CHARACTERIZATION OF NON-NUTRITIVE FACTORS OF FEEDS FOR MODEL DEVELOPMENT

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INTRODUCTION

Protein is one of the most expensive macronutrients in dairy cattle rations, and overfeeding degradable protein relative to supply results in excessive N losses to the environment (Huhtanen and Hristov, 2009). 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 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. One such byproduct of commercial amino acid production is Fermenten (Arm and Hammer Animal Nutrition, Princeton, NJ). 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 vs. 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).

Lack of agreement between in vitro fermentation responses and in vivo metabolism and performance responses is not a new issue. Many compounds have been tested in vitro and found to have potent selective antimicrobial effects, however when moved to the cow, the effects disappear. This is likely due to differences in the environment, especially the concentrations of microbes to substrate and closed nature of the system. Even products that are known to have lasting effects on rumen fermentation do not always demonstrate the same mode of action in vitro as is observed in vivo, as was discussed by Recktenwald et al. (2014). In many diet formulation programs, in vitro results can be used in conjunction with performance studies to create surface level, semi-empirical response profiles, however more detailed in vivo studies are necessary to model the effects in a more mechanistic manner.

Mathematical models such as the Cornell Net Carbohydrate and Protein System (CNCPS) (Higgs et al., 2015; Van Amburgh et al., 2015) have been successfully used to quantify rumen microbial output and meet animal nutrient requirements while reducing N losses to the environment (Tylutki et al., 2008). The mechanistic elements of the

rumen sub-model in the CNCPS require appropriate experimental data to evaluate and develop equations to predict metabolizable AA outflows from the rumen. A new, dynamic version of the CNCPS (v. 7) was recently developed (Higgs, 2014; outlined in these proceedings) that describes rumen degradation of substrates with mechanistic representations of growth of bacteria and protozoa and includes interactions among protozoa and bacteria such as predation and intra-ruminal microbial N turnover. Evaluations of this model indicated a strong ability to predict the partitioning between microbial and non-microbial nitrogen flows; however the partitioning between protozoa and bacteria along with individual AA predictions might require some refinement (Fessenden, 2016). Further, the dynamic nature of v. 7 might allow the non-nutritive elements of some feeds to be described more completely compared with previous versions of the CNCPS. As with most model development, evaluations of the rumen sub-model with independent data can be helpful for determining areas for improvement.

Considering these factors, we identified the need to perform more quantitative studies investigating the non-nutritive aspects of some feedstuffs to better understand how to best characterize the differences between a nutrient driven effect on microbial behavior compared to a non-nutritive outcome. Given the importance of AA to the cow, feedstuffs with possible effects on rumen protein synthesis and flows were determined to be prime candidates for study. To maximize the value of the data generated during an intensive study, several quantitative techniques were combined to provide insight into rumen function. Omasal sampling and rumen evacuations were used to estimate pools and flows for kinetic digestion parameters, improved protozoa isolation techniques allowed for investigation of microbial metabolism, and more thorough AA analysis were used to more accurately quantify AA flows and improve model predictions when compared against a larger literature dataset.

QUANTITATIVE METHODOLOGY

To isolate the non-nutritive aspects of the fermentation byproduct, careful consideration was needed when designing treatments. Our goal in formulation of the control diet, which contained no fermentation byproduct, was to simulate as closely as possible the nutrient composition of the feedstuff. To achieve this, a control protein premix containing wheat midds and urea was used, which allowed for diet formulation to be iso-nitrogenous, iso-soluble protein, iso-NDF, and iso-energetic. Other minor differences included some shifts in mineral sources to account for the high sulfur content of the fermentation byproduct. This allowed for two treatments diets: one with the fermentation byproduct at 3% inclusion rate (EXP), and a control consisting of wheat-midds and urea (CON). Beyond the feedstuffs mentioned above, the rest of the diet was identical between the treatments. Diets differed only in the protein pools, with the EXP diet containing 18 g more non-ammonia soluble N than the EXP diet. This shift was intentional and given the feed chemistry of fermentation byproduct, the additional N was assumed to be in the form of soluble AA and peptides. The differences represented ~ 3.3 % of total N intake.

Eight ruminally cannulated multiparous Holstein cows averaging (mean ± SD) 60 ± 10 d in milk and 637 ± 38 kg of body weight were stratified by pre-trial milk production and randomly assigned to one of two treatment sequences in a switchback trial with three 28 d periods. In this design, each cow was fed each diet at least 1 time, allowing the variation associated with each cow to be controlled. Each period provided 21 d for diet adaptation and 7 d of data and sample collection.

Omasal Sampling

During the sample collection period, the omasal sampling technique was used to quantify post-rumen flows. Sampling though omasal cannulas has been performed since the 1960's (Oyaert and Bouckaert, 1961), however routine sampling was improved by Huhtanen et al. (1997) using a device that, once inserted into the omasum through a rumen cannula, would allow for repeated sampling over a longer time period without the need for more intensive omasal cannulation. This method was adapted by the University of Wisconsin researchers for a series of studies on omasal flows of nutrients (Reynal and Broderick, 2005). The technique has been validated against duodenal sampling (Ahvenjärvi et al., 2000; Ipharraguerre et al., 2007) and these evaluations demonstrated that when combined with a triple marker method (France and Siddons, 1986), the technique can allow for fairly small coefficients of variation in measurement of ruminal digestion variables. Omasal sampling experiments have provided useful data from which to build and evaluate field applicable models of rumen fermentation. Broderick et al. (2010) demonstrated the NRC (2001) overestimated RUP by 21%, and underestimated microbial-N flow by 26%. This series of studies also provided much needed data for evaluation of the CNCPS, through which post ruminal N and AA flows could be compared to model predictions (Higgs, 2014; Van Amburgh et al., 2015).

Partitioning of Post-ruminal N Flows

To better understand the different sources of AA flowing from the rumen, N must be partitioned between microbial and non-microbial sources. To do this, a NPN compound enriched with ^{15}N isotope was provided to the rumen via the blood as a marker. Microbes in the rumen take up the N and synthesize amino acids. Therefore, any ^{15}N amino acid measured in the rumen or omasum is assumed to be of microbial origin. By measuring the ^{15}N content of isolated microbes and the ^{15}N content of the rumen outflow, we can determine microbial protein synthesis. Many previous omasal studies have used this marker system, as it holds distinct advantages over other methods like purines.

Several aspects of rumen fermentation can be determined using the omasal flow method, including dry matter, organic matter and NDF digestion, VFA flows, and N flows. Using the data from the omasal experiment with fermentation byproduct (Table 1), it is evident that cows fed the EXP diet 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). Total NAN flow from the rumen

was well predicted by CNCPS v. 6.5, however the partition between microbial and non-microbial N demonstrates the need for further investigation, most likely related to the current inability to predict robust rates of digestion of protein.

Table 1. Effect of rumen available nitrogen source on omasal nitrogen flow and digestibility

Item[2]	Diet[1]		SEM	*P*
	CON	EXP		
N intake, g/d	603	613	18	0.70
CNCPS fraction PA1	61	43	-	-
CNCPS fraction PA2	171	183	-	-
CNCPS fraction PB1	304	310	-	-
Flow at omasal canal				
Total N, g/d	664	693	25	0.37
Total N flow predicted by CNCPS v. 6.5, g/d	664	674	-	-
Ammonia N, g/d	21.5	22.4	1.5	0.67
NAN				
g/d	642	670	25	0.38
% of N intake	106.6	109.1	3.4	0.58
NANMN				
g/d	191	256	26	0.09
% of N intake	31.3	41.7	3.5	0.05
Microbial NAN				
g/d	450	409	28	0.31
% of total NAN	69.9	61.5	3.5	0.11
Microbial N flow predicted by CNCPS v. 6.5, 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, OTDR = organic matter truly digested in the rumen.

The information on flows and partitioning of N also demonstrates that flow alone does not give a strong indication of the processes happening within the rumen (Table 1). In this case, 20 g more non-ammonia soluble N was provided by EXP diets, however 65 g additional non-ammonia non-microbial N were flowing out. This indicates that the soluble portion of the fermentation byproduct was not simply flowing out with the liquid phase. Instead, some aspect of the feed was exerting associative effects on the degradation of proteins from other feedstuffs. To fully understand this effect for model characterization, we need to better understand the dynamics within the rumen, not just the outflow. This is achieved by leveraging other data collected during the trial, namely the partitioning of protozoa flows and rumen pool sizes of microbial biomass and

digestible substrate. This is not data usually reported in other omasal flow studies, but can be very useful data for modeling purposes.

Protozoa Isolation

Protozoa flow has been quantified using a variety of methods (Ahvenjärvi et al., 2002; Sylvester et al., 2005). As investigators became more interested in protozoa and bacteria interactions, it was found that protozoa typically take up less of the ^{15}N microbial marker (Brito et al., 2006) primarily due to the lack of direct incorporation of ammonia by protozoa. The ultimate effect of this is that reported microbial AA flows are likely underestimated by approximately 10% in the literature datasets that do not quantify the protozoa flow. The most common issues to address in the isolation protocol are feed particle and bacterial contamination (Volden et al., 1999). A typical isolation procedure relies on filtration and/or centrifugation to isolate biomass that is assumed to be representative of protozoa. One of the early studies of microbial composition isolated protozoa only through repeated centrifugation (Czerkawski, 1976). For large scale separations, Storm and Ørskov (1983) used a large filtration and separation system to examine microbial biomass from animals coming into abattoirs, however feed and bacterial contamination was likely high. To address this, researchers began using flocculation and sedimentation to remove large feed particles, followed by centrifugation and filtration on nylon cloth to wash away bacteria (Williams and Strachan, 1984; Martin et al., 1994). Glucose was used to enhance flocculation, although this likely altered microbial composition as a result of competition for growth substrate. For a protozoal isolation to be representative of the population in the rumen, techniques must strive to be rapid, have limited addition of any growth promoting substances, and avoid lysis of microbial cells. Many of the previously reported studies have suffered from weaknesses in one or more of these areas.

More recent work with microbial populations has necessitated the development of a rapid technique to isolate mixed protozoa cultures with viability enough to culture. The techniques are described in the paper by Denton et al. (2015) and might provide useful data when combined with the omasal sampling technique. The procedure uses a combination of flocculation, sedimentation, and filtration to recover much of the protozoa in a sample in a form that has high viability, low feed contamination, and no addition of substrate that is known to appreciably change cell composition. For the omasal study, protozoa were isolated as quantitatively as possible from omasal fluid, and the marker system was used to calculate the flow of protozoa in the fluid phase (Table 2). Partitioning of the microbial flows also allowed for estimation of the predation of protozoa on bacteria under a couple assumptions: 1) Protozoa acquire almost all of the ^{15}N through consumption of bacterial AA (Newbold et al., 2005), and 2) protozoa retain approximately 50% of consumed AA in cell biomass (Hristov and Jouany, 2005).

Predictions using the dynamic version of the CNCPS v. 7 demonstrated the ability of the model to predict microbial flows (Table 2). Compared to the predictions from v6.5 (Table 1), microbial flow is much closer to the actual measured value. The output from v7 also predicts protozoa flow although the values appear to be slightly

under predicted in this comparison. The predicted values for CON and EXP also demonstrate that the model is not necessarily sensitive to the associative effect of the fermentation byproduct—an expected finding given the structure of the CNCPS.

Table 2. Microbial nitrogen flows and protozoa predation in lactating dairy cattle fed two different sources of rumen available nitrogen

	Diet[1]			
Item	CON	EXP	SEM	*P*
Total microbial NAN flow, g/d	450	409	28	0.31
Bacteria NAN	378	337	23.0	0.22
% of microbial NAN flow	84.2	82.1	1.0	0.12
Protozoa NAN	72.1	73.9	7.3	0.84
% of microbial NAN flow	15.8	17.9	1.0	0.12
Protozoa NAN consumed	90.6	76.3	12.9	0.45
% of bacterial N flow	23.4	22.2	2.4	0.70
CNCPS v. 7 output				
Predicted microbial N flow, g/d	412	417	-	-
Bacteria N flow	371	375	-	-
Protozoa N flow	41	42	-	-
% of microbial N flow	9.9	10.1	-	-
Predation estimate, bacterial N consumed, g/d	75	76	-	-

[1]CON = 3% of diet DM as urea control mix; EXP = 3% of diet DM as fermentation byproduct.

Microbial Growth and Turnover

To improve the predictions of microbial growth and turnover, we have to move past looking at post-ruminal flows alone, and start to understand how microbial populations are interacting with their substrate. Therefore, rumen evacuations and measurements of pool sizes were critical to determine digestion kinetics and evaluate the effects of the fermentation byproduct. For this study, rumen contents were evacuated, weighed, and subsampled to get a representative sample from the rumen. This sample was analyzed for DM, OM, N, NAN, aNDFom, uNDFom, and the microbial marker, ^{15}N. Using these values, we are able to determine the pool sizes of these nutrients in the rumen. Total fermentable carbohydrate was calculated in a similar manner to the traditional non-fiber carbohydrate fraction in feeds, however potentially digestible NDF was added back to the equation. Using the same approach on the flows, it is possible to calculate the fractional rate of degradation of the digestible pools (Table 3). This value, albeit subject to some compiled error, can be evaluated against the predicted rate of degradation in CNCPS v. 7. To obtain model predictions of carbohydrate availability, samples of the forages and corn grains were analyzed for in vitro aNDFom and starch digestion rates using commercially available methods. These values were then entered into the model, and feed library digestion rates were used for

all other rates to reflect the data that would be available when using the model in the field.

Rumen microbial pool size and flows can be used to calculate growth rates of microbes in the rumen. To calculate fractional rate of microbial growth, omasal flow (g/h) is divided by the pool size in the rumen (g). Since flows are measured post ruminal values, the result is a fractional rate of growth that accounts for lysis and turnover (Wells and Russell, 1996). However, since protozoa pool size was not directly measured in the rumen fluid, and protozoa are thought to be selectively retained in the rumen, it becomes difficult to partition bacterial and protozoal N pools. Reported rumen protozoa retention in rumen vs. post-ruminal measurements vary widely, and range from < 5 % (Sylvester et al., 2005) to over 70% (Punia et al., 1992). Luckily, the total ^{15}N pool in the rumen can be measured, making it possible to evaluate the effect of several theoretical levels of selective retention on rumen pool sizes. In this way, at 0 % selective retention, we expect the protozoa to account for the same proportion of total microbial N as measured in the omasal flow. At greater levels of retention, protozoa account for larger portions of the microbial pool. Therefore, rumen protozoa ^{15}N proportion of the total rumen ^{15}N pool was calculated at 4 different levels representing 0 to 75 % retention (Table 3).

To assess which level of selective retention of protozoa is likely most correct, it is possible to use pool size and flow to estimate fractional rates of growth (Table 3). Recognizing that the main energy substrate for rumen bacteria is CHO (Russell et al., 1992), and assuming the maximum yield of cell DM / g of CHO degraded ($\mathbf{Y_g}$) is 0.5 (Isaacson et al., 1975), one can quickly determine which retention values allows for realistic growth rates. In this instance, selective retention at 50 % indicate that bacteria would have to grow at a fractional rate of 0.07 h^{-1}, corresponding to a CHO degradation rate of 0.14 h^{-1} (0.07 / 0.5). Given the estimated pool size (g) and digestion (g/h), the fractional rate of CHO availability in this study averaged 0.138 h^{-1} of the available pool; therefore theoretical maximal fractional growth rate was estimated at 0.138 x 0.5, or ~ 0.069 h^{-1}. Using the measured total microbial pool at 25 % selective retention, it was calculated that the fractional growth rate of all microbes in the rumen was 0.061 h^{-1}. This corresponds to an estimated Y_g of 0.44 g / g of CHO degraded. This is close to the theoretical maximums for individual species reported in pure cultures (Russell and Baldwin, 1979; Theodorou and France, 2005). In vitro measurements of mixed rumen microbes often give yields on the high range of those observed in pure culture (Russell and Wallace, 1997).

Also, the calculations allow for comparisons of the model predicted vs. study estimated Y_g (Table 3). This comparison serves primarily to verify several aspects of the rumen sub-model in CNCPS v. 7. When provided with feed chemistry data that is available in the field, the CNCPS was able to fairly accurately predict the fractional rate of CHO degradation. The model relates cell growth directly to CHO availability, so accurate estimates of CHO degradation are key to accurately predicting microbial yield and eventually AA supply.

Table 3. Fractional rates of microbial growth, nutrient digestion, and rumen fermentation parameters in lactating dairy cattle fed two different sources of rumen available nitrogen

	Diet[1]			
Item	CON	EXP	SEM	*P*
Fractional growth rate of bacteria[2], h^{-1}				
0% selective retention	0.061	0.061	0.004	0.99
25% selective retention	0.064	0.064	0.005	0.99
50% selective retention	0.070	0.070	0.006	1.00
75% selective retention	0.108	0.103	0.012	0.74
Fractional growth rate of protozoa[2], h^{-1}				
0% selective retention	0.061	0.061	0.004	0.99
25% selective retention	0.046	0.046	0.003	0.99
50% selective retention	0.030	0.030	0.002	0.99
75% selective retention	0.015	0.015	0.001	0.99
Omasal flows and ruminal digestion parameters				
True OM flow, kg/d	7.08	7.19	0.47	0.87
Microbial NAN flow, g/d	450	409	28	0.31
Ruminal true OM digestion rate, g/h	626	619	17	0.77
Ruminal true CHO digestion rate, g/h	518	526	15	0.72
Fractional rate of OM digestion[3], h^{-1}	0.101	0.094	0.008	0.54
Fractional rate of CHO digestion[3], h^{-1}	0.139	0.138	0.011	0.91
Microbial growth parameters				
Fractional growth rate of all microbes, h^{-1}	0.060	0.060	0.004	0.94
Theoretical maximum CHO allowable growth[4], h^{-1}	0.070	0.069	0.005	0.91
Observed Y_g, g of cells / g of CHO degraded[5]	0.44	0.44	0.03	0.99
% of theoretical maximum Y_g	88.4	88.3	6.6	0.99
CNCPS v. 7 output				
Predicted CHO degradation, g/h	484	487	-	-
Predicted fractional rate of CHO digestion, h^{-1}	0.124	0.124	-	-
Predicted Y_g, g of cells / g of CHO degraded	0.45	0.45	-	-

[1]CON = 3% of diet DM as urea control mix; EXP = 3% of diet DM as fermentation byproduct.

[2]bacteria or protozoa daily flow (g/h) / bacteria or protozoa pool size (g) at 4 levels of protozoa selective retention

[3]Measured microbial NAN flow (g/h) / measured rumen microbial NAN pool (g)

[4]Fractional rate of CHO digestion x 0.5

[5]Fractional microbial growth rate / fractional rate of CHO digestion

By dividing predicted yield of all microbes by carbohydrate degradation, we can calculate an apparent Y_g used by the model and compare it to the measured values obtained from the omasal study. The agreement between predicted vs. independently measured values indicates the structure of the model is likely adequate to provide accurate estimates of microbial yield from substrate degradation. This provides a strong basis from which to improve AA supply predictions, as microbial N represents a large portion of MP flowing from the rumen.

Overall, the model guided research approach to the non-nutritive aspects of feeds has allowed for a better understanding of how fermentation byproducts might be characterized. Investigation of the feeding effects on kinetic aspects of rumen fermentation allowed us to better understand that the byproduct did not stimulate microbial growth, but rather changed the way microbial populations interact with their substrate. Fractional rates of digestion and growth indicate that bacteria were not negatively influenced by fermentation byproduct inclusion. By studying and modeling the dynamics within the rumen, not just the outflows, we gain a deeper understanding of the system. Models, while inherently wrong, can help a great deal in guiding the research question. For complex models to be improved, a stepwise evaluation is usually necessary to identify and address offsetting errors. In this case, the stepwise evaluation demonstrated that effects observed in vitro did not occur in vivo. Further, the data generated in this study allowed us to updated AA profiles of microbial protein, and evaluate the model's ability to predict post-ruminal flows of AA when compared with a larger dataset, as described in the next two sections.

AMINO ACID PROFILES OF MICROBIAL PROTEIN

The CNCPS uses a factorial approach to calculate AA supply, so accurate profiles of AA in undegraded feed, bacteria, protozoa, and endogenous portions of post rumen protein flows are important. For the purposes of this study, it was necessary to understand principally the microbial portions, as limited data exist on microbial (especially protozoa) AA profiles from high producing lactating cows. Amino acid content of protein has historically been determined by single time point hydrolysis, as this represents a compromise between maximal release of AA from the matrix while minimizing the loss of acid labile AA (Rutherfurd, 2009). Determination at multiple time points followed by least-squares non-linear regression provides more accurate estimates of the true amino acid profile (Darragh and Moughan, 2005). To our knowledge, AA determination after multiple hydrolyses times has not been performed on rumen microbial biomass.

Microbial samples obtained from the omasum were used to determine the AA content after multiple time point hydrolysis. The AA content of all samples was determined by HPLC following hydrolysis at 110°C in a block heater (Gehrke et al., 1985) for 2, 4, 6, 12, 18, 21, 24, 30, 48, 72, 120 and 168 h. All AA except Trp were determined using 6N HCl hydrolysis, with Met and Cys undergoing an additional pre-oxidation step. Tryptophan was determined using florescence detection after hydrolysis in barium hydroxide at the same time points as the acid hydrolysis. The entire time

course was performed twice for each sample, and the reported values are the mean of the two determinations.

Table 4. Comparison of measured AA composition after single hydrolysis time point vs. estimated AA composition determined using least-squares non-linear regression after multiple hydrolysis times for omasal bacteria and protozoa isolates from trial B.

	Bacteria			Protozoa		
Item	24 h[1]	Mult[2]	% Δ	24 h[1]	Mult[2]	% Δ
Essential AA, % of AA						
ARG	4.96	4.88	1.6	5.37	5.41	-0.7
HIS	2.24	2.17	3.0	2.50	2.59	-3.6
ILE	4.25	4.77	-12.4	4.03	4.51	-12.0
LEU	5.48	5.47	0.3	6.83	6.43	5.8
LYS	7.52	7.40	1.6	8.90	8.79	1.2
MET	4.71	4.81	-2.0	3.44	3.87	-12.6
PHE	6.15	5.94	3.4	6.79	6.76	0.4
TRP	5.51	5.93	-7.7	4.26	5.49	-29.1
THR	5.67	5.70	-0.5	4.84	5.09	-5.1
VAL	6.58	7.14	-8.4	4.67	4.88	-4.6
Total EAA	53.07	51.73	2.5	51.61	51.01	1.2
Non-essential AA, % of AA						
ALA	6.68	7.15	-7.0	5.36	5.17	3.6
ASP	10.46	11.13	-6.3	9.65	10.42	-7.9
CYS	1.43	1.45	-1.4	2.37	2.22	6.5
GLU	11.25	11.39	-1.3	12.94	13.40	-3.5
GLY	5.01	4.98	0.6	4.67	4.53	2.9
PRO	2.00	1.97	1.2	2.99	2.97	0.7
SER	4.48	5.03	-12.2	5.14	5.43	-5.8
TYR	5.61	5.82	-3.6	5.27	4.83	8.3
Total NEAA	46.93	48.90	-4.2	48.39	49.22	-1.7
Total AA, % of DM	346.6	339.0	2.2	295.0	290.7	1.4

[1]AA composition after 24 h hydrolysis time
[2]AA composition determined from least-squares non-linear regression from multiple hydrolysis times.

The comparison of the multiple time point vs. single time point indicates that the AA profile is affected by the rate at which AA are hydrolyzed in the assay. This means that when using a single time point hydrolysis at 21 or 24 h, the acid labile and slower releasing AA will be underestimated, while the faster releasing and acid stable AA would be overestimated. In a quantitative sense, this might not account for much of the rumen-undegraded portion individual feed ingredient AA. However, when assigning a profile of AA to the microbial flows, error in the analysis will have a large effect on

predicted AA flows when using the factorial approach, as the microbial portion is usually responsible for 40-60 % of the total AA supply.

MODEL EVALUATION

To determine the effect of the updated AA profiles on prediction from the microbial sub-model of CNCPS v.7, an evaluation was performed in a similar matter as previous evaluations of the CNCPS (Higgs, 2014; Van Amburgh et al., 2015). In a separate paper in these proceedings, Higgs and Van Amburgh reported the v.7 predicted vs. observed values for microbial N, undegraded feed N, and total non-ammonia N from the evaluations of Higgs (2014). Amino acid predictions were also evaluated using 11 publications with 43 treatment means of individual AA flows at the omasal canal. Full descriptions of the criteria used to select and enter the studies into the database have previously been reported by Higgs (2014).

The updated bacteria and protozoa AA profiles were entered into CNCPS v.7, and the evaluation was re-run. The results from the original evaluation (Higgs, 2014) were compared with values from the updated evaluation. The regressions for Lys, Met, and His are displayed in Figure 1. A full reporting of the results of the evaluation is beyond the scope of this paper, however overall AA predictions were improved from the previous evaluation of the model. Average reported concordance correlation coefficient (CCC; a simultaneous measure of accuracy and precision) and root mean squared prediction error (RMSPE) for the Higgs (2014) evaluation was 0.66 and 28.5, respectively; while the current evaluation averaged 0.69 and 23.8 for CCC and RMSPE, respectively; indicating overall improvement in AA flow predictions. Of the AA considered most often to be first limiting in lactating dairy cattle, Lys and His predictions were improved, while Met predictions were not improved. Met analysis is technically challenging, and pre-oxidation recoveries are rarely reported in the literature. It is important to note that reported AA flows in the literature are from a single time point hydrolysis, which would likely contribute additional mean and/or systematic bias when values are compared to the predictions from the CNCPS when using the updated profile. Nonetheless, this evaluation demonstrated that as with all model development, improvements in some areas leads can lead to the realization of shortcomings in others.

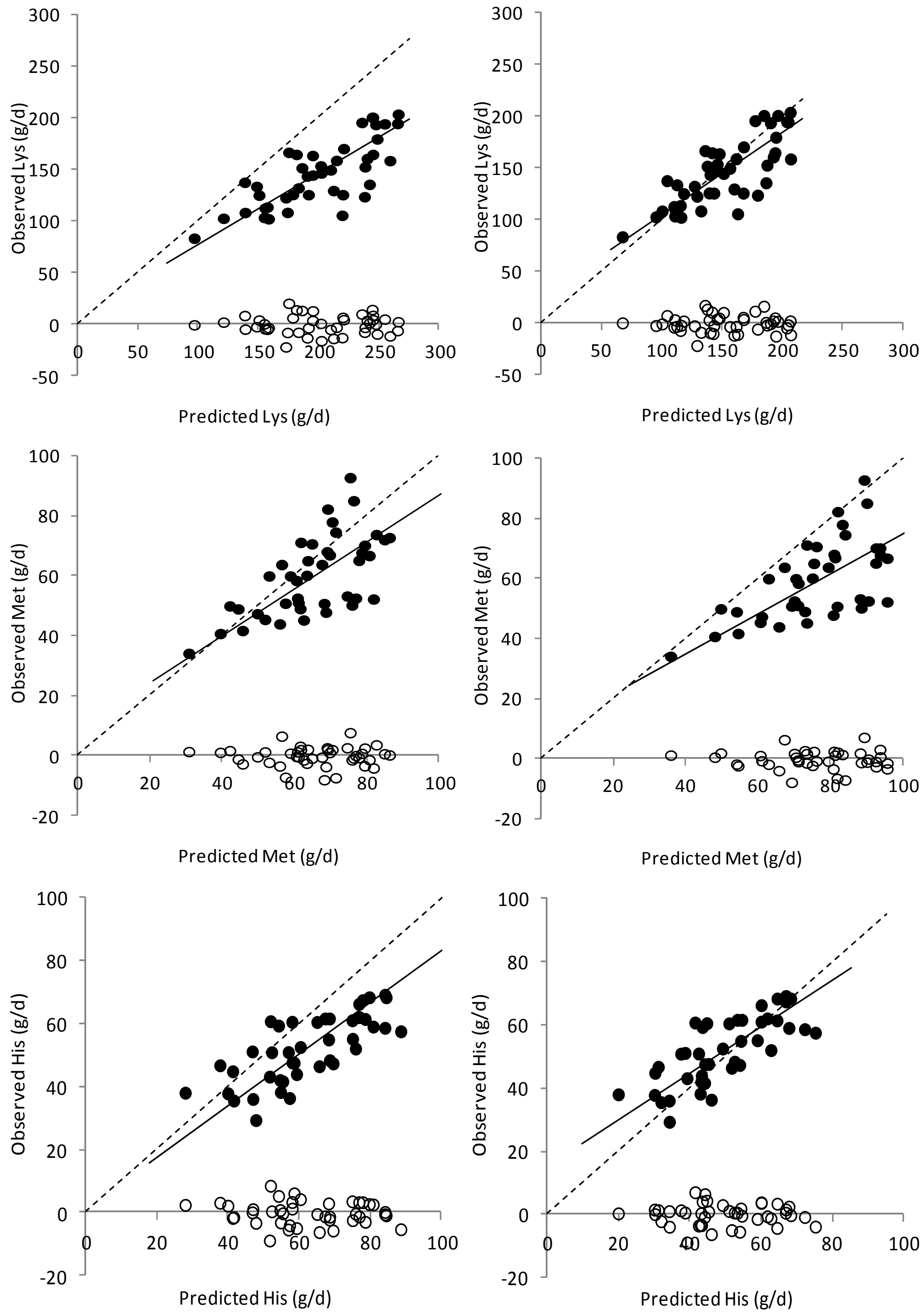

Figure 1. Predicted vs. observed values for Lys, Met, and His flow at the omasal canal (g/d). Values (●) and residuals (○) from a mixed model analysis, along with lines representing the regression (─) and unity (---) are displayed.

CONCLUSIONS

Evaluation of the AA profiles indicated the CNCPS has a good ability to predict post-ruminal AA flow in lactating dairy cattle. Further work is needed to improve predictions of some AA, especially Met. Re-evaluation of AA ratios and relationships to other dietary or animal parameters used in practical ration formulation will likely occur as supply predictions improve. Overall, the methods detailed in this paper, including omasal sampling, improved isolation of protozoa, and more accurate determination of post ruminal flows of digestible AA can allow for further development of mechanistic elements that describe the non-nutritive aspects of feedstuffs. Using the model to guide research can lead to large advances in our knowledge of the ruminant animal. This is often done through the leverage of specific techniques to better understand a complex system. However, modelers can often become quite enamored with their work when models perform well, and can fail to recognize structural issues when the models fail. This can, and often does lead to excessive complexity and decreased applicability --- a fatal outcome for any model. At all times in model development, application of the model must be considered. If more complex models are to be used in the field, training, support, and most of all, usability must be a top priority at all times.

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