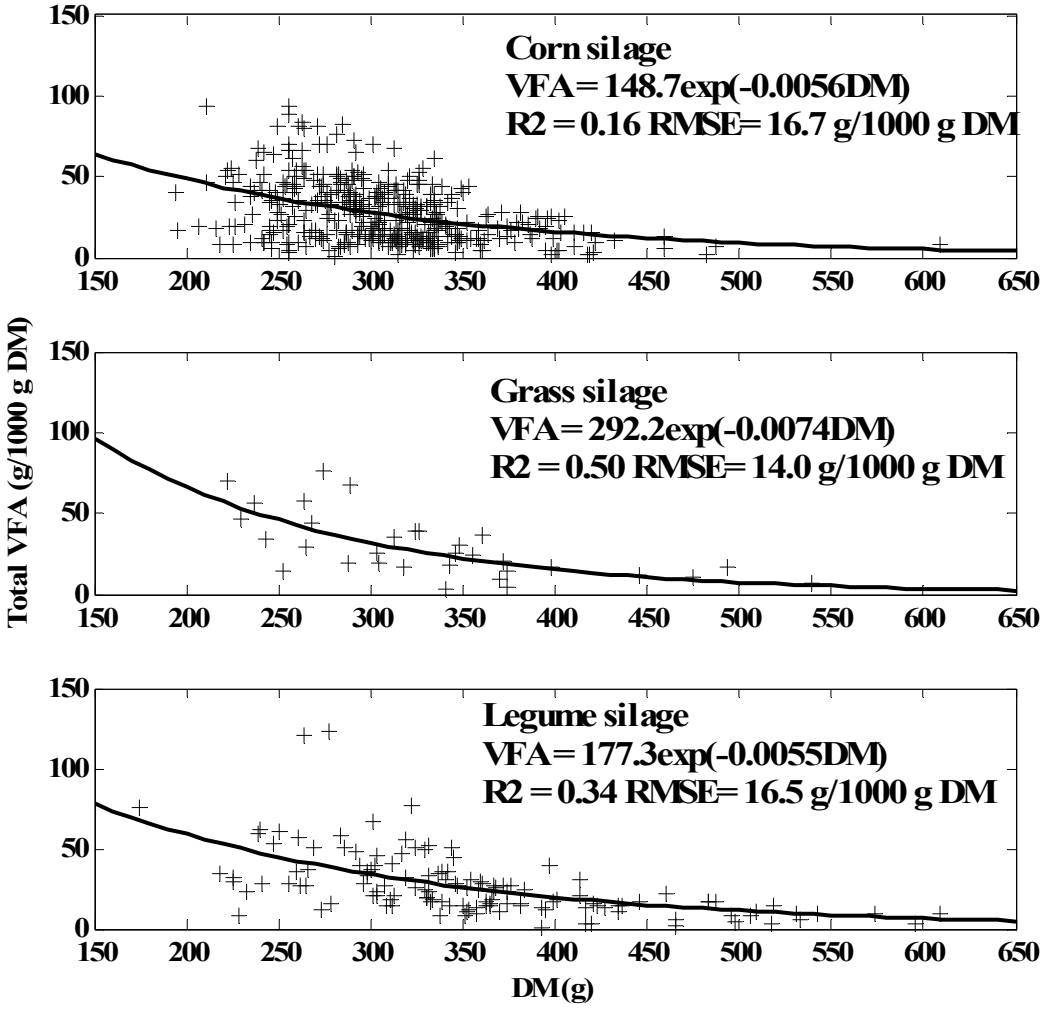


Figure 2.1. Relationship between total volatile fatty acids and dry matter of corn silage (N = 440), grass silage (N=34), and legume silage (N= 131).

Organic acids are generally analyzed by gas or high-pressure liquid chromatography (Amin, 1980, Russell and Van Soest, 1984) or can be estimated indirectly as NFC minus ethanol insoluble residue (adjusted for CP) and sugar content (Hall, et al., 1999). Because of difficulties in measuring organic acids as a group, model users will have to rely on feed library values for the CA3 fraction more than for other fractions. In the CPM dairy model, non-silage acids were included within the soluble fiber fraction (CPM CB2), while we included the other organic acids as a separate fraction to account for their fermentation characteristics. Although they provide some fermentable energy, it is considerably less than the other components that were included in the CPM soluble fiber fraction. Dicarboxylic acids (i.e., aspartate, fumarate, and malate) stimulate lactate utilization by the predominant ruminal bacteria, *Selenomonas ruminantium* (Evans and Martin, 1997, Martin and Streeter, 1995). In the feed library, the highest organic acid concentrations were allocated in pastures and fresh forages (Table 2. 2); (Callaway, et al., 1997, Martin, 1970, Mayland, et al., 2000). In forages, organic acids decline with maturity and age (Martin, 1970). In silages, other organic acids were assumed to be degraded during fermentation during ensiling (McDonald, et al., 1991), and therefore were assigned a value of 0 (Table 2. 2).

The sugar fraction represents a heterogeneous fraction and most sugar measurements in commercial laboratories are based on ethanol/water extractions (Hall, 2003), which may extract different components depending on the proportion of ethanol (Hall, et al., 1999, Smith and Grotelueschen, 1966), the standard used (e.g., glucose, fructose or sucrose) and the feedstuff matrix. Some of the differences in the sugar composition have been accounted for by using different ruminal degradation rates (Table 2). The proportion of ethanol used in the extraction may affect partition of components between sugar and soluble fiber. For example, in temperate cool season

grasses, variable amounts of fructans are extracted depending on the ethanol concentration (Smith and Grotelueschen, 1966). Fructans are classified as dietary fiber since they are not digested by mammalian enzymes (Nilsson, et al., 1988). Even so, the VFA profile of fructans is similar to sugars because sucrose is the precursor for fructan synthesis (Marounek, et al., 1988, Pollock, 1986) and their release from the plant cells is similar to that of free sugars (Boudon, et al., 2002). Therefore, in predicting nutrient availability for ruminants, it may be more appropriate to associate fructans with sugars rather than with soluble fiber.

In the expanded scheme, the soluble fiber fraction is calculated by difference. Thus, it contains errors from other component assays. Knudsen (1997) measured β-glucans, and other soluble polysaccharides, for selected energy and protein-rich concentrate feeds. For cereal grains, values for soluble fiber calculated by difference were similar to measured soluble fiber as the sum of β-glucans and other soluble polysaccharides. For example, calculated and measured values for corn grain, barley grain and wheat middlings were 8 vs 10, 73 vs 98, and 98 vs 97 g/kg DM, respectively. For protein-rich feeds, calculated values were not consistently related to measured values (e.g., soybean meal, 63 vs 141; cottonseed meal, 24 vs 18; linseed meal, 521 vs 138; white lupins, 131 vs 134 g/kg DM, respectively). Several factors may contribute to underprediction of the soluble fiber fraction (Eq. 14) for some feeds. While VFA's (CA1) are expressed on a DM basis, they are typically measured in 'wet' feeds because they are partly volatilized during oven drying. For acetic acid, drying losses can be as high as 53 % for grass silages, and 83 % for corn silages (Sorensen, 2004). This may especially contribute to the underprediction of CB2 in legume silages because both CA1 and CB2 fractions can be a substantive proportion of the CHO. Based on the assumption that protein contains 16 % N, the conversion factor of 6.25 is used as an average to convert N into CP for all the feeds. However,

when non-protein compounds and variations in their AA composition are considered, the conversion factor for most common feeds are consistently lower than 6.25 (e.g.,soybean meal, 5.49; barley, 5.17; fish meal, 4.75) (Boisen, et al., 1987). Ash contamination may result in insoluble ash being recovered in aNDF, overpredicting available FC. In contrast, over-estimation may result from correcting NDF assayed with sodium sulfite in the ND for NDICP assayed without sodium sulfite in the ND. The NDF method approved by the Association of Official Analytical Chemists International (Mertens, 2002) uses sodium sulfite, which removes most N the insoluble fiber. For most feeds, the difference in NDICP with and without sodium sulfite is less than 10 g/kg DM, but, for protein-rich feeds, the difference can be as high as 90 g/kg DM (Hintz, et al., 1996). The CB2 pool size was very sensitive to NDICP adjustment for canola and sunflower meals, distillers' grains and whole soybean (results not shown). Correcting aNDF for NDICP and ash is the most accurate way to estimate FC and NFC. However, because of the inconsistency of method used to measure NDF among feed analysis laboratories, we assumed that the NDICP fraction is in the NDF fraction.

2.4.2. Ruminal degradation rates and microbial yield

Although *in vitro* gravimetric and gas measurements have been extensively used to measure degradation rates, no *in vitro* method has been proven to be appropriate to measure rates in all CHO fractions. The rates used are a mixture of rates for fermentation and hydrolysis. Rates for the CA2, CA3, and CA4 fractions have been updated from data based on gas production measurements (Doane, et al., 1998, Molina, 2002). Gas production systems can be used to determine rates of ruminal fermentation. Sugars are the most rapidly degraded CHO, with rates of hydrolysis as high as 10/h (Weisbjerg, et al., 1998). Despite their high rates of hydrolysis, fermentation rates for sugars are several magnitudes lower (Van Kessel and Russell,

1997). Part of the discrepancy between hydrolysis and fermentation rates is because sugars can be partially stored as microbial glycogen and used later for endogenous metabolism (Van Kessel and Russell, 1997). Thus the rates for these fractions are lower than the values for the A fraction in the original CNCPS scheme (Sniffen, et al., 1992), which overpredicted fluctuations in ruminal pH (Pitt and Pell, 1997) and microbial yield for the A fraction (Alderman, 2001).

Some of the starch degradation rates for the new scheme have also been updated based on *in vivo* and *in vitro* data (Lanzas, 2003, Monteils, et al., 2002, Remond, et al., 2004, Richards, et al., 1995, Tothi, et al., 2003, Yang, et al., 2000). In contrast, *in situ* rates have not been used for the starch fraction because the *in situ* method divides starch into a soluble fraction which is considered to be degraded instantaneously and completely, and an insoluble fraction which is degraded exponentially. As *in situ* results measure the digestion rate for the slowly degradable pool, while starch in our fractionation scheme is treated as single fraction with a rate for the entire degradable pool, values for the starch degradation rates (Table 2. 2) are generally higher than those derived from *in situ* (Offner, et al., 2003). Because of variability in starch degradation rates in feeds due to processing and starch sources, starch degradation rates are feed specific and a method to estimate them routinely is needed.

2. 4. 3. Variability of feed carbohydrate fractions

Table 2.3 lists the expected variation and probability density functions used to describe the feeds used in the simulations. They represent variability within the population of the feedstuff since they were derived from an extensive database, and distributions for a large proportion of the feeds were not normal (Table 2.3).

In silages, sugars and VFAs were the fractions that varied the most as indicated by their high coefficients of variations (Table 2.3). Distributions for corn silage sugars

and grass and corn silage VFA were not symmetrical in that some VFA had an exponential distribution, in which the probability of a given value decreased as values departed from 0, with a negative rate (Evans, et al., 2000). Ensiling adds variability to the forage composition because it adds a wide range of factors, including forage quality, silo type, particle size, packing and covering (McDonald, et al., 1991). In addition, pre-harvest and weather conditions can affect forage quality. Although corn silage starch and aNDF had symmetrical distributions (Table 2.3), both components had long tails and a subpopulation of corn silages had low starch (< 150 g/kg) and high fiber (>580 g/kg) contents. Drought conditions, or high plant densities, decreases grain content to less than 270 g/kg of DM (Woody, 1978). High moisture corn grain had the lowest nutrient variation of all the feeds. In by-product feeds and soybean meal, the inputs with the largest variability are the nutrients influenced by processing. For soybean meal, EE had the largest variation because of differences in oil extraction (Table 2.3). For distillers' grains, lignin(sa), sugar and NDICP were the fractions with the largest variation due to differences in heat damage and content of solubles among samples (Table 2.3).

When variability in feed inputs was considered in the simulated diet, the CHO fractions varied in a decreasing order as: VFA's, soluble fiber, lactic acid, sugar, aNDF, starch, and total NFC (Table 2.5). Volatile fatty acids, soluble fiber and lactic acids are small proportions of the total CHO. Variation in calculated NFC, CA and CB1 fractions causes variation in the soluble fiber fraction. Feeds in the simulated diet were generally low in soluble fiber, and it was sensitive to aNDF and CP content of grass silage, since grass silage provided the greatest amount of CB2 of all the feeds in the simulated diet. Variability in the sugar fraction was due mainly to variation in sugar content of the silages (Table 2.5) and it may be a highly variable fraction among dairy cattle diets. In fresh forages, the sugar fraction is a highly labile pool, which

accumulates and depletes through out the day (Pollock, 1986). In silages, sugar fractions vary with the ensiling process (Table 2.6). Analytical variability may occur due to differences in extraction conditions and methods used to analyze sugars (Hall, 2003). Although NFC is calculated by difference (Eq. 8), variation in the inputs used to calculate NFC offset each other to some extent, thereby decreasing the uncertainty range of the NFC fraction. The moderate correlations among the grass silage aNDF, the most influencing input (Table 5) and other inputs used to calculate NFC (i.e., grass silage CP and EE), may contribute to decreasing the NFC variation (Table 2. 4).

2. 4. 4. Model behavior and sensitivity analysis

Model predictions for MP from bacteria and ruminal NFC digestibility were assessed with the original and expanded schemes (Table 2.6). The expanded CHO scheme decreases mean predicted microbial CP with 43 g difference in MP between the schemes. Assuming an efficiency of MP utilization of 0.65 for milk production and 30 g true protein per kg of milk, the difference would represent approximately 1 kg in predicted MP allowable milk (MP milk = 43×0.65/30) (Table 2.6). The decrease in MP from bacteria is due mainly to a decrease in the microbial yield supported by the CA fraction; the rates for the A fractions have been reduced compared to the rates for the original scheme. In the original scheme, the CA rate for the entire pool in silages was set at an intermediate rate (e.g., 0.10/h) to account indirectly for the presence of organic acids. The expanded scheme may not always result in lower rumen microbial growth than the original scheme for a silage-based diet. For immature corn silages with high water soluble CHO and low VFA content, the expanded scheme predicts greater MP from bacteria than the original scheme (results not shown).

Table 2.5. Variation of carbohydrate (CHO) fractions (g/kg ration DM) when all the feed inputs were varied.

CHO fraction	Mean	SD	Minimum	Maximum
NDF [1]	307	23.4	234	388
NFC [2]	403	27.4	303	403
Lactic acid [3]	28	8.1	4	60
Starch [4]	284	19.8	192	339
Sugar [5]	57	9.8	30	99
Soluble fiber [6]	40	13.9	4	114
VFAs [7]	14	5.3	2	40

[1] The inputs that had the most influence (regression coefficient in brackets) were grass silage aNDF (0.77) and corn silage aNDF (0.58).
[2] The inputs that had the most influence (regression coefficient in brackets) were grass silage aNDF (-0.64) and corn silage aNDF (-0.5).
[3] The inputs that had the most influence (regression coefficient in brackets) were grass silage acetic (0.67) and corn silage acetic (0.64).
[4]The inputs that had the most influence (regression coefficient in brackets) were corn silage starch (0.89) and HMCG starch (0.37).
[5] The inputs that had the most influence (regression coefficient in brackets) were grass silage sugar (0.75) and corn silage sugar (0.41).
[6] The inputs that had the most influence (regression coefficient in brackets) were grass silage aNDF (-0.70) and grass silage CP (-0.43).
[7] The inputs that had the most influence (regression coefficient in brackets) were grass silage lactic (0.78) and corn silage lactic (0.55).

Predicted ruminal NFC digestibility is similar between the two schemes (Table 2. 6). The prediction of site of digestion is less sensitive to CHO degradation rates than microbial CP production. With the first-order approach used to predict site of digestion, the model is sensitive to degradation rates that are closer to its ruminal passage rate.

The expanded fractionation scheme also repartitions impact of the different inputs on model predictions (Table 2.6). Predictions with the original scheme are more sensitive to NFC rates and inputs used to calculate CHO than predictions with the expanded scheme, which were more sensitive to NFC fractions and their corresponding rates (Table 2.6). For MP from bacteria, for both schemes, the fractional degradation rates for fiber had the biggest effect (Table 6).The use of the expanded CHO scheme increases the number of inputs, as listed in Table 2.6, and thus the risk of use of the model may increase if the inputs to the model are sensitive and have not been measured. The SD for model predictions when all inputs were varied was greater for the expanded scheme (Table 2.6). Despite this, the individual feed inputs that contributed most to variability in MP from bacteria were similar for both schemes (Figure 2.2). The same four variables had the highest regression coefficients in both schemes (i.e., corn silage starch, grass silage NDF rate, high moisture corn grain starch rate, and corn silage NDF rate). The only important change in the regression coefficient was a much higher value for variation in the corn silage starch pool in the expanded CHO scheme. This is likely due to removing soluble fiber from this pool. The grass and corn silage CA rates (0.10/h) were sensitive in the original scheme but none of the CA fraction rates were sensitive in the expanded scheme. In the expanded scheme, the CA1, CA2, and CA3 had low microbial yields and CA4 had high degradation rates, which makes the model more sensitive to their pool size, rather than their degradation rates. Although the sugar fraction was highly variable (Table

2.5), the sensitivity of the model to sugar content of silages was moderate (Figure 2.2). The uncertainty due to feed composition may be important in predictions of the nutritional model used for formulating rations. Feed inputs that vary the most within a feed may not necessarily be the ones that the model is most sensitive to.

The feed inputs with moderate or large variability and those that the model is sensitive to should be analyzed most frequently. Both accuracy and precision should be considered when problems associated with undertainty of feed composition are addressed. Low accuracy occurs when values reported from a laboratory differ from known reference values and may result in systematic bias in the model predictions. Low precision results from random variation and can be overcome by increasing analysis frequency.

Table 2.6. Impact of varying the inputs used to calculate carbohydrate fractions with the original and expanded scheme and their corresponding rates on metabolizable protein (MP) from bacteria, and ruminal non-fiber carbohydrates (NFC) digestibility. Means or standard deviation (SD) with different superscripts within a column (for each scheme).

	Original CHO scheme		Expanded CHO scheme	
	Mean	SD	Mean	SD
MP from bacteria, g/day				
Calculated CHO[1]	1633[a]	36.4[a]	1574[a]	28.1[a]
FC vs NFC[2]	1632[a]	27.4[b]	1587[b]	30.1[b]
NFC fractions[3]	1629[b]	29.4[c]	1581[c]	50.1[c]
NFC rates[4]	1619[c]	46.2[d]	1543[d]	42.5[d]
FC rate[5]	1617[c]	54.3[f]	1540[d]	53.4[e]
All inputs[6]	1613[d]	88.3[g]	1570[a]	91.5[f]
Rumen NFC digestibility, g/g				
Calculated CHO[1]	0.82[a]	0.007[a]	0.81[a]	0.020[a]
FC vs NFC[2]	0.82[ab]	0.010[b]	0.82[b]	0.032[b]
NFC fractions[3]	0.82[b]	0.010[b]	0.81[c]	0.030[b]
NFC rates[4]	0.81[c]	0.017[c]	0.79[d]	0.015[c]
FC rate[5]	0.82[d]	0.000[d]	0.79[d]	0.000[d]
All inputs[6]	0.81[e]	0.021[e]	0.81[c]	0.035[e]

[1] The inputs need to compute CHO (CP, EE, and ash, Eq. 1) were varied.
[2] The inputs needed to partition FC and NFC were varied (Eq. 2.2, 2.3, 2.4 for the original scheme, and Eq. 2.2, 2.7, 2.8 for the expanded scheme).
[3] The inputs needed to fractionate NFC were varied (Eq. 2.5 and 2.6 for the original scheme, and Eq. 2.9, 2.10, 2.11, 2.12, 2.13, 2.14 for the expanded scheme).
[4] The rates for the NFC fractions were varied (A, and B1 for the original scheme, and A2, A3, A4, B1, and B2 rates for the expanded scheme).
[5] The rates for the FC fraction were varied.
[6] All the inputs were varied (Eq. 2.1, 2.2, 2.3, 2.4, 2.5, 2.6 and corresponding rates for the original scheme, and Eq. 2.1, 2.2, 2.7, 2.8, 2.9, 2.10, 2.11, 2.12, 2.13, 2.14 and corresponding rates for the expanded scheme).

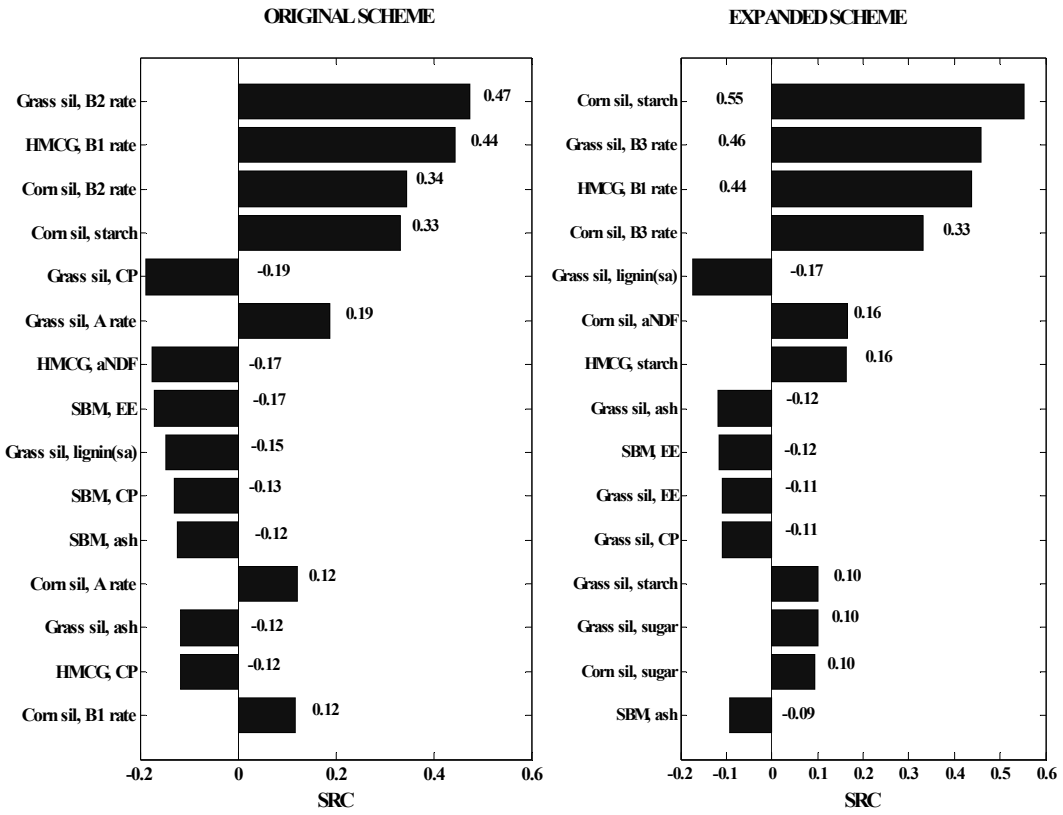


Figure 2.2. Standard regression coefficients (SRC) for the inputs ranked as the most influential in predicting microbial growth with the original carbohydrate scheme (Panel A) and expanded scheme (Panel B).

[CP=crude protein; EE= ether extract; HMCG= High moisture corn grain; NDF= neutral detergent fiber; SBM= soybean meal.]

2. 4. 5. Applications of the expanded carbohydrate scheme

The expanded CHO scheme increases the ability of the CNCPS model to account for variation in animal production due to differences in feed composition, including accounting for silage quality, assessing production responses to changes in diet NFC composition and sugar supplementation.

2. 4. 5. 1. Supplementing silages

Extent of silage fermentation is highly variable (Table 2.3), and it can be stimulated by adding inoculants such as lactic acid bacteria, enzymes and added fermentable CHO, while wilting or formic acid addition reduces the extent of silage fermentation (Huhtanen, 1998). The expanded scheme accounts for more variation in silage fermentation and table 6 summarizes CNCPS model predictions with the expanded CHO scheme for grass silages derived from the same crop, but with different fermentations (i.e., inoculated vs restricted fermentation). When silages are fed alone, the model predicts protein to be first limiting for both silages, with lower MP allowable milk for cows fed the inoculated silage. The model with the expanded CHO scheme predicted milk responses to increased MP supply for both silages with predicted responses for both fermentable CHO and CP supplementation larger for the inoculated silage (Table 2.7). Histidine was predicted to be the first limiting AA, in agreement with previous reports (Korhonen, et al., 2000). The content of some AA (i.e., histidine and leucine) in microbial CP is lower than in milk protein, which may attenuate the responses to sources of fermentable CHO to the diet when one of these AA is first limiting in the ration. The model with the original CHO scheme did not predict differences due to extent of silage fermentation (results not shown).

Table 2.7. CNCPS predictions with the expanded carbohydrate scheme for un-treated grass silage or inoculated with lactic acid bacteria with supplements (formulated for a lactating dairy cow 650 kg BW, intake: 24.9 kg).

	MP allowable milk	ME allowable milk	First limiting AA
Untreated Grass silage alone[1]	22.3	35.9	Histidine
Grass silage (500 g/kg diet DM) and cracked corn (500g/kg diet DM)	30	45.9	Isoleucine
Grass silage (840 g/kg diet DM) and extruded SBM (160 g/kg diet DM)	46.3	36.7	Leucine
Inoculated grass silage alone[2]	15.6	30.2	Histidine
Inoculated silage (500 g/kg diet DM) and cracked corn (500 g/kg diet DM)	26.6	43.3	Valine
Inoculated silage (840 g/kg diet DM) and extruded SBM (160 g/kg diet DM)	40.7	32.1	Leucine

[1] Grass silage composition (g/kg): sugar 160, lactic acid 35, volatile fatty acids 14

[2]Grass silage inoculated with lactic acid bacteria composition (g/kg): sugar 61, lactic acid 132, volatile fatty acids 5.

2. 4. 5. 2. Balancing for NFC

While the NRC (2001) provides few guidelines for balancing total diet NFC, altering the proportions of the types of NFC can alter recommendations for total NFC, and other components, of the ration since interactions among NFC components and fiber and protein fractions have been described (Hall, 2002). Table 2.8 shows changes in CHO fractions and model predictions using the expanded scheme replacing HMCG, a high starch concentrate, with the high soluble fiber by-product beet pulp in a ration. Replacing HMCG with beet pulp causes an increase in the content of sugar, soluble fiber and NDF of the ration and a decrease in the starch content. With increasing levels of beet pulp, the model predicts a reduction in both ME and MP allowable milk. The ME allowable milk decreases more sharply than MP allowable milk because of the higher content of NDF of the beet pulp, which reduces the total digestible nutrients derived from the ration. Metabolizable protein allowable milk also decreases due mainly to a decrease in the microbial CP supply (Table 2.8). A small repartitioning of N excretion was also predicted. Beet pulp changed some of the N excretion from urine to feces. With beet pulp, indigestible DM intake increases, which in turn increases predicted metabolic fecal N. Van Vuuren (1993) observed a similar trend in N partition when replacing a corn grain based diet with a beet pulp based diet. The original model also predicted a decrease in ME allowable milk when beet pulp content was increased because this effect was caused by an increase in diet FC; however predicted microbial MP and MP allowable milk were rather insensitive to changes in the percentage inclusions of beet pulp (results not shown).

Table 2.8. Effect of replacing high moisture corn grain (HMCG) with beet pulp (BP) in dietary carbohydrate composition on CNCPS predictions with the expanded carbohydrate scheme.

	100 HMCG: 0 BP[1,2]	75 HMCG: 25 BP[2]	50 HMCG: 50 BP[2]	25 HMCG: 75 BP[2]	0 HMCG: 100 BP[2]
Diet composition, g/kg					
Sugar	38	49	59	70	80
Starch	333	273	213	153	93
Soluble fiber	71	95	119	143	167
NDF	237	264	290	317	344
CNCPS predictions					
Pred DMI, kg/day[3]	23.2	23.2	23.2	23.2	23.2
Pred DMI, kg/day[4]	25.5	25.5	25.5	25.5	25.5
ME allowable milk, kg/day	44.7	42.4	40.1	37.9	35.6
MP allowable milk, kg/day	44.6	44.1	43.3	42.0	40.7
Microbial MP, g/day	1491	1492	1478	1441	1361
Fecal N, g/day	244	253	261	267	273
Urinary N, g/day	406	399	394	390	386

Table 2.8 (Continued)

[1] Diet formulated for a lactating dairy cow 650 kg BW consuming 24.8 kg DM. Diet composition (g/kg): 360 HMCG, 200 corn silage, 200 alfalfa silage, 150 solvent soybean meal, 40 corn distillers' grains with solubles, 10 blood meal, and 40 mineral vitamin mixture.

[2] Beet pulp substituted for HMCG as 0, 25, 50, 75 g/100 g of the HMCG of the ration. All diets were 188 g CP/kg DM

[3] Fox et al. (2004)

[4] NRC (2001)

Some differences in animal responses when they are fed different sources of CHO are mediated through changes in DM intake. Voelker and Allen (2003) reported a decrease in DM intake when beet pulp constituted 240 g /kg of the ration DM, which they attributed this to a physical fill effect. Changes in DM intake have also been observed when HMCG is replaced with dried molasses (Broderick and Radloff, 2004). Predictions of DM intake (NRC, 2001, Roseler, et al., 1997) were insensitive to changes in the NFC composition of the ration (Table 2.8). Empirical equations used to predict DM intake account for body weight, fat-corrected milk, ambient temperature, mud depth and early lactation lag in intake (Fox, et al., 2004, NRC, 2001), but dietary factors are not considered. Mechanistic predictions of changes in DM intake due to changes in dietary factors are an important addition to nutritional models needed to account for difference in CHO utilization.

Prediction of the amount and profile of VFA in the rumen due to variation in CHO fractions is important in relating feed composition to milk production and composition, as well as to changes in body composition (Dijkstra, 1994, Pitt, et al., 1996). While total VFA production is acceptably predicted by many models, proportions of the VFA have been poorly predicted (Dijkstra, et al., 1992, Pitt, et al., 1996). Description of the nutrient profile of the diet and substrate availability affects the profile of VFA produced in the rumen. While the original CNCPS scheme divided CHO based on the rate of degradation, it combines CHO fractions that differ in their ruminal VFA profile (e.g. pectin and starch). Therefore, the expanded scheme would be more suitable to provide dietary inputs for a VFA production pH rumen submodel (Fox, et al., 2004).

2. 5. Conclusions

The expanded CHO scheme for the CNCPS model that is outlined in this paper divides feed CHO in fractions that more accurately relate to ruminal fermentation characteristics. It is practical to use this scheme for quantifying CHO fractions in feeds because most of the fractions are now being provided by some commercial laboratories. Shortcomings in the current analytical methodology to measure some of the fractions (e.g. sugars) and their corresponding ruminal degradation rates complicate full characterization of feed CHO. Nevertheless, the proposed fractionation provides a framework for applying this information, and may stimulate research to develop appropriate laboratory methods to measure them.

CHAPTER 3

EVALUATION OF PROTEIN FRACTIONATION SYSTEMS USED IN FORMULATING RATIONS FOR DAIRY CATTLE[3]

3.1. Abstract

Production efficiency decreases when diets are not properly balanced for protein. Sensitivity analyses of the protein fractionation schemes used by the National Research Council Nutrient Requirement of Dairy Cattle (NRC) and the Cornell Net Carbohydrate and Protein System (CNCPS) were conducted to assess the influence of the uncertainty in feed inputs and the assumptions underlying the CNCPS scheme on metabolizable protein (MP) and amino acids (AA) predictions. Monte Carlo techniques were used. Two lactating dairy cow diets with low and high protein content were developed for the analysis. A feed database provided by a commercial laboratory and published sources were used to obtain the distributions and correlations of the input variables. Both models behaved similarly when variation in protein fractionation was taken into account. The maximal impact of variation on MP from RUP was 2.5 (CNCPS), 3.0 (NRC) kg/d of allowable milk for the low protein diet, and 3.5 (CNCPS), and 3.9 (NRC) kg/d allowable milk for the high protein diet. The RUP flows were sensitive to ruminal degradation rates of the B protein fraction in NRC and of the B2 protein fraction in the CNCPS for protein supplements, energy concentrates and forages. Absorbed Met and Lys flows were also sensitive to intestinal digestibility of RUP, and the CNCPS model was sensitive to the acid detergent insoluble crude protein (ADICP) and its assumption of complete unavailability. Neither the intestinal digestibility of the RUP fraction nor the protein degradation rates are measured

[3] Lanzas, C., L. O. Tedeschi, S. Seo, and D. G. Fox. 2006. Evaluation of protein fractionation systems used in formulating rations for dairy cattle. J. Dairy Sci. Accepted.

routinely. Approaches need to be developed to account for their variability. Research is needed to provide better methods for measuring pool sizes and ruminal digestion rates for protein fractionation systems.

3.2. Introduction

Livestock enterprises in developed countries are significant contributors to non-point sources of environmental N pollution because of their contributions to ammonia emissions and nitrate contamination of surface and ground water (NRC, 1993, NRC, 2003). Purchased feed, especially protein supplements, is a major source of imported nutrients and farm expenses on dairy farms (Klausner, et al., 1998). Under these economic and environmental constraints, improving the efficiency of N utilization and reducing N excreted are very important to maintain the sustainability of dairy farms, and nutrition models have become an effective farm management tool to accomplish these tasks (Dinn, et al., 1998, Wattiaux and Karg, 2004b).

Feedstuffs vary widely in non-protein nitrogen (NPN), the rate and extent of ruminal protein degradation, intestinal digestibility and essential amino acid (EAA) supply (Broderick, et al., 1989, NRC, 2001). Milk production will be reduced when protein supplied by the diet is below the energy allowable milk production, which is affected by protein degradation rates (Fox et al., 2004). Feed protein fractionation systems have been integrated into nutrition models to account for differences in protein availability and utilization. *In situ* techniques and schemes based on solubility in buffers, and detergent solutions have been adopted by the NRC (2001) and the Cornell Net Carbohydrate and Protein System (CNCPS, Fox et al., 2004) to measure protein fractions in feeds.

Sensitivity analysis identifies key sources of variability and uncertainty and quantifies their contribution to the variance of model outputs (Saltelli, 2000), helping to establish research and data collection priorities for further improvement of nutrition

models. Evaluations of the ability of nutrition models to predict duodenal flow of N and animal performance have been conducted (Bateman, et al., 2001a, Bateman, et al., 2001b, Fox, et al., 2004, Kohn, et al., 1998, NRC, 2001, Offner and Sauvant, 2004). However, few evaluations based on sensitivity analysis have been conducted. Fox et al (1995) assessed the impact of feed carbohydrate and protein fractions and microbial composition on animal performance predictions. Tylutki (2002) determined the inputs that routinely need to be analyzed to reduce risk of use of the CNCPS model in field conditions. However, the impact of feed protein variability and model assumptions on metabolizable protein (MP) and AA predicted flows have not been assessed. Reliable predictions of nutrient supply are critical for mathematical models to predict the effects of nutrients absorbed on milk composition and N efficiency, since any intermediary metabolism model would rely on rumen models for their substrates (Fox, et al., 2004, Offner and Sauvant, 2004). The objective of this study was to conduct a series of sensitivity analysis of the protein fractionation schemes of the NRC (2001) and CNCPS (Fox, et al., 2004) to assess their impact on variation in MP and absorbed AA predictions due to feed composition variability. A second objective was to assess the impact of assumptions underlying the CNCPS feed protein fractionation scheme. The overall objective of both analyses is to establish research priorities for increasing the robustness of the models.

3.3. Materials and Methods

3.3.1. Protein fractionation

The NRC (2001) and the CNCPS (Fox, et al., 2004) differ in the schemes used to predict MP and AA supply and requirements. The NRC (2001) adopted the *in situ* method to partition feed N fractions into rumen degradable protein (RDP) and rumen undegradable protein (RUP). The *in situ*-A fraction includes NPN, solubilized protein, and protein in particles sufficiently small to pass from the nylon bag. The *in situ*-B

fraction is potentially degradable in the rumen, depending on the competition between digestion and passage, and the *in situ*-C fraction is the unavailable protein, which is estimated as the remaining nitrogen after incubation for a predetermined time. Intestinal digestibilities of RUP are based on the mobile bag technique (Hvelplund, et al., 1992) and *in vitro* estimates (Calsamiglia and Stern, 1995). A regression approach is used to determine essential amino acid (EAA) composition of duodenal protein.

The CNCPS fractionates N into five fractions based on solubility; the A fraction is NPN, the B fraction is true protein and C is unavailable protein (Van Soest, et al., 1981b). The B fraction is further sub-divided into three fractions with different digestion rates (B1, B2, and B3). The B1 fraction is both soluble in borate phosphate buffer, precipitated by tricholoracetic acid. The B3 fraction is insoluble in neutral detergent but is soluble in acid detergent. The C fraction is insoluble in acid detergent solution. The B2 fraction is calculated by difference. The extent of degradation of the B fractions are based on the competition between fractional rates of degradation and passage. The A fraction is assumed to be completely degraded, while the C fraction is assumed completely undegraded. Intestinal digestibility is assumed to be 100 % for B1, and B2, 80% for B3, and 0% for C. A factorial approach is used to estimate EAA supply (O'Connor, et al., 1993)

3.3.2. Sensitivity analyses

3.3.2.1. Animals and diets.

Two different scenarios were chosen to test the sensitivity of the models. A low CP protein diet (12-14 % CP, 43 % NDF) with grass hay and corn silage as forage sources (named low protein diet) was formulated with each model to meet requirements for 20 kg milk per day. A second diet (18 % CP, 30 % NDF) with alfalfa and corn silage as forage sources was formulated with each model to meet requirements for 38 kg milk per day (named high protein diet). Both scenarios were

chosen because they represent situations in which a lactating dairy cow would likely be responsive to protein. Feedstuffs commonly used in diets of dairy cows in North America (Mowrey and Spain, 1999) were used (Table 3.1).

Table 3.1. Diets used in the simulations

Feeds in low protein diet	kg DM/day	Feeds in high protein diet	kg DM/day
Grass hay	7.0	Corn silage	7.0
Corn silage	6.0	High moisture corn grain	5.5
Dried shelled corn	4.5	Alfalfa silage	4.0
Soybean meal	0.4	Soybean meal	2.8
Urea[1]	0.2	Distiller grains	2.0

[1] Urea was added when the diet was formulated for the NRC to supply the required ruminally degraded protein.

3.3.2.2. Simulation procedures.

Global sensitivity analysis based on Monte Carlo techniques have been used in modeling simulations (Helton and Davis, 2003). In a Monte Carlo analysis, model inputs are described as probability density functions from which samples are drawn to feed the model and derive the probabilities of possible solutions for the model (Law and Kelton, 2000). The Monte Carlo analysis was done with @Risk version 4.5 (Palisade Corp., Newfield, NY) with spreadsheet versions of the CNCPS model version 5.0 as described by Fox et al. (2004) and the NRC model (NRC, 2001). Several sampling techniques that are suitable to Monte Carlo simulation are available. The sampling technique chosen for drawing the samples from the distributions was the Latin Hypercube (McKay, et al., 1979). The probability distribution is stratified in the Latin Hypercube sampling. This stratification divides the cumulative curve into

intervals of equal probability; from each interval, a sample is randomly taken. Sampling is forced to represent values at each interval. Because of the stratification, the Latin Hypercube is more efficient and provides more stable analysis of the model outcomes than random sampling (Helton and Davis, 2003). Ten thousand samplings for simulation were carried out. Convergence was set to be less than 1.5% of change in output statistics; it was achieved in all simulations.

3.3.2.3. Uncertainty and sensitivity measures

The model outputs generated by the simulations are presented as box plots. In a box plot, the box contains the middle 50 % of the data. The middle line in the box represents the median, and the upper edge of the box indicates the 75^{th} percentile, and the lower edge indicates the 25^{th} percentile. The range between the 75^{th} and the 25^{th} is the inter-quartile range. The vertical lines extend to a maximum of 1.5 times the inter-quartile range; the points outside the ends of the vertical lines are outliers. For comparative purposes, the inter-quartile range was expressed as MP or essential EAA allowable milk, using the efficiency coefficients of MP and EAA utilization of the CNCPS model (Fox, et al., 2004).

In order to relate the variation in the model outputs to the different sources of inputs, a stepwise regression analysis was used. The standard regression coefficients (SRC) were used to rank the inputs. They provide a measure of importance based on the effect of moving each input away from its mean value by a fixed fraction of its SD while retaining all other inputs at their mean values (Helton and Davis, 2002).

To assess differences in precision of the models, Bonferroni confidence intervals were computed for the SD of the simulated outputs (Ott and Longnecker, 2001).

Sensitivity analysis 1: Assessment of the impact of feed protein and EAA composition variability

A first series of simulations were conducted to assess the impact of feed protein and EAA composition variability on the N flows. For each model and scenario, the following simulations were conducted: (1) only the CP values of the feedstuffs were varied, (2) the inputs necessary to describe protein fractions and their corresponding rates and intestinal digestibilities were varied, (Cobelli and DiStefano) both CP and protein fraction inputs were varied, and (4) EAA composition was varied. The following outputs of the models were assessed: for simulations 1 to 3, MP from microbial crude protein (MCP) and RUP, absorbed Lys and Met flows and for simulation 4, absorbed EAA flows.

In order to describe inputs as probability density functions (Table 3. 2), a data base provided by a commercial laboratory (Dairy One, Ithaca, NY) was used to obtain the feed chemical composition measurements (CP, soluble protein, neutral detergent insoluble CP (NDICP), ADICP). Feed composition data were fit to a normal distribution. When feed inputs were not statistically normal, the distribution with the best fit to the data was assigned. The goodness of fit was assessed with several statistics (Chi-squared, Kolmogorov-Smirnov, and Anderson-Darling statistical tests) and graphical methods (distribution function differences plots and probability plots) (Law and Kelton, 2000). Minimum and maximum values in the data base were used to truncate the distributions and a correlation matrix was incorporated to take into account the correlation among inputs within feed when sampling. For the CNCPS, a normal distribution with a SD proportional to the mean of the degradation rate was used to take into account the fact that the variability in the rate estimates increases as the mean value increases for the degradation rates (Weiss, 1994). A triangular distribution was used for the intestinal digestibility coefficients for B1, B2, and B3. For the NRC model, *in situ* inputs were described as a normal distribution with mean

and SD as reported in the NRC (2001). Similarly, the NRC (2001) intestinal RUP digestibilities were also described by triangular distributions.

For the feed EAA composition (Table 3. 3), a normal distribution with mean and SD as reported in the NRC (2001) was used. For the grass hay and alfalfa silage, the NRC data were supplemented with other published sources (Givens and Rulquin, 2004, Muscato, et al., 1983, Ross, 2004, Tedeschi, et al., 2001) because the NRC database contains single observations. The CNCPS model uses EAA as a percentage of buffer insoluble protein. Muscato et al (1983) and Tedeschi et al (2001) concluded that the EAA profile of the original forage could be used to predict the EAA profile of the undegraded intake protein instead of using the buffer insoluble protein profile. Therefore, the EAA profile from the original feedstuff was also used for the CNCPS.

Sensitivity analysis 2: Assessment of the impact of the assumptions underlying the solubility based protein fractionation scheme in the CNCPS (Fox et al., 2004)

A second series of simulations was conducted to test the sensitivity of the model to the assumptions about N utilization underlying the solubility based protein fractionation scheme used in the CNCPS as described above. The following assumptions were tested: (1) the true soluble protein (B1 fraction) is nearly completely degraded in the rumen, (2) the buffer insoluble CP is composed of two kinetically distinct fractions (the NDICP corrected for ADICP (B3 fraction), which represents a slowly degradable fraction across feeds, and the B2 fraction that represents an intermediate degradable fraction), and (3) ADICP is assumed to be undegradable in the rumen and indigestible in the small intestine. For testing the assumptions, the following modifications were incorporated into the model spreadsheet and simulations in which CP and protein composition were varied were carried out:

(1) The degradation rates for B1 fraction were adjusted to available published data, and the fraction was linked to the liquid passage rate. Current feed library values

for the degradation rates for the B1 fraction exceed most of the published values for soluble proteins (Broderick, et al., 1989, Hedqvist and Udén, 2006, Mahadevan, et al., 1980, Peltekova and Broderick, 1996) (Table 3. 5).

(2) The impact of assuming two potentially degradable fractions within the insoluble protein was tested by collapsing both fractions into a single fraction, with a weighted average degradation rate (Table 3. 5).

(3) The effect of partial intestinal digestibility of ADICP of protein supplements on model predictions was assessed by assigning partial digestibilities based on published data (Table 3. 5). For unheated forages, ADICP coefficients of digestion are assumed to be zero (Goering, et al., 1972). However, additional ADICP produced by heating was partially digested in steamed treated alfalfa (Broderick, et al., 1993), distiller's grains (Nakamura, et al., 1994, Van Soest, 1989), and plant proteins (Hussein, et al., 1995, Nakamura, et al., 1994, Schroeder, et al., 1995).

3. 4. Results and discussion

3. 4. 1. Sensitivity Analysis 1: Influence of Feed Composition Variation on Model Predictions

3. 4. 1. 1. Input variability

The observed variability of each feedstuff is based on a broad population of the feeds with observations from extensive databases. The range in values for the CP and protein inputs (Table 3. 2) were similar to those previously reported for other data bases (Cromwell, et al., 1999, Kertz, 1998). Table 2 shows the distributions used to describe the feed protein composition. Although the normal distribution was the first choice and the number of samples available to fit the distributions were in all cases large ($100 < N < 1300$), not all the inputs were normally distributed. Some feed components (e.g. ADICP of grass hay and HMCG) had distributions skewed to the right (e.g. Pearson and gamma). These skewed distributions have zero as a limit of the

function and few observations with high values (Law and Kelton, 2000). Some other inputs (e.g. CP of soybean meal) were narrower around the mean than the normal distribution; thus they were better represented by log and logistic distributions (Law and Kelton, 2000). This is in agreement with the findings of Kertz (1998), who reported low coefficients of variation (< 2%) for CP in soybean meal. A consequence of the non-normality of the feed composition is that the mean and SD are less appropriate as measures of centrality and dispersion of the population (Law and Kelton, 2000). For skewed distributions, the mean overestimates the measure of centrality. Both models are deterministic, and in a deterministic model, the solutions of the model represent an average (Baldwin, 1995). However, when variability is taken into account, the mean value of the solutions are not necessarily coincident with the deterministic solution (Matis and Tolley, 1980). As the need for reducing safety factors for nutrients increases, accounting for feed composition variability may become more critical.

Table 3.2. Mean, SD and distributions for the feeds used in the simulations

	Grass hay		
	Mean	SD	Distribution [1]
CP, % DM	10.7	3.62	Gamma (5.0, 1.6)
Soluble CP, %DM	3	1.29	Gamma (4.2, 0.6)
NPN, % Soluble CP[2]	95	3.00	Normal (95.0, 3.0)
NDICP, %DM[2]	3.5	1.20	BetaGeneral (7.0, 14.6)
ADICP, %DM[2]	0.9	0.37	PearsonV (47.8, 117.8)
In situ A, %CP	28.4	13.9	Normal (28.4, 13.9)
In situ C, %CP	18.7	12.00	Normal (18.7, 12.0)
Rate of *in situ* B, h^{-1}	5	3.30	Normal (5.0, 3.3)
RUP digestibility,%	50		Triangular (40,60)
Rate of CNCPS B1, h^{-1}	135	20.00	Normal (135.0, 20.0)
Rate of CNCPS B2,h^{-1}	11	4.00	Normal (11.0, 4.0)
Rate of CNCPS B3,h^{-1}	1.2	1.00	Normal (1.2, 1.0)
ID of CNCPS B1, %[2]	100		Triangular (90,100)
ID of CNCPS B2,%[2]	100		Triangular (90,100)
ID of CNCPS B3,%[2]	80		Triangular (70,90)
	Corn silage		
	Mean	SD	Distribution [1]
CP, % DM	8.5	1.06	Loglogistic (2.1, 6.2, 11.3)
Soluble CP, %DM	4.2	1.05	Weibull (3.8, 4.0)
NPN, % Soluble CP[2]	95	3.00	Normal(95.0, 3.0)
NDICP, %DM[2]	1.4	0.33	Loglogistic (0.3, 1.1, 6.1)
ADICP, %DM[2]	0.7	0.16	Loglogistic (0.05, 0.61, 7.6)
In situ A, %CP	51.3	16.9	Normal (51.3, 16.9)
In situ C, %CP	18.5	5.30	Normal (18.5, 5.3)
Rate of *in situ* B, h^{-1}	4.4	1.50	Normal (4.4, 1.5)
RUP digestibility,%	55		Triangular (45, 65)
Rate of CNCPS B1, h^{-1}	150	20.00	Normal (150.0, 20.0)
Rate of CNCPS B2,h^{-1}	15	4.00	Normal (15.0, 4.0)
Rate of CNCPS B3,h^{-1}	0.2	1.00	Normal (0.2, 1.0)
ID of CNCPS B1, %[2]	100		Triangular (90,100)
ID of CNCPS B2,%[2]	100		Triangular (90, 100)
ID of CNCPS B3,%[2]	80		Triangular (70,90)

Table 3.2. (Continued)

	Alfalfa silage		
	Mean	SD	Distribution[1]
CP, % DM	21	2.91	Normal (21.0, 2.9)
Soluble CP, %DM	12.4	2.75	Logistic (12.4, 1.6)
NPN, % Soluble CP[2]	67	3.00	Normal(67.0, 3.0)
NDICP, %DM[2]	3.1	0.95	Loglogistic (-0.05, 3.0, 6.0)
ADICP, %DM[2]	1.5	0.55	Loglogistic (0.4, 1.0, 4.9)
In situ A, %CP	57.3	10.20	Normal(57.3, 10.2)
In situ C, %CP	7.4	2.30	Normal (7.4, 2.3)
Rate of *in situ* B, h^{-1}	12.2	7.10	Normal (12.2, 7.1)
RUP digestibility,%	65	--	Triangular (55, 75)
Rate of CNCPS B1, h^{-1}	150	20.00	Normal (150,20)
Rate of CNCPS B2,h^{-1}	15	4.00	Normal (15,4)
Rate of CNCPS B3,h^{-1}	1.8	1.00	Normal (1.8,1)
ID of CNCPS B1, %[2]	100	--	Triangular(90,100)
ID of CNCPS B2,%[2]	100	--	Triangular (90, 100)
ID of CNCPS B3,%[2]	80	--	Triangular (90, 100)

	Dried shelled corn		
	Mean	SD	Distribution [1]
CP, % DM	9.5	1.31	Normal (9.5, 1.3)
Soluble CP, %DM	1.9	0.59	Normal (20.1, 6.2)
NPN, % Soluble CP[2]	73	3.00	Normal (73.0, 3.0)
NDICP, %DM[2]	1	0.36	Normal (10.1, 3.8)
ADICP, %DM[2]	0.9	0.20	Normal (9.7, 2.1)
In situ A, %CP	23.9	12.50	Normal (23.9, 12.5)
In situ C, %CP	3.6	8.30	Normal (3.6, 8.3)
Rate of *in situ* B, h^{-1}	4.9	2.00	Normal (4.9, 2.0)
RUP digestibility,%	75	--	Triangular (75, 95)
Rate of CNCPS B1, h^{-1}	150	20.00	Normal (150,20)
Rate of CNCPS B2,h^{-1}	6	3.00	Normal (6.0, 3.0)
Rate of CNCPS B3,h^{-1}	0.1	1.00	Normal (0.1, 1.0)
ID of CNCPS B1, %[2]	100	--	Triangular (90,100)
ID of CNCPS B2,%[2]	100	--	Triangular (90,100)
ID of CNCPS B3,%[2]	80	--	Triangular(70,90)

Table 3.2. (Continued)

	High moisture corn		
	Mean	SD	Distribution[1]
CP, % DM	9.7	1.03	Pearson(53.5,387.4)
Soluble CP, %DM	2.8	1.06	Extreme value (2.3,0.7)
NPN, % Soluble CP[2]	95	3.00	Normal (95.0, 3.0)
NDICP, %DM[2]	0.8	0.19	Logistic (0.8, 0.1)
ADICP, %DM[2]	0.4	0.10	Gamma (53.8, 0.01)
In situ A, %CP	27.9	2.90	Normal (27.9, 2.9)
In situ C, %CP	0.7	0.90	Normal (0.7, 0.9)
Rate of *in situ* B, h^{-1}	5.1	2.50	Normal (5.1, 2.5)
RUP digestibility,%	90	--	Triangular (80,100)
Rate of CNCPS B1, h^{-1}	150	20.00	Normal (150.0, 20.0)
Rate of CNCPS B2,h^{-1}	15	4.00	Normal(15.0, 4.0)
Rate of CNCPS B3,h^{-1}	1.8	1.00	Normal (1.8, 1.0)
ID of CNCPS B1, %[2]	100	--	Triangular (90,100)
ID of CNCPS B2,%[2]	100	--	Triangular(90,100)
ID of CNCPS B3,%[2]	80	--	Triangular (70,90)

	Solvent soybean meal		
	Mean	SD	Distribution[1]
CP, % DM	51	3.19	Logistic (51.4, 1.7)
Soluble CP, %DM	10.1	3.98	BetaGeneral (1.9, 2.6)
NPN, % Soluble CP[2]	55	3.00	Normal (55.0, 3.0)
NDICP, %DM[2]	5.5	3.38	Normal (10.7, 6.6)
ADICP, %DM[2]	1.6	1.34	Normal (3.2, 2.6)
In situ A, %CP	15	6.20	Normal (15.0, 6.2)
In situ C, %CP	0.6	1.90	Normal (0.6, 1.9)
Rate of *in situ* B, h^{-1}	4.4	1.50	Normal (4.4, 1.5)
RUP digestibility,%	80	--	Triangular (70, 90)
Rate of CNCPS B1, h^{-1}	230	30.00	Normal (230.0, 30.0)
Rate of CNCPS B2,h^{-1}	11	4.00	Normal (11.0, 4.0)
Rate of CNCPS B3,h^{-1}	0.2	1.00	Normal (0.2, 1.0)
ID of CNCPS B1, %[2]	100	--	Triangular (90,100)
ID of CNCPS B2,%[2]	100	--	Triangular (90,100)
ID of CNCPS B3,%[2]	80	--	Triangular (90,100)

Table 3.2. (Continued)

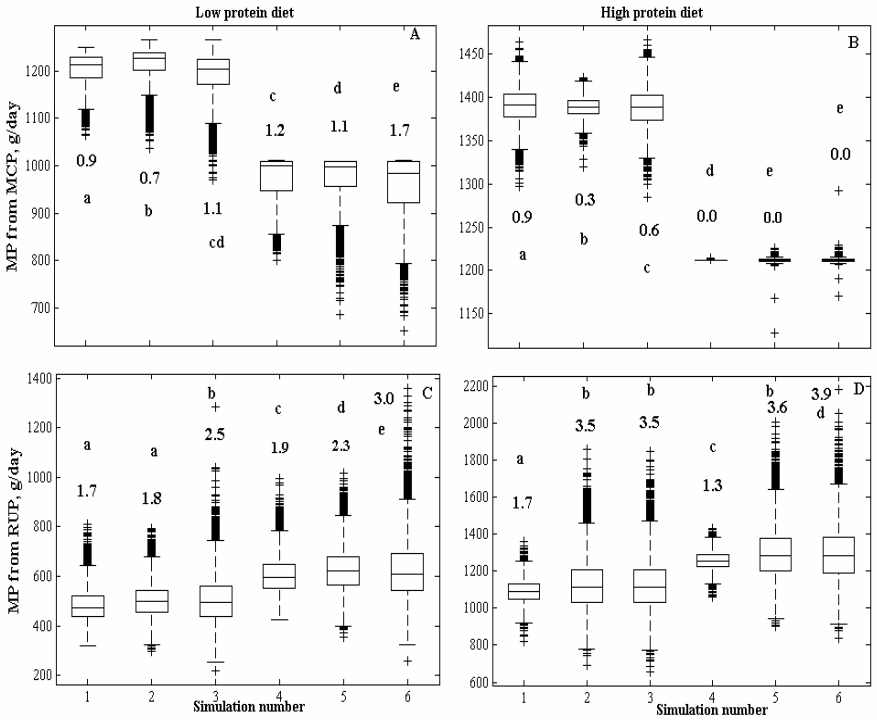
	Distillers Grains		
	Mean	SD	Distribution [1]
CP, % DM	31.4	2.40	Normal (31.4, 2.4)
Soluble CP, %DM	14.7	8.76	Loglogistic (-0.4, 4.6, 5.3)
NPN, % Soluble CP[2]	67	3.00	Normal (67.0, 3.0)
NDICP, %DM[2]	31	9.46	Normal (31.0, 9.5)
ADICP, %DM[2]	17.5	5.50	Logistic (5.5, 0.9)
In situ A, %CP	18.3	7.90	Normal (18.3, 7.9)
In situ C, %CP	17.1	10.30	Normal (17.1, 10.3)
Rate of *in situ* B, h-1	4.7	1.40	Normal (4.7, 1.4)
Rate of CNCPS B1, h-1	150	20.00	Normal (150, 20)
Rate of CNCPS B2,h-1	8	3.00	Normal (8.0, 3.0)
Rate of CNCPS B3,h-1	0.5	1.00	Normal (0.5, 1.0)
ID of CNCPS B1, %[2]	100	--	Triangular (90, 100)
ID of CNCPS B2,%[2]	100	--	Triangular (90, 100)
ID of CNCPS B3,%[2]	80	--	Triangular (70, 90)

[1] The parameters needed to characterize the distribution are indicated between brackets. An α parameter indicates shape of the distribution, a β parameter indicates scale (e.g. σ for the normal distribution), and a γ parameter indicates location (i.e. μ for the normal distribution). The distributions are beta general (α_1, α_2), extreme value (γ, β), gamma (α, β), logistic (α, β), loglogistic (γ, α, β), normal (μ, σ), PearsonV (α, β), and Weibull (α, β). The triangular distribution (a, b) was used in absence of data; a is the minimum value and b is the maximum value.
[2] ADICP= Acid detergent insoluble crude protein, ID= Intestinal digestibility, NDICP= Neutral detergent insoluble crude protein, NPN= Non-protein nitrogen.

3.4.1.2 Microbial crude protein predictions.

The impact of the protein inputs on MP predictions is shown in Figure 3.1. Although each diet was formulated for the same MP allowable milk, the models differed in the amounts and proportions that MCP and RUP contributed to MP supply (Figure 3.1). For comparative purposes, the variation in MP and AA flows was expressed in milk responses using a constant efficiency; it is plausible that this approach over predicts responses to protein since marginal conversion decreases as supply approaches the requirements (Doepel, et al., 2004). Predictions for MCP had different distributions between diets (Figure 3. 1, Panels A and B). The MCP distributions of the low protein diet were strongly skewed to the left (Figure 3.1, Panel A). For the NRC predictions, the upper bound corresponded to the maximum RDP requirement. These skewed distributions for both models are due to the discontinuity of the equations used to estimate microbial growth. In both models, predictions of microbial growth are based on the assumption that the most limiting nutrient restricts growth by calculating both energy and N-allowable growth and using the lower of the two values (NRC, 2001, Tedeschi, et al., 2000). A consequence of this discontinuity in the calculation may be an increased risk of use of the models when safety factors are reduced for RDP. The accuracy of MCP predictions relies on those inputs that provide fermentable organic matter when energy is first limiting and degradable protein when N is first limiting (Ruiz, et al., 2002). Equations with smooth or continuous transitions from situations in which N or energy limits growth would make the models more robust and biologically correct. The estimation of N requirements for microbes is an area that needs further refinements in

Figure 3.1. Box plots for the variability in predicted metabolizable protein from microbial protein (Panel A: low protein diet, Panel B: high protein diet) and from rumen undegradable protein (Panel C: low protein diet, Panel D: high protein diet) due to feed protein variation for the following simulations: 1) CNCPS, CP, 2) CNCPS, protein fractions, 3) CNCPS, CP and protein fractions, 4) NRC, CP, 5) NRC, protein fractions, and 6) NRC, CP and protein fractions. The middle line in the box represents the median, and upper and lower areas of the center box indicate the 75^{th} and 25^{th} percentiles (50% of the values are included; The inter-quartile range (H) is the difference between the two percentiles). The whiskers on the lines are extreme values, and indicate values that fall within 1.5H. For comparative purposes, H is expressed in MP allowable milk (assuming an efficiency of 0.65). Predictions within a panel with different variance have different letters ($P < 0.05$).


Low protein diet
High protein diet
A
B
C
D
MP from MCP, g/day
MP from RUP, g/day
Simulation number
Simulation number

both the NRC and CNCPS models. The inaccuracy in prediction of microbial N requirements is well illustrated by Schwab et al (2005); milk protein yields were predicted better when MP supply was predicted from available energy only, rather than from both available energy and nitrogen. Biases in predicting microbial growth when N is first limiting may result from not adequately accounting for N supplied by recycling (both intraruminal and urea recycling), inaccurate predictions of RDP supply, and/or efficiency of microbial use of RDP. If RDP requirements are over predicted, the risk of overfeeding RDP and increasing N excretion increases. If RDP requirements are under predicted, the risk of not maximizing microbial growth increases, and MP supply decreases. For the high protein diet, the impact of protein variability on MCP predictions of the NRC model was negligible with no predicted milk responses (Figure 3. 1, Panel B). At high protein levels, the CNCPS microbial growth predictions were more sensitive to protein (Figure 3.1, Panel B). This is due to the peptide stimulation adjustment factor and the indirect impact that varying protein has on prediction of the size of the non-fiber carbohydrates pool (Fox, et al., 2004). Non-fiber carbohydrates are calculated by difference and the amount of carbohydrate fermented in the rumen dictates microbial growth (Fox, et al., 2004). The CNCPS adjusts the yield of the bacteria that ferment nonstructural carbohydrates with an empirical function of amino N stimulation that enhances microbial yield up to 18 % at any given carbohydrate fermentation rate. Although *in vivo* responses to amino N has been variable, improvements in microbial growth and efficiency greater than 18 % have been reported (Chikunya, et al., 1996, Hume, 1970). Van Kessel and Russell (1996) demonstrated that peptides and amino acids had little impact on the yield of carbohydrate limited, ammonia-excess cultures, but they improved the growth rate and yield in excess-energy conditions. Amino-N helps to match anabolic and catabolic rates, decreasing the waste of energy in spilling reactions (Russell, 1993, VanKessel

and Russell, 1996). Therefore the sensitivity of microbial growth to protein supply may be over predicted when the rate of carbohydrate fermentation is low, but may be under predicted at high fermentation rates (VanKessel and Russell, 1996).

3.4.1.3.Metabolizable protein from RUP.

Overall, both models predicted wide ranges in amounts of RUP (Figure 3. 1, Panels C and D). The SD for predicted RUP within the high protein diet was approximately 200 g/d for both models when CP and protein fractions were varied. Ipharraguerre and Clark (2005) summarized intestinal flow data from 57 studies. In their database, a variety of protein sources were represented; DMI ranged from 10.8 to 26.8 kg/d and dietary CP ranged from 11.3 to 23.1 %. Despite their extensive database, they reported a SD for the nonammonia, nonmicrobial N intestinal flow of 87.1 g (544 g CP) which was only 2.7 fold greater than models predicted for a single diet. Similarly, in an evaluation of the NRC model, the range in RUP supply was overestimated (Huhtanen, 2005).

The protein inputs that contributed most to the MP from RUP variability are presented in Figure 3. 2. Ruminal degradation rates were highly ranked among the inputs in all the simulations (NRC B rate and CNCPS B2 rate). In the high protein diet, RUP flow was very sensitive to digestion rates of soybean meal. In addition, the models were sensitive to protein B fraction degradation rates for energy concentrates (dried corn and HMCG) and forages (grass hay and alfalfa silage) (Figure 3. 2). Grains provide a substantial amount of protein since their inclusion rate is high in most mixed dairy rations (Mowrey and Spain, 1999). Protein has been described as a first limiting nutrient for rations based on alfalfa silage (Cadorniga and Satter, 1993; Dhiman and Satter, 1993), and grass silage (Aston et al., 1994). If heated appropriately, RUP content of forages can be increased (Broderick, 1995). Heat treatment at harvest decreased rumen protein degradation and increased the N of dietary origin flowing to

the intestines (Charmley and Veira, 1990). *In situ* data on protein degradation for grains is limited and *in vivo* or *in vitro* data is practically non-existent (Herrera-Saldana, et al., 1990, Lykos and Varga, 1995).

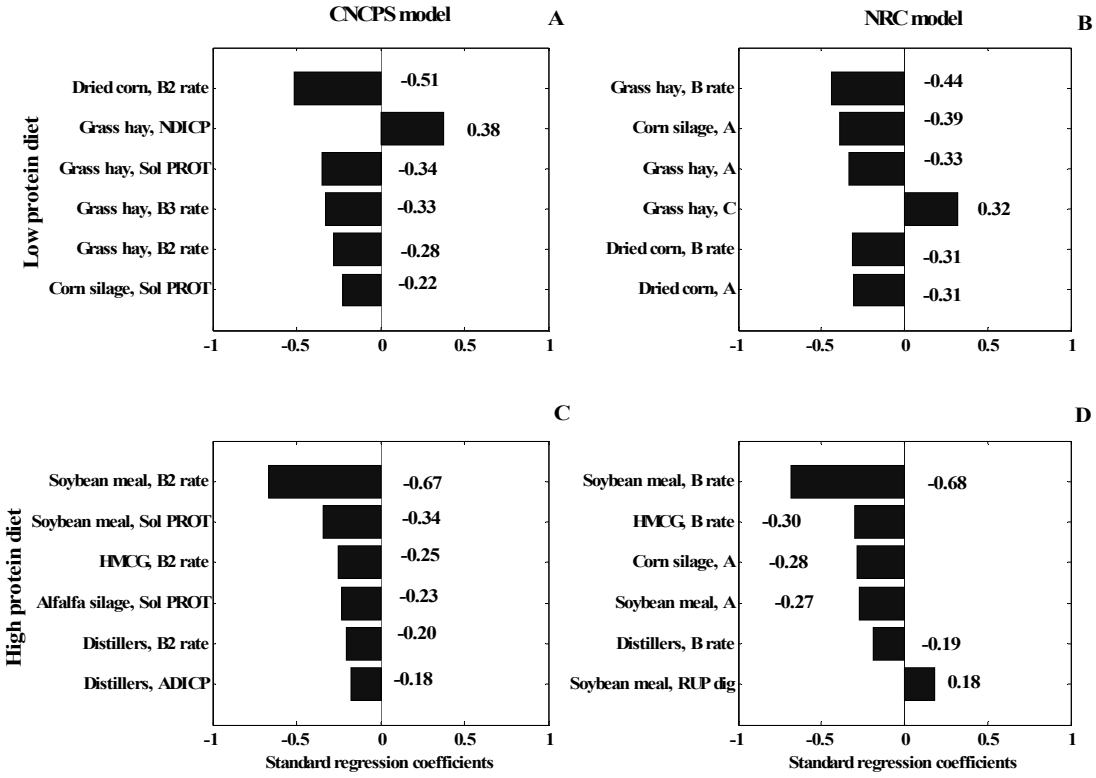


Figure 3. 2. Standard regression coefficients (SRC) ($P < 0.05$) for the protein inputs ranked as the most influential in predicting metabolizable protein from rumen undegradable protein in the CNCPS (Panels A and C) and NRC (Panels B and D) models.

[ADICP= Acid detergent insoluble crude protein, NDICP= Neutral detergent insoluble crude protein, SOL PROT= Soluble protein]

The imprecision of the RUP flows may result from the sensitivity of the models to the degradation rates used in the models. With the first-order approach used for both models, the closer the degradation rate is to the passage rate, the larger the changes in the model predictions are with small deviations in the rates. Most of the rates for the *in situ* B and CNCPS B2 fractions are close to the passage rate predicted by these models (Fox, et al., 2003, NRC, 2001). However, Reynal and Broderick (2003b) found that the *in vivo* rates were consistently higher than *in vitro* and *in situ* estimates (e.g. for expeller soybean meal, the *in vivo* rate was 17.9 %/h while the *in vitro* rate was 4 %/h). Thus *in vivo* protein degradation rates may be several-fold greater than the passage rate, which may make the RUP flows less sensitive to degradation rates than predicted by the models. Another contributing factor to the imprecision of predicting the RUP flows may be a lack of accuracy of predicted passage rates. Empirical equations used to predict passage rates explained at most 40 % of the variability when evaluated against an independent database (Seo, et al., 2006b). Methodological factors such as choice of marker and kinetic model may bias the estimates of passage rates. None of the markers are uniformly distributed across digesta phases. Ahvenjärvi et al. (2003) found that N flowing in the omasal canal was concentrated in small particulate matter. Ytterbium infused in the rumen had greater affinity for small particles (Siddons, et al., 1985), and thus the accuracy of measurements of N flows was linked to the accuracy of ytterbium as a marker (Ahvenjärvi, et al., 2003). Reynal and Broderick (2003b) obtained rates of passage with ytterbium infused in the rumen of the range of 12 to 14 %/h, while rates with ytterbium adsorbed in feed particles were between 2.5 and 6 %/h (Ellis, et al., 2002, Hristov and Broderick, 1996).

The low accuracy and repeatability of the methods used to estimate degradation rates compromise the robustness of the models. The intrinsic limitations of the *in situ* technique results in inconsistent underestimation of degradation rates.

The loss of particles from the bag causes an underestimation of the rate parameter, since the lost particles, which have different chemical composition and surface area than those in the bag, generally have faster digestion rates (Noziere and Michalet-Doreau, 2000). In addition, the N of microbial origin can make up 60 % of the N in the residue (Beckers, et al., 1995), and removing attached microbes is difficult. Similarly, *in vitro* methods tended to underpredict protein digestion rates (Reynal and Broderick, 2003b). Advances in this area will rely upon a better understanding of the sources of variation in the techniques (Broderick, et al., 2004c), and greater efforts in modeling and understanding of *in vitro* digestion. Although proteolysis is assumed to be a first-order process, *in vitro* methods deviate from first-order kinetics for several reasons: (1) substrate-limiting conditions are difficult to maintain through the incubation, (2) when proteolytic enzymes are used, the enzymatic activity may decline over time, and may be subject to end-product inhibition (Broderick and Clayton, 1992, Kohn and Allen, 1995), and (3) microbial growth in a batch follows well-defined phases, namely, lag, exponential growth, and stationary phase, not observed *in vivo*.

Along with the problems encountered in estimating digestion and passage rates, the kinetic models used to integrate both passage and digestion (Orskov and McDonald, 1979, Waldo, et al., 1972) may be too simplistic to appropriately mimic rumen digestion. For example, the assumption that the rumen is a single compartment in which materials are instantaneously and completely mixed is biologically incorrect and leads to incorrect model predictions.

The RUP flows were also sensitive to *in situ* A and soluble protein fractions (Figure 3. 2). They were negatively linked to RUP supply because both are assumed to be completely degraded in the rumen. High correlations have been found for *in situ* A (soluble in water) and soluble protein measurement (soluble in borate phosphate buffer, fractions A and B1 in CNCPS) ($r = 0.90$) since they measure essentially the

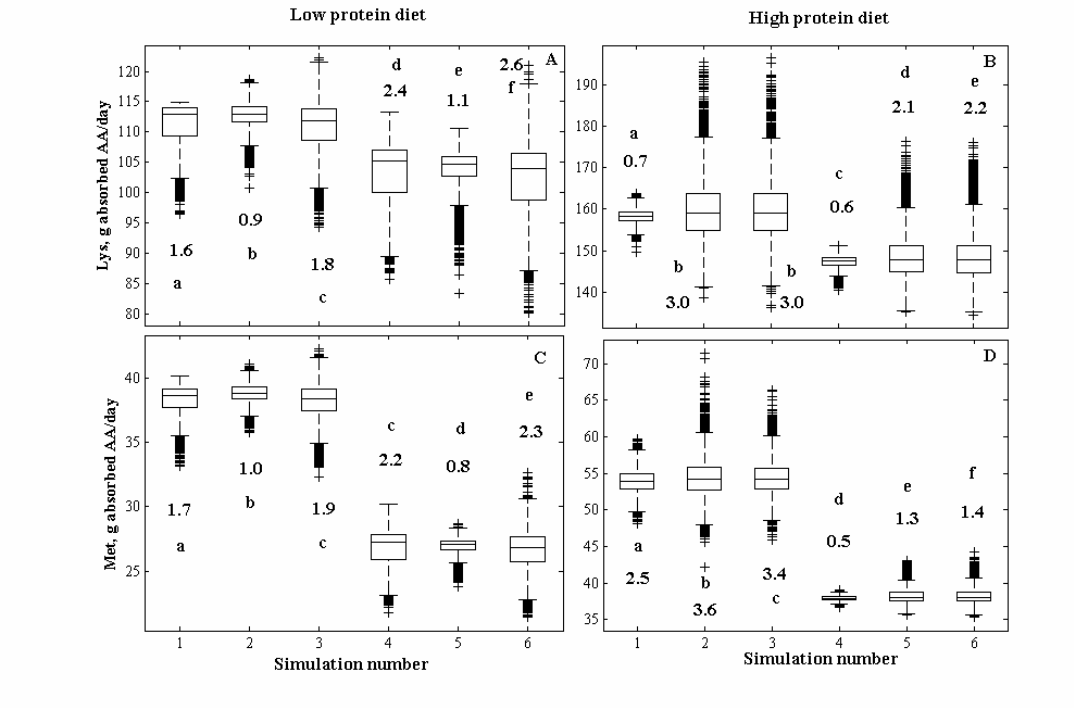
same protein fraction (Hoffman, et al., 1999). For the low protein diet, the RUP flows were also positively related to grass silage NDICP (SRC= 0.38) and grass silage *in situ* C (SRC= 0.32) (Figure 2). For the high protein diet, RUP flows were sensitive to distillers ADICP (SRC= -0.18) and soybean meal RUP intestinal digestibility (SRC= 0.18).

3.4.1.4. Absorbed methionine and lysine flows.

Lysine and Met are most frequently first limiting EAA for milk production in lactating dairy cows fed corn-based rations (Schwab, et al., 1992), and the impact of variability in protein fractionation on their flows is presented in Figures 3.3 and 3.4.

For the low protein diet, the NRC predicted flows of Lys and Met were more sensitive to feed variability than were CNCPS predictions because the main contributor was the MCP, which was more variable in the NRC model predictions (Figure 3.3, Panels A and C). The sensitivity in the low protein diet was distributed among several inputs similarly ranked (Figure 3. 4, Panels A, B, E and F). The NRC model was sensitive to those inputs that increase the amount of RDP. Because of the regression approach used in the NRC to predict amino acid rumen outflows from feeds, those inputs that increased the main source of MP, MCP for the low protein diet, were positively related to AA flows. An exception was the *in situ* C fraction for grass hay. The *in situ* C fraction was negatively related with AA flows (SRC= -0.22), but it was positively related with MP supply (SRC= 0.32), which suggests a disconnection between the AA and MP predictions. With the factorial approach used in the CNCPS, AA predictions were sensitive to inputs that increase RUP flow or RDP supply when the diet was deficient in RDP, depending on the AA profile of the feeds.

Figure 3.3. Box plots for the variability in absorbed Lysine (Panel A: low protein diet, Panel B: silage diet) and methionine (Panel C: low protein diet, Panel D: silage diet) predictions due to feed protein variation for the following simulations: 1) CNCPS, CP, 2) CNCPS, protein fractions, 3) CNCPS, CP and protein fractions, 4) NRC, CP, 5) NRC, protein fractions, and 6) NRC, CP and protein fractions. The middle line in the box represents the median, and upper and lower areas of the center box indicate the 75^{th} and 25^{th} percentiles (50 % of the values are included; the inter-quartile range (H) is the difference between the two percentiles). The whiskers on the lines are extreme values, and indicate values that fall within 1.5H. For comparative purposes, H is expressed in Lys or Met allowable milk (assuming an efficiency of utilization of 0.82 for Lys and 1 for Met). Predictions within panel with different variance have different letters ($P < 0.05$).


Low protein diet
High protein diet
A
B
C
D
Lys, g absorbed AA/day
Met, g absorbed AA/day
Simulation number
Simulation number

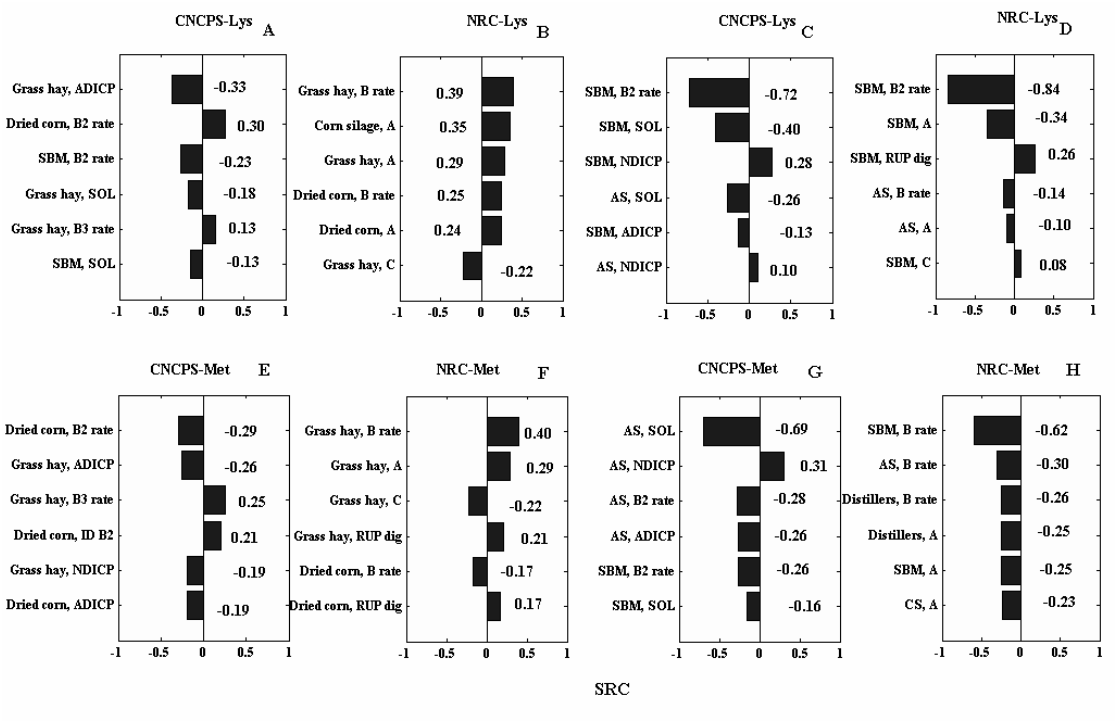


Figure 3.4. Standard regression coefficients (SRC) ($P < 0.05$) for the protein inputs ranked as the most influential in predicting absorbed lysine and methinone in the CNCPS (panels A, C, E, and G) and the NRC (panels B, D, F, and H) models for low (Panel A, B, E, and F) and high protein (Panel C, D, G, and H) diets.

[ADICP= Acid detergent insoluble crude protein, ID= Intestinal digestibility, NDICP= Neutral detergent insoluble crude protein, SOL= Soluble protein].

For example, the B2 rate for dried corn was positively related to Lys flows (SRC = 0.30) and negatively related to Met flows (SRC = -0.29). The NRC predictions were less sensitive to feed variation with the high protein diet. In the high protein diet (Figure 3. 4, panels C, D, G, and H), the soybean meal B2 rate and *in situ* B rate were highly ranked for their influence on Lys flows and NRC Met flows. Otherwise, several fractions in various feeds had similar effects on Met and Lys flows. Overall, Met flows were particularly sensitive to intestinal RUP digestibilities (Figure 3. 4, Panel E, F, and G) since Met contents of the feeds vary considerably (NRC, 2001) . The importance of protein intestinal digestibility was highlighted by Notfstger and St-Pierre (2003); when low digestible RUP (< 0.60) was replaced by high digestible RUP sources (>0.90), dry matter intake increased two kg/d and milk responses as great as 6 kg/d were reported. When a low protein diet (17 % CP) with a high digestible RUP source was supplemented with Met, dry matter intake increased less than 1 kg/d, but milk responses greater than 4 kg/d were observed (Noftsger and St-Pierre, 2003).

3.4.1.5. Amino acid supply

The EAA composition of feeds and its impact on duodenal flows are presented in Tables 3. 3 and 3. 4, respectively. Despite the statistical differences in their variance, with the exception of the Leu flows and to some extent Thr, the EAA flows had numerically similar ranges in EAA allowable milk, indicating similar sensitivity (Table 4) across the NRC (2001) and CNCPS models and diets. The large responses of milk predicted for some EAA (e.g. Leu) result from the use of a constant efficiency of conversion of EAA to milk protein assumed in the models. For the absorbed Lys and Met predictions for both models, the impact of the variation in Lys and Met content (Table 4) was greater than the impact of protein fractions in the low protein diet (Figure 3, Panel A and C) and greater than the impact of the CP variation (Figure 3, Panel B and D) in the high protein diet.

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Table 3. 3. Essential amino acids composition (% CP) of the feeds used in the simulations (mean ± SD).

	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Val
Alfalfa silage[1]	4.1±0.21	1.7±0.13	4.2±0.39	6.8±0.69	4.6±0.90	1.2±0.11	4.4±0.25	4.0±0.16	1.9±0.88
Corn silage[2]	2.0±0.41	1.8±0.30	3.3±0.23	8.6±0.91	2.5±0.35	1.5±0.12	3.8±0.23	3.2±0.30	4.5±0.28
Distillers[2]	4.1±0.28	2.5±0.21	3.7±0.13	9.6±2.80	2.2±0.39	1.8±0.21	4.9±0.37	3.4±0.34	4.7±0.27
Dry corn[2]	4.5±0.05	3.1±0.05	4.1±0.04	11.2±0.14	2.8±0.03	2.1±0.02	4.6±0.05	3.6±0.03	4.0±0.04
Grass hay[3]	3.6±0.59	1.4±0.25	3.3±0.63	6.0±1.26	3.6±0.68	1.3±0.46	3.8±0.75	3.5±0.78	4.3±0.92
HMCG[2]	3.9±0.74	2.5±0.22	3.4±0.25	11.6±0.93	2.6±0.41	2.1±0.28	4.6±0.33	3.7±0.30	4.9±0.38
Soybean meal[2]	7.3±0.36	2.8±0.17	4.6±0.22	7.8±0.24	6.3±0.27	1.4±0.09	5.3±0.21	4.0±0.14	4.6±0.26

[1] Givens and Rulquin (2004), NRC (2001), and Ross (2004).
[2] NRC (2001), HMCG: high moisture corn grain.
[3] Muscato et al. (1983), NRC (2001), Tedeschi et al. (2001).

Table 3. 4. Variation in absorbed essential amino acids (EAA) due to variability in EAA composition of the feeds[1].

	Low protein, CNCPS		Low protein, NRC		High protein, CNCPS		High protein , NRC	
	Mean (g/day)	EAA allowed (kg milk/day)	Mean (g/day)	EAA allowed (kg milk/day)	Mean	EAA allowed (kg milk/day)	Mean (g/day)	EAA allowed (kg milk/day)
Arg	106	1.0^{b}	79	0.9^{c}	155	1.2^{a}	115	0.8^{d}
His	44	0.8^{d}	36	1.3^{c}	65	1.6^{a}	54	1.6^{b}
Ile	91	1.3^{b}	86	1.8^{a}	126	1.3^{c}	119	1.3^{d}
Leu	133	2.7^{d}	153	4.1^{c}	200	8.2^{b}	215	8.9^{a}
Lys	122	1.7^{d}	122	2.1^{c}	160	2.3^{a}	154	2.2^{b}
Met	44	1.4^{c}	33	2.0^{a}	60	1.9^{b}	44	1.2^{d}
Phe	85	2.2^{b}	84	1.9^{c}	125	2.3^{a}	125	1.6^{d}
Thr	86	1.8^{b}	86	3.1^{a}	119	1.5^{d}	117	1.7^{c}
Val	97	1.7^{b}	95	1.9^{a}	136	1.9^{a}	134	1.3^{c}

[1]Difference between the 75th and 25th percentiles are expressed in essential amino acid (EAA) allowable milk. Predictions with different variance within row have different superscripts ($P < 0.05$).

3. 4. 2. Sensitivity analysis 2: Impact of assumptions underlying the CNCPS protein fractionation scheme

Tables 3.5 and 3.6 summarize the changes and results of the evaluations of CNCPS protein digestion rates and ADICP digestibility. The MP supply was rather insensitive to changes in the assumptions underlying the fractionation scheme. The changes on predicted allowable milk were less than 0.5 kg milk/day. The Met and Lys flows were more sensitive to changes in the assumptions.

3. 4. 2. 1. Soluble protein degradation.

Degradation rates for the B1 fraction were reduced to reflect available published data (Table 3.5) and integrated with liquid rather than particle passage rate as assumed in the CNCPS. The MP supply for both diets was insensitive to these changes, because the B1 fraction represented a small proportion of the total protein supply (< 8 % of the total CP). Although the rates were lowered, they were still much greater than the predicted liquid passage rates by the CNCPS passage rate equations (9.8 $\%h^{-1}$ for the low protein diet, and 11.8 $\%h^{-1}$ for the high protein diet), which resulted only in small changes in extent of B1 degradation. *In vivo* studies have shown similar effects. When Choi et al. (2002b) supplemented a grass silage-based diet with protein concentrates with high and low *in situ*-A fractions, soluble non-amino N omasal flow was not significantly different among treatments. However, these modifications resulted in an increase in the Lys and Met flows, especially for the high protein diet (Table 3.6), because Lys and Met flows were more sensitive to the variation in B1 fraction than total RUP flows (Figure 3.2, Panel C and Figure 4, Panels C and G). Assuming constant efficiencies, the increase in Lys and Met were predicted to increase milk production (Table 3.6).

Table 3.5. Variations in digestion rates and intestinal digestibilities used to evaluate assumptions underlying the CNCPS protein fractionation scheme.

	kd of CNCPS B1[1], % h^{-1}		kd of CNCPS B2+B3[2], % h^{-1}		Id of CNCPS C[3], %		
	Mean	SD	Mean	SD	Mean	Min	Max
Alfalfa silage	28	5	10.1	4	--	--	--
Corn silage	28	5	9.9	3	--	--	--
Distillers grains	50	7	4.7	2	30	0	60
Dried shelled corn	50	7	5.7	3	--	--	--
Grass hay	49	6	4.9	2	--	--	--
High moisture corn	50	7	8.9	3	--	--	--
Soybean meal	46	6	9.1	3	40	0	80

[1] B1 rates are based on several published sources (Broderick, et al., 1989, Hedqvist and Udén, 2006, Peltekova and Broderick, 1996).
[2] B2 and B3 rate were collapsed into a single fraction, by assigning the same rate, using a weighted average of the original degradation rates.
[3] The intestinal digestibility coefficients (Id) for the C fraction of protein supplements (triangular distributions) are based on Hussein et al (1995), Nakamura et al (1994), Schroeder (1995) and Van Soest (1989).

Table 3. 6. Impact of varying the assumptions underlying the CNCPS protein fractionation scheme on model predictions. The change in the model predictions (prediction with the modified assumption – base prediction) are expressed as g/day and allowable milk.

	Base	Lower[1] B1 rates		Collapsed B2 and B3[2] fractions		Partial [3] Id for C fraction	
Low protein diet	Mean (g/day)	as g/day	as kg milk/day	as g/day	as kg milk/day	as g/day	as kg milk/day
MP from MCP	1194	-4	0	-4	0	--	--
MP from RUP	504	11	0.2	-22	-0.4	0	0
Absorbed Lys	111	1	0.4	2	0.8	1	0.4
Absorbed Met	38	1	1.2	1	1.2	1	1.2
High protein diet							
MP from MCP	1388	0	0	1	0	--	--
MP from RUP	1127	-2	0	-5	-0.1	0	0
Absorbed Lys	160	4	1.6	2	0.8	2	0.8
Absorbed Met	54	3	3.5	1	1.2	1	1.2

[1] The degradation rates for CNCPS B1 fraction were adjusted to available published data, and the fraction was linked to the liquid passage rate.
[2] B2 and B3 fractions were collapsed into a single fraction, with a weighted average degradation rate.
[3] Partial intestinal digestibility coefficients (Id) for the C fraction of protein supplements were assigned.

3. 4. 2. 2. Degradation rates for the insoluble protein.

Collapsing B2 and B3 fractions had a greater effect on the RUP flows for the low protein diet, since the B3 fraction represents a greater proportion of the total protein. The assigned degradation rates for the B fraction were based on the number of pools and rates identified by the curve peeling technique described by Jacquez (1985), using data from *in vitro* incubations with protease from *Streptomyces griseus* (Pichard, 1977). The low rates for the protein B3 fraction are not always supported by data (Coblentz, et al., 1999, Lagunes, et al., 1999). Because the curve peeling approach causes the errors to propagate from the slow component into the faster components (Jacquez, 1985), protein B2 rates may have also been inaccurately estimated. The partition of the insoluble protein into two distinguishable fractions may not be necessary.

3. 4. 2. 3. Partial intestinal digestibility of ADICP.

Assuming partial intestinal digestibility of the ADICP fraction in protein supplements (distillers' grains and soybean meal) had a similar impact on Lys and Met flows than the previous tested assumptions. These results are consistent with the observation that Lys and Met flows were very sensitive to intestinal digestibilities. Because no data were available on ruminal digestion rates of ADICP, the impact of partial ruminal digestion of ADICP could not be assessed. However, Hussein et al., (1995) found that ADICP from roasted soybean meals were partially digested in both rumen and small intestine. Some of the components recovered in the ADICP fraction may be Maillard products from the early stages of the reaction that are available.

3.5. Conclusions

Sensitivity analysis can be used to prioritize which protein fractions require frequent analysis and to identify research priorities to improve nutritional models for accurately predicting MP and AA supply. Despite the differences in the protein

schemes, both NRC and CNCPS predictions of MP supply were similar in sensitivity to variation in protein fractions and their degradation rates because both models are based on common principles, such as the competition between digestion and passage to predict site of digestion and using the first limiting nutrient to estimate microbial growth. Metabolizable protein and AA flows were sensitive to the degradation rates of the B protein fraction in the NRC and the B2 fraction in the CNCPS and intestinal digestibilities. Neither the degradation rates nor the intestinal digestibilities are routinely measured. In addition, the low accuracy of *in vitro* and *in situ* degradation rates may cause an overprediction of the ranges in RDP-RUP flows. Both laboratory methods and a better approach to integrate protein degradation rates are necessary. While predicted flows for diets with supplemented protein were very sensitive to the feed inputs of the supplements, decreasing the supplemented protein resulted in an increase of the number of inputs that needed to be measured. For accurate predictions of low protein diets, more data is needed on protein fractionation and their digestion rates for both forages and energy supplements, since forages and energy supplements represent the largest proportion of MP derived from the diet.

CHAPTER 4

IMPROVED FEED PROTEIN FRACTIONATION SCHEMES FOR FORMULATING RATIONS WITH THE CORNELL NET CARBOHYDRATE AND PROTEIN SYSTEM

4.1. Abstract

Accurate predictions of rumen degradable protein (RDP) and rumen undegradable protein (RUP) supplies are necessary for precision feeding to minimize excess N losses from ruminants while optimizing performance. The objectives of this study were to revise and evaluate the original Cornell Net Carbohydrate Protein System (CNCPS) protein fractionation scheme and alternatives designed to improve its accuracy in predicting RDP and RUP. Model predictions were evaluated with studies with N flow data from the omasum. The N fractionation scheme in version 5 of the CNCPS explained 78 % of the variation in RDP with a root mean square prediction error (RMSPE) of 275 g/d, and 51 % of the RUP variation with RMSPE of 248 g/d. Neutral detergent insoluble CP (NDICP) flows were overpredicted with a mean bias of 128 g/d (40 % of the observed mean). The greatest improvements in the accuracy of RDP and RUP predictions were obtained with the following alternative schemes: (1) A= non-protein N (NPN), B1= true soluble protein, B2= insoluble protein, C= unavailable (RDP, R^2= 0.84, RMSPE= 167 g/d, RUP, RUP, R^2 = 0.61, RMSPE= 209 g/d) and the use of the inhibitory in vitro (IIV) system for the B2 fraction and (2) the A and B1 fractions were redefined as the non amino-N and amino-N in the soluble fraction respectively (RDP R^2 = 0.79 with RMSPE= 195 g/d and RUP R^2 = 0.54 with RMSPE= 225 g/d).

4.2. Introduction

Systems to fractionate feed N have been integrated into nutrition models to predict the amount of RDP and RUP supplied by the diet. *In situ* techniques and schemes based on solubility in buffers, and detergent solutions have been adopted by the NRC (2001) and the CNCPS (Fox et al., 2004). Despite the differences in methodology used to fractionate protein, both schemes shared similar limitations in predicting RPD and RUP (Lanzas, et al., 2006b, Schwab, et al., 2005); including the following: (1) the range of RDP and RUP was over predicted, (2) the assumptions underlying the kinetic models were too restrictive to appropriately mimic rumen digestion, (3) the methods used to estimate some of the inputs, such as degradation rates, had low accuracy and repeatability. The assumptions that the N insoluble in neutral detergent and acid detergent represent slowly degradable and undegradable protein respectively does not hold for all feeds (Coblentz, et al., 1999, Nakamura, et al., 1994, Waters, et al., 1992). In addition, the assumption that the NPN fraction enters the ammonia pool directly and does not provide amino N that can stimulate microbial growth or escape rumen digestion caused under prediction of microbial protein (Aquino, et al., 2003) and ignores the fact that free amino acid (AA) and peptides contribute to the RUP flows (Choi, et al., 2002a, Volden, et al., 2002).

The objective of this study is to use existing literature and currently available methodology to evaluate and revise the original CNCPS protein fractionation system to improve its ability to predict RDP and RUP accurately. Alternative schemes to predict *in vivo* RDP and RUP were assessed.

4. 3. Materials and methods

4.3.1. Feed protein fractionation schemes

4.3.1.1. Original CNCPS protein fractionation scheme

The original CNCPS protein fractionation divides feed protein into five fractions (Sniffen, et al., 1992). The A fraction represents the soluble non-protein N

times 6.25 and contains peptides, free amino acids, ammonia, amides, ureides, nucleotides and nitrates (Reid, 1994). It is determined as the N soluble in buffer and non-precipitated by protein precipitating agents, such as tricholoracetic acid (TCA);

$$PA_j = NPN_j \times (SolCP_j /1000) \times (CP_j/1000) \quad (g / kg\ DM) \quad [4.1]$$

Where: CP_j is the crude protein content of the j^{th} feed, g/kg DM; NPN_j is the non-protein content of the j^{th} feed, g/kg SolCP; PA_j is the protein A fraction content of the j^{th} feed, g/kg DM; and $SolCP_j$ is the buffer soluble CP content, g/kg CP.

The fraction B1 is the soluble true protein, which is assumed to be very rapidly degraded in the rumen with degradation rates greater than 1.0/h. It is measured as the buffer soluble protein that is precipitated by protein precipitating agents;

$$PB1_j = (SolCP_j /1000) \times (CP_j/1000) - PA_j \quad (g / kg\ DM) \quad [4.2]$$

Where: CP_j is the crude protein content of the j^{th} feed, g/kg DM; PA_j is the protein A fraction content of the j^{th} feed, g/kg DM; $PB1_j$ is the protein B1 fraction content of the j^{th} feed, g/kg DM; and $SolCP_j$ is the buffer soluble CP content, g/kg CP.

The fraction C is the unavailable N, which when multiplied by 6.25, is assumed to be the protein associated with lignin, tannin-protein complexes, and Maillard products because they are highly resistant to degradation, and are insoluble in acid detergent (AD) solution times 6.25. Ruminal degradation rates and intestinal digestibility for the C fraction are 0;

$$PC_j = ADICP_j \times (CP_j/1000) \quad (g / kg\ DM) \quad [4.3]$$

Where: $ADICP_j$ is the acid detergent insoluble crude protein content of the j^{th} feed, g/kg CP; CP_j is the crude protein content of the j^{th} feed, g/kg DM; and PC_j is the protein C fraction content of the j^{th} feed, g/kg DM.

The B3 fraction is the CP insoluble in neutral detergent (ND) solution, but soluble in AD;

$$PB3_j = (NDICP - ADICP_j) \times (CP_j/1000) \quad (g / kg\ DM) \quad [4.4]$$

Where: $ADICP_j$ is the crude protein insoluble in AD solution content of the j^{th} feed, g/kg CP; CP_j is the crude protein content of the j^{th} feed, g/kg DM; NDICP is the neutral detergent insoluble crude protein content of the j^{th} feed, g/kg CP; and $PB3_j$ is the protein B3 fraction content of the j^{th} feed, g/kg DM.

It is assumed that the protein associated with the cell wall is very slowly degraded (< 0.02/h) and thus a high percentage escapes degradation in the rumen.

The B2 fraction represents the intermediate degradable protein with rates of degradation within the range 0.03- 0.16/h, and it is calculated by difference;

$$PB2_j = CP_j - PA_j - PB1_j - PB3_j - PC_j \quad (g / kg\ DM) \quad [4.5]$$

4.3.1.2. Modifications of the original feed protein fractionation system.

Both the original and alternative schemes tested in this study are listed in Table 4.1. These alternatives contain combinations of modifications of the original scheme described below.

1. Accounting for amino N in the soluble protein fraction.

The NPN fraction contains both amino N (AAN) and non-amino N (NAAN). Recent studies showed that peptides and free AA contributed to the RUP flows (Choi, et al., 2002a, Volden, et al., 2002). For corn- and alfalfa-silage based diets, the amount of N flowing as free AA out of the rumen exceed the outflow of N insoluble in ND (Olmos Colmenero and Broderick, 2006c). In addition, peptides and amino acids (AA) may stimulate microbial growth more than ammonia (VanKessel and Russell, 1996). Therefore, the distinction between the fraction containing non-amino N and amino-N (soluble true protein, peptides and free AA) is important in predicting both RUP and microbial protein flows. The A and B1 fractions were redefined as the non amino-N and amino-N in the soluble fraction.

$$PA_j = (1000 - AAN_j) \times (SolCP_j / 1000) \times (CP_j / 1000) \quad (g / kg\ DM) \quad [4.6]$$

Where: CP_j is the crude protein content of the j^{th} feed, g/kg DM; AAN_j is the amino N content of the j^{th} feed, g/kg SolCP; PA_j is the protein A fraction content of the j^{th} feed, g/kg DM; and $SolCP_j$ is the buffer soluble CP content, g/kg CP.

Because the ranges of reported fractional degradation rates of soluble protein and peptides degradation are similar (Volden, et al., 2002), and factors affecting peptide recoveries with precipitating agents used to separate true protein have not been fully investigated (Hedqvist, 2004), the aggregation of soluble true protein, peptides, and free AA in one fraction seems justified. In addition, the B1 fraction was assumed to pass at the same rate as liquid leaving the rumen. *In vivo* studies using the pulse dose technique reported degradation rates similar to the original B1 rates (Mangan, 1972, Volden, et al., 2002). However degradation rates of the B1 fraction in the CNCPS feed library rates exceed most of the published values for *in vitro* soluble proteins (Broderick, et al., 1989, Hedqvist and Udén, 2006, Mahadevan, et al., 1980, Peltekova and Broderick, 1996). The effects of adjusting the B1 rates to reflect those observed *in vitro* rates was also investigated (Table 4.1).

2. Insoluble protein fractions

Degradation rates for the neutral detergent insoluble crude protein. Recent studies of the kinetics of NDICP disappearance has been determined indicated that the digestion rates for the NDICP are considerably higher than the rates found in the CNCPS feed library for the B3 fraction (Coblentz, et al., 1999, Juarez, 1998, McBeth, et al., 2003, Rossi, et al., 1997). Values reported for NDICP degradation rates were similar or slightly higher than NDF degradation rates (Pichard, 1977). The impact of adjusting the B3 rates was assessed (Table 4.1).

Aggregation of the insoluble protein B2 and B3 fractions. From the results of the sensitivity analysis, we know that unless the rates for fractions within the insoluble protein differed by several magnitudes, the model predictions were insensitive to the

presence of different pools (see Chapter 3). Therefore, the aggregation of the B2 and B3 pools was assessed. In this scheme, the B2 fraction becomes,

$$PB2_j = CP_j - PA_j - PB1_j - PC_j \qquad \text{(g / kg DM)} \qquad [4.7]$$

Rates for the combined fraction were obtained using the inhibitory *in vitro* (IIV) method. In the IIV, developed by Broderick (1987), proteins are incubated with ruminal inoculum containing metabolic inhibitors to obtain quantitative recovery of the end-products of protein degradation. The IIV is one of the most studied and evaluated method to estimate protein degradation (Broderick, 1987, Broderick and Clayton, 1992, Broderick, et al., 2004b, Broderick, et al., 2004c).

Table 4.1. List of alternative protein fractionation schemes

Scheme	Modifications
1	Original scheme
2	Original scheme with adjusted B3 rates
3	A fraction as NAAN[1]
4	A fraction as NAAN[1] and adjusted B1 rates
5	A fraction as NAAN[1] and adjusted B3 rates
6	A fraction as NAAN[1] and adjusted B1 and B3 rates
7	Aggregated insoluble fraction[3], A fraction as NPN[2]
8	Aggregated insoluble fraction[3], A fraction as NAAN[1]
9	Aggregated insoluble fraction[3], A fraction as NPN[2], adjusted B1
10	Aggregated insoluble fraction[3], A fraction as NAAN[1], adjusted B1

[1] NAAN= Non amino nitrogen, A fraction computed as indicated in Eq. 4.6
[2] NPN= Non protein nitrogen, A fraction computed as indicated in Eq. 4.1
[3] B2 fraction computed as indicated in Eq. 4.7

4.3.2. Evaluation of the feed protein fractionation schemes

4.3.2.1. Data base description

Five studies designed to test the effect of dietary protein content and supplementation on N metabolism and animal performance in lactating dairy cows in which omasal flows were determined were used to evaluate the ability of the protein fractionation schemes to predict RDP supply and RUP flows (Brito and Broderick, 2004a, Brito and Broderick, 2004b, Brito and Broderick, 2006, Brito, et al., 2006, Olmos Colmenero and Broderick, 2006b, Olmos Colmenero and Broderick, 2006c, Reynal and Broderick, 2003a, Reynal and Broderick, 2005, Reynal, et al., 2003, Reynal, et al., 2005) (Table 4.2). The advantages of using omasal data for estimating N fractions include (Ahvenjarvi, et al., 2000): (1) there is substantially less endogenous N secreted into the rumen than into the duodenum, and (2) rumen microbes are measured before they reach the abomasum, and therefore they are not digested, which allow the digesta N to be separated into particle- and liquid-associated bacteria, protozoa and soluble and insoluble dietary N fractions.

4.3.2.2. Simulations and evaluation

A spreadsheet version of the rumen submodel of the CNCPS as described by Fox et al (2004) that incorporates new passage rates equations developed by Seo et al. (2006b) and a revised feed carbohydrate fractionation scheme (Lanzas, et al., 2006a) (Chapter 2) was used for the simulations. The following predicted outputs were evaluated against the *in vivo* data;

1. Total CP flows out of the rumen substracting NH_3 outflow (g/d),

   Observed CP flows = NAN × 6.25

   Predicted CP flows =

$$\sum REPB1_j + REPB2_j + REPB3_j + REPC_j + REBTP_j + REBCW_j + REBNA_j$$

2. RUP flows (g/d)

Observed RUP flows = Total CP flows – Microbial NAN × 6.25

Predicted RUP flows = $\sum REPB1_j + REPB2_j + REPB3_j + REPC_j$

3. RDP supply (CP intake – RUP flows) (g/d)

Observed RDP supply = Total CP intake – RUP flow

Predicted RDP flows = $\sum RDPA_j + RDPB1_j + RDPB2_j + RDPB3_j$

4. NDICP flows (g/d)

Observed NDICP flow= NDIN flow × 6.25

Predicted NDICP flow = $\sum REPB3_j + REPC_j$

Where NAN is non ammonia nitrogen, NDIN is the neutral detergent insoluble nitrogen, RDPAj is ruminally degraded protein A fraction of the j^{th} feedstuff , the $RDPB_{ij}$ is the ruminally degraded protein B_i fraction of the j^{th} feedstuff, $REBCW_j$ is the ruminally escaped bacterial cell wall protein of the j^{th} feedstuff, $REBNA_j$ is the ruminally escaped bacterial nucleic acids of the j^{th} feedstuff, $REBTP_j$ is the ruminally escaped bacterial true protein of the j^{th} feedstuff, REPBij is the ruminally escaped protein B_i fraction of the j^{th} feedstuff, $REPC_j$ is the ruminally escaped protein C fraction of the j^{th} feedstuff.

To assess the model predictions, the following statistical tests were used. For assessing accuracy and precision, regression coefficients of determination (R^2), mean square error (MSE), mean square prediction error (MSPE) and its partition into three independent and additive components (Theil, 1961), mean bias (U^M), slope bias (U^R), and random unexplained errors (U^D) , and linear regression were performed.

Table 4.2. Descriptive statistics for the studies used to evaluate the ability of the protein fractionation schemes to predict rumen degradable protein supply and flow of rumen undegradable protein

	Descriptive statistics				
	N	Mean	SD	Min	Max
Diet composition and intake					
DM intake, kg/d	22	23.9	1.55	21.4	26.8
NDF, g/kg DM	22	250	2.4	22.4	30
N, g/kg DM	22	27.8	2.63	21.6	32.5
NEl, MJ/kg DM	22	6.28	0.251	5.94	6.90
Production and N excretion					
BW, kg	22	602	27.5	561	634
DIM, d	22	91	19	72	120
Milk, kg/d	22	39	2.9	32.9	42.8
Fat yield, kg/d	22	1.3	0.12	1	1.6
True protein yield, kg/d	22	1.2	0.11	0.9	1.3
Urine N, g/d	17	154	48.7	63	240
Fecal N, g/d	17	211	28.7	154	275
Omasal N flows					
Total N, g/d	22	562	163.7	233	709
Free AAN, g/d	18	42.1	19	16	70
Total NAN, g/d	22	551	161.8	226	695
Dietary NAN, g/d	22	236	80.8	74	403
Bacterial NAN, g/d	22	397	87.2	238	480
NDIN, g/d	17	25	7.7	14	45
ADIN, g/d	18	20	23.6	3	66

AAN= Amino acid nitrogen, ADIN= Acid detergent insoluble nitrogen, DIM= Days in milk, NAN= Non ammonia nitrogen, NDIN= Neutral detergent insoluble nitrogen.

4.4. Results

Table 4.3 presents the average values for the protein feed fractions of the feeds included in the evaluation. The NPN fraction was assayed with TCA. For the protein concentrates, the NPN fraction represented approximately 500 g/ kg of the soluble CP. When the soluble protein was corrected for its amino N content, the average amino N content was greater than 800 g/kg of soluble CP. Table 4.4 lists the current feed library rates and the adjusted rates for the B1 and B3 fractions. *In vitro* estimates for the soluble protein fraction are approximately 30 % of the rates of the original scheme. While the B3 rates of the CNCPS feed library were close to 0/h, the adjusted rates based on published data were between 0.01 to 0.14/h (Table 4.4). The IIV rates were within the range of 0.01 (blood meal) to 0.17/h (soybean meal) and did not necessarily rank the feeds in the same order as the feed library rates.

Table 4.3. Feed protein fractions in the feeds included in the evaluation

	CP (g/kg DM)	Soluble CP (g/ kg CP)	NPN (g/kg Sol CP)	True protein (g/kg Sol CP)
Alfalfa silage	224.8	496.2	829.5	170.5
Blood meal	1000.0	50.0	60.0	940.0
Canola meal	427.0	323.2	652.2	347.8
Corn gluten meal	651.9	41.4	740.7	259.3
Corn silage	72.7	565.3	889.4	110.6
Cottonseed meal	484.0	200.4	402.1	597.9
Expeller SBM	489.4	61.3	533.3	466.7
Lignosulfonate SBM	496.6	48.3	500.0	500.0
Roasted soybeans	400.0	57.5	1000.0	0.0
Rolled HMSC	86.4	321.9	935.4	64.6
Solvent SBM	530.8	199.7	537.8	462.2
	NAAN (g/kg Sol CP)	AAN (g/kg Sol CP)	NDICP (g/ kg CP)	ADICP (g/ kg CP)
Alfalfa silage	136.4	863.6	92.2	28.9
Blood meal	10.0	990.0	64.0	12.0
Canola meal	170.0	830.0	71.7	40.3
Corn gluten meal	10.0	990.0	81.0	64.0
Corn silage	199.2	800.8	74.3	13.5
Cottonseed meal	180.0	820.0	27.3	19.5
Expeller SBM	10.0	990.0	107.0	23.0
Lignosulfonate SBM	20.0	980.0	323.6	74.6
Roasted soybeans	20.0	980.0	82.5	34.4
Rolled HMSC	103.5	896.5	34.7	6.1
Solvent SBM	20.0	980.0	15.2	5.2

AAN= Amino acid nitrogen, ADICP= Acid detergent insoluble crude protein, HMSC= High moisture shelled corn, NAAN= Non amino acid nitrogen, NDICP= Neutral detergent insoluble crude protein, NPN= Non-protein nitrogen, SBM= Soybean meal.

Table 4.4. Degradation rates for the protein fractions of the feeds used in the evaluation.

	CNCPS B1 rates /h	AdjB1 rates[1] /h	CNCPS B3 rates /h	AdjB3 rates[2] /h	IIV rates[3] /h
Alfalfa silage	1.5	0.28	0.0180	0.14	0.04
Blood meal	1.35	0.2	0.0009	0.01	0.01
Canola meal	2.3	0.46	0.0002	0.05	0.12
Corn gluten meal	1.5	0.2	0.0050	0.02	0.02
Corn silage	1.5	0.28	0.0180	0.03	0.04
Cottonseed meal	1.75	0.46	0.0175	0.04	0.10
Expeller SBM[4]	2.3	0.46	0.0020	0.05	0.04
Lignosulfonate SBM	2.3	0.46	0.0020	0.04	0.04
Roasted soybeans	2.3	0.46	0.0020	0.04	0.05
Rolled HMSC[4]	1.5	0.5	0.0200	0.02	0.02
Solvent SBM	2.3	0.46	0.0100	0.06	0.17

[1] AdjB1 rates were based on several published sources (Broderick, et al., 1989, Hedqvist and Udén, 2006, Peltekova and Broderick, 1996).
[2] AdjB3 rates were based on several published sources (Coblentz, et al., 1999, Juarez, 1998, McBeth, et al., 2003, Ogden, et al., 2006, Pichard, et al., 2005, Rossi, et al., 1997).
[3] Corn silage, rolled HMSC and canola meal rates were assigned based on relative ranking compared to the other feeds.
[4] HMSC= High moisture shelled corn, SBM= Soybean meal.

Figure 4.1 summarizes the evaluation of RDP and RUP for the original scheme. The original scheme over predicted RDP, with a mean bias of 150 g/d (5 % of the predicted mean). The regressed residuals (observed – predicted) against predicted RDP had significant intercept and slope (Y= -148.7 – 0.28(X-3050.8); indicating the presence of significant slope and mean bias and 86 % of the observations were over predicted. The original scheme explained more variation in the RDP supply (R^2 = 0.78) than for the RUP flows (R^2= 0.51) (Table 4.5). It underpredicted RUP flow, with a mean bias of 152 g/d (12 % of the predicted mean). The regressed residuals against predicted RUP flow had significant intercept and slope (Y=151.8 – 0.39 (X-1086.7)).

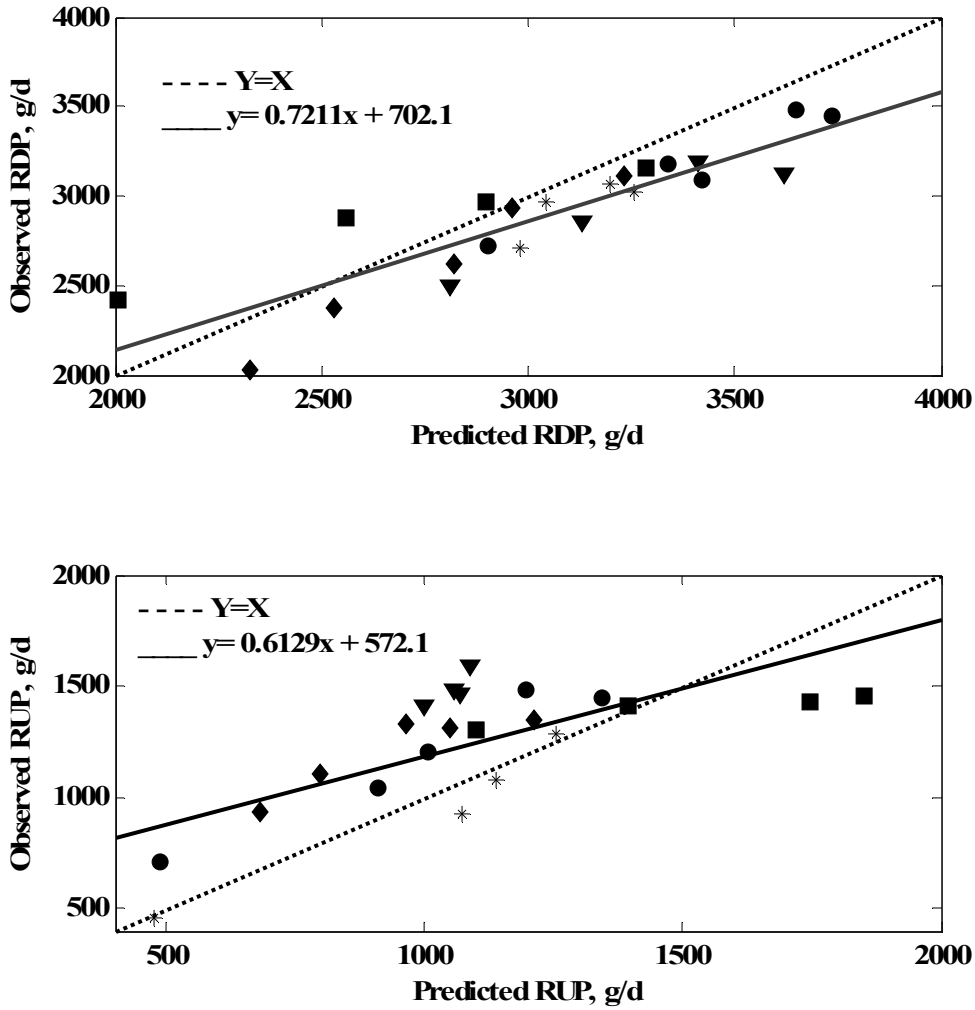


Figure 4.1. Predictions of the rumen degradable protein (RDP) supply and rumen undegradable protein (RUP) flow using the original CNCPS protein scheme for the following studies Reynal et al (2003) (●), Reynal and Broderick (2005) (■), Colmerero and Broderick (2006c) (♦), Brito and Broderick (2004b) (*), and Brito et al (2006) (▼).

Four studies also measured NDICP flows (Brito and Broderick, 2004b, Brito, et al., 2006, Olmos Colmenero and Broderick, 2006c, Reynal and Broderick, 2005). The original scheme over predicted the flow of NDICP out of the rumen (Table 4.5), with a mean bias of 62.3 g/d, which represented 28.5 % of the predicted mean and 40 % of the observed mean. For the study with the greatest proportion of protein as B3 and C fraction (Reynal and Broderick, 2005), the averaged mean bias for the study was as great as 204 g/d, representing 40 % of the predicted mean and 97 % of the observed mean. Adjusting the B3 rates to reflect available data (scheme 2) resulted in a decrease in the RMSPE and lower mean bias (21 g/d) (Table 4.5). However, the NDICP flows were still overpredicted when the adjusted rates were used. Overall, the predicted contribution of the NDICP to the RUP flows was greater than observed because the NDICP fraction was more extensively degraded in the rumen (Table 4.2).

Statistical measures for the evaluation of the protein fractionation schemes as listed in Table 4.1 are summarized in Table 4.6. As a general trend, after adjusting for the AAN in the soluble protein (schemes 3 and 5) (Eq. 4.6), RDP supply was still over predicted as in the original scheme, but scheme 3 resulted in the lowest mean bias. Aggregating B2 and B3 pools (schemes 7 to 10) resulted in an under prediction of the RDP supply and over prediction of RUP flows.

Schemes 3 (A fraction as NAAN), 7 (aggregated insoluble fraction and A fraction as NPN), and 9 (aggregated insoluble fraction, A fraction as NPN, and adjusted B1 rates) were the schemes that resulted in an overall improvement in the accuracy of both RDP supply and RUP flows predictions. The scheme that performed the worst was scheme 10, in which A fraction and B1 rates were adjusted, and the insoluble fraction was aggregated. It over predicted the amount of escaping soluble and insoluble protein fractions.

Table 4.5. Evaluation of the predictions of the escape of the neutral detergent crude protein using the original protein fractionation scheme with the either default feed library B3 rates or adjusted B3 rates based on published data (N = 17).

	Default B3 rates	Adjusted B3 rates
Intercept	96.4 (P<0.0001)	101 (P<0.0001)
Slope	0.27 (P<0.0001)	0.31 (P<0.0001)
R^2	0.77	0.78
RMSE	24.0	24.0
Mean bias (MB)[1]	-62.31	-21
MB as % of predicted mean	28.5	11.8
MB as % of observed mean	39.8	13.4
MSPE	16281.8	9604.0
Partition of MSPE		
% mean bias (U^M)	23.8	4.5
% slope not equal to 1 (U^R)	73	90.3
% lack of correlation (U^D)	3.2	5.2
RMSPE	127.6	98

RMSE= root mean square error, MSPE= mean square prediction error, RMSPE= Root mean square prediction error.
[1] Mean bias= Observed - Predicted

Table 4.6. Evaluation of the ability of alternative protein fractionation schemes to predict rumen degradable protein (RDP) supply and rumen undegradable protein flow (RUP) (N= 22).

	Schemes[1]									
RDP	1	2	3	4	5	6	7	8	9	10
Intercept	702.1	723.0	771.4	957.7	1061.0	742.0	447.5	488.3	422.0	675.5
Slope	0.72	0.70	0.73	0.72	0.63	0.78	0.87	0.90	0.89	0.89
R^2	0.78	0.79	0.79	0.78	0.70	0.85	0.84	0.88	0.86	0.77
RMSE[2]	167.7	163.7	164.7	170.6	197.7	139.9	144.8	123.7	134.4	174.2
Mean bias (MB)[3]	-148.7	-198.7	-59.6	210.1	-28.4	123.0	80.4	210.0	125.0	411.7
MB as % of predicted mean	5	7	1	8	1	5	3	8	4	17
MB as % of observed mean	5	6	1	8	1	5	3	7	4	14
MSPE[2]	61653	80769	38103	44142	65536	41209	27761	59363	33599	198292
Partition of MSPE										
% mean bias (U^M)	35.8	48.9	1.1	52.3	1.2	37.1	23.3	74.2	46.7	85.4
% slope not equal to 1 (U^R)	22.6	21.0	34.1	16.2	44.6	20.0	8.0	2.0	4.4	0.1
% lack of correlation (U^D)	41.6	30.1	64.8	31.5	54.2	42.9	68.7	23.8	48.9	14.5
RMSPE[2]	248.3	284.2	195.2	210.1	256.0	203.0	166.6	243.6	183.3	445.3

Table 4.6 (Continued)

	Schemes[1]									
RUP	1	2	3	4	5	6	7	8	9	10
Intercept	572.1	563.9	483.4	396.9	527.3	401.2	237.8	206.3	257.5	112.0
Slope	0.61	0.63	0.62	0.58	0.59	0.60	0.75	0.72	0.72	0.68
R^2	0.51	0.53	0.54	0.53	0.47	0.53	0.61	0.56	0.54	0.54
RMSE[2]	201.8	197.8	196.1	198.9	209.4	197.3	181.2	191.8	195.1	196.4
Mean bias (MB)[3]	151.8	194.1	20.5	-209.5	43.0	-163.0	-94.6	-201.5	-127.2	-416.9
MB as % of predicted mean	12	16	2	17	13	3	7	14	9	25
MB as % of observed mean	14	19	2	14	12	4	8	16	10	34
MSPE[2]	75625	85264	50850	100679	58516	80486	43890	80698	571667	217902
Partition of MSPE										
% mean bias (U^M)	30.5	44.1	0.8	43.6	3.2	33.1	20.4	50.3	28.3	79.7
% slope not equal to 1 (U^R)	20.6	14.3	5.1	20.7	28.7	23.0	11.6	8.3	11.2	4.2
% lack of correlation (U^D)	48.9	41.6	94.1	35.7	68.1	43.9	68.0	41.4	60.5	16.1
RMSPE[2]	275.0	292.2	225.5	317.3	241.9	283.7	209.5	284.1	239.1	466.8

[1]Schemes description: 1 = Original, 2 = Original scheme with adjusted B3 rates, 3 = A fraction as non amino nitrogen (NAAN), 4 = A fraction as NAAN and adjusted B1 rates, 5 = A fraction as NAAN and adjusted B3 rates, 6 = A fraction as NAAN and adjusted B1 and B3 rates, 7= Aggregated insoluble fraction, A as non-protein N (NPN), 8 = Aggregated insoluble fraction, A as NAAN, 9 = Aggregated insoluble fraction, A fraction as NPN, and adjusted B1 rates, and 10 = Aggregated insoluble fraction, A fraction as NAAN, and adjusted B1 rates.

[2] RMSE= root mean square error, MSPE= mean square prediction error, RMSPE= Root mean square prediction error.

[3] Mean bias = Observed- Predicted.

Table 4.7 ranks the schemes by their accuracy in predicting RDP and RUP. The original scheme ranked 7th and 5th in predicting RDP and RUP, respectively, while scheme 7 (in which the insoluble fraction was combined into one fraction, and fraction A = NPN) was the best.

Table 4. 7. Ranking of the protein fractionation schemes based on their ability to predict rumen degradable protein (RDP) supply, and rumen undegradable protein (RUP) flow as assessed by their root mean square prediction error (RMSPE).

Schemes[1]	RDP		RUP	
	RMSPE	Ranking		Ranking
1	248.3	7	275	5
2	284.2	9	292.2	8
3	185.2	3	225.5	2
4	210.1	5	317.3	9
5	256	8	241.9	4
6	203	4	283.7	6
7	166.6	1	209.5	1
8	243.6	6	284.1	7
9	183.3	2	239.1	3
10	445.3	10	466.8	10

[1]Schemes description: 1 = Original, 2 = Original scheme with adjusted B3 rates, 3 = A fraction as non amino nitrogen (NAAN), 4 = A fraction as NAAN and adjusted B1 rates, 5 = A fraction as NAAN and adjusted B3 rates, 6 = A fraction as NAAN and adjusted B1 and B3 rates, 7 = Aggregated insoluble fraction, A as non-protein N (NPN), 8 = Aggregated insoluble fraction, A as NAAN, 9 = Aggregated insoluble fraction, A fraction as NPN, and adjusted B1 rates, and 10 = Aggregated insoluble fraction, A fraction as NAAN, and adjusted B1 rates

4.5. Discussion

The original scheme over predicted RDP supply and under predicted RUP flows when compared against omasal flow data. Evaluations using previous versions of the CNCPS model reported the same directionality for biases (Bateman, et al., 2001b, Kohn, et al., 1998), but the RMSPE in this study are considerable lower than previously reported (Bateman, et al., 2001a, Kohn, et al., 1998). Greater accuracy is probably the result of a more homogenous data base and the use of feed analyses when available rather than reliance on the feed library. Likely contributing factors to the over prediction of RDP supply in the original scheme are the predicted high degradability of the B2 fraction, and the almost complete degradation of the soluble protein (B1+A). For most feeds, the B2 fraction represents the largest protein pool size (Sniffen, et al., 1992) and the default degradation rates for the B2 fraction are greater than most of the *in situ* and *in vitro* estimates (NRC, 2001). In addition, for most feeds, the B1 fraction represents a small percentage of the total soluble protein (Table 4.3), and most of the soluble protein is allocated into the A fraction, which is assumed to be immediately converted to ammonia. As a result, and similar to results with the *in situ* method, almost no soluble protein is predicted to be in the RUP. On average, the predicted RUP contained mostly B2 protein (~ 75 %), B3 + C fractions (~ 20 %), and small amounts of B1 (~ 5 %). However, for the studies included in the evaluation, the free AA-N was represented in the RUP in a proportion similar to the NDIN (Table 4.2). In other studies, the peptide-N was identified to be the most important amino N flowing out from the rumen in the liquid phase (Choi, et al., 2002a). Within the insoluble fraction, the contribution of the B3 and C fractions were also over estimated. The original scheme over predicted NDICP flow out of the rumen (Table 4.5). The CNCPS feed library values for the degradation rates of the B3 fraction are virtually 0, and therefore it almost completely escapes. When values for the degradation rates for

the B3 fraction were reassessed and adjusted (Table 4.4), the predictions of NDICP were improved (Table 4.5). However, adjusting rates for the B3 fraction with no other changes in the fractionation (scheme 2) increased the bias in RDP and RUP predictions. The CNCPS model was only sensitive to NDICP measurements for feeds that contain a high proportion of protein as NDICP (Chapter 3), but it is for those feeds (i.e. tropical forages) that rates consistently higher than CNCPS B3 feed library values have been reported (Coblentz, et al., 1999, Juarez, 1998, Ogden, et al., 2006).

Changes in the fractionation scheme were proposed to address some of the issues indicated previously. The contribution of the soluble N fractions to the RUP flows was improved by accounting for all the AAN pool in the soluble protein and adjusting B1 rates. Adjusting the B1 fraction to represent the AAN pool (scheme 3) resulted in the lowest bias in RDP and RUP of all the schemes. From a nutritional point of view, the AAN fraction represents a more homogenous fraction than the NPN fraction. In addition, AAN may be a less variable than the current B1 fraction, and therefore it may be more robust for use as default feed library values. Silages are the feeds with the greatest variation in the composition of the soluble protein fraction (McDonald, et al., 1991). In well fermented silages, with predominantly lactic acid fermentation, free AAN is the main fraction within the NPN since lactic acid bacteria have limited ability to ferment AA, with the exception of serine and arginine (Givens and Rulquin, 2004). Although differences in *in vivo* degradation rates of long peptides, short peptides, and free amino acids have been reported (Volden, et al., 2002), all reported values were greater than >1.5/h, and the original CNCPS protein fractionation scheme is rather insensitive to differences in such high rates (see Chapter 3).

Aggregating the insoluble fractions and using the IIV rates for the combined fraction (scheme 7) resulted in the scheme with the greatest accuracy for both RDP

and RUP (Table 4.7). It also resulted in a change of the sign of the bias (over predicting RUP, and under predicting RDP), but did not address the under representation of the soluble N fractions in the RUP flows. Predicted RDP, RUP and amino acids flows were very sensitive to protein B2 degradation rates (Chapter 3). Combining both insoluble fractions (B2 + B3) makes the currently infeasible task of measuring degradation rates much easier. An implicit assumption in using the IIV rates for the insoluble fraction is that the rate for the insoluble fraction is directly proportional to the overall rate. For most feed, the true soluble protein B1 represents a small percentage of the total protein. An approach not tested but that would likely increase the contribution of the soluble protein and reduce the over prediction of the RUP flow is defining the A fraction as NAAN, and the using of the Michaelis-Menten variant of the IIV method (Broderick and Clayton, 1992) to obtain rates for the combined insoluble fraction.

4.6. Implementation

In order to implement the best ranked scheme (7, Aggregated insoluble fraction, A as non-protein N (NPN)), the following aspects should be considered:

(1) To implement the scheme within the current feed library, the new insoluble rate should be applied to both the B2 and B3 fractions, which would in practice collapse the two fractions into one fraction in the current versions of CNCPS versions 5 and 6.

(2) The IIV method can be simplified by determining total N of the TCA-supernatants with either the combustion assay or Kjeldahl (Broderick, et al., 2004c).

(3) For some groups of feeds the method may be less accurate, and modifications or alternative methods should be considered. Degradation rates for feeds containing high levels of ammonia and free amino acids (e.g. grass and legume silages) are less accurate (Broderick, 1994). For those feeds, incubation of the

insoluble residue in buffer could reduce the background levels of the ammonia and free amino acids background levels. The method also is not very accurate for tannin-containing forages, and for those forages the Michaelis-Menten variant of the IIV method may be the more feasible method (Broderick, 1994).

4.7. Conclusions

Improvements in the accuracy of RDP and RUP predictions of the original CNCPS protein fractionation scheme were obtained when the insoluble fractions B2 and B3 were combined resulting in a single pool and degradation rate, which can be measured with the IIV method. Evaluations of the NDICP flows indicated that the escape of the NDICP was over predicted, and thus the concept that the N insoluble in ND represents the slow degradable protein needs further revision. Improvements in the accuracy of the predictions also were achieved when AA-N was accounted for in the soluble fraction.

CHAPTER 5

A MODEL TO DESCRIBE THE DYNAMICS OF UREA RECYCLING AND EXCRETION IN DAIRY CATTLE

5.1. Abstract

Reducing protein in the diet by formulating diets that more accurately meet rumen nitrogen (N) and animal requirements is an important goal in cattle nutrition in developed countries. Urea recycled to the rumen represents an N source for microbes, while urinary urea N excretion must be accounted for in predicting ammonia losses from a dairy herd. This chapter describes a dynamic mechanistic model developed to be used as a component of ration formulation models to predict N recycling to the GIT and urinary urea N. The model was developed with emphasis on the feedback structure of the system. Recycling processes were modeled as positive feedbacks, while renal excretion was modeled as a negative feedback. Both processes were assumed to be regulated primary by N intake. Model simulations suggested that the CNCPS underestimated the amount of urea recycling to the rumen for lactating dairy cows.

5.2. Introduction

Reducing protein in the diet by formulating diets that more accurately meet rumen nitrogen and animal requirements is an important goal in cattle nutrition, since dairy farming is an important contributor to non-point source of environmental pollution (NRC, 2003). Recently, ammonia volatilization has become an important environmental issue because of the impact of ammonia emissions on the soil and surface water acidification and eutrophication (Bussink and Oenema, 1998). On a

global scale, animal farming systems represent about 50 % of the total NH3 emissions from terrestrial systems (NRC, 2003).

Dairy waste is a major source of NH_3 emissions, with urinary urea being the compound with the highest NH_3 volatilization potential (Bussink and Oenema, 1998). Ruminal ammonia is the main substrate for liver ureagenesis (Lapierre and Lobley, 2001). When dietary protein is degraded faster than the rate at which ammonia can be assimilated by microbes, ruminal ammonia concentration increases. Ammonia is absorbed across all sections of the digestive tract and converted into urea in the liver. Once released into blood, urea is excreted in urine or re-enters the digestive tract by diffusion into saliva or directly across the gut wall. The partition of urea between recycling into the gastrointestinal tract (GIT) and excretion is highly variable and depends on physiological processes and diet conditions (Lapierre and Lobley, 2001). How urea is partitioned and excreted has multiple practical implications. Dietary changes that reduce urinary urea concentration are effective tools to decrease ammonia volatilization (Monteny, et al., 2002). Increasing the anabolic use of recycled urea can improve nitrogen efficiency.

The objective of this study was to use accumulated research knowledge to (1) identify variables related to the partition of urea outflows between GIT and kidney, and (2) conceptualize and develop a dynamic mechanistic model of nitrogen fluxes in dairy cows that can be used to characterize and predict the partition between urea recycling and excretion.

5. 3. Materials and methods

5. 3. 1. Identifying variables related to urea partition

The urea flows to the GIT and kidney (g/d) can be described as the function of urea concentration (g/L) times the renal or GIT clearance (L/d) (C_R and C_{GIT}, respectively). Clearance of a substance from the body is defined as the volume of

distribution that is completely cleared per unit of time (Koeppen and Stanton, 1997). Urea clearance depends on changes in the permeability of the kidney and GIT to urea (Koeppen and Stanton, 1997). Therefore, variables linked to clearance are candidates to be involved in the regulation of urea metabolism. Both C_R and C_{GIT} were computed from experimental studies as the rate of urea flow divided by urea concentration, and expressed in metabolic weight (L/(d×kg $^{0.75}$)):

$$C = \frac{ureaflow}{[Urea] \times BW^{0.75}} \qquad [5.1]$$

Data from studies that were mostly designed to test the effect of protein supplementation on nitrogen metabolism and animal performance were used to model renal clearance of urea (Table 5.1) (Broderick, 2003, Broderick and Radloff, 2004, Gonda, et al., 1996, Haig, et al., 2002, Maltz and Silanikove, 1996, Olmos Colmenero and Broderick, 2006a, Olmos Colmenero and Broderick, 2006b, Reynal and Broderick, 2005, Sannes, et al., 2002, Valadares Filho, et al., 2000, Wattiaux and Karg, 2004a).

Table 5.1. Descriptive statistics for the studies used to describe renal urea clearance for dairy cows.

	Descriptive statistics				
	N	Mean	SD	Min	Max
Urea metabolism					
BUN, g/L[1]	48	0.169	0.043	0.107	0.33
MUN, g/L[1]	42	0.141	0.040	0.077	0.257
Urea excretion, g/d	48	176	51.2	63	342
Renal urea clearance, L/d	48	1067	293	424	1739
Renal urea clearance, L/(kg $^{0.75}$ d)	48	8.47	2.23	3.66	13.8
Diet composition and intake					
DM intake, kg/d	48	23.58	2.71	17.16	28.1
OM intake, kg/d	48	21.77	2.45	15.88	26.1
NDF, %	48	29.67	4.67	22.4	43.6
NDF intake, kg/d	48	6.97	1.19	4.9	9.64
N, %	48	2.77	0.27	2.16	3.52
N intake, g/d	48	654	95	480	834
N intake, g/(kg$^{0.75}$ d)	48	5.2	0.67	4.04	6.8
NFC, %[1]	46	43.73	6.6	24.5	55
NFC intake, kg/d	46	10.5	2.1	5.32	13.3
Na intake, g/d	33	66.7	15.5	31.5	106
Na+K+Cl intake, g/d	33	540.8	97.7	392.5	783
Production and nutrient supply					
Body weight, kg	48	629	39.7	549	690
Milk, kg/d	46	36.43	5.93	22.8	45.5
FCM, kg/d[1]	46	34.9	4.57	25.7	43.7
True protein yield, g/d	46	1062	168.8	667.9	1358
MP balance, g/d[2]	33	117.5	259.8	-435.6	704
ME balance, Mcal/d[2]	33	9.04	3.84	-0.63	14.8

[1] BUN= Blood urea nitrogen, FCM= Fat corrected milk, MUN= Milk urea nitrogen, NFC= Non-fiber carbohydrates,

[2] As predicted by the Cornell Net Carbohydrate Protein System version 6.0 (Broderick, 2003, Broderick and Radloff, 2004, Olmos Colmenero and Broderick, 2006a, Olmos Colmenero and Broderick, 2006b, Reynal and Broderick, 2005, Sannes, et al., 2002, Valadares Filho, et al., 2000, Wattiaux and Karg, 2004a).

Most of the information available on GIT urea entry for dairy has been derived from net mass transfer estimates based on veno-arterial measurements across splachnic tissues (Lapierre and Lobley, 2001). Studies reporting blood flow measurements of the portal-drained viscera were used to model GIT urea entry through the gut wall which does not consider salivary contributions because salivary glands do not drain to the portal vein (Table 5. 2) (Bach, et al., 2000, Benson, et al., 2002, Berthiaume, et al., 2006, Blouin, et al., 2002, Casse, et al., 1994, Delgado-Elorduy, et al., 2002a, Delgado-Elorduy, et al., 2002b, Raggio, et al., 2004, Reynolds, et al., 2003, Reynolds, et al., 1988).

Both linear and quadratic relationships among variables related to diet composition, nutrient supply, and production and nutrient clearances were explored (Table 5.1 and 5.2). The MIXED procedure of SAS (2002) was used (Littell, et al., 1996). A random coefficients model was fitted with study as a random variable. No pattern in the covariance (unstructured) was assumed. If interactions among variance components were not significant, the simple variance component covariance was used (Littell, et al., 1996). If study effect was not significant, the GLM procedure of SAS (2002) was used.

Table 5.2. Descriptive statistics for the studies used to describe gastrointestinal (GIT) urea clearance for dairy cows.

	N	Mean	SD	Min	Max
Body Weight, kg	28	598	78.1	434	684
Dry matter intake, kg/d	28	20	2.9	14.5	23.7
DIM, d[1]	28	83	53	11	210
Milk, kg/d	26	33.6	7.63	15.9	47.7
True protein yield, g/d	26	1047	237.8	668	1464
N intake, g/d	28	576	122.3	363	925
N intake, g/(kg0.75d)	28	4.9	1.4	2.8	9.3
OM intake, kg/d[1]	26	18.7	2.4	13.6	21.5
NDF intake, kg/d	24	6.5	1.20	4.8	8.9
BUN, g/L[1]	28	0.19	0.061	0.07	0.32
Net portal urea flow, g N/d	28	166	89.9	30	408
GIT urea clearance, L/d[1]	28	982	475.9	168	2204
GIT urea clearance, L/(kg $^{0.75}$ d)	28	8.1	3.7	1.3	17

[1] BUN= Blood urea nitrogen, DIM= Days in milk, GIT= Gastrointestinal tract, OM= Organic matter

5. 3. 2. Dynamic model

5.3.2.1. Conceptual model

A dynamic mechanistic model was developed with emphasis on the underlying feedback structure and the effect of the feedback loops on the behavior of the state variables (Franklin, et al., 1991, Milhorn, 1966). Both homeostatic and homeorhetic regulations involve multiple feedbacks. In a feedback process, some flow or information of the output of a system is passed to the input with the objective of smoothing and adjusting nutrient and metabolic flows (Milhorn, 1966). The behavior of a system arises from the interaction between two types of feedbacks, positive and negative. Negative feedbacks cause the influence of a disturbance to a regulator to be minimized, so that the system maintains, within limits, a constant output (Milhorn,

1966). Positive feedback causes the output to increase or decrease continually due to an initial disturbance, and gives the system the ability to reach new points of equilibrium (Milhorn, 1966). Figure 5.1 identifies the main feedback mechanisms included in the model. Similar feedbacks have been described for the hindgut (not shown in figure 5.1). For the loops involved directly in the partition of urea, the open loop gain was calculated. The gain of a feedback loop is the strength of the signal return by the loop (Sterman, 2000). It was calculated by breaking the loops (renal urea excretion, hindgut wall recycling, rumen wall recycling, and saliva recycling loops) at the body urea pool and calculating the change in the size of body urea pool as it returns to itself with the chain rule from the gains of the individual links of the loops (Sterman, 2000):

$$\text{Open loop gain} = \left(\frac{\partial x_1^O}{\partial x_n}\right) \times \left(\frac{\partial x_n}{\partial x_{n-1}}\right) \times \ldots \times \left(\frac{\partial x_2}{\partial x_1^I}\right) \qquad [5.2]$$

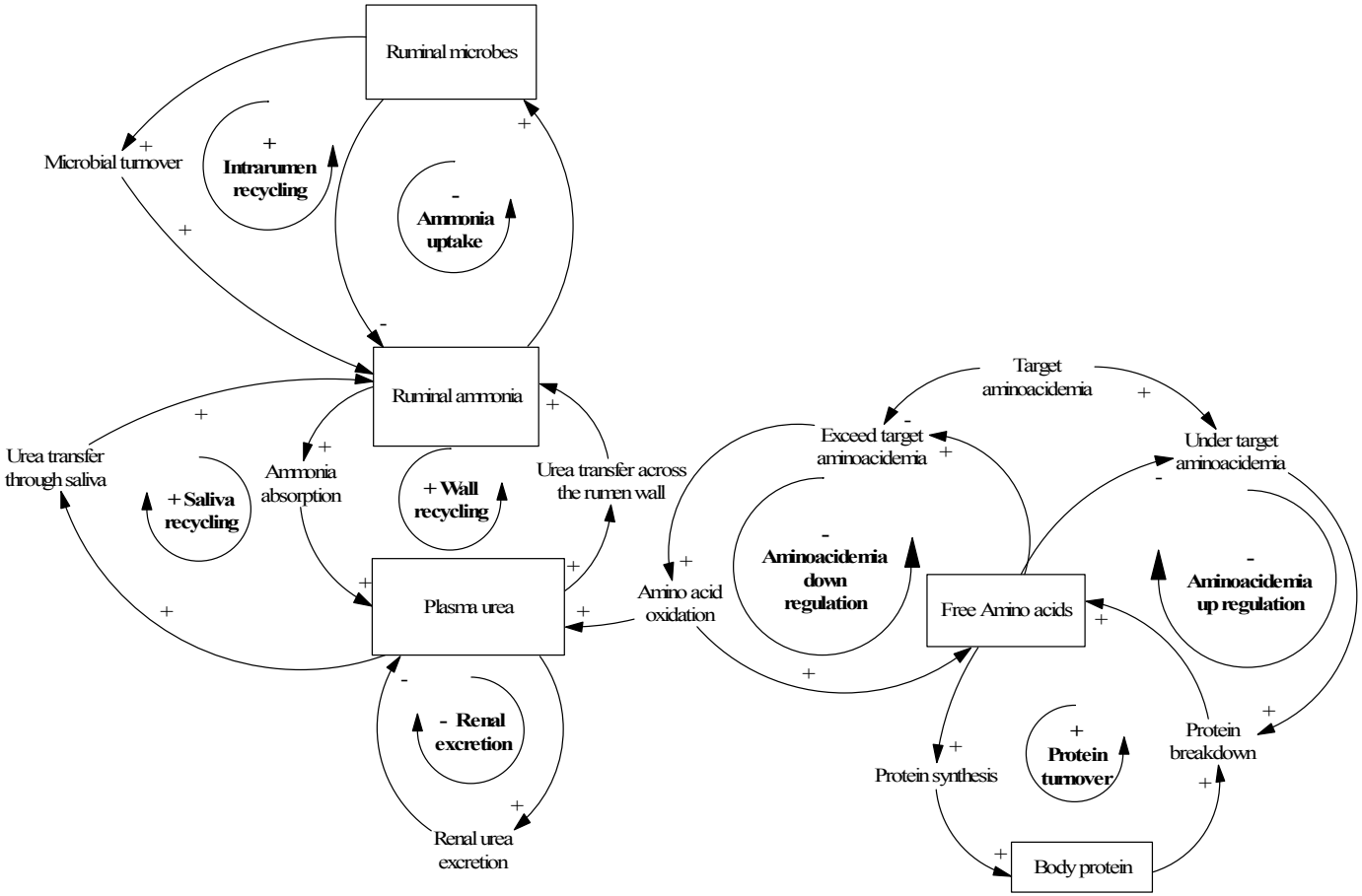
5.3.2.2. Model description

To represent the feedback loops presented in Figure 5.1, three main subcomponents were included in the model; (1) the flows of carbohydrates, protein, and microbes within the rumen and hindgut as needed to describe the anabolic use of urea, (2) the flows of non-protein N between rumen, hindgut, and body pools, and (3) a simple representation of the body amino acids (AA) transactions to account for AA oxidation.

Ruminal and hindgut carbohydrate, and protein flows and microbial growth

Dietary carbohydrates were divided into available fiber (FC), unavailable fiber (UC), and non-fiber carbohydrates (NFC) (Table 5.3).

Figure 5.1. Schematic representation of the positive and negative loops affecting the dynamics of urea metabolism included in the model. Arrows represent causal links between variables. The positive sign at the arrowheads indicates that both variables have the same directionality, while the negative sign indicates that as one of the variables increase, the dependent variable decreases or vice versa. Positive and negative feedback loops are represented by positive and negative signs within the semi-circle arrow. Variables within a box are state variables.


Ruminal microbes
Microbial turnover
+ Intrarumen recycling
- Ammonia uptake
Ruminal ammonia
Urea transfer through saliva
+ Saliva recycling
Ammonia absorption
+ Wall recycling
Urea transfer across the rumen wall
Plasma urea
- Renal excretion
Renal urea excretion
Target aminoacidemia
Exceed target aminoacidemia
Under target aminoacidemia
- Aminoacidemia down regulation
- Aminoacidemia up regulation
Amino acid oxidation
Free Amino acids
Protein synthesis
+ Protein turnover
Protein breakdown
Body protein

Dietary protein was divided into unavailable N (UN), protein (PROT), free AA and peptides (AAN) and non-AA N (NAAN) (Table 5.4). Protein degradation into peptides and AA, which could be passed to the small intestine with liquid, be taken up by the microbes to either for direct incorporation into microbial protein or deamination, which was described as a first order process. The proportion of microbial protein derived from amino N was assumed to be directly proportional to available amino N (Eq.5.56) (Atasoglu, et al., 1999). Microbial turnover provided amino N also (Firkins, et al., 1992).

Microbial N includes N from two microbial groups; the FC and NFC digesters (Table 5.5). Degraded carbohydrates are divided into those used for non-growth functions and for growth (biomass increases) (Pirt, 1982). Microbial growth can be limited by energy or N. Preformed AA allow NFC bacteria to better match their anabolic and catabolic rates and spill less energy, improving microbial yield when energy is in excess (VanKessel and Russell, 1996). Therefore, it was assumed that if energy is in excess, microbial yield for NFC digesters could be improved up to 54 % by the presence of amino N (Eq. 5.75) (Atasoglu, et al., 1999, Russell and Sniffen, 1984).

The rumen structure was used as a basis for describing the hindgut fermentation. Inputs to the large intestine are the rumen outflows modified to account for digestion in the small intestine. Ruminally unavailable FC escaping the rumen were assumed to pass undegraded in the small intestine, while the ruminally available NFC escaping the rumen are assigned an intestinal digestibility of 70 %, Mean retention time for all feed fractions in the hindgut was set to 13 hours for cattle (Vanhatalo and Ketoja, 1995). But selective retention for hindgut microbes takes place in the hindgut (Van Soest, 1994), so microbial passage rates were 0.80 times the digesta passage rate.

Table 5.3. List of the equations for the gastrointestinal carbohydrates compartments.

Eq.	Mathematical statement	Description
	Differential equations	
5.3	$dFC_R/dt = FCintake - FC_Rpas - FC_Rmain - FC_Rgrowth$	Ruminal fiber CHO pool, g CHO
5.4	$dNFC_R/dt = NFCintake - NFC_Rpas - NFC_Rmain - NFCRgrowth$	Ruminal non-fiber CHO pool, g CHO
5.5	$dUC_R/dt = UCintake - UC_Rpas$	Ruminal unavailable CHO pool, g CHO
5.6	$dNFC_H/dt = NFC_Hinput - NFC_Hpas - NFC_Hmain - NFCHgrowth$	Hindgut fiber CHO pool, g CHO
5.7	$dUC_H/dt = UC_Hinput - UC_Hpas$	Hindgut non-fiber CHO pool, g CHO
		Hindgut unavailable CHO pool, g CHO
	Flows	
5.8	$FCintake = FCdiet \times feed\ intake$	FC intake, g CHO/h
5.9	$NFCintake = NFCdiet \times feed\ intake$	NFC intake, g CHO/h
5.1	$UCintake = UCdiet \times feed\ intake$	Unavailable CHO intake, g CHO/h
5.12	$FC_Rmain = Min(FC_R/dt, MIC_{FCR} \times Me)$	Degraded FC for maintenance, g CHO/h
5.13	$NFC_Rmain = Min(NFC_R/dt, MIC_{NFCR} \times Me)$	Degraded NFC for maintenance, g CHO/h
5.14	$FC_Rgrowth = FC_R \times kd_{FC} - Fcmain$	Degraded FC for growth, g CHO/h
5.15	$NFC_Rgrowth = NFC_R \times kd_{NFC} - NFCmain$	Degraded NFC for growth, g CHO/h
5.16	$FC_Rpas = FC_R \times kp_{SR}$	Ruminal FC escape, g CHO/h
5.17	$NFC_Rpas = NFC_R \times kp_{SR}$	Ruminal NFC escape, g CHO/h
5.18	$UC_Rpas = UC_R \times kp_{SR}$	Ruminal UC escape, g CHO/h
5.19	$FC_Hinput = FC_Hpas$	Hindgut FC input, g CHO/h
5.2	$NFC_Hinput = (1 - NFCid) \times NFC_Rpas$	Hindgut NFC input, g CHO/h
5.21	$UC_Hinput = UC_Hpas$	Hindgut UC input, g CHO/h
5.22	$FC_Hmain = Min(FC_H/dt, MIC_{FCH} \times Me)$	Hindgut FC for maintenance, g CHO/h
5.23	$NFC_Hmain = Min(NFC_H/dt, MIC_{NFCH} \times Me)$	Hindgut NFC for maintenance, g CHO/h
5.24	$FC_Hgrowth = FC_H \times kd_{FC} - FC_Hmain$	Hindgut FC for growth, g CHO/h
5.25	$NFC_Hgrowth = NFC_H \times kd_{NFC} - NFC_Hmain$	Hindgut NFC used for growth, g CHO/h

Table 5.3. (Continued)

Eq.	Mathematical statement	Description
5.26	$FC_Hpas = FC_H \times kp_{SH}$	Hindgut FC escape, g CHO/h
5.27	$NFC_Hpas = NFC_H \times kp_{SH}$	Hindgut NFC escape, g CHO/h
5.28	$UC_Hpas = UC_H \times kp_{SH}$	Hindgut UC escape, g CHO/h
	Auxiliary equations	
5.29	Feed intake= dry matter intake × n meals	Feed intake flow, kg DMI/h

Table 5.4. List of the equations for the gastrointestinal amino-N compartments.

Eq.	Mathematical statement	Description
	Differential equations	
5.30	$dPROT_R/dt = PROTintake - PROT_Rpas - PROT_Rdeg$	Ruminal protein pool, g N
5.31	$dAA_R/dt = AAintake + PROT_Rdeg + TurnoverN_{FCR} + TurnoverN_{NFCR} - UptakeAAMIC_{NFCR} - AA_Rdeam - AA_Rpas$	Ruminal Amino acid + peptides pool, g N
5.32	$dUN_R/dt = UNintake - UN_Rpas$	Ruminal unavailable N pool, g N
5.33	$dPROT_H/dt = PROT_Hinput - PROT_Hpas - PROT_Hdeg$	Hindgut protein pool, g N
5.34	$dAA_H/dt = PROT_Hdeg + TurnoverN_{FCH} + TurnoverN_{NFCH} - UptakeMICNFCH - AA_Hdeg - AA_Hpas$	Hindgut amino acid + peptides pool, g N
5.35	$dUN_H/dt = UN_Rpas - UN_Hpas$	Hindgut unavailable N, g N
	Flows	
5.36	PROTintake= (Dietary N-NAAN-UN) × Feed intake	Available AAN intake, g N/h
5.37	$PROT_Rpas = PROT_R \times kp_{RS}$	Ruminal protein escape, g N/h
5.38	$PROT_Rdegr = PROT_R \times kd_{PROT}$	Ruminal protein degradation, g N/h
5.39	$TurnoverN_{FCR} = Turnover_{FCR} \times Nmic$	N from turnover of FC microbes, g N/h
5.40	$TurnoverN_{NFCR} = Turnover_{NFCR} \times Nmic$	N from turnover of NFC microbes, g N/h

Table 5.4. (Continued)

Eq.	Mathematical statement	Description
5.41	$UptakeAAMIC_{NFCR} = NFC_R growth \times Nmic \times cAAuptake_R$	AAN uptake by NFC microbes, g N/h
5.42	$Aaintake = AAN \times Feed\ intake$	Free amino acids and peptides intake, g N/h
5.43	$AA_R deam = AA_R \times kd_{AA}$	Amino acid N deamination, g N/h
5.44	$AA_R pas = AA_R \times kp_{LR}$	Amino acid N escape, g N/h
5.45	$UNintake = UN \times Feed\ intake$	Unavailable N intake, g N/h
5.46	$UN_R pas = UN_R \times kp_{RS}$	Unavailable N escape, g N/h
5.47	$PROT_H input = PROTid \times (PROT_R pas + MICNFCRpas \times Nmic + MICFCRpas \times Nmic)$	Hindgut AAN input, g N/h
5.48	$PROT_H pas = PROT_H \times kp_H$	Hindgut AAN escape, g N/h
5.49	$PROT_H degr = PROT_H \times kd_{PROT}$	Hindgut protein degradation, g N/h
5.5	$TurnoverN_{FCH=} Turnover_{FCH} \times Nmic$	N from turnover of FC microbes, g N/h
5.51	$TurnoverN_{NFCH=} Turnover_{NFCH} \times Nmic$	N from turnover of FC microbes, g N/h
5.52	$UptakeMIC_{NFCH} = NFC_H growth \times Nmic \times cAAuptake_H$	Uptake by NFC microbes, g N/h
5.53	$AA_H deam = AA_H \times kd_{AA}$	AAN degradation, g N/h
5.54	$AA_H pas = AA_H \times kp_H$	AAN escape, g N/h
5.55	$UN_H pas = UN_H \times kp_H$	Unavailable N escape, g N/h
	Auxiliary equations	
5.56	$cAAuptake_{RorH} = 0.0119 + 0.6997 \times ([AA]_{R\ or\ H}/([AA]_{R\ or\ H} + [NH_3]_{R\ or\ H}))$	Proportion of N uptake as amino N, dmnl

Table 5.5. List of the equations for the gastrointestinal microbial compartments.

Eq.	Mathematical statement	Description
	Differential equations	
5.57	$dMIC_{FCR}/dt = Growth_{FCR} - Turnover_{FCR} - Passage_{FCR}$	Ruminal FC microbes, g MIC/h
5.58	$dMIC_{NFCR}/dt = Growth_{NFCR} - Turnover_{NFCR} - Passage_{NFCR}$	Ruminal NFC microbes, g MIC/h
5.59	$dMIC_{FCH}/dt = Growth_{FCH} - Turnover_{FCH} - Passage_{FCH}$	Hindgut FC microbes, g MIC/h
5.60	$dMIC_{NFCH}/dt = Growth_{NFCH} - Turnover_{NFCH} - Passage_{NFCH}$	Hindgut NFC microbes, g MIC/h
	Flows	
5.61	$Growth_{FCR} = MIN(\mu_{FCRN},\mu_{FCRE}) \times MIC_{FCR}$	FC microbial growth, g MIC/h
5.62	$Turnover_{FCR} = MIC_{FCR} \times kt_{MIC}$	FC microbial turnover, g MIC/h
5.63	$Passage_{FCR} = MIC_{FCR} \times kp_{SR}$	FC microbial escape, g MIC/h
5.64	$Growth_{NFCR} = MIN(\mu NFCRN,\mu NFCRE) \times MICNFCR \times ImpAAR$	NFC microbial growth, g MIC/h
5.65	$Turnover_{NFCR} = MIC_{NFCR} \times kt_{MIC}$	NFC microbial turnover, g MIC/h
5.66	$Passage_{NFCR} = MIC_{NFCR} \times kp_{MICR}$	NFC microbial escape, g MIC/h
5.67	$Growth_{FCH} = MIN(\mu_{FCHN},\mu_{FCHE}) \times MIC_{FCH}$	FC microbial growth, g MIC/h
5.68	$Turnover_{FCH} = MIC_{FCH} \times kt_{MIC}$	FC microbial turnover, g MIC/h
5.69	$Passage_{FCH} = MIC_{FCH} \times kp_{H\times selret}$	FC microbial escape, g MIC/h
5.70	$Growth_{NFCH} = MIN(\mu NFCHN,\mu NFCHE) \times MICNFCH\times ImpAAH$	NFC microbial growth, g MIC/h
	Auxiliary equations	
5.71	$\mu_{FCRN} = UptakeNH_{3MICFCR}/Nmic/ MIC_{FCR}$	N limited specific growth FC microbes, h^{-1}
5.72	$\mu_{FCRE} = Ymax\times FCRgrowth / MICFCR$	E limited specific growth FC microbes, h^{-1}
5.73	$\mu_{NFCRN} = (UptakeAA_{MICNFCR} + UptakeNH_{3MICNFCR})/Nmic/ MIC_{FCR}$	N limited specific growth NFC microbes, h^{-1}
5.74	$\mu_{NFCRE} = Ymax \times NFC_Rgrowth / MIC_{NFCR}$	E limited specific growth NFC microbes, h^{-1}
5.75	$ImpAA_R = 1 + 0.49\times (UptakeAA_{MICNFCR/(}UptakeAA_{MICNFCR} + UptakeNH_{3MICNFCR))}$	For μNFCRE > μNFCRN , Yield improvement due to AA availability, dmnl

Non-protein nitrogen flows

Non protein N is described by three compartments; ruminal ammonia, hindgut ammonia and body urea (Figure 5. 2).

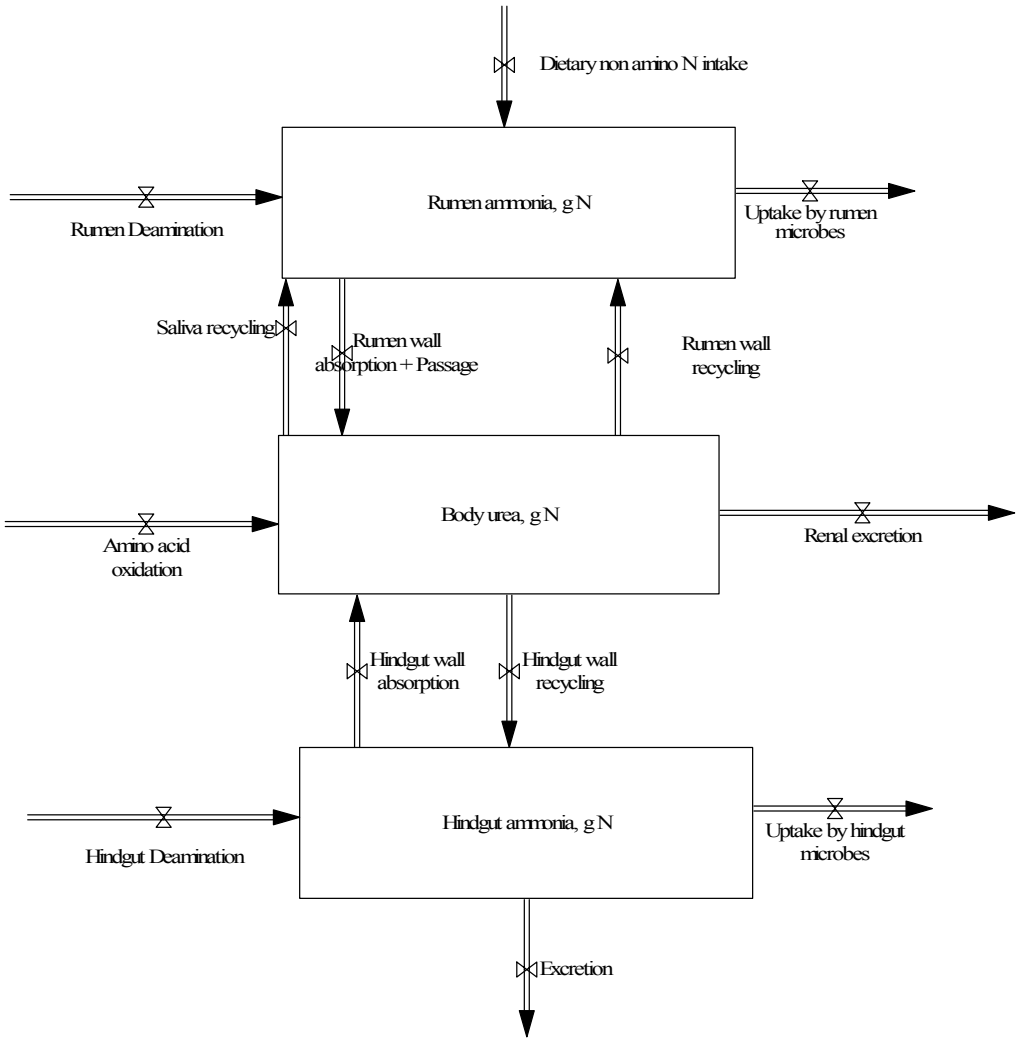


Figure 5.2. Representation of the inflows and outflows of the non-protein nitrogen compartments.

With the exception of the salivary urea transfer, the remaining flows were represented as first-order processes (Table 5.6). Saliva urea flow was saliva flow times saliva urea concentration (Eq.5.81); EAT, RUM, and RES are Boolean variables that have values of 0 or 1. A value of 1 indicates that the action (EAT=eating, RUM=ruminating, RES=resting) took place during the current interval of time. The saliva flow depends on the chewing activity of the animal (Beauchemin, 1991). Rumination activity follows a circadian pattern, with the greatest proportion of rumination occurring at night (Beauchemin, et al., 1990, Murphy, et al., 1983). In order to account for differences in the rumination frequency during the day, a sinusoidal function, derived from the spectral analysis of rumination data, was used to describe the probability that the animal ruminates within a daily cycle (Eq. 5.96).

Fractional rates of urea excretion and GIT recycling were described as functions of N intake. The fractional rate of urea excretion (Eq. 5.97) was described as a linear function of N intake (g/d) using the database described in Table 5.1. A segmented-linear model was used to describe the rate of urea entry to the GIT ($k_{git,}\ h^{-1}$) in relation to N intake (Eq.5.90 and 5.91). It was assumed that the amount of urea returning to the GIT was partitioned between the rumen and hindgut in relation to the amount of carbohydrate fermented in each site (Eq. 5.89).

Table 5.6. List of the equations for the non protein nitrogen compartments

Eq.	Mathematical statement	Description
	Differential equations	
5.76	$dNH_{3R}/dt = NAANintake + DeamAA_R + RecWall_R + RecSal – UptakeNH3MICFCR-UptakeMICNFCR – AbsorpNH3R$	Rumen ammonia pool, N g
5.77	$dNH_{3H}/dt = DeamAAH + RecWallH - UptakeMICFCH-Uptake_{MICNFCH} – Absorp_{NH3H-} Passage_{NH3H}$	Hindgut ammonia pool, N g
5.78	$dUrea/dt= Absorp_{NH3R} + Absorp_{NH3H} + AAoxidation – RecWallR – RecSal - RecWallH – Renalexc$	Body urea pool, N g
	Flows	
5.79	$NAANintake = NAAN \times feed\ intake$	Non-amino N intake, N g/h
5.80	$RecWall_R= Site\times k_{git} \times Urea$	Urea recycling through rumen wall, N g/h
5.81	$RecSal= EAT\times SFchew \times BW^{0.75}\times [urea]s + RES \times SFres \times BW^{0.75} [urea]s+ RUM \times SFchew \times BW0.75 \times [urea]s$	Urea recycling through saliva, g N/h
5.82	$Absorp_{NH3R} = kabs_{NH3} \times NH3_R$	Ammonia absorption, g N/h
5.83	$RecWall_H= (1\text{-}Site)\times k_{git} \times Urea$	Urea recycling through hindgut wall, g N/h
5.84	$Uptake_{MICFCH} = Growth_{FCH}\times Nmic$	Ammonia uptake by FC microbes, g N/h
5.85	$Uptake_{MICNFCH} =(1\text{-}cAAuptake)\times Growth_{NFCH}\times Nmic$	Ammonia uptake by NFC microbes, g N/h
5.86	$Passage_{NH3H} = NH3H \times kpH$	Ammonia passage, g N/h
5.87	$AAoxidation= ([AA]b\times Vb – [AA]b_{target}\times Vb)/AT$	for $[AA]b > [AA]b_{target}$
5.88	$Renalexc= k_{exc} \times Urea$	Renal urea excretion, g N/ h

Table 5.6 (Continued)

Eq.	Mathematical statement	Description
	Auxiliary equations	
5.89	Site = ($FC_R \times kd_{FC} + NFC_R \times kd_{NFC}$)/(FCR × kdFC + NFCR × kdNFC + FCH × kdFC + NFCH × kdNFC)	Site of urea entry, dmnl
5.90	$k_{git=}$ 0.131	Rate of urea transfer to the GIT for Nint > 417 g N/d
5.91	kgit= 0.7983 – 0.0016 × Nint	Rate of urea transfer to the GIT for Nint < 417 g N/d
5.92	[urea]s= 0.53 × [urea]b	Urea concentration in saliva, g N/L
5.93	Chew time= 2.86 + 0.281 × NDF	Daily hours spend chewing, h
5.94	Rum time= chew time – eating time	Daily ruminating time, h
5.95	Res time= 24 h – chew time	Daily resting time, h
5.96	P(ruminating| no eating) =(Rum time / (Rum time + resting time))× A × cos (wt + θ)	Probability of ruminating given that the animal is not eating
5.97	kexc = 0.0001874 × Nint (g N/d)	Fractional rate of urea excretion, h^{-1}

Body amino acids flows

An aggregate representation of the amino acid flows based on the concepts described by Waterlow (1999, 1978) was developed (Table 5.7). Two AA pools were included; the free AA pools and a body AA pool. The inflows to the free AA pools were the dietary and microbial AA inputs and the AA from the turnover of body protein. Flows from the free AA pool included the export of AA as milk, the synthesis of body protein and AA oxidation. Aminoacidemia is maintained within a tide range. A target average of 0.025 g N/L was assumed (Lobley, 2003, Waterlow, 1999). When the free AA pool deviates from its target, two negative feedback mechanisms come

into play; if AA are in excess, oxidation activates. If AA are deficient, an increase in the breakdown of body AA occurs. For dairy cows, protein synthesis required for functions other than milk output was found to be fairly constant, and independent of the stage of lactation (Lapierre, et al., 2002, Lapierre, et al., 2005), and therefore basal rates of synthesis and breakdown are assumed to be constants.

Table 5.7. List of the equations for the body amino acids compartments

Eq.	Mathematical statement	Description
	Differential equations	
5.98	$dFreeAAb/dt = PROTsupply + Break_{AAb} - Synt_{AAb} - Mamgland\_AA\ oxidation$	Blood free AA pool, g N
5.99	$dBodyAA/dt = Synt_{AAb} - Break_{AAb}$	Body AA pool, g N
	Flows	
5.100	$PROTsupply = PROTid \times (Passage_{NFCR} \times Nmic + PassageFCR \times Nmic - Nnuc + PROTRpas + AARpas)$	AA nitrogen supply, g N/h
5.101	$Break_{AAb} = k_{break} \times BodyAA + \lvert([AA]b \times Vb - [AA]btarget \times Vb)\rvert / AT$	Breakdown body protein, g N/h, for [AA]b < [AA]btarget
5.102	$Synt_{AAb} = k_{synt} \times BodyAA$	Synthesis body protein, g N/h
5.103	$Mamgland = (Cmgsyn \times Milk \times Protmilk)/6.38$	Mammary gland protein synthesis, g N/h

Table 5.8. Definition and numerical value of parameters

Parameter	Description	Parameter value	Reference
Θ	Phase	1.89 h	Beauchemin et al (1990)
$[AA]b_{target}$	Target blood amino acids concentration	0.025 g N/L	Lobley (2003)
A	Amplitude	0.128 h	Beauchemin et al (1990)
AT	Adjustment time	0.1 h	
Cmgsyn	Ratio mammary gland protein synthesis-protein output	1.35 dmnl	Bequette et al (1996)
$kabs_{NH3}$	Fractional rate ammonia absorption	0.75 h^{-1}	Oldick et al. (2000)
k_{break}	Basal fractional rate of protein breakdown	0.00151 h^{-1}	Lobley et al. (1980)
kd_{AA}	Fractional rate of AA degradation	1.35 h^{-1}	Oldick et al. (2000)
kd_{FC}	Fractional rate of FC degradation	0.05 h^{-1}	
kd_{NFC}	Fractional rate of NFC degradation	0.15 h^{-1}	
kd_{PROT}	Fractional rate of protein degradation	0.15 h^{-1}	
kp_{LR}	Fractional rate of liquid passage in the rumen	0.14 h^{-1}	
kp_{micR}	Fractional rate of microbial passage in the rumen	0.08 h^{-1}	
kp_{SH}	Fractional rate of digesta passage in the hindgut	0.08 h^{-1}	Vanhatalo and Ketoja (1995)
kp_{SR}	Fractional rate of solid passage in the rumen	0.05 h^{-1}	
k_{synt}	Basal fractional rate of protein synthesis	0.0019 h^{-1}	Lapierre et al. (2005)
kt_{MIC}	Fractional rate of microbial turnover	0.05 h^{-1}	
Me	Microbial maintenance	0.05 g CHO/ (g MIC×h)	Russell and Baldwin (1979)
NFCid	Small intestinal digestibility of NFC	0.70 dmnl	
Nmic	Nitrogen content of microbes	0.10 g N/ g MIC	Clark et al (1992)
Nnuc	Nitrogen content of microbes as nucleic acids	0.01 g N/ g MIC	Clark et al (1992)

Table 5.8 (Continued)

Parameter	Description	Parameter value	Reference
PROTid	Digestibility of ruminal escape protein in the small intestine	0.80 dmnl	
Selret	Microbial selective retention coefficient in the hindgut	0.80 dmnl	Van Soest (1994)
SFchew	Saliva flow during chewing	0.115 L/(h×$BW^{0.75}$)	Seo et al (2006a)
SFres	Saliva flow during resting	0.05 L/(h×$BW^{0.75}$)	Seo et al (2006a)
W	Wavelength	1 h	Beauchemin et al (1990)
Ymax	Maximum microbial yield	0.5 g MIC/g CHO	Issacson et al (1975)

5.3.2.3. Model sensitivity and evaluation

The model was implemented and simulated with Vensim professional version 5.0a (Ventana Systems Inc., Harvard, MA). Several time steps (between 0.0156 to 0.25) and integration methods (Euler, and Runge-Kutta methods) were tested. A Euler method with integration step of 0.0625 hour was selected. The sensitivity of the model to selected parameters was assessed in a base run with a dairy cow of 650 kg BW, DMI of 26 kg and 38 kg milk/d, and a ration with 300 g NDF/kg DM and 176 g CP/kg DM (Table 5.9). The sensitivity analysis was conducted by describing each parameter as a uniform distribution with ± 15 % from the mean as the minimum and maximum values (Table 5.8). All parameters tested were varied simultaneously using a Monte Carlo simulation. Rank correlations were used to assess the strength of the relationship between the parameters and the model outputs.

Table 5.9. Definition of inputs and initial values used for the sensitivity analysis

Inputs	Description	Values
AAN	Dietary free amino acids and peptides, g N/kg DM	3
Dietary N	Dietary nitrogen concentration, g N/ kg DM	28.2
DMI	Dry matter intake, kg/d	26.4
Duration meal	Duration of a meal, h	1
Eating time	Daily time spending eating h	12
FCdiet	Dietary fiber carbohydrate concentration, g CHO/kg DM	225
Milk	Milk production, kg/d	38
NAAN	Dietary non-amino nitrogen, g N/kg DM	9.2
NFCdiet	Dietary non-fiber carbohydrate concentration, g CHO/kg DM	400
Nmeal	Number of meals a day, meals/d	12
Protmilk	Milk true protein content, g / kg milk	30.5
UC	Dietary unavailable carbohydrate concentration, g CHO/kg DM	75
UN	Dietary unavailable nitrogen, g N/kg DM	5

Model predictions for urea GIT entry and urea excretion at steady state were compared to observations from studies of urea kinetics with double labeled urea (Lapierre, et al., 2004, Ruiz, et al., 2002). Root mean square prediction error (RMSPE) and coefficients of determination were estimated. Mean square deviations were partitioned into three independent and additive components (Theil, 1961); mean bias, slope bias, and random unexplained errors.

5. 4. Results and discussion

5.4.1. Identifying variables related to urea partition

Statistics describing the data and linear relationships between dietary and productive variables and renal urea clearance are presented in Tables 5.1 and 5.3. Both

renal urea clearance and urea excretion varied considerably, and had similar coefficients of variation (27 and 29 %, respectively). The average renal urea clearance was 1067 L/d (8.47 L/($kg^{0.75}$ × d)) (Table 5.1). Several studies have estimated renal urea clearance rates by regressing total urinary N against milk or plasma urea N concentrations (Jonker, et al., 1998, Kauffman and St-Pierre, 2001, Kohn, et al., 2002). Since they used total N excretion rather than urea excretion, they reported greater renal clearances. For example, for a 500 kg dairy cow, total N renal clearance ranged from 1254 to 1295 L/d (Jonker, et al., 1998, Kauffman and St-Pierre, 2001), while renal urea clearance in our data base for a 500 kg dairy cow was 894 L/d (Table 5. 1). Urinary N contains urea, which accounts for 50-90 % of the total N excreted, and other N-compounds, including creatinine, purine derivatives, and AA (Bristow, et al., 1992); renal clearances of each of the N components differ depending on the processes the component undergoes at the renal tubular level. For example, creatinine has tubular secretion, and for that reason its renal clearance is close to or greater than the glomerular filtration rate (Koeppen and Stanton, 1997). However, some purine derivatives have partial reabsorption (Surra, et al., 1997). The slope of the equation urinary N = β × MUN and its relationship to urea clearance may change with the relative proportion of N components in the urine. Nitrogen intake and N content of the ration were the only variables that were significantly related to clearance (Table 5.10). Urea is freely filtered at the glomerulus and partly reabsorbed at the collective tube and renal pelvis (Cirio and Boivin, 1990). Changes in the reabsorption of urea, mediated by changes in the expression of urea transporters, take place in response to variable N loads and salvage N needs (Bagnasco, 2005). Mineral intakes were not significantly related to clearance. However, because urea and non-urea solutes excretion are interdependent in ruminants (Schmidt-Nielsen, et al., 1961), under

situations of heat stress, or water deprivation, in which the maximum urine concentration is reached, mineral intakes may affect renal urea clearance.

Table 5.10. Linear relationships between dietary and productive parameters and renal urea clearance.

Relationship of renal urea clearance (L/dBW$^{0.75}$) with diet and production variables				
	a(S.E)	b (S.E)	P-value	RMSE
Diet composition and intakes				
DM intake, kg/d	6.21 (±2.43)	0.09 (±0.10)	0.35	2.11
OM intake, kg/d	5.59 (±0.13)	0.13 (±0.10)	0.21	2.17
NDF, %	7.32 (±1.35)	0.03 (±0.04)	0.33	2.16
NDF intake, kg/d	7.01 (±1.33)	0.20 (±0.16)	0.21	1.95
N, %	4.46 (±1.43)	1.40 (±0.42)	< 0.001	2.14
N intake, g/d	4.76 (±1.26)	0.005 (±0.0016)	< 0.001	1.94
N intake, g/(kg $^{0.75}$ d)	4.46 (±1.30)	0.76 (±0.207)	< 0.001	1.99
NFC, %	10.95 (±1.31)	-0.05 (±0.25)	0.06	2.05
NFC intake, kg/d	10.16 (±1.22)	-0.13 (±0.09)	0.18	2.09
Na intake, g/d	9.57 (±1.25)	-0.002 (±0.01)	0.8	2.27
Na+K+Cl intake, g/d	9.81 (±1.62)	-0.0008 (±0.002)	0.74	2.32
Production and nutrient supply				
Milk, kg/d	8.38 (±1.62)	0.013 (±0.04)	0.74	1.97
FCM, kg/d	8.90 (±1.79)	-0.001 (±0.04)	0.98	2.04
True protein yield, g/d	8.60 (±1.46)	0.0002 (±0.0001)	0.84	1.99
MP balance, g/d	9.45 (±1.03)	-0.0006 (±0.0008)	0.49	2.32
ME balance, Mcal/d	9.87 (±1.08)	-0.057 (±0.055)	0.31	1.92

The GIT clearance was derived from net transfers based on veno-arterial measurements across splanchnic tissues (Table 5.2). The average GIT urea clearance was 976 L/d (8.1 L/(kg$^{0.75}$ × d)), with a coefficient of variation of 48 % (Table 5.2). None of the variables presented in Table 5.2 were significantly related to urea clearance through the GIT wall; study effect explained most of the variability

observed (Results not shown). The variability and low precision of the veno-arterial data may contribute to the lack of relationships. In addition, the data base did not include low protein diets. For sheep and growing cattle, changes in GIT urea clearance when N content of diet was varied have been reported (Kennedy and Milligan, 1980, Marini and Van Amburgh, 2003). High urea clearance through the rumen wall has been also related to low ruminal ammonia concentrations and highly rumen fermentable organic matter (Kennedy, 1980, Kennedy, et al., 1981, Obara and Dellow, 1993, Obara and Dellow, 1994). Few studies of the splanchnic metabolism for dairy cows reported rumen fermentation and digestion characteristics, which limit the ability to integrate rumen and splanchnic metabolism.

5. 4. 2. Dynamic model

5. 4. 2. 1. Feedback loop analysis and sensitivity analysis

The gain of the loops involved in the recycling and excretion of urea were calculated for the steady state when N content of the diets were varied (Figure 5.3). The relative importance of the recycling and excretion loops changed as N intake varied. At low N intakes, the rumen wall recycling loop returns a gain as high as 0.40 for each cycle around the loop, while for high N intakes, the negative feedback of urea excretion had the greatest gain. For the loops presented in Figure 5.3, the sum of the gain for the recycling loops was greater than the gain for the excretion loop for all N intakes. High gains for the recycling loops may be necessary for animal to preserve N. In the rumen, extensive proteolysis and deamination occurs. Consequently, considerable cycling of the BUN to the digestive tract may be needed for positive N balance (Waterlow, 1999).

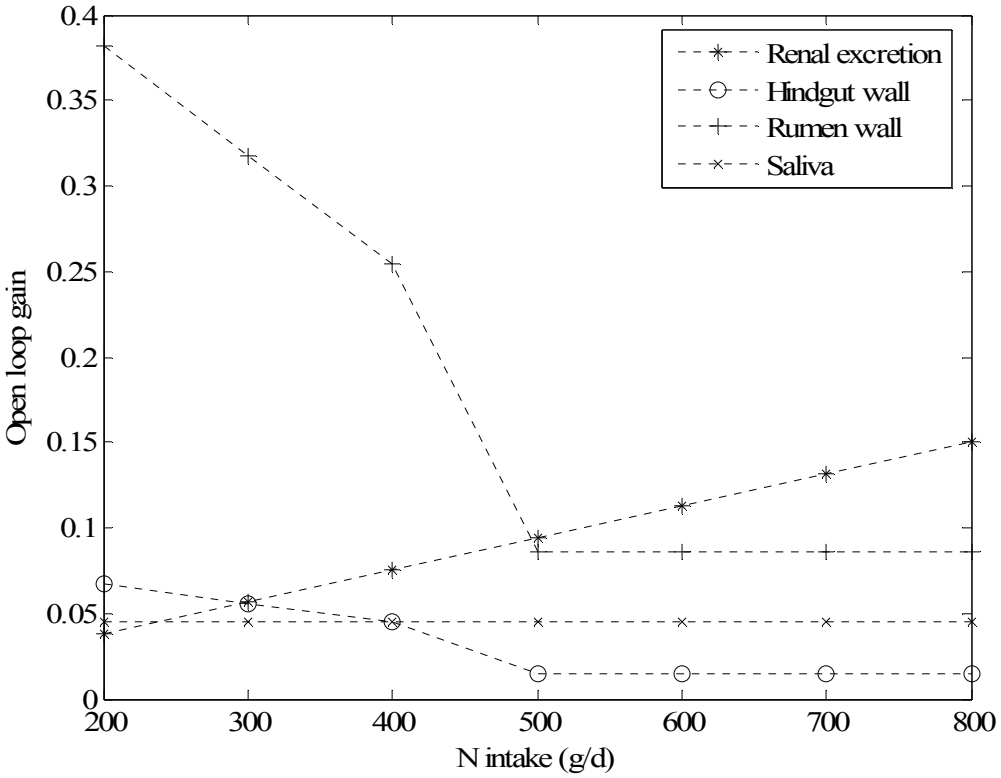


Figure 5.3. Open loop gain for the feedback loops of renal urea excretion[1], hindgut wall recycling[2], rumen wall recycling[3], and saliva recycling[4] at different N intakes.

[1]The sign of the gain for the renal urea excretion loop is negative.
[1, 2, 3, 4] All the loops were open at the body urea pool.

As a result of the change in the strength of the feedback loops (Figure 5.3), the model predicted a repartition of urea at different N intakes. If the fractional rates of urea excretion and GIT entry were constant and the GIT urea entry and excretion were only functions of the urea pool size, the strength of the feedback loops would have been the same regardless of the N intake (Milhorn, 1966). This implies that although the absolute flows would have varied with the N intake, the relative partitioning between recycling and excretion would have remained constant, supporting the idea that GIT entry and excretion are coordinated. Renal responses to varying dietary protein included changes in renal plasma flow, glomerular filtration rate, and renal pelvis and tubular urea reabsorption (Boldizarova, et al., 1999, Cirio and Boivin, 1990, Tebot, et al., 2002). All these physiological changes represent changes in the strength of the renal excretion feedback. The mechanisms by which the GIT entry is coordinated and the actual regulators that act as intermediate between amino acid availability and the physiological responses remain elusive (Marini, et al., 2004b).

The impact of varying the parameter values for the percentage of rumen ammonia derived from urea recycling, rumen NPN net entry (calculated as urea entry-ammonia absorption + passage), and urinary urea N is presented in Figure 5.4.

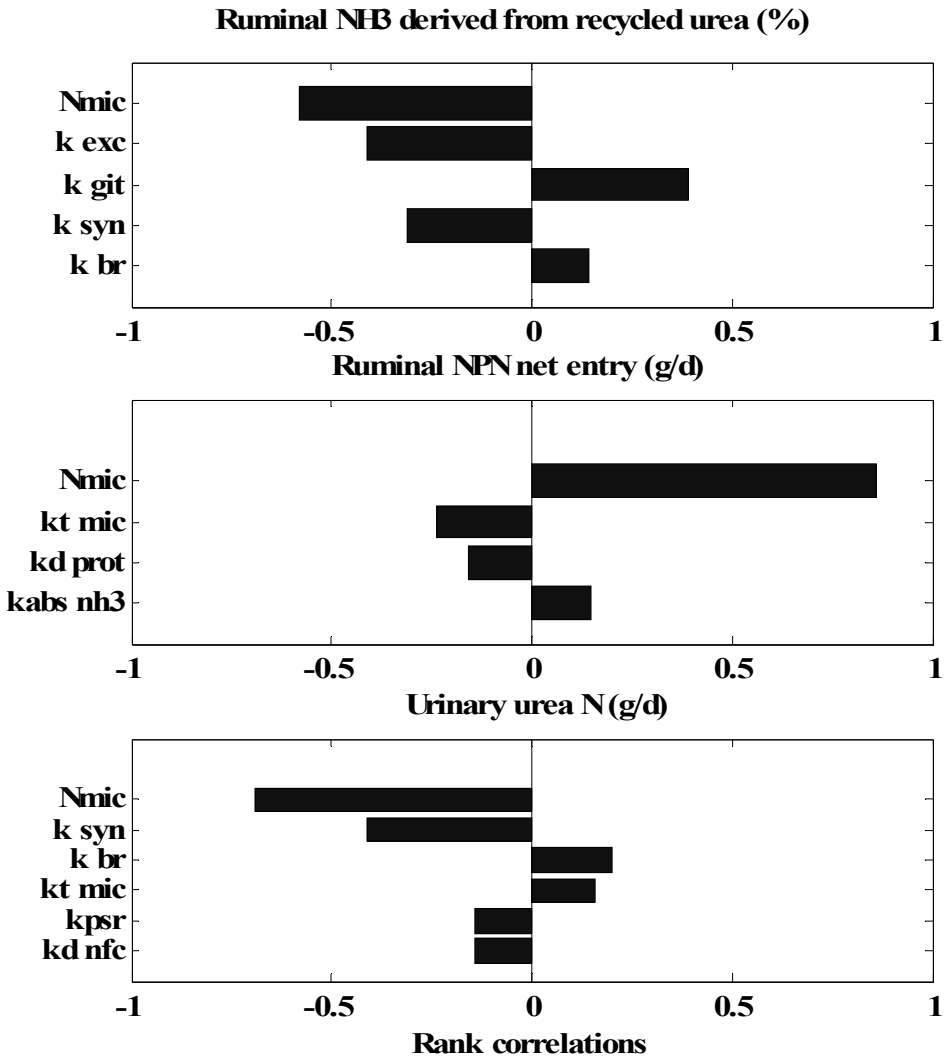


Figure 5.4. Rank correlations between the parameters ranked as the most influential in predicting ruminal NH_3 derived from recycled urea, ruminal non-protein N (NPN) net entry (calculated as rumen urea entry minus ammonia absorption), and urinary urea N.

k abs nh3 = rate of ammonia absorption+passage; k br = rate of protein breakdown; kd nfc = rate of non-fiber carbohydrate degradation; kd prot = rate of protein degradation; k exc = rate of urea excretion; k git = rate of urea entry to the gastrointestinal tract; kpsr = rate of solid passage in the rumen; k syn = rate of protein synthesis; kt mic = rate of microbial turnover; Nmic = nitrogen content of microbes.

The net entry was sensitive to parameters related to microbial efficiency (N content of microbes, rate of microbial turnover) (Figure 5.4). Low fractional rates of protein degradation also were related to positive net entry values. Faster NH_3 absorption rates strengthened the wall recycling loop, increasing the net entry. Highly fermentable diets have been associated with increased urea recycling (Kennedy, et al., 1981). Volatile fatty acids facilitate ammonia absorption (Bodeker, et al., 1992), and may enhance urea recycling by means of increasing ammonia absorption.

The percentage of NH_3 derived from recycled urea was also very sensitive to microbial N content: higher microbial N content resulted in greater N uptake and microbial turnover and lower NH_3 absorption. Therefore, as N microbial content increased, the proportion of NH_3 from intra-ruminal recycling and dietary protein degradation increased. Fractional rates for synthesis and breakdown affected the proportion of urea derived from amino acid catabolism. Increasing the urea derived from sources other than rumen NH_3 increased the percentage of rumen NH_3 derived from urea recycling. For urinary urea N, the two most influential variables were related to the anabolic use of N (microbial N uptake, and body protein synthesis).

5. 4. 2. 2. Validation of model predictions of renal excretion and recycling

Studies in which both renal urea excretion and GIT entry are simultaneously measured are scarce for dairy cows. For two studies using double labeled urea (Lapierre, et al., 2004, Ruiz, et al., 2002), the variation accounted for by model predictions were acceptable for GIT urea entry ($R^2 = 0.70$) and renal urea excretion ($R^2 = 0.95$) (Table 5.11). The model overestimated urea excretion and underpredicted GIT entrance, suggesting recycling loops are stronger than those represented in the model.

Table 5.11. Root mean square prediction (RMSPE) and mean square error (MSE) partition for urea excretion and gastrointestinal (GIT) urea entry

	N	Observed mean	Predicted mean	RMSPE	R^2	Mean Bias (%)	Systematic Bias (%)	Random errors (%)
Renal urea excretion, g/d	5	66.5	77.9	13.3	0.95	47.1	14.3	38.5
GIT urea entry, g/d	5	164.6	158.9	33.9	0.70	3.8	11.6	84.5

5. 4. 2. 3. Model applications

The efficiency of use of recycled nutrients in a system depends on several combined factors, including the pool of entry, the total nutrient system through-flow, the proportion of the nutrient attributed to cycling, and the intensity of use (Finn, 1976, Groot, et al., 2003).This section summarizes the results of simulations with the model when used to explore the effect of changes in diet fermentability on urea cycling and its use by microbes, and to compare the predictions of the dynamic model with the equation used to predict recycled N in the Cornell Net Carbohydrate and Protein System (CNCPS).

Urea recycling and its anabolic use

The urea that re-enters the rumen was more likely to be used for anabolic purposes than urea that re-enters the hindgut. On average, the simulated urea entry to the hindgut represented only approximately 15 % of the total urea recycled, increasing as the FC:NFC ratio increased. The inflows for the hindgut NH_3 were AAN deamination and urea recycling (Figure 5.2). The proportion of the NH_3 derived from each flow was approximately 50:50. For sheep, Dixon and Nolan (1986) reported similar ratios between digesta N flow and urea as sources of caecal NH_3. The hindgut

urea net entry, calculated as hindgut urea entry minus hindgut ammonia absorption + passage was positive for a wide range of simulated diet compositions. The net entry increased as intestinal protein digestibility increased (r = 0.9) and as the rumen solid passage rate increased (r= 0.23). Increasing ruminal passage rate increased the amount of fermentable organic matter reaching the hindgut, and thus enhanced hindgut microbial growth, and diverted N from urine into fecal excretion.

The overall total N flows and the anabolic use of N interacted to determine total recycled urea flow and its efficiency of use (Table 5.12). The total amount of urea recycled increased as the N intake increased. Higher diet fermentability resulted in greater ruminal N efficiency. For the low protein diet, up to 87 % of the NH_3 was taken up by microbes and the uptake of both ammonia and amino N by microbes increased (Table 5.12) and ammonia escape from the rumen decreased. Reducing ruminal NH_3 absorption resulted in a lower urea production and therefore the amount of urea returning to the rumen was lower,. For the high protein diet, more ammonia was absorbed, both as a percentage of the total NH_3 produced (microbial growth was limited by carbohydrate availability), and as absolute values, increasing urea flows. The proportion of NH_3 derived from recycled urea was 19 and 22 % of the NH_3 for the high and low fermentability diets (Table 5.12).

Table 5.12. Model predicted urea flows and its anabolic use in diets varying in protein content and fermentability

	Low protein diet[1]		High protein diet[2]	
	High ferm	Low ferm	High ferm	Low ferm
Total urea recycled, g/d	61	125	192	244
% ruminal NH3 derived from recycled urea	13.5	21	19	22
% microbial uptake/(uptake + absorption)	87	64	50	43
Microbial N derived from AAN	162 (50 %)	75 (27.3 %)	133.4 (31 %)	82.8 (22.6 %)
Microbial N derived from NH3	162 (50 %)	199.2 (72.7 %)	296.9 (69 %)	282.9 (77.4 %)
Microbial N derived from recycled urea	22 (6.7 %)	41 (15.3 %)	40.9 (13.8 %)	48.6 (17.2 %)

[1] Low protein diet: 12.5 % CP
[2] High protein diet: 18.7 % CP

Comparison with models that predict rumen urea recycling as a source of N for microbes

Urea recycling to the rumen represents an important source of N for microbes. However, the most recent Nutrient Requirements for Dairy Cattle (NRC, 2001) only includes dietary rumen degradable protein (RDP) as source of N for microbes; it assumes the average net recycling N to the rumen was close to zero. In contrast, the CNCPS includes urea recycling as a source of N for microbes. An equation based on the crude protein content of the diet is used to predict urea recycling (NRC, 1985). Predictions of our model were compared with those predicted by the CNCPS for seven diets varying in protein content, intakes and milk supported (Figure 5.5). The shape of the curve for urea entry was similar for both the CNCPS and dynamic model predictions. However, the NRC (1985) equation in the CNCPS predicted lower rumen

urea entry than the dynamic model. The NRC (1985) equation was developed from the sheep data of Kennedy and Milligan (1980), and therefore even with N intakes adjusted to metabolic body weight, the overall flows and clearances for dairy cows may be underpredicted when using equations based on sheep data. The CNCPS rumen N balance is the difference between dietary RDP and urea recycling and microbial use and do not include absorption and passage (Fox, et al., 2004). Failure to account for both urea entry and ammonia loss from the rumen resulted into an overprediction of rumen available N. At low N intakes, when the urea entry was expressed as the difference between urea entry and ammonia absorption and passage, the urea entry exceeded ammonia escaping. However at N intakes greater than 500 g/d the loss of ammonia from the rumen was greater than urea entry (Figure 5.5).

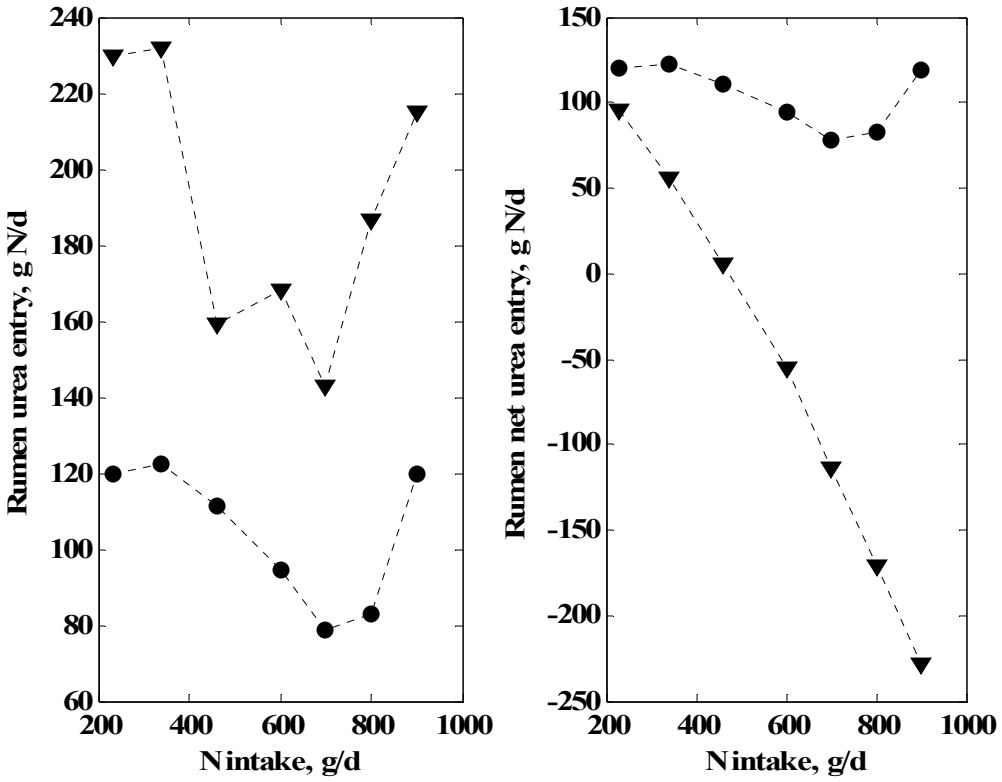


Figure 5.5. Rumen urea entry (g N/d) and net urea entry (calculated as rumen recycled urea entry – ammonia absorption + passage) (g N/d) as recycled N for diets varying in percentage of CP (7.2 to 21. 6 % CP) and milk production supported (12 to 40 kg/d) using the NRC (1985) equation (●) and the dynamic model (▼).

5. 5. Conclusions

A model is presented that can be used as a component of ration formulation models to predict N recycling to the GIT and urinary urea N. Reducing N excretion to meet emerging ammonia emission regulations requires decreasing excess N in the diets and accurate prediction of urinary N. Insuring rumen N requirements are met requires accurate accounting for the recycling N mechanisms in ruminants and the

potential for their manipulation in order to improve its transformation into anabolic products. At low protein intakes, urea recycling represents an important mechanism for N conservation. At high protein intakes, urinary N excretion increases at an increasing rate and must be accounted for in predicting ammonia losses from a dairy herd.

CHAPTER 6

SUMMARY AND FURTHER RESEARCH

Since the first description of the Cornell Net Carbohydrate and Protein System (CNCPS) carbohydrate (CHO) (Sniffen, et al., 1992) and protein (Van Soest, et al., 1981a) fractionation schemes, methodology to measure feed fractions and knowledge of ruminal nitrogen and CHO metabolism have advanced, making the revision of the schemes timely. The main limitations of the CHO fractionation scheme described by Sniffen et al (1992) were (1) fractions could not be precisely defined or assayed, and (2) although CHO were fractionated based on the rate of degradation, it combined CHO that differ in their ruminal volatile fatty acid (VFA) profile (e.g. pectin and starch). The scheme outlined in Chapter 2 divides feed CHO into fractions that more accurately relate to ruminal fermentation characteristics. However, improvements in the analytical methodology to measure some of the fractions (e.g. sugars) and their corresponding ruminal degradation rates are still necessary. Predictions of microbial protein yield were especially sensitive to rates of fiber and starch degradation. Better estimates of degradation rates are not only limited by the relative low accuracy and precision of the current methods, but also by the structure of the model itself. The CNCPS rumen submodel assumes that the growth rate of microorganisms is directly proportional to the rate of CHO digestion (Russell, et al., 1992). However, degradation rates and microbial growth rates do not always coincide. The maximum degradation of the crystalline cellulose is approximately 0.08/h, but rates of fiber digestion of forages by mixed ruminal bacteria rarely approach the maximum rate for crystalline cellulose (Weimer, 1996). However, there are growth rates for cellulolytic bacteria exceeding 0.08/h (Lynd, et al., 2002). Rates necessary to reflect microbial growth rates can be

greater than degradation rates necessary to predict extent of digestion, and therefore simultaneous accurate prediction of both extent of digestion and microbial protein yield may be difficult. This issue has been demonstrated in model evaluations. Aquino et al (2003) showed that in order to predict milk production of cows fed alfalfa silage diets when protein was first limiting, rates of approximately 0.11/h for fiber digestion were necessary. Pitt et al (1996) pointed out that degradation rates for starch lower than the CNCPS feed library values were necessary to improve predictions of VFA production and pH, but that would penalize accuracy in microbial protein yield predictions. Rates for the CHO fractions in Chapter 2 were assigned to better account for the differences in microbial protein yields. To fully account for differences in feed CHO utilization, inclusion of dietary factors in dry matter intake predictions, and prediction of ruminal VFA production and pH are necessary. A reassessment of CHO degradation rates and microbial growth submodel may be a necessary first step before integrating these other factors.

Evaluation of the protein fractionation schemes in Chapter 3 showed that despite the differences in the methodology used to obtained protein fractions, both NRC and CNCPS predictions of metabolizable protein supply had very similar sensitivity to variation in protein fractions and degradation rates because these two models rely on common principles, such as competition between digestion and passage to predict site of digestion basing microbial growth estimates on the first limiting nutrient (energy or protein) and almost complete rumen degradation of the soluble N fractions. As indicated in Chapter 1, there are several disconnects present in the CNCPS protein scheme that force us to question the validity of some of the assumptions of the scheme, especially regarding the use of detergent solutions to fractionate N. In Chapter 4, the ability of the original CNCPS protein scheme to predict rumen undegradable protein (RUP) for corn and alfalfa silage based diets was

rather moderate and neutral detergent insoluble crude protein (NDICP) flows were largely over predicted, suggesting the need to reexamine the appropriateness of using detergent solutions to fractionate N. At the same time, the flows were very sensitive to the rates for the B2 fraction. Dividing the available insoluble true protein (B fraction) into two fractions (B2 and B3) complicates the development of methodology to estimate rates. Predictions of rumen degradable protein and RUP were improved by assigning rates obtained with the inhibitory in vitro system to a combined insoluble protein B fraction. Advances in this area will rely upon a better understanding of the sources of variation in the techniques (Broderick, et al., 2004c), and greater efforts in modeling and understanding of *in vitro* digestion.

In Chapter 5, a dynamic mechanistic model was developed to integrate urea recycling and excretion. The model was developed with emphasis on the feedback structure of the system. Recycling processes were modeled as positive feedbacks, while renal excretion was modeled as a negative feedback. Both recycling and excretion were very sensitive to parameters related to microbial efficiency; highlighting once more the importance of how microbial growth is represented in rumen models. Model simulations suggested that the use of the NRC 1985 empirical equation to predict urea recycling to the rumen may greatly underestimate urea recycling in lactating dairy cows, and that the CNCPS prediction of ruminal N balance needs further revision. Not taking into account simultaneously urea entry and ammonia loss from the rumen may result into an overprediction of rumen available N. In addition, it does not address the impact that N recycling within the rumen has on ruminal N availability.

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