

EVALUATION OF CNCPS PREDICTIONS OF MILK PRODUCTION OF DAIRY COWS FED ALFALFA SILAGE

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INTRODUCTION

The Cornell Net Carbohydrate and Protein System (**CNCPS**) model has a mechanistic ruminal submodel that uses carbohydrate and protein degradation rates to predict the amount of feed that is fermented in the rumen and the amount that escapes undigested to the lower gut (Russell et al., 1992; Sniffen et al., 1992). The CNCPS has been modified as new information becomes available (Fox et al., 2003a). Overall, model validations indicated that it can provide realistic estimates of animal performance over a wide range of dietary ingredients (Fox et al., 2003a). However, preliminary analyses indicated the CNCPS did not provide realistic predictions of milk production when alfalfa silage was the sole dietary ingredient; it underpredicted nutritive value of diets based on alfalfa silage in lactating dairy cows with a mean bias as great as 50%.

The amount of inputs necessary to perform simulations using the CNCPS is high. In addition, it requires methods of feed analysis that are not always currently available, such as non-protein N (**NPN**). The use of feed library information to characterize feeds when their chemical composition is not available is a known constraint in adequate modeling and simulation. The objective of this evaluation work was to identify limiting factors that caused this under-prediction of the nutritive value of diets based on alfalfa silage. As a first step, CNCPS feed library values were adjusted to predict observed productions. Then, changes were evaluated with more rations.

ADJUSTMENT OF ALFALFA SILAGE INPUTS FOR THE CNCPS MODEL

Alfalfa silage inputs for the CNCPS v. 4.0 were initially calibrated using diets based solely on alfalfa silage. Because a large portion of alfalfa silage CP is NPN and much of the carbohydrate is NDF, these parameters were adjusted in stepwise fashion to evaluate their impact on predictions of milk production. These initial analyses used four published datasets (Cadorniga and Satter, 1993; Dhiman et al., 1993; Dhiman and Satter, 1993, 1997; Tessman et al., 1991).

Predicting Milk Production from Diets Based on Only Alfalfa Silage

In the 1990's, L. D. Satter and his colleagues conducted a series of lactation trials to evaluate alfalfa silage based rations and to determine the nutrient category (energy versus protein) that was most limiting milk production (Cadorniga and Satter, 1993; Dhiman and Satter, 1993; Tessman et al., 1991). In these experiments, alfalfa silage supplied 98.2% of diet DM (19.3% CP and 43.9% NDF). The feeding trials were relatively short term (average of 14 wk). The Holstein cows in the studies were early lactation animals with a high potential for milk production (Table 1). Some of the cows were rumen cannulated and energy sources (e.g. glucose, propylene glycol or propionate) or amino acids (casein) were infused directly into the abomasum to determine which nutrient category was limiting (energy versus protein). Because abomasal infusion of casein resulted in a much greater increase in milk production than the energy sources, it appeared that protein was the first limiting nutrient category. This conclusion was consistent with the observation that fish meal and roasted SBM (supplements that would escape ruminal fermentation) increased milk production (Dhiman et al., 1993; Dhiman and Satter, 1993). All of the studies had a feed description that included ADF, NDF, CP, NPN and NEI, but NDF digestion rates were not provided. The CP of these rations varied from 16.7 to 20.6% DM and the NDF values ranged from 41.8 to 45.5% DM. The studies had 46 early lactation Holstein dairy cows. Table 2 lists the ration ingredients.

Table 1. Description, milk production and DMI of dairy cows (mean \pm SD)

Item	Alfalfa Silage (AS) ^a	AS+HMEC ^a	AS+HMEC+ SBM ^a	AS+HMEC+ FM ^a
Animal Description				
No. of Cows	46	55	69	20
Body weight, kg	553 \pm 19.9	601 \pm 19.8	608 \pm 30.8	608 \pm 11.1
Body condition score	3 \pm 0.3	3 \pm 0.2	3 \pm 0.0	3 \pm 0.0
No. of lactation	2 \pm 0.9	3 \pm 0.5	3 \pm 0.3	3 \pm 0.4
Days in milk	73 \pm 5.3	42 \pm 5.0	43 \pm 4.7	40 \pm 5.5
Change in BW, kg	-0.05 \pm 0.3	0.02 \pm 0.3	0.24 \pm 0.2	0.07 \pm 0.1
Milk Production				
Milk yield, kg/d	22.7 \pm 5.4	32.4 \pm 3.0	33.0 \pm 3.2	35.5 \pm 2.8
3.5% FCM, kg/d	23.1 \pm 5.3	32.5 \pm 2.1	33.2 \pm 2.9	35.4 \pm 2.8
Milk Fat, %	3.7 \pm 0.1	3.6 \pm 0.2	3.6 \pm 0.3	3.4 \pm 0.1
Milk Protein, %	2.8 \pm 0.2	2.9 \pm 0.1	3.0 \pm 0.1	3.3 \pm 0.5
DMI, kg/d	18.1 \pm 1.6	21.7 \pm 1.2	22.7 \pm 2.1	22.4 \pm 2.0

^a AS is alfalfa silage, HMEC is high moisture ear corn, SBM is soybean meal, and FM is fish meal.

The CNCPS feed library (CNCPS v. 4.0) did not have silages that were exactly the same, but we were able to adjust these values to closely mimic the ones used by Satter and his colleagues. In some cases, Dairy NRC (2001) was used as a source of information. The NPN content of the derived alfalfa silages averaged 51.0% of total CP. The CNCPS inputs were obtained so that the animal description, production, and environment effects were accounted for. In these initial evaluations, the average shrunk BW was 553 kg and the BCS was 3 (Table 1). Cows were in their second lactation, were approximately 73 DIM, produced 22.7 kg/d (3.7 and 2.8 % fat and protein, respectively), consumed 18.1 kg DM/d, and lost 0.05 kg BW/d. The cows were kept in tie stall barns, were fed *ad libitum* once a day, and were milked twice a day. Milk samples were collected weekly and analyzed for milk fat and crude protein contents.

Table 2. The ration ingredients (%) used in the CNCPS (mean \pm SD)

Ingredient	Alfalfa Silage (AS) ^a	AS+HM EC ^a	AS+HMEC +SBM ^a	AS+HMEC+ FM ^a
Alfalfa silage	98.2	66.7	58.7	67.6
High moisture corn		26.5	35.9	28.2
Corn grain		5.4		
Soybean meal			4.52	
Fish meal				2.93
Ca ₂ PO ₄	1.1	0.62	0.40	0.7
Mono CaPO ₄		0.26	0.05	
Na(CO ₃) ₂		0.05	0.03	
MgO			0.03	
Limestone			0.03	
Salt	0.7	0.45	0.33	0.45
Vitamin + minerals		0.02	0.06	0.10

^a AS is alfalfa silage, HMEC is high moisture corn, SBM is soybean meal, and FM is fish meal.

Analyzing the NPN Content of Alfalfa Silages

The CP of alfalfa silages in the standard CNCPS feed library ranges from 16 to 25 % in the DM. This CP is partitioned into three fractions: A = NPN, B= true protein and C= unavailable protein (Van Soest et al., 1981). The B fraction is further sub-divided into B1, B2, and B3. The B1 fraction is soluble in buffer (Roe et al., 1990), precipitated by TCA (Krishnamoorthy et al., 1983; Van Soest et al., 1981) and is mostly available to ruminal microorganisms. The B2 fraction has a slower degradation rate than B1 fraction and some of it escapes to the lower gut. The B3 fraction is insoluble in neutral detergent but is soluble in acid detergent and has an even slower ruminal degradation rate (Goering and Van Soest, 1970; Krishnamoorthy et al., 1982). The C fraction is insoluble in acid detergent solution and is considered to be unavailable.

In the CNCPS feed library, the NPN values of alfalfa silage range from 70 to 95%, expressed as % of soluble CP. Based on total CP, the NPN values are 28 to 67%. The NPN content of CP ranged from 38% (Dhiman and Satter, 1993) to 76.4% (Broderick et al., 1990) (Table 3). Recently, Makoni et al. (1997) reported that alfalfa silage CP could have as little as 55.5% NPN and as much as 66% of this CP was peptides and amino acids.

In the CNCPS, NPN is assumed to be converted rapidly to ammonia and does not contribute to the ruminal peptide pool, which is derived from the degraded true protein fractions. NPN is determined as the nitrogen passing into the filtrate after precipitation with a protein specific reagent (Licitra et al., 1996). When trichloroacetic acid (TCA) is used as a protein precipitant, peptides of less than ten amino acid units are not precipitated. Therefore, they are allocated to the NPN fraction. Because peptides and amino acids can stimulate microbial growth to a greater extent than ammonia (Russell et al., 1992), these solubilized peptides contribute to microbial growth, and allocating them to the NPN pool results in the underestimation of microbial growth and MP allowable milk.

Table 3. Nutrient composition of alfalfa silage (mean \pm SD)

Nutrient composition	Alfalfa silage ^a	AS+HMEC ^a	AS+HMEC +SBM ^a	AS+HMEC +FM ^a
DM, %	46.5 \pm 1.8	41.2 \pm 6.9	41.4 \pm 4.3	40.0 \pm 1.1
CP, %	19.3 \pm 1.3	20.9 \pm 3.6	21.4 \pm 2.9	20.9 \pm 3.1
NDF, %	43.9 \pm 1.4	42.5 \pm 0.6	44.6 \pm 2.9	39.9 \pm 0.7
ADF, %	36.3 \pm 1.5	33.7 \pm 4.1	36.5 \pm 3.1	38.8 \pm 2.3
Lignin, %	7.1 \pm 0.6	9.6 \pm 0.4	8.4 \pm 1.8	11.1 \pm 1.0
Ash, %	11.5 \pm 0.0	11.6 \pm 1.4	11.9 \pm 2.4	8.4 \pm 1.0
NPN, %CP	51.0 \pm 7.1	57.0 \pm 9.0	63.9 \pm 5.2	51.4 \pm 2.2
NH ₃ -N, % CP	8.9 \pm 1.3	9.1 \pm 3.1	11.3 \pm 4.0	8.7 \pm 0.0
AA-N, % CP	36.3 \pm 4.1	39.4 \pm 6.1	43.9 \pm 1.9	41.7 \pm 0.0
PH	4.7 \pm 0.1	4.7 \pm 0.1	4.7 \pm 0.0	4.7 \pm 0.0
NEL, Mcal/kg	1.4 \pm 0.0	1.4 \pm 0.1	1.3 \pm 0.1	1.4 \pm 0.2

^a AS is alfalfa silage, HMEC is high moisture corn, SBM is soybean meal, and FM is fish meal.

Estimating the CHO B2 Degradation Rate of Alfalfa Silage

The CHO B2 fraction can be determined by subtracting the C fraction (Lignin \times 2.4) from ash-free NDF once the NDF has been corrected for associated protein, but the rate of CHO B2 degradation is much more difficult to estimate. The CNCPS feed library indicates that alfalfa silage CHO B2 degradation rates vary from 4.0 to 5.5 %/h (Fox et al., 2003b), but these rates are somewhat peculiar. When the CP content of the alfalfa silage is high (e.g. less mature material), the degradation rate is often low and vice

versa. This examination indicated that at least some currently used CHO B2 degradation rates probably are inconsistent.

The CHO B2 degradation rates typically are estimated from either *in situ* nylon bag experiments or *in vitro* digestion, and these methods do not always give realistic estimates of lag time, fermentation rate or the extent of digestion (Varel and Kreikemeier, 1995). If the nylon bags have very small pores, bacteria cannot easily colonize the fiber, and conversely, feed materials can leak from the bags if the pore size is too great (Dewhurst et al., 1995). When the bacteria are exposed to oxygen prior to *in vitro* digestion, there can be a significant lag time before digestion is observed, standard log transformations are confounded, and it is difficult to estimate a first order rate (Vanzant et al., 1998). These studies indicated that *in vitro* degradation rates consistently were slower than *in situ* (*in sacco*) methods by more than 3%/h (Dewhurst et al., 1995; Varel and Kreikemeier, 1995).

In vitro fermentations indicate that the CHO B2 of legumes is degraded faster than that of grasses (Van Soest, 1994; Varga and Hoover, 1983). Early work indicated that the degradation rate of coarsely chopped alfalfa silage was only 4 to 7%/h, but finely chopped silage appears to have a higher CHO B2 degradation rate (5 to 9 %/h) (Ewing and Johnson, 1987; Krishnamoorthy et al., 1983; Mertens, 1973; Mertens and Ely, 1979; Van Soest et al., 1981; Waldo et al., 1972; Williams et al., 1992). If the dry matter disappearance is not corrected for indigestible residues, the estimated rate can be too low. More recently, *in vitro* gas production has been used to estimate rates, and the correlation between the gas production and NDF disappearance was high (Pell and Schofield, 1993; Prasad et al., 1994; Schofield and Pell, 1995a, b). Gas production measurements are not confounded by indigestible residues, but they can be confounded by pH changes and variations in molar proportions of VFA. When Doane et al. (1997) used gas measurements to estimate the NDF digestion rate of alfalfa silages, the values were as high as 12%/h. Additionally, Van Soest et al. (2000) reported values ranging from 5.9%/h to 12.5%/h, which are based on data of Mertens (1973).

Inputs and Adjustments

Using the feed description of L. D. Satter and his colleagues, average NPN was initially set at 51.0% of total CP (90% of the soluble CP) and the NDF degradation rate was initially set at 5.5%/h based on CNCPS feed library values (Fox et al., 2003b). When these inputs were used, the ME allowable milk was 16.1 kg/d, but MP allowable milk was only 10.1 kg/d (Figure 1). Given the observation that the actual milk production values averaged 22.7 kg/d, it appeared that the feed library was not giving an accurate estimate of nutrient availability for milk production.

Our first attempt in adjusting the alfalfa silage inputs for the CNCPS to more correctly represent the pools and digestion rates involved a reduction in conventional NPN and the movement of some of this N to the B1 protein fraction (amino acids). When we moved 70% of this N to the B1 fraction, only 16% of the total N went directly

to ruminal ammonia. Prior to this adjustment, the B1 fraction accounted for less than 6% of the total CP, but thereafter, the B1 fraction increased to approximately 37.6% of the CP. When this adjustment was made, predicted ME and MP allowable milk productions were 16.3 and 13.3 kg/d, respectively.

Our second attempt to adjust the alfalfa silage inputs involved increasing NDF degradation rate from 5.5 to 11%/h. When this adjustment was made (and NPN was left unchanged), ME and MP allowable milk productions were 18.8 and 15.0 kg/d, respectively. When the NDF degradation rate was 11%/h and 70% of the NPN was moved to protein B1, ME and MP allowable milk estimates were 19.0 and 18.6 kg/d, respectively, and these values were similar to the actual milk production. When both of the changes in CNCPS inputs were employed, predicted ME and MP allowable milk was in agreement with the predictions with the Dairy NRC (2001) model (Figure 1).

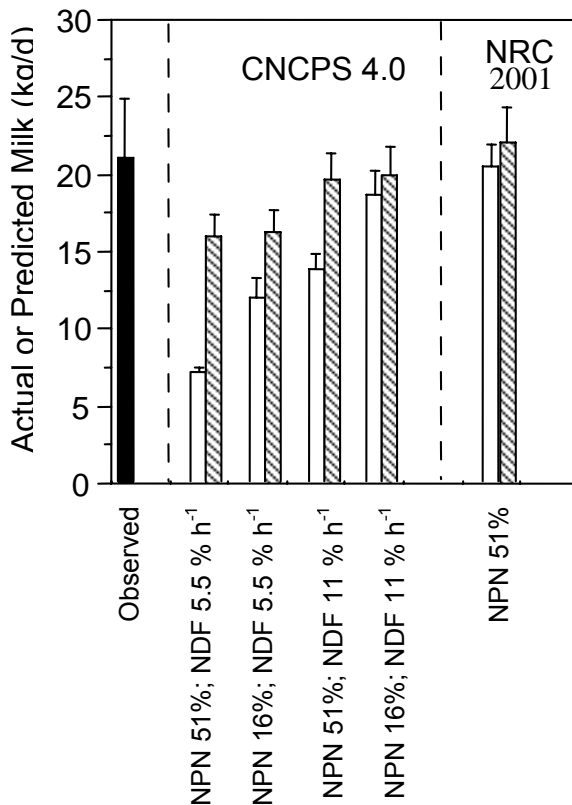


Figure 1. CNCPS prediction using only alfalfa silage diets. Solid black bar is the actual milk production, empty bar is MP allowable milk, and diagonal bar is ME allowable milk.

EVALUATION OF THE CNCPS WITH OTHER ALFALFA SILAGE BASED DIETS

Preliminary results indicated that alfalfa silage inputs for the CNCPS could be modified to obtain more accurate predictions of milk production when alfalfa silage was the sole ration ingredient, but the question then arose: *Would these adjustments be useful over a wider and more realistic range of rations?*

The CNCPS model predictions were evaluated with 9 studies that had 25 different rations, containing corn, soybean meal, fish meal, and alfalfa silage. These studies had a total of 144 lactating Holstein cows. Each ration description had sufficient information to provide necessary inputs for the CNCPS. Predictions of ME or MP allowable milk were compared with the actual milk productions. The rations were grouped as: 1) alfalfa silage (AS) plus high moisture ear corn (HMEC); 2) AS plus HMEC plus Soybean meal (SBM) or 3) AS plus HMEC plus fish meal (Fish meal) (Table 2).

Group 1 rations had from 54-70% AS and 20-40% HMEC (Broderick, 1995; Broderick et al., 1990; Broderick et al., 2000; Dhiman and Satter, 1993; Valadares Filho et al., 2000). These experiments used 55 animals, the average body weight was 601 kg, and the body condition score was 3.0. The animals were in the early stage of their third lactation. The animals consumed a TMR of 21.7 kg DM/d and produced 32.4 kg milk/d with 3.6 % fat and 2.9% CP. The lactation period lasted for 86 days. The AS had an average of 20.9% CP; 42.5% NDF and 57.0% NPN (% of total N) (Table 3).

Group 2 rations had 54-64% AS, 25-38% HMEC, and 2-8% SBM (Broderick, 1985; Broderick et al., 1990; Broderick et al., 2000; Jerred et al., 1990; Petit and Veira, 1991). The AS had an average CP of 21.4%; 44.5% NDF and an NPN content of 63.9% (% total CP). Sixty-nine lactating cows were used, the average body weight was 608 kg, and animals were housed in tie stall barns. They were on their third lactation, milk production averaged 33.0 kg milk/d (3.6% fat and 3.0% crude protein), and the lactation period was 86 d.

Group 3 rations were derived from the experiments of Broderick et al. (1995; 2000). AS ranged from 64-70%, HMEC was 25-31% and fish meal was 2.8-3% of the ration DM. The average CP, NDF and NPN values for the AS were 20.9%, 39.9% and 51.4%, respectively. These studies had 20 cows. The average body weight was 608 kg, the body condition score was 3.3, the lactation trials lasted 100 d, and the DMI averaged 22.4 kg DM/d. The average milk production was 35.5 kg (3.4% fat and 3.3% CP).

The CNCPS indicated that the MP allowable milk was always lower than the ME allowable milk (Figures 2A and 2B), and the adjustments did not significantly affect the ME allowable milk values. The non-adjusted MP allowable milk values were highly correlated with actual milk production ($r^2 = 71.2\%$) but there was a very large bias (approximately 9.2 kg/d). When the adjustments were used, there was only a small increase in the r^2 (76.7%), but the bias was greatly reduced (approximately 2.8 kg/d). Paired *t*-test comparisons indicated that the adjustments significantly improved the prediction of milk production ($P < 0.05$), and predicted MP allowable milk was closer to the actual milk production.

Adjustments based on NPN and NDF degradation rate improved the CNCPS prediction, but it should be realized that these corrections were based on high quality alfalfa silages. When alfalfa matures, there is often an increase in NDF and lignin, and the CHO B2 degradation rate may decrease. Because poor quality alfalfa silage has less CP, the need for an NPN adjustment would decrease. If alfalfa lacks sugar to promote adequate silage fermentation, and the decline in pH is slow, a larger fraction of the amino-N (peptides and amino acids) would be converted to non-amino-N, and the need for moving NPN to the B1 protein pool would also decline. Further work is needed to accommodate maturity and quality effects, but the overall strategy of modifying NPN and CHO B2 degradation rates is likely to improve the prediction of dairy cow performance.

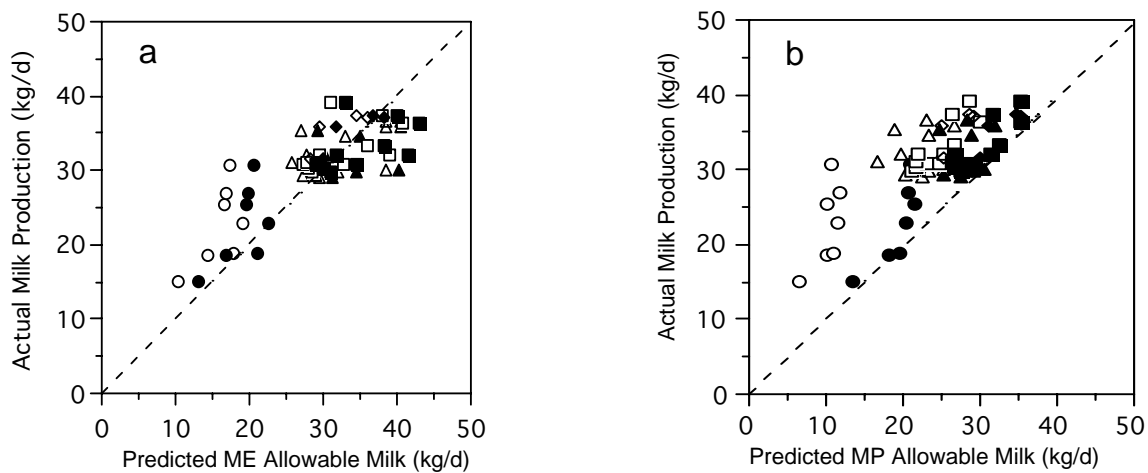


Figure 2. Predicted ME (a) and MP (b) allowable milk of the validation distribution. The symbols indicate rations based on alfalfa silage (AS) alone (●○), AS plus high moisture ear corn (HMEC) (▲△), AS plus HMEC plus SBM (■□), and AS plus HMEC plus fish meal (◆◇). Open and closed symbols are values before and after the adjustment, respectively.

CONSIDERATIONS FOR FUTURE RESEARCH ON PROTEIN

Concerns about the impact of livestock on the environment have increased in recent years, and will continue to be a concern (Fox et al., 2002). The prediction of N utilization and its main routes of excretion (fecal or urinary) and forms have been targeted as one of the areas that need further refinement in the CNCPS (Fox et al., 2002). The CNCPS has a complex feed N fractionation with five chemically distinguished fractions and their corresponding digestion rates (Sniffen et al., 1992), but evaluations indicate that the simple use of values of the feed library decreased the accuracy in predictions. The question then arises: *Can our current fractionation scheme be modified to decrease the number of inputs while maintaining or enhancing accuracy?*

Degradation rates for the true protein fractions (B1, B2, and B3) are not always easy to estimate, and may not always have the same first order degradation rate. The unavailable N (C fraction) may also need to be re-evaluated. The C fraction is chemically defined as the N recovered with ADF (ADIN) (Sniffen et al., 1992). However, Lucas's test has shown that for some feeds, ADIN does not behave as a completely indigestible entity. Van Soest (1994, p. 295) indicates that as much as 60% of ADIN of distillers' grain can be degraded and absorbed, but it is poorly metabolized. Nakamura et al. (1994) found poor relationships between ADIN and N indigestibility for protein concentrates. *In situ* protein fractionation is often used in protein evaluation systems (AFRC, 1993; NRC, 2001), but *in situ* N models have a greater aggregation than the CNCPS N fractions. The A *in situ* fraction includes not only NPN but also rapidly solubilized protein, and protein in particles of smaller size than the porosity of the nylon bag (NRC, 2001). Distinction between NPN (CNCPS A fraction) from true protein (CNCPS B fractions) is necessary because NFC and FC bacteria have different patterns of N utilization (Russell et al., 1992).

Current feed library values for protein degradation rates are largely based on enzymatic studies (Sniffen et al., 1992), but these methods do not follow always first order kinetics (Kohn and Allen, 1995). If the ratio of feed sample to enzyme concentration is inappropriate, the reaction may not be an enzyme-excess, substrate-limited system. Furthermore, given the complexity of the ruminal proteolysis, it seems unlikely that one commercial protease will be able to mimic microbial proteolysis. A complex mixture of commercial proteases with activities similar to those found in the rumen and/or microbial-cell preparations is probably more desirable, but such mixtures have not been systematically evaluated (Kohn and Allen, 1995; Luchini et al., 1996). Luchini et al. (1996) used a mixture of commercial enzymes (trypsin, carboxypeptidase B, chymotrypsin, and carboxypeptidase A), but this mixture could not detect differences due to heat damage or mimic the digestion rates obtained with strained ruminal fluid.

Near-infrared reflectance spectroscopy (NIRS) is routinely performed by a variety of commercial laboratories, and it is conceivable that this method could be used to determine protein digestion rates. NIRS has the potential to account for physicochemical properties, 3-dimensional structures, inert barriers (cell walls) and anti-nutritional factors (e.g. tannins). Hoffman et al. (1999) used NIRS to calibrate the degradation rate of the true protein fractions (B) with *in situ* methods for legume and grass silage samples ($SEP = 1.4 \text{ h}^{-1}$) and the R^2 was 87%. Further work will be needed to see if NIRS can be used to develop rates for the CNCPS.

Electrophoresis separates proteins via differences in charge and molecular weight. This technique can be used to assess the effect of chemical and physical treatments on ruminal protein degradation, but it should be noted that only soluble proteins can be examined (Makoni et al., 1993; Messman and Weiss, 1994; Messman et al., 1994). Many proteins (e.g. corn gluten meal) have a large fraction of insoluble proteins, and the recovery of this fraction is often incomplete (Messman and Weiss, 1994). The CNCPS currently uses a scheme that employs borate-phosphate buffer to determine the amino acid profile of the protein insoluble proteins that escape ruminal degradation (O'Connor et al., 1993). These intestinal digestion coefficients are then used to estimate

percentage of absorbed amino acids, (100 % for A, B1, and B2 protein fractions, 80 % for B3 fraction, and 0 % for C fraction). The use of amino acid profiles for each feed N fraction could improve the accuracy of prediction of the amino acid flow.

Intestinal metabolism and its transport mechanisms play a crucial role in the profile of available amino acids, and it is now clear that amino acids can have different efficiencies of utilization (Reeds et al., 2000). The recovery of intestinally infused essential amino acids (EAA) at the portal vein ranged from 0.61 for histidine to 0.83 for valine (MacRae et al., 1997). Enterocytes are polarized cells with unique amino acid transport mechanisms in the apical and basolateral membranes (Matthews, 1991). The rate of absorption of amino acids is related to their concentrations and to the overall kinetic characteristics of their intestinal transports (Matthews, 1991). The prediction of available portal amino acids rather than intestinal amino acids may improve the accuracy in predicting of amino acid available for growth and lactation. If the affinity for each transporter in the apical and basolateral membranes could be determined, the likelihood of an amino acid arriving to the portal bloodstream can be computed from the concentration of amino acids in the intestine (Fox and Tedeschi, 2003).

CONCLUSION

Previous feed descriptions of high quality AS led to an under-prediction of milk production. These inputs can be easily changed. When the NPN and CHO B2 degradation rates were modified, the ability of the CNCPS model to predict milk production was improved ($r^2 = 71.2$ to 76.7%) and the bias was decreased from 9.2 to 2.8 kg/d. This comparison indicated that NPN and CHO B2 degradation rate are crucial information for alfalfa based diets.

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