Updates to the Cornell Net Carbohydrate System v7 and Updates to Ruminal Pool Characterizations and Requirements to Facilitate Feed Additives

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Introduction

The Cornell Net Carbohydrate and Protein System **(CNCPS)** has been updated with a dynamic rumen sub-model and full gastrointestinal model (Higgs, 2014; Higgs and Van Amburgh, 2016; Higgs et al., 2023). Although this system is meant to provide a more comprehensive report on the nutrients supplied than its previous versions, the influence with which various feed additives have on the metabolism of the microbial population and their host animal continue to be parameterized. This paper will review pertinent updates to the model which are necessary to understand how particular feed additives with possible non-nutritional actions can be modeled.

Updated Nutrient Supply Predictions

Nutrient supply predictions within CNCPS v7 build upon ruminal and intestinal transactions that are reported in previous versions and further describe their dynamic flow starting at the mouth, ending at the rectum, and providing pool size and flux predictions for the rumen, omasum, and small and large intestines (Table 1). This disaggregation of compartmental modeling will utilize a similar feed fractionation scheme, with a greater description of fiber carbohydrates and revisions on how intestinal digestibility of protein in feeds which contain little to no fiber are calculated. A more descriptive report becomes useful during formulation as it will allow the user to understand total tract digestibility of fiber and if feed inventory and costs allow, make modifications to enhance digestibility and energy availability. This will also provide useful information about ruminal digestibility of aNDFom as its digestion will be explicitly quantitative. The total tract digestibly estimations have been tested on four prospective studies, three of which were formulated to North American specifications and one using an Irish grazing system. On average, the resolution of predicted aNDFom total tract digestibility was within 7%, or 2.9 units, of observed total tract digestibility. This group will continue to use future studies to evaluate the accuracy of these predictions and will modify equations when biases present themselves under varying fiber feeding conditions.

There are two aspects to this pool size data on aNDFom which will become relevant to the user as the steady state rumen pool size of the potentially digestible aNDFom and the uNDFom will be a determinant of potential dry matter intake **(DMI)** for the animal (Table 2). This approach is meant to complement existing equations provided within previous versions of the CNCPS, in addition to an equation published in the (NASEM, 2021) model, providing users with an additional tool to troubleshoot and reconcile predicted and observed DMI on farm. The recommended intake and rumen fill values

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are based on the work conducted at Miner Institute, University of Bologna, and Cornell University (Cotanch et al., 2014) using the intake metrics developed by Mertens (2010). This information was one of the outcomes of the "Informal Fiber Working Group" that has been meeting at the Cornell Nutrition Conference for over 10 years.

The model will provide predictions for bacterial protein flows, as in previous versions, based on the fiber (Feed fractions CHO B3 and CHO C; FC) vs non-fiber carbohydrate (Feed fractions CHO A1, A2, A3, A4, B1, and B2; NFC) characteristics, with many of the existing metabolic coefficients, including maintenance and growth potentials, remaining intact. Ruminal protozoal relationships have been studied, quantified, and published, including the uptake of free peptides and amino acids **(AA)**, predation and engulfment of bacteria, and lysis/excretion of nutrients back into their environment. The CNCPS v.7 can capture these relationships, where predictions for protozoal growth and flow will be quantified as a source of microbial nitrogen, carbohydrates, and fatty acids (Table 3 and Table 5). Recreation of previously fed diets and formulation of prospective studies have elucidated a supply of protozoal MP that ranges between 10 and 20% of the total metabolizable microbial supply in most Northeastern US diets. In the study by Dineen et al. (2020), cattle were fed high quality Irish pasture grass, resulting in protozoal contributions representing 23% of microbial supply. It is plausible that cattle fed these highly degradable grasses, with high sugar content, maximize microbial growth and thereby represent the upper limit of protozoal contributions between 22 to 25% of total microbial yield. The addition of protozoal metabolism also provides insights on the microbial yield response when varying the supply of other carbohydrate fractions to a diet, particularly regarding protozoal growth, and subsequent microbial MP supply, when sugar is increased in a diet. Previous versions of the CNCPS were not sensitive enough to capture the full microbial yield response when sugar was added, only modestly improving NFC degrading bacteria growth. Further efforts to quantify microbial metabolism in the rumen will refine the effect other carbohydrates have on the proliferation of varying microbial communities.

In this version of the model, rumen ammonia levels are estimated based on a sub-model which predicts ammonia production, subsequent hepatic urea production and full urea recycling back to the gastrointestinal tract. This updated approach has at least two benefits. First, it will provide a more stochastic approach to estimating rumen ammonia as the flux generally displays a large amplitude throughout the day, but recycling of nitrogen into the rumen is generally constant (Reynolds and Kristensen, 2008). It is important to note that behavioral patterns, including meal frequency and cow time budgets, in conjunction with dietary composition, including carbohydrate digestibility and nitrogen solubility, can interact to cause large swings in rumen ammonia, which can be problematic throughout periods of the day where its concentration could drop below 5.5 mg/dL and cause microbial growth depression. Figure 1 describes the rumen ammonia concentration for a North American based diet that is formulated for 68% forage DM which uses various concentrate feedstuffs to provide other required nutrients. Two of these ingredients, soybean meal and canola meal, are fed at varying levels to provide a different soluble and degradable protein supply in the rumen. As with previous versions of the CNCPS, version 7 can calculate an average ammonia concentration for this diet; however, a static evaluation of this concentration may not provide a meaningful explanation if microbial growth is depressed. For instance, the diet which splits 2.5 kg of DM

into equal parts of soybean meal and canola meal has an average ammonia concentration of 6.5 mg/dL, which can raise some concerns but does not flag microbial growth depression within the model. Conversely, if a user was to describe the feeding behavior of the target animal, in this case an 8 meal/day behavior was designated, the model would provide a more dynamic form of rumen ammonia concentration that would indicate periods throughout the day where this concentration would fall below 6.0 md/dL and microbial growth would be marginally depressed. Users will also be provided with a summarized table (Table 4) indicating both average and range of rumen ammonia concentration and microbial growth depression. Depression of microbial growth will become more pronounced with the associated decrease in carbohydrate digestion, specifically regarding potentially digestible aNDFom **(pdNDFom**), as we expect the fiber degradation to be disproportionately decreased under N limiting conditions.

Another quantitative addition to the updated version of CNCPS is the inclusion of endogenous transactions which occur ubiquitously throughout the gastro-intestinal tract (Ouellet et al., 2007; Ouellet et al., 2010). The inclusions of these flows do not add an appreciable increase in the supply of metabolizable protein **(MP)**, as most endogenous secretions that are quantified in the model are offset by the maintenance requirement calculated for the loss of these endogenous fractions. This, however, does not mean that these fractions should be left unquantified, given that the remains of salivary proteins, ruminal secretions, and sloughed cells can all be utilized by microbial populations within the rumen to proliferate and further alter the supply of amino acids flowing out of the rumen. Contributions of endogenous proteins within the CNCPS v.7 include salivary proteins (Yisehak et al., 2012), sloughed ruminal, omasal, and abomasal cells (Larsen et al., 2000), omasal and abomasal secretions (Ørskov et al., 1986), pancreatic secretions (Hamza, 1976; Larsen et al., 2000), bile secretions (Larsen et al., 2000), and small and large intestinal sloughed cells and secretions (Larsen et al., 2000; Jansman et al., 2002).

Feed Additives and Meeting Other Ruminal Requirements

With this information as a background, two "feed additives" will be briefly described.

Feed Additive 1: Byproduct of MSG and amino acid production with anti-proteolytic behavior

Efficient use of feed N can be achieved by first meeting the requirements of the rumen microbial population, followed by balancing diets to meet the amino acid **(AA)** requirements of the cow. To decrease competition for quality protein that could otherwise be fed to humans, dairy cattle can be fed byproducts of human food production, thereby converting waste product streams into highly valuable milk protein. Commercial AA production is performed using bacterial cultures, resulting in a waste stream with high amounts of soluble nitrogenous compounds. A meta-analysis of in-vitro data from continuous culture fermenters using these fermentation byproducts demonstrated an almost 16% increase in microbial nitrogen output versus a control with no fermentation byproduct addition (Lean et al., 2005). The response in that paper was attributed to a stimulation of microbial protein synthesis by AA and peptides contained in the fermentation byproduct (Cotta and Russell, 1982). However, in vivo results have been more varied, with some studies showing limited effect on rumen metabolism and cattle performance (Broderick et al., 2000), or effects mediated by other dietary components, such as sugar (Penner et al., 2009).

An omasal flow study was conducted (Fessenden et al., 2019) evaluating the effects of a fermentation byproduct on the flow of microbial and non-microbial AA in lactating dairy cattle. Using the data from the omasal experiment with a fermentation byproduct (Table 5), it is evident that cows fed the byproduct did not show an increase in microbial flow, as has been shown in vitro (Lean et al., 2005). Instead, there was a 15% decrease in rumen degraded N (68.7 vs. 58.3% of dietary N intake). When evaluated against CNCPS predictions from v6.5.5, non-ammonia nitrogen **(NAN)** flow from the rumen was well predicted; however, the partition between microbial and non- microbial N demonstrated the need for modification as the apparent rate of N disappearance and movement into the microbial pool was decreased, suggesting that when feeding the byproduct, the rates of soluble protein degradation and related microbial growth must be modified.

To ensure the omasal flow data were repeatable, a follow-up study was conducted in lactating dairy cattle to examine, through differences in dietary soluble true protein formulation if milk yield and energy-corrected milk **(ECM)** yield followed the observations from the omasal flow study (Fessenden et al., 2020). The cattle in the lactation study responded to the formulation differences by producing 1.7 kg/d more ECM (45.3 kg vs 43.6 kg), about a 4% increase, demonstrating that feeding the byproduct provided more MP, most likely from undegraded feed as the byproduct diet was formulated to be lower in rumen escape protein. In addition, in the lactation study, plasma urea nitrogen was lower on the byproduct diet, suggesting a reduction in ammonia production and hepatic urea production supposedly due to reduced feed protein degradation (Fessenden et al., 2020).

There was an approximate 30 g/d increase in N flow to the omasum in cattle fed the byproduct, and the primary source of this increased flow was non-microbial, NAN (more feed N escaping the rumen). In the study, they observed an increase total N flow out of the rumen of 28 g/d comprised of an increase of approximately 64 g/d NAN, non-microbial N, and a decrease of 41 g/d of microbial NAN. Thus, rates of digestion of feed N and microbial growth rates require modification to accurately model this outcome. The two feed chemistry factors which need to be considered are the solubility of the true protein fractions Protein A2 and B1, and the amount of Fermenten added to the diet as the effect on the rate of disappearance is likely non-linear with increasing dietary inclusion rate. Modeling the outcome, the optimum response in reduced protein disappearance is observed at approximately 3% dietary inclusion of Fermenten and the associated decrease in the rate of disappearance is ~10 to 12%. Therefore, if the rate of disappearance for Protein A2 is 45%/h and the B1 is 15%/h without the byproduct, inclusion of the byproduct will result in a rate of disappearance of 39.6%/h for A2 and 13.2% for B1, thus reducing the transfer of N into the microbial compartment and to ammonia. Depending on the size of the fractions and the passage rate, this adjustment could result in a small, but biologically significant, change in microbial yield and feed protein escape. Using a 1,650 lb cow yielding 98 lb/d milk, consuming 61 lb/d dry matter intake of a 16.1% CP diet, there is 3,099 g MP available from the diet, of which 1,699 g are from bacterial yield and 1,400 g are from undegraded feed. Applying the optimum inclusion of byproduct, the reduction in microbial yield will be approximately 236 g/d, whereas the increase in escape feed protein will be approximately 380 g/d for an overall increase of approximately 144 g/d of MP, which is adequate for a 4 to 6 lb increase in MP allowable milk. This approach would suggest

the byproduct would improve the efficiency of use of N in diets which are high in soluble true protein, like diets containing high amounts of alfalfa silage and soybean meal.

Feed Additive 2: Branched chain volatile fatty acids (BCVFA)

It has been long known that cellulolytic bacteria, including *Fibrobacter succinogenes*, *Ruminococcus albus*, and *Ruminococcus flavefaciens*, exhibit requirements for BCVFA (Andries et al., 1987) with the intention of using them to synthesize branch chain amino acids **(BCAA)** through reductive carboxylation (Allison et al., 1962b; Allison, 1969; Robinson and Allison, 1969) or branch chain fatty acids **(BCFA)** through chain elongation (Allison et al., 1962a). The majority of BCVFA found in the rumen are by-products of BCAA metabolism from other dominant species of bacteria, whereby the deamination of the amino group or transamination of the corresponding keto acid occurs. Ironically, it has been observed that the amylolytic bacteria, *Prevotella ruminicola*, which does not require BCVFA to synthesize BCAA, will preferentially carboxylate them back to BCAA, down regulating de novo BCAA production and reducing cross feeding to bacteria who cannot synthesize BCAA without BCVFA as the precursor (Allison et al., 1984). An argument could be made that the reduction in aNDFom digestibility observed in dairy cattle with higher levels of dietary starch (de Souza et al., 2018) is due to this resource competition. Bacteria that digest starch have the capacity to take up peptides and amino acids (Russell and Sniffen, 1984; Chen et al., 1987) which provides them a competitive advantage over fiber digesting bacteria which can only utilize ammonia and have a much slower growth rate (Bryant, 1973). Exogenous supplementation of these BCVFA has shown improved aNDFom disappearance in batch cultures (Russell and Sniffen, 1984; Cummins and Papas, 1985; Roman-Garcia et al., 2021), providing necessary growth factors for primary colonizers in fibrotic material; however, in diets where true rumen degraded protein (RDP less ammonia concentration) is provided in sufficient amounts, no such improvements in disappearance are observed (Copelin et al., 2021). Further, supplying these iso-acids has demonstrated improvements in dry matter intake, milk volume (Andries et al., 1987), and milk components (Wang et al., 2019).

Under the current structure of CNCPS v6.5.5 (Fox et al., 2004; Tylutki et al., 2008; Van Amburgh et al., 2015), predictions for the ruminal disappearance of carbohydrate and proteins adhere to first order kinetics which are calculated using rates of degradation and passage for each ingredient in percent per hour, establishing the maximum potential with which a particular feed fraction would be degraded. Additionally, a microbial yield coefficient is also calculated for each carbohydrate fraction in the diet, using the intrinsic rate of degradation for each feed ingredient in percent per hour, the maintenance rate of bacteria in grams of CHO per gram of bacteria, and growth potential of that bacteria in grams of bacteria per gram of CHO. Each yield coefficient is applied to the corresponding feed fraction to estimate microbial pool size for a given feed. Summation of the microbial yields for CHO A2, A3, A4, B1, and B2 feed fractions represents the potential NFC degrading bacteria pool, whereas CHO B3 is the sole carbohydrate fraction used to estimate the potential fiber carbohydrate **(FC)** degrading bacteria pool size (Fox et al., 2004). These estimates of carbohydrate disappearance and microbial pool size are predicated on the assumption that the rumen is not limited in other growth factors, including nitrogenous substrates. The CNCPS estimates total rumen N content by summating dietary ammonia intake **(PRO A1)**, recycled N (Recktenwald et al.,

2014), and dietary peptides and degraded feed N from microbial action. This pool is used to estimate the potential microbial yield from rumen N content by factoring the protein content of NFC and FC degrading bacteria (Russell et al., 1992). Taken with the carbohydrate allowable microbial yields, the system considers whether a limitation of carbohydrate or nitrogen exists, using the lesser amount of these pools to estimate the potential yield. If the predicted allowable N pool is inadequate to complement the potential carbohydrate degradation, the system will also restrict the level of carbohydrate degraded, prioritizing a limitation of fiber carbohydrate degradation, based on metabolic rates of NFC and FC bacteria.

As the industry looks to reduce protein feeding in lactating diets, the likelihood that a nutritionist would encounter a scenario where predicted rumen N is not sufficient to meet potential carbohydrate degradation is greater than previously seen. When such scenarios present themselves, it is imperative to understand the context of this limitation. Presently, the CNCPS amalgamates all nitrogenous substrates into one N pool, considering them all equal when reconciling the necessary substrates for proper microbial metabolism, proliferation, and feed degradation. Inclusion of dietary urea can often be used as a method to improve rumen N pool size, giving the appearance that sufficient N is present to realize potential carbohydrate degradation and microbial yield. This solution creates a fallacy, as rumen ammonia content may not be the prevailing cause for this limitation in carbohydrate degradation. Branch chain amino acids, which are not only considered in the rumen N pool, but because they are precursors for BCVFA, also have a unique carbon backbone used for other metabolic processes, become limited in scenarios when dietary protein is concomitantly limited. This presents an opportunity to disaggregate the rumen N pool, allowing for the consideration of BCAA/BCVFA sufficiency. To account for BCAA/BCVFA adequacy in the CNCPS, an approach similar to Tedeschi et al. (2000) will be considered for future iterations of the model. In brief, the supply of BCAA and any exogenous sources of BCVFA will be estimated using provided feed chemistry. Because oxidative deamination of BCAA by microbes is still the primary source of ruminal BCVFA (Allison et al., 1962b), the rate of BCAA deamination, release of BCVFA by bacteria, and assimilation of BCVFA by other bacteria to be used for metabolism are currently under investigation with the aim to include them in supply calculations. The primary fates of BCVFA are defined in the CNCPS as BCAA or BCFA, with the understanding that a small proportion of BCVFA may be used for branch chain keto acid production (Firkins, 2021). Using literature data to define the BCAA and BCFA composition of bacteria will create recommended feeding rates of RDP, BCAA, and exogenous BCVFA. Similar to the concept of the total rumen N pool, in situations where rumen degraded BCAA and exogenous BCVFA are not sufficient enough to meet the potential microbial growth from degraded carbohydrates, the system will restrict carbohydrate degradation and subsequent microbial growth based on what is supplied. Independent of the rumen N pool, a user of the CNCPS would be able to troubleshoot whether a low protein diet was limited in either rumen N, BCAA/BCVFA, or a combination of both. As such, limitation born from BCVFA deficiencies cannot be overcome with the supplementation of other nitrogenous compounds, such as urea, and will only be reconciled when either true RDP with an appropriate level of BCAA or a concomitant substitution of exogenous BCVFA are provided.

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Table 1. Intake, digestion, and excretion by digestive compartment of carbohydrate pools from both forage and concentrate sources according to CNCPS v7 calculations.

	Nutrient digestion by compartment[1] (g/day)					
				Neutral Detergent Fiber		
	Sugar	Starch	Soluble Fiber	Fast Degrading	Slow Degrading	Undegradable
Proportion of diet, % DM	4.2	30.5	3.7	18.5	5.0	7.1
Forages, g						
Intake	181	6212	424	3481	1122	1629
Rumen degraded	105	5037	340	2954	615	0
Rumen pool[2]	15	488	35	1241	1193	3802
Rumen escape	76	1175	84	528	507	1629
Small intestine digested	76	877	0	0	0	0
Small intestine passed	0	298	84	528	507	1629
Large intestine degraded	0	207	57	226	71	0
Fecal excretion	0	91	27	302	437	1629
Apparent total tract digestion, %	100	98.5	93.7	91.3	61.1	0
Concentrates, g						
Intake	998	3078	626	1706	283	358
Rumen degraded	730	2116	445	1290	172	0
Rumen pool	50	329	62	821	216	709
Rumen escape	269	961	180	416	110	358
Small intestine digested	269	754	0	0	0	0
Small intestine passed	0	208	180	416	110	358
Large intestine degraded	0	120	107	131	22	0
Fecal excretion	0	88	73	285	88	358
Apparent total tract digestion, %	100	97.2	88.3	83.3	68.8	0

[1]Cattle consumed an average of 28.0 kg of DMI from this diet.

[2]Defines the residual quantity of each carbohydrate fraction which resides in the rumen and has not been degraded or passed.

Table 2. Output from CNCPS v.7 describing the flux and pool size of fiber fractions within the rumen. Outcomes aid in the determination of dry matter intake according to pdNDFom or uNDFom fill limits.

Fiber Fraction	Flux, g·d-1	Flux, kg BW-1·d-1	Rumen pool size, g	Rumen pool Size, kg BW-1
CHO B3; Fast	5187	0.69%	2070	0.28%
CHO B3; Slow	1405	0.19%	1421	0.19%
CHO B3; Total	6593	0.88%	3318	0.47%
NDF Recommendations[1]	-	1.27 - 1.47%	-	-
CHO C (uNDFom)	1987	0.26%	4596	0.61%
uNDFom Recommendations[1]	-	0.39 - 0.48%	-	0.48 - 0.62%

[1]Recommendations according to Cotanch et al. (2014)

Table 3. Metabolizable protein **(MP)** predictions from feed, bacteria, and protozoa under CNCPS v.7 predictions.

MP flows	Quantity
Feed MP, g	1349
Bacterial MP, g	1343
Protozoal MP, g	325
Feed MP, %	45.0%
Microbial MP, %	55.0%
Protozoal MP, % microbial supply	19.5%

Table 4. Rumen ammonia concentrations and associated microbial growth depression, both with provided minimum and maximums predicted over a day.

Rumen N concentrations	Mean	Min	Max
Rumen ammonia, mg/dL	9.3	8.1	11.1
Microbial growth depression			% Depression
Mean depression			0.0%
Minimum depression			0.0%
Maximum depression			0.1%

Table 5. Effect of rumen available nitrogen source on omasal nitrogen flow and ruminal digestibility.

Item[2]	Diet[1] CON	EXP	SEM	*P*-Value
Feed nitrogen intake, g/d	603	613	18	0.70
CNCPS fraction Pro A1	61	43	-	-
CNCPS fraction Pro A2	171	183	-	-
CNCPS fraction Pro B1	304	310	-	-
Flow at omasal canal				
Total nitrogen flow, g/d	664	693	25	0.37
Predicted total nitrogen, g/d	664	674	-	-
Ammonia nitrogen, g/d	21.5	22.4	1.5	0.67
Non-ammonia nitrogen, g/d	642	670	25	0.38
Non-ammonia nitrogen, % N intake	106.6	109.1	3.4	0.58
Non-ammonia, non-microbial nitrogen, g/d	191	256	26	0.09
Non-ammonia, non-microbial nitrogen, % N intake	31.3	41.7	3.5	0.05
Microbial NAN				
g/d	450	409	28	0.31
% NAN	69.9	61.5	3.5	0.11
Microbial N flow predicted by CNCPS v6.5, g/d	351	352	-	-
Microbial efficiency				
g of microbial CP/kg of OTDR	28.9	26.1	1.7	0.26
True ruminal N digestibility, %	68.7	58.3	3.5	0.05
aNDFom digested/g of dietary CP degraded	0.97	1.23	0.1	0.02

[1]CON = 3% of diet DM as urea control mix; EXP = 3% of diet DM as fermentation byproduct.
[2]NANMN = non-ammonia non-microbial N and OTDR = organic matter truly digested in the rumen.

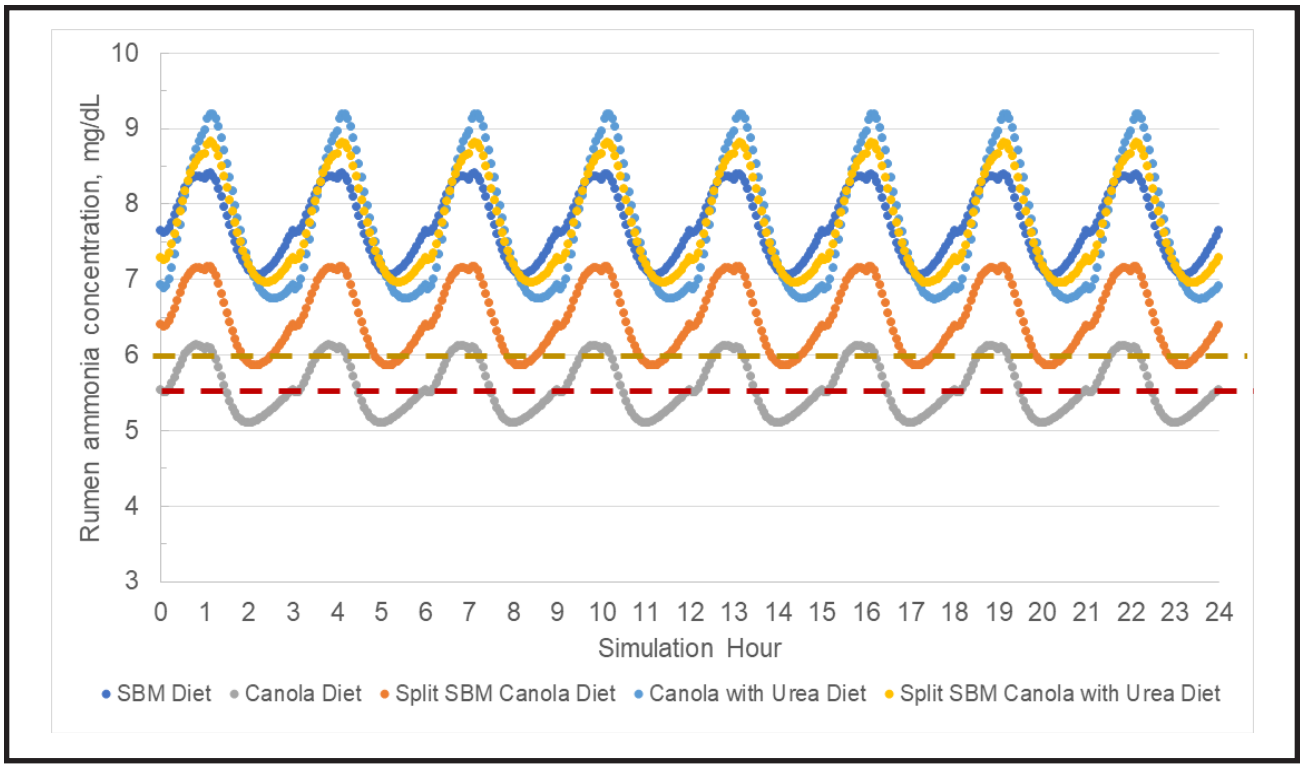

Figure 1. Rumen ammonia concentration after feeding a high forage diet (68% DM) with either: A. 2.5 kg of soybean meal (SBM) included; B. 2.5 kg of canola meal included; C. 1.25 kg of SBM and 1.25 kg of canola meal included; D. 2.5 kg of canola meal with 125 grams of urea included; and E. 1.25 kg of SBM and 1.25 kg of canola meal with 125 grams of urea included. Within CNCPS v.7, microbial growth depression begins when ammonia concentration falls below 6.0 mg/dL and is significantly impactful when falling bellowing 5.5 mg/dL. Feed library values from the CNCPS were used to describe all feeds within this ration.