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Variation in urea kinetics associated with ruminant species, dietary characteristics, and ruminal fermentation: A meta-analysis

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ABSTRACT

The objective of this meta-analysis was to quantitatively summarize variations in urea kinetics related to ruminant species, diet composition, and ruminal fermentation. A database of 31 studies measuring urea recycling kinetics were used to derive 2 sets of linear mixed-effects regression models. Study was used as a random intercept and regressions were weighted by 1 divided by the standard error of the mean observation. Models were compared, when appropriated, using the concordance correlation coefficient, root estimated variance associated with study ($\hat{\sigma}_s$) and error ($\hat{\sigma}_e$), and corrected Akaike information criterion values. From a dietary standpoint, most response variables were affected by measures reflecting dietary crude protein [(CP; e.g., N-NH_3 or rumen-degradable protein (RDP)] and by variables reflecting dietary energy content [e.g., total digestible nutrients (TDN), dietary starch, or ruminal pH]. Dietary CP, N-NH_3, and TDN typically had positive slopes on urea N entry rate (UER; g/d and g/$kg^{0.75}$), whereas starch and TDN/RDP had negative slopes on UER (g/$kg^{0.75}$). On the other hand, increasing TDN increased gastrointestinal entry rate (GER; g/$kg^{0.75}$), whereas an opposite effect was observed for RDP. Increasing diet RDP content reduced the urea N returned to ornithine cycle (ROC; g/$kg^{0.75}$) in most models. Ruminal variables also reflected the importance of N and energy supplies. Ruminal ammonia concentration significantly affected ROC (g/d and g/$kg^{0.75}$), used for anabolism (UUA; g/$kg^{0.75}$), ROC:GER, UUA:GER, and the incorporation of recycled urea N into microbial N relative to gastrointestinal entry rate of urea. Ruminal pH significantly affected GER:UER and ROC:GER ratios. Total digestible nutrients had a positive slope on UUA (g/$kg^{0.75}$). Increasing the ratio of energy to protein (TDN:RDP) increased the GER:UER ratio, decreased the ROC:GER ratio, and increased the UUA:GER ratio and the incorporation of recycled urea N into microbial N relative to gastrointestinal entry rate of urea N. Comparison among models revealed that species was an important explanatory variable affecting most response variables. However, whether these differences are related to the intrinsic N metabolism of each species or due to the diet variation remains unclear. Understanding these differences could lead to improvements in N use efficiency in ruminant diets by formulating more precise low-N diets considering the particularities for each species.

Key words: nitrogen cycling, ration formulation, meta-analysis

INTRODUCTION

Urea recycling is a physiological process common in several mammal species (Lobley et al., 2000). In ruminant animals, urea recycling provides an important advantage over other species because it yields a source of N for microbial protein synthesis in the rumen (Reynolds and Kristensen, 2008). The importance of urea recycling as an N source is increased in situations where animals are consuming low-N diets (Marini and Van Amburgh, 2003). Despite the importance of urea recycling for ruminants, the majority of nutritional models do not consider the amount of N that is recycled through the gastrointestinal tract (**GIT**) or its contribution to RDP supply. Even in the current Beef Cattle Nutrient Requirements Model, the equation for estimating urea recycling is a part of the mechanistic level solution and is only recommended for growing cattle consuming forage-based diets (NASEM, 2016).

The influence of dietary factors on urea recycling has been discussed in previous reviews and meta-analysis (Lapierre and Lobley, 2001; Reynolds and Kristensen, 2008; Batista et al., 2017; Silva et al., 2019). Understandably the primary focus of the majority of these studies was evaluating the association between urea recycling and dietary protein levels. Despite the importance of this focused relationship, the literature contains several studies using jugular infusion of ($^{15}N^{15}N$)-urea (Lobley

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et al., 2000) done across ruminant species and using a variety of diets that could be used to evaluate a broader spectrum of relationships governing urea recycling. A better understanding of the factors affecting urea kinetics and microbial usage of recycled urea would allow animal scientists to improve the estimation of N supply and N requirements used in future protein models for ruminant ration formulation.

The objective of this study was to quantitatively summarize the relationships among urea kinetics measured by the doubly labeled urea technique, ruminant species, ruminal fermentation, and dietary characteristics. Because of the depth of previous work evaluating the relationship between dietary protein and urea kinetics, we focused specifically on additional dietary factors, particularly energy supply. We hypothesized that urea kinetics are affected by ruminant species, ruminal fermentation, and dietary factors other than protein content, and these factors should be accounted for in future predictive models. Because we focus exclusively on measurements made by the doubly labeled urea technique, the findings of this paper are best interpreted in the context of that technique, and it is possible that other measurement methods may reveal different relationships and dynamics.

MATERIALS AND METHODS

The database used in the present study was constructed from peer-reviewed journal articles that evaluated urea kinetics in sheep, beef, and dairy cattle. Studies were eligible for inclusion in the database if they were: (1) published in English in a peer-reviewed journal, (2) used sheep or cattle, (3) presented least squares means and standard errors of the means (**SEM**) for response variables of interest, (4) presented a complete list of diet ingredients and their inclusion rates, and (5) used the dual-labeled urea technique as described by (Lobley et al., 2000). From that set of eligible studies, studies were excluded if they included: (1) inadequate diet descriptions; (2) treatments with nonconstant dietary CP concentrations; or (3) use of anabolic implants or jugular or ruminal infusion of substances that could affect urea kinetics (e.g., urea, AA, VFA). Papers were sourced by a comprehensive search through PubMed, Google Scholar, and Science-Direct from summer 2019 to fall 2019. Key words included urea recycling, urea kinetics, nitrogen metabolism, sheep, beef, and dairy cattle. Key words were used alone or in several combinations during the search process. From this search, a total of 38 studies were identified for inclusion. Of those, 7 were excluded, and the resulting database included 31 studies (sheep = 9, beef = 12, dairy = 10), presenting 124 treatment means measured from 565 animals. A description of the papers used in this meta-analysis, as well as the criteria for rejection of some studies, are included in Supplemental Tables S1 and S2, respectively (http://hdl.handle.net/10919/101353).

In cases where relevant information was not reported in the paper, an inquiry was sent to the corresponding author to obtain those data, if possible. In cases where dietary chemical composition was not reported and could not be obtained from the corresponding author, it was calculated using average nutrient values for individual feed ingredients sourced from the NASEM (2016) and NRC (2001). For complementary information about feed composition we also consulted the Dairy One Feed Composition Library (http://dairyone.com/), Feedipedia (https://www.feedipedia.org), or online product information for certain commercial feed supplements. Means and standard errors (**SE**) were collected for all response variables, and when SE were not directly reported, they were calculated using error propagation (Roman-Garcia et al., 2016).

Model Derivation

Sets of Models Derived. In this study we derived 2 sets of models to understand factors that drive variability in response variables. The response variables in set 1 included urea N entry rate (**UER**; g/d) and its different fates in the body, such as recycling (gastrointestinal entry rate, **GER**; g/d), urea N returned to ornithine cycle (**ROC**; g/d), more commonly referred to as the urea cycle, and urea N used for anabolism (**UUA**; g/d). To allow a reliable comparison between the response variables of different ruminant species used in this meta-analysis the explanatory variables from set 1 were also expressed on a metabolic body weight ($BW^{0.75}$) basis, considering that metabolic process in the body, including urea kinetics, can be described as a function of $BW^{0.75}$ (Kleiber, 1975). Despite being used in previous studies, it is important to note that metabolic body weight is a measure of body surface area, and although it provides a reasonable means of scaling biological measures across species with largely different body sizes, it is unlikely to be a direct biological driver of N dynamics in the way that it is for energy dynamics. As such, the models scaled by metabolic body weight should be considered as scaling by body size, and not as an attempt to describe causal biological associations between body surface area, N dynamics, and other experimental variables evaluated. Response variables in set 2 included proportion (%) of gastrointestinal entry rate relative to urea N entry rate (GER:UER), urea N returned to ornithine cycle relative to gastrointestinal entry rate of urea N (ROC:GER), urea N used for anabolism relative to gastrointestinal entry

rate of urea N (UUA:GER), incorporation of recycled urea N into microbial N (**INC**) relative to gastrointestinal entry rate of urea N (INC:GER). Between these 2 sets of variables, a total of 8 response variables were considered.

Eight models were generated to predict each response variable (90 models total), where data from ruminant species (**RS**), dietary characteristics (**DC**), ruminal factors (**RF**), and experimental variables (**EV**) data sets were evaluated individually and in combinations.

The explanatory variables included were categorized as those associated with RS, DC, RF, and EV. Ruminant species was a single variable with unique levels reflecting individual species (beef cattle, lactating dairy cows, nonlactating heifers, and sheep). Dietary characteristics included OM (% DM), CP (% DM), RDP (% CP), TDN (% DM), TDN/RDP ratio, ME (Mcal.kg^{-1} DM), starch (% DM), NDF (% DM), ADF (% DM), and lignin (% DM). Ruminal factors included ruminal pH, N-NH_3 (mg/dL), VFA production (mM), acetate (mol/100 mol), propionate (mol/100 mol), butyrate (mol/100 mol), and the acetate:propionate ratio. Experimental variables we considered were DMI (kg/d or g/kg$^{0.75}$), OM, and DM digestibilities (%).

Models were derived using linear mixed-effects regression with a random intercept for study. Error normality was checked at each step of the model derivation process through evaluation of residual plots. The weight used in the meta-regression was calculated as 1/SEM, as performed by Roman-Garcia et al. (2016).

Statistical Approach. All data were analyzed using R version 3.4.1 (R Core Team, 2017). The R package lme4 version 3.4.3 was used for model derivation (Bates et al., 2015). All response variables in the data set were checked to determine whether the statistical method (Latin square design or completely randomized design) used affected their respective SEM by running ANOVA analysis. In cases where the statistical method affected the SEM, the mean SE for each type of experimental design was estimated. The standardized errors within the statistical method were then calculated by dividing by the mean for that method. Extremely small SE were truncated to half the mean of the observed SE to prevent overweighting.

Variable Selection. Models were generated using a backward elimination approach following the method described by Gleason and White (2018). Briefly, all variables within 1 or more explanatory variable sets were included in an initial model derivation, and variables were removed from the model 1 by 1 in order of least significance ($P > 0.10$). When variables within a model were reduced to only those with a significant effect ($P \leq 0.05$) or that exhibited a trend toward significance ($0.05 < P \leq 0.10$), variables that had previously been eliminated were reintroduced and evaluated for significant effects in reverse order of removal.

Model Evaluation

Fit Statistics and Interpretation. After derivation by the above procedure, a model's variables were evaluated for multicollinearity using variance inflation factors (**VIF**) as described by Akinwande et al. (2015). Variables were required to have a VIF <10 to remain in the model. Importantly, VIF was not used as a model selection criterion per se; rather, it was used to affirm that final models did not suffer from multicollinearity. During this checking procedure, we did not find final models with elevated VIF, and most VIF were less than 3. Model quality was assessed on the basis of the standard deviation for study ($\hat{\boldsymbol{\sigma}}_{\boldsymbol{s}}$), as well as the standard deviation for error ($\hat{\boldsymbol{\sigma}}_{\boldsymbol{e}}$). The ratio of $\hat{\sigma}_s$ to $\hat{\sigma}_e$ was also calculated. In cases where $\hat{\sigma}_s$ is much greater than $\hat{\sigma}_e$, the model may be acceptable on average but would not be expected to predict new, single observations well because of the substantial error variance associated with individual studies. Root mean square error was not considered in model evaluation because a random study effect was used (Boerman et al., 2015).

The corrected Akaike information criterion was used to evaluate the relative quality of the models when derived against identical data sets. Finally, Lin's concordance correlation coefficient was used to evaluate the agreement between variables (Lin, 1989). Coefficients were obtained using predicted values that were calibrated for study effects (concordance correlation coefficients, **CCC**). For final comparisons of models using different derivation data sets, CCC was the main tool used for model comparison.

Cross-Validation. Models were tested for probable competency at predicting new observations using Monte Carlo cross-validation. During this cross-validation procedure, the data set was split so 60% of the treatments were used for model derivation and 40% of the data were used for model evaluation. The data splitting, model derivation, and model evaluation was repeated 100 times using unique strings of random numbers to identify which treatments were used for evaluation and which were used for derivation.

RESULTS AND DISCUSSION

Descriptive statistics of the explanatory data are available in Table 1 and Supplemental Table S15 (http://hdl.handle.net/10919/101353). Descriptive statistics for the response variable data are included in Table 2 and Supplemental Table S16. Parameter estimates for

Table 1. Summary statistics of explanatory variables

Variable[1]	N[2]	Mean	SD	Minimum	Maximum
Diet characteristics					
OM, % DM	124	92.4	2.19	83.4	96.5
CP, % DM	124	13.0	4.59	4.70	25.7
RDP, % CP basis	124	58.9	7.20	38.27	71.0
NDF, % DM	124	45.4	17.0	12.1	80.1
ADF, % DM	124	26.0	10.0	6.23	46.5
Lignin, % DM	124	1.64	0.991	1.64	5.80
Starch, % DM	124	20.3	15.8	0.574	65.5
TDN, % DM	124	63.7	10.1	40.0	82.4
TDN/RDP ratio, g/g	124	9.70	4.14	2.46	21.8
ME, Mcal/kg	124	2.25	0.446	0.776	2.98
Ruminal factors					
pH	65	6.19	0.330	5.59	6.85
N-NH_3, mg/dL	69	9.75	7.09	0.231	32.2
VFA total, m*M*	61	93.6	21.2	52.2	143.3
Acetate, % molar	61	64.9	7.95	41.2	77.1
Propionate, % molar	61	20.6	7.16	12.0	48.4
Butyrate, % molar	61	10.8	3.39	5.34	23.2
A:P ratio	61	3.54	1.26	0.852	6.42
Experimental variables					
DMI, kg/d	124	9.10	9.11	0.30	28.7
DMI, g/$kg^{0.75}$	124	102.7	51.0	18.0	211.4
DMD, %	54	67.2	6.09	57.9	80.5
OMD, %	74	65.1	9.71	36.8	81.6

[1]A:P ratio = acetate:propionate ratio; DMD = dry matter digestibility; OMD = organic matter digestibility.
[2]N = number of treatment means.

models along with fit statistics can be found in Tables 3 to 14. Cross-validation results are presented in the correspondingly numbered Supplemental Tables S3 to S14 (http://hdl.handle.net/10919/101353).

Urea N Entry Rate

The urea N entry rate is the amount of the urea produced in the liver (Lobley et al., 2000). Including DMI in model 5 allowed us to account for specific variation in UER to be partitioned to the CP term, which was the only dietary characteristic that was identified as significant ($P \leq 0.003$; Table 3). The improvement in model 5 by the inclusion of DMI compared with model 2 was expected (CCC = 0.906 vs. 0.168; Table 3), because it has been shown that DMI plays an important role in UER production (Sarraseca et al., 1998). Accounting for DMI is particularly important because DMI drives intake of all nutrients, including N. Changes in N intake are known to be the major factor affecting urea production in the liver (Reynolds and Kristensen, 2008). Batista et al. (2017) showed in a meta-analysis that UER increased exponentially as CP level increased in the diet. Although nonlinear

Table 2. Summary statistics of response variables

Variable[1]	N[2]	Mean	SD	Minimum	Maximum
UER, g/d	124	136.9	152.4	2.40	5.31
UER, g/$kg^{0.75}$	124	1.59	1.01	0.25	3.77
GER, g/d	124	93.0	106.1	1.80	390.0
GER, g/$kg^{0.75}$	124	1.04	0.71	0.12	2.69
ROC, g/d	115	58.5	79.4	1.80	295.0
ROC, g/$kg^{0.75}$	115	0.60	0.56	0.05	2.09
UUA, g/d	104	37.5	41.2	2.11	213.8
UUA, g/$kg^{0.75}$	104	0.45	0.29	0.09	1.59
GER:UER, %	124	68.2	18.3	6.50	98.9
ROC:GER, %	113	48.7	18.4	15.2	88.6
UUA:GER, %	102	43.7	16.7	7.20	75.0
INC:GER, %	43	28.5	18.5	5.90	64.0

[1]UER = urea N entry rate; GER = gastrointestinal entry rate; ROC = urea N returned to ornithine cycle; UUA = urea N used for anabolism; INC = incorporation of recycled urea N into microbial N.
[2]N = number of treatment means.

Table 3. Parameter estimates in the UER (urea N entry rate, g/d) models using ruminant species (RS), dietary characteristics (DC), ruminal factors (RF), and experimental variables (EV) data sets individually and in combination (*P*-values are given in parentheses)

Item[1]	RS	DC	RF	DC+RF	DC+EV	DC+RS	RS+RF[2]	RS+DC+RF+EV[3]
Model	1	2	3	4	5	6	7	8
Intercept	79.2 (0.001)	−296.6 (0.016)	136.4 (0.004)	−644.5 (0.007)	−64.6 (<0.001)	−217.8 (0.036)	49.9 (0.155)	20.0 (0.707)
Sheep	−56.0 (0.109)					−86.2 (0.013)	−85.0 (0.191)	
Nonlactating heifers	−9.12 (0.874)					−58.6 (0.293)		
Lactating dairy cows	263.1 (<0.001)					203.3 (<0.001)	245.8 (<0.001)	
DMI, kg/d					14.6 (<0.001)			15.8 (<0.001)
CP, % DM		8.40 (<0.001)		7.84 (0.003)	4.95 (<0.001)	7.60 (<0.001)		4.88 (0.002)
Lignin, % DM		27.1 (0.021)				22.7 (0.028)		
TDN, % DM		3.40 (0.016)		6.38 (0.005)		2.20 (0.066)		
ME, Mcal/kg								80.0 (0.012)
OMD, %								−4.40 (0.004)
N-NH_3, mg/dL			5.10 (<0.001)	2.93 (0.031)			5.05 (<0.001)	2.88 (0.003)
Acetate, % molar				4.47 (0.022)				
Fit statistics								
n	124	124	69	61	124	124	69	58
Mean (model)	136.8	136.9	198.2	176.9	136.9	136.9	198.2	178.4
$\hat{\sigma}_s$	72.3	127.4	161.6	141.3	41.7	67.00	85.8	34.1
$\hat{\sigma}_e$	25.1	21.24	25.7	22.2	18.8	21.0	25.6	17.5
$\hat{\sigma}_s/\hat{\sigma}_e$	2.88	6.00	6.29	6.36	2.23	3.18	3.35	1.95
$\hat{\sigma}_s$/mean	52.8	93.1	81.5	79.9	30.5	48.9	43.3	19.1
$\hat{\sigma}_e$/mean	18.3	15.5	13.0	12.6	13.7	15.4	12.9	9.84
AICc	1,315.7	1,333.3	748.5	642.0	1,253.8	1,272.4	711.9	549.2
CCC	0.776	0.168	0.049	0.231	0.906	0.797	0.642	0.917

[1]There was no adjustment for beef cattle species, OM, RDP, NDF, ADF, starch, TDN/RDP ratio, DM digestibility, pH, VFA total, propionate, butyrate, and acetate:propionate ratio; $\hat{\sigma}_s$ = SD for study; $\hat{\sigma}_e$ = SD for error; AICc = corrected Akaike information criterion; CCC = concordance correlation coefficient.

[2]There was no adjustment for nonlactating heifers.

[3]There was no adjustment for any ruminant species.

Table 4. Parameter estimates in the UER (urea N entry rate, g/kg$^{0.75}$) models using ruminant species (RS), dietary characteristics (DC), ruminal factors (RF), and experimental variables (EV) data sets individually and in combination (*P*-values are given in parentheses)

Item[1]	RS	DC	RF	DC+RF	DC+EV	DC+RS	RS+RF[2]	RS+DC+RF+EV[3]
Model	1	2	3	4	5	6	7	8
Intercept	1.18 (<0.001)	−4.00 (<0.001)	1.07 (<0.001)	−3.87 (<0.001)	−5.66 (<0.001)	−3.77 (<0.001)	0.607 (0.011)	−2.71 (<0.001)
Sheep	0.182 (0.568)					−0.351 (0.211)	0.112 (0.762)	
Nonlactating heifers	−0.039 (0.944)					−0.835 (0.086)		
Lactating dairy cows	1.41 (<0.001)					0.387 (0.218)	1.27 (0.001)	
DMI, g/kg$^{0.75}$/d					0.009 (<0.001)			0.009 (<0.001)
CP, % DM		0.081 (<0.001)		0.065 (<0.001)	0.117 (<0.001)	0.081 (<0.001)		0.056 (<0.001)
ADF, % DM					0.048 (<0.001)			
Lignin, % DM		0.241 (0.016)				0.236 (0.017)		
Starch, % DM		−0.017 (0.075)		−0.048 (<0.001)		−0.017 (0.063)		−0.034 (<0.001)
TDN/RDP ratio, g/g		−0.040 (0.054)				−0.039 (0.065)		
TDN, % DM		0.068 (<0.001)		0.083 (<0.001)	0.054 (<0.001)	0.065 (<0.001)		
ME, Mcal/kg								1.31 (<0.001)
N-NH_3			0.074 (<0.001)	0.055 (<0.001)			0.068 (<0.001)	0.055 (<0.001)
Fit statistics								
n	124	124	69	69	124	124	69	69
Mean (model)	1.58	1.59	1.87	1.87	1.58	1.59	1.87	1.87
$\hat{\sigma}_s$	0.681	0.643	0.785	0.518	0.498	0.570	0.500	0.318
$\hat{\sigma}_e$	0.301	0.205	0.249	0.206	0.192	0.205	0.252	0.192
$\hat{\sigma}_s/\hat{\sigma}_e$	2.27	3.14	3.14	2.51	2.60	2.78	1.99	1.65
$\hat{\sigma}_s$/mean	42.9	40.5	41.9	27.7	31.4	35.9	26.7	17.0
$\hat{\sigma}_e$/mean	18.9	13.0	13.3	11.0	12.1	12.9	13.5	10.3
AICc	217.9	175.6	94.9	88.2	149.4	174.7	86.3	74.5
CCC	0.368	0.567	0.268	0.659	0.739	0.656	0.431	0.816

[1]There was no adjustment for beef cattle species, OM, RDP, NDF, DM digestibility, OM digestibility, pH, VFA total, acetate, propionate, butyrate, and acetate:propionate ratio; $\hat{\sigma}_s$ = SD for study; $\hat{\sigma}_e$ = SD for error; AICc = corrected Akaike information criterion; CCC = concordance correlation coefficient obtained in the Monte Carlo analysis.

[2]There was no adjustment for nonlactating heifers.

[3]There was no adjustment for any ruminant species.

Table 5. Parameter estimates in the GER (gastrointestinal entry rate, g/d) models using ruminant species (RS), dietary characteristics (DC), ruminal factors (RF), and experimental variables (EV) data sets individually and in combination (*P*-values are given in parentheses)

Item[1]	RS	DC	RF	DC+RF	DC+EV	DC+RS	RS+RF[2]	RS+DC+RF+EV[3]
Model	1	2	3	4	5	6	7	8
Intercept	−56.6 (0.001)	201.9 (<0.001)	93.0 (0.005)	194.9 (0.003)	13.7 (0.248)	183.8 (<0.001)	38.6 (0.138)	191.3 (0.024)
Sheep	−44.1 (0.077)					−67.8 (0.010)	−71.3 (0.136)	
Nonlactating heifers	−22.7 (0.577)					−66.3 (0.112)		
Lactating dairy cows	174.2 (<0.001)					132.9 (<0.001)	165.9 (<0.001)	
DMI, kg/d					10.2 (<0.001)			12.0 (<0.001)
RDP, % CP basis		−1.43 (0.020)		−1.76 (0.075)		−1.35 (0.022)		
NDF, % DM		−2.08 (0.017)				−1.70 (0.025)		
ADF, % DM								−2.89 (0.012)
Lignin, % DM		26.1 (0.023)				22.0 (0.029)		
Starch, % DM								−1.38 (0.083)
TDN/RDP ratio, g/g		−3.43 (<0.001)			−1.85 (0.001)	−3.39 (<0.001)		
OM digestibility, %								−1.77 (0.084)
N-NH_3, mg/dL			3.52 (0.001)	4.05 (<0.001)			3.34 (0.001)	1.13 (0.099)
Fit statistics								
n	124	124	69	69	124	124	69	58
Mean (model)	93.0	93	136.7	136.7	93.0	93.0	136.7	120.9
$\hat{\sigma}_s$	52.2	90.7	114.1	115.4	33.8	48.2	62.5	25.3
$\hat{\sigma}_e$	15.0	14.0	17.5	17.0	12.5	13.9	17.4	12.2
$\hat{\sigma}_s/\hat{\sigma}_e$	3.48	6.48	6.53	6.77	2.71	3.47	3.58	2.08
$\hat{\sigma}_s$/mean	56.1	97.5	83.5	84.4	36.3	51.8	45.7	20.9
$\hat{\sigma}_e$/mean	16.1	15.1	12.8	12.5	13.4	15.0	12.8	10.1
AICc	1,196.6	1,232.5	707.5	704.8	1,159.2	1,174.2	673.2	528.4
CCC	0.735	0.137	0.020	0.022	0.853	0.783	0.668	0.913

[1]There was no adjustment for beef cattle species, OM, CP, TDN, ME, DM digestibility, pH, VFA total, acetate, propionate, butyrate, and acetate:propionate ratio; $\hat{\sigma}_s$ = SD for study; $\hat{\sigma}_e$ = SD for error; AICc = corrected Akaike information criterion; CCC = concordance correlation coefficient obtained in the Monte Carlo analysis.

[2]There was no adjustment for nonlactating heifers.

[3]There was no adjustment for any ruminant species.

relationships were not evaluated in this assessment due to the number of models tested and the complexity of those models, the previous work of Batista et al. (2017) suggests that a saturating effect of CP should be considered in future analyses.

Inclusion of RS in models 6 and 7 enabled explanation of additional variation (CCC = 0.797 and 0.642, respectively) in UER compared with models 2 and 3 (CCC = 0.168 and 0.049, respectively), which omitted the species terms. The improvement in CCC associated with the categorical species effect suggests variation exists among species in terms of their baseline UER rates. The same trend was observed when the variation in metabolic body size was accounted for (Table 5). In support of our findings, Lapierre and Lobley (2001) showed a different correlation coefficient for cattle and sheep (r^2 = 0.58 vs 0.33, respectively) between N intake and UER. Also, more recently Batista et al. (2017) showed higher UER for sheep than for cattle when compared on a $BW^{0.75}$ basis. Although these differences could be due to baseline differences in species physiology, they could also reflect dietary or management differences between species. For example, species have notably different dietary characteristics, ruminal ammonia concentration, and DM and OM digestibility coefficients (Supplemental Table S15; http://hdl.handle.net/10919/101353). According to our models, these factors are also important in governing UER (Tables 3 and 4). In addition, in our database N intake (g/kg $BW^{0.75}$) was much higher for lactating dairy cows (4.50 g/$kg^{0.75}$) compared with beef cattle, sheep, and nonlactating heifers (1.37, 1.36, and 2.45 g/$kg^{0.75}$, respectively). However, whether the variation in UER production across species is a function of diet composition differences or particularities in N metabolism intrinsic to each species remains unclear. Further studies are necessary to understand this complex mechanism governing UER, and so that it can be better leveraged to enhance N utilization among different species.

It has been suggested that one strategy to increase N use efficiency is to reduce the amount of urea produced in the liver (Lapierre and Lobley, 2001). Therefore, understanding factors governing UER is essential to improve N use efficiency in ruminants. Dietary CP was significant ($P \leq 0.003$) in all models (g/d or g/$kg^{0.75}$) that included diet characteristics, which was expected due to the positive effect CP has on UER (Batista et al., 2017). Ruminal ammonia N concentrations significantly affected UER ($P \leq 0.031$) in all models (g/d or g/$kg^{0.75}$) that included rumen variables, suggesting a consistent and repeatable relationship among these factors. This relationship is sensible because rumen ammonia is acknowledged to play an important role as a substrate for UER production (Reynolds and Kristensen, 2008). There are limited data evaluating the role of energy supply on urea kinetics. In this study the inclusion of energy density variables such as starch, TDN/RDP ratio, and TDN affected UER, mainly when UER was expressed on a metabolic BW basis (Table 4). Increasing starch concentration in the diet decreased UER production in models 2, 4, 6, and 8 (Table 4). It has been suggested that the capture of ammonia N by rumen bacteria is improved by the increase in energy availability (Bach et al., 2005). In cases of high energy availability, ruminal ammonia concentration and absorption are reduced, and less substrate is available for urea synthesis in the liver (Reynolds and Kristensen, 2008). As such, the decreased UER production on diets with elevated starch concentration may be biologically sensible. Interestingly, TDN content of the diet was positively associated with UER production, which is somewhat paradoxical and may reflect the insensitivity of TDN as an energy metric or the inclusion of digestible protein as an important part of the TDN calculation. An alternative hypothesis is that the increased TDN reflected increased truly fermentable organic matter then it would be logically that greater microbial protein was synthesized if ruminal ammonia N was not limiting. Consequently, greater TDN would likely be correlated with greater flows of microbial crude protein. Diets used by most the studies included in the model development were not likely limited by metabolizable protein supply. Hence, increased absorption of metabolizable protein from greater flows of microbial crude protein could then provide a logical basis whereby UER was increased (e.g., increased hepatic oxidation of excess AA from metabolizable protein). Either way, additional work is needed to evaluate how individual energy substrates (e.g., starch, fiber, fat), total energy supply, and dietary protein interact to govern UER.

Gastrointestinal Entry Rate

Gastrointestinal entry rate can be defined as the portion of UER that returns to the GIT, which includes inflow via saliva (Lobley et al., 2000). Unfortunately, the method developed by Lobley et al. (2000) does not allow separation of recycling into the compartments of GIT (e.g., rumen, small intestine, hindgut). Thus, GER should not be interpreted as urea N recycling to the rumen. Inclusion of RS in models 6 and 7 enabled explanation of additional variation (CCC = 0.783 and 0.668, respectively) in GER compared with models 2 and 3 (CCC = 0.137 and 0.020), which omitted the species terms (Table 5). The improvement in CCC associated with the categorical species effect suggests that variation exists among species in terms of their baseline GER rates. The same trend was observed when

the variation in metabolic BW sizes was accounted for (Table 6).

The effects that CP exerts on GER are well known (Reynolds and Kristensen, 2008; Batista et al., 2017), and it is not a surprise that most studies have focused on the effects of N on urea kinetics. By comparison, few studies have focused on energy effects on GER in ruminants (Bailey et al., 2012a,b). In our study, when GER was standardized for metabolic BW, significant ($P \leq 0.03$) associations with TDN and RDP were identified in 4 out of 5 models that included dietary characteristics (Table 6). Increasing TDN increased GER, whereas an opposite effect was observed for RDP. Urea recycling to the rumen is considered a survival strategy for ruminants because it provides a source of N for microbial protein synthesis in protein-restricted diets (Silva et al., 2019). Therefore, at least within biological limits, ruminants can increase N recycling to sustain microbial protein synthesis in the rumen. Our data suggest that in situations where the energy concentration is increased the ruminant can increase GER to provide a source of N for rumen microbial protein synthesis, although an unknown amount of urea N will be also recycled to the postruminal portions of the GIT. At present, most nutritional models do not account for urea recycling to estimate the RDP requirements (Prates et al., 2017), and current models are of limited application. Future adjustments to predict RDP supply should be made to account for the urea recycling to the rumen. Although the relationships with TDN confer with biological expectation, the relationship between GER and the TDN/RDP ratio identified in the models was directionality opposite of our expectation. There are several possibilities for this result. First, it is important to acknowledge that our understanding of this phenomenon is limited. Another explanation is that forcing of a linear relationship between these variables is inappropriate because it is a ratio and reflects changes in either TDN or RDP or both (Eisemann and Tedeschi, 2016). Irrespectively, additional work is needed to better understand how TDN and RDP interact to influence GER. This would likely best be accomplished through targeted factorial experiments evaluating this interaction.

Urea N Returned to Ornithine Cycle

Predominantly, ROC arises from the recycled urea that is hydrolyzed to ammonia, with the ammonia absorbed from the gut (Reynolds and Kristensen, 2008), therefore being a measure of the recycled urea that is not captured by the rumen microbes and likely to return to the GIT or be eliminated in urine (i.e., UUE). Inclusion of RS in models 6 and 7 improved the ability of the models to explain the variation (CCC = 0.646 and 0.490, respectively) in ROC compared with models 2 and 3 (CCC = 0.137 and 0.024), which did not include the species terms (Table 7). The improvement in CCC associated with the categorical species effect suggests variation exists among species in terms of their baseline ROC. The same trend was observed when the variation in metabolic BW sizes was accounted (Table 8).

According to Lapierre and Lobley (2001) part of the efficient reuse of N in ruminants is because urea N atoms can return to the gut multiple times, which increases the probability of urea N sequestration toward anabolic use. In this study, dietary factors associated with energy supply and also those related to diet quality affected ROC (Table 7). In terms of N supply, our results indicate that protein degradability plays an important role in the amount of ROC. Increasing RDP content in the diet reduced ROC in models 2 and 6 (Table 7) and in models 2, 4, 5, 6, and 8 (Table 8). Lignin content in the diet has a negative association with diet digestibility (Van Soest, 1994). In our study, increasing lignin content increased ($P \leq 0.02$) ROC (g/d and g/kg$^{0.75}$) in models 2, 5, and 6 and in models 2 and 6, respectively, which likely exemplifies the capacity of the ruminants fed low-quality diets to recycle metabolic N back to the GIT as an attempt to sustain rumen microbial protein synthesis (Wickersham et al., 2009; Batista et al., 2016). Inclusion of EV in model 5 improved the capacity of the model to explain the variation (CCC = 0.685) in ROC (g/d) compared with model 2 (CCC = 0.137; Table 7). The improvement in CCC associated with the EV was mainly due to the differences in DMI across RS (Supplemental Table S15, http://hdl.handle.net/10919/101353). Ruminal ammonia concentration was significant or tended to be ($P \leq 0.08$) in 3 out of 4 ROC models that included ruminal factors in both bases (g/d and g/kg$^{0.75}$). When ruminal ammonia concentration is high, then a greater portion of ammonia is expected to be absorbed and returned to the ornithine cycle for urea synthesis (Holder et al., 2015), which can explain the general positive effect of ammonia on ROC. Looking into the energy factors, starch was significant ($P \leq 0.01$) in all models that included dietary characteristics and TDN was significant ($P \leq 0.02$) in 3 out of 5 models when response variables were corrected for the variation in metabolic BW (Table 8). Increasing starch reduced ROC, probably due to the increased capture of ammonia by rumen bacteria, which reduces the ammonia absorption by the ruminal wall (Bach et al., 2005). As with UER, an opposite effect of TDN was observed. This lack of agreement between energy terms (starch vs. TDN) requires further experimental investigation to be fully understood.

Table 6. Parameter estimates in the GER (gastrointestinal entry rate, g/kg$^{0.75}$) models using ruminant species (RS), dietary characteristics (DC), ruminal factors (RF), and experimental variables (EV) data sets individually and in combination (*P*-values are given in parentheses)

Item[1]	RS	DC	RF	DC+RF	DC+EV	DC+RS	RS+RF[2]	RS+DC+RF+EV[3]
Model	1	2	3	4	5	6	7	8
Intercept	0.855 (<0.001)	0.720 (0.348)	0.894 (<0.001)	2.23 (0.013)	−1.64 (0.359)	1.00 (0.191)	0.619 (0.005)	1.14 (0.035)
Sheep	−0.086 (0.714)					−0.232 (0.327)	−0.343 (0.328)	
Nonlactating heifers	−0.294 (0.468)					−0.613 (0.127)		
Lactating dairy cows	0.878 (0.001)					0.516 (0.053)	0.881 (0.004)	
DMI, g/kg$^{0.75}$					0.010 (<0.001)			0.009 (<0.001)
OM, % DM					−0.033 (0.052)			
CP, % DM					0.036 (<0.001)			0.020 (0.088)
RDP, % CP basis		−0.016 (0.013)		−0.026 (0.003)		−0.020 (0.001)		−0.016 (0.004)
Lignin, % DM						0.101 (0.092)		
Starch, % DM		−0.013 (0.052)			−0.018 (0.001)			
TDN/RDP ratio, g/g		−0.064 (<0.001)		−0.071 (<0.001)		−0.069 (<0.001)		−0.033 (0.021)
TDN, % DM		0.033 (0.002)		0.011 (0.034)	0.022 (0.008)	0.023 (0.012)		
N-NH_3, mg/dL			0.029 (0.001)				0.026 (0.002)	
Propionate, % molar				−0.017 (0.082)				
Fit statistics								
n	124	124	69	61	124	124	69	124
Mean (model)	1.04	1.04	1.24	1.13	1.04	1.04	1.24	1.04
$\hat{\sigma}_s$	0.506	0.712	0.674	0.590	0.427	0.481	0.461	0.395
$\hat{\sigma}_e$	0.332	0.596	0.335	0.274	0.229	0.265	0.336	0.241
$\hat{\sigma}_s/\hat{\sigma}_e$	1.53	2.29	2.01	2.15	1.85	1.81	1.37	1.64
$\hat{\sigma}_s$/mean	48.5	57.1	54.2	52.1	40.9	46.0	37.1	37.8
$\hat{\sigma}_e$/mean	31.7	24.9	27.0	24.2	22.0	25.4	27.0	23.0
AICc	123.8	118.7	72.5	71.1	88.2	112.2	65.3	83.6
CCC	0.474	0.221	0.033	0.216	0.661	0.525	0.496	0.647

[1]There was no adjustment for beef cattle species, NDF, ADF, ME, DM digestibility, OM digestibility, pH, VFA total, acetate, butyrate, and acetate:propionate ratio; $\hat{\sigma}_s$ = SD for study; $\hat{\sigma}_e$ = SD for error; AICc = corrected Akaike information criterion; CCC = concordance correlation coefficient obtained in the Monte Carlo analysis.

[2]There was no adjustment for nonlactating heifers.

[3]There was no adjustment for any ruminant species.

Table 7. Parameter estimates in ROC (urea N returned to ornithine cycle, g/d) models using ruminant species (RS), dietary characteristics (DC), ruminal factors (RF), and experimental variables (EV) data sets individually and in combination (*P*-values are given in parentheses)

Item[1]	RS	DC	RF	DC+RF	DC+EV	DC+RS	RS+RF[2]	RS+DC+RF+EV[3]
Model	1	2	3	4	5	6	7	8
Intercept	26.9 (0.098)	119.2 (<0.001)	68.8 (0.009)	99.2 (0.002)	49.5 (0.049)	102.0 (0.001)	19.1 (0.4619)	−3.71 (0.782)
Sheep	−18.5 (0.503)					−35.2 (0.189)	−26.1 (0.661)	
Nonlactating heifers	−17.8 (0.672)					−51.7 (0.208)		
Lactating dairy cows	126.0 (<0.001)					94.3 (0.001)	122.5 (0.004)	
DMI, kg/d					6.08 (<0.001)			6.51 (<0.001)
CP, % DM								1.15 (0.013)
RDP, % CP basis		−0.909 (0.012)				−0.930 (0.009)		
NDF, % DM		−1.58 (0.004)			−1.70 (0.001)	−1.33 (0.015)		
Lignin, % DM		22.7 (0.002)			14.7 (0.015)	20.4 (0.005)		
Starch, % DM				1.84 (0.004)	−1.39 (<0.001)			−0.853 (<0.011)
TDN/RDP ratio, g/g		−2.12 (0.002)				−2.15 (<0.001)		
N-NH_3			1.87 (0.005)	−1.50 (0.042)			1.80 (0.007)	
Fit statistics								
n	115	115	66	66	115	115	66	115
Mean (model)	58.5	58.5	87.8	87.8	58.5	58.5	87.8	58.5
$\hat{\sigma}_s$	53.6	70.0	89.8	97.1	40.7	49.5	65.3	42.5
$\hat{\sigma}_e$	8.95	8.15	10.7	10.2	7.45	8.11	10.7	7.70
$\hat{\sigma}_s/\hat{\sigma}_e$	5.99	8.59	10.7	9.55	5.47	6.09	6.10	5.53
$\hat{\sigma}_s$/mean	91.6	119.7	102.2	110.6	69.6	84.6	74.4	72.7
$\hat{\sigma}_e$/mean	15.3	13.9	12.2	11.6	12.7	13.9	12.2	13.2
AICc	1,028.9	1,048.1	626.1	623.4	1,003.7	1,007.9	600.8	1,015.0
CCC	0.577	0.137	0.024	0.007	0.685	0.646	0.490	0.660

[1]There was no adjustment for beef cattle species, OM, ADF, TDN, ME, DM digestibility, OM digestibility, pH, VFA total, acetate, propionate, butyrate, and acetate:propionate ratio; $\hat{\sigma}_s$ = SD for study; $\hat{\sigma}_e$ = SD for error; AICc = corrected Akaike information criterion; CCC = concordance correlation coefficient.

[2]There was no adjustment for nonlactating heifers.

[3]There was no adjustment for any ruminant species.

Table 8. Parameter estimates in the ROC (urea N returned to ornithine cycle, g/kg$^{0.75}$) models using ruminant species (RS), dietary characteristics (DC), ruminal factors (RF), and experimental variables (EV) data sets individually and in combination (*P*-values are given in parentheses)

Item[1]	RS	DC	RF	DC+RF	DC+EV	DC+RS	RS+RF[2]	RS+DC+RF+EV[3]
Model	1	2	3	4	5	6	7	8
Intercept	0.395 (0.006)	0.679 (0.169)	0.254 (0.209)	0.383 (0.519)	1.56 (0.053)	0.762 (0.118)	0.039 (0.844)	−4.11e-2 (0.925)
Sheep	0.125 (0.586)					−0.081 (0.696)	−0.126 (0.727)	
Nonlactating heifers	−0.247 (0.490)					−0.700 (0.035)		
Lactating dairy cows	0.730 (0.002)					0.293 (0.166)	0.674 (0.018)	
DMI, g/kg$^{0.75}$					0.005 (0.001)			3.78e-3 (<0.001)
RDP, % CP basis		−0.010 (0.018)		−0.013 (0.025)	−0.010 (0.051)	−0.010 (0.016)		−1.06e-2 (0.004)
NDF, % DM		−0.015 (0.006)			−0.010 (0.062)	−0.016 (0.006)		
Lignin, % DM		0.151 (0.017)				0.158 (0.018)		
Starch, % DM		−0.013 (0.011)		−0.017 (0.010)	−0.021 (0.004)	−0.014 (0.007)		−1.47e-2 (<0.001)
TDN/RDP ratio, g/g		−0.035 (<0.001)		−0.040 (<0.001)	−0.038 (<0.001)	−0.034 (<0.001)		−3.83e-2 (<0.001)
TDN, % DM		0.019 (0.004)				0.018 (0.006)		2.40e-2 (<0.001)
ME, Mcal/kg				0.793 (0.001)	0.813 (<0.001)			
OM digestibility, %					−0.021 (0.023)			
N-NH_3, mg/dL			0.025 (0.001)	0.011 (0.081)			0.023 (0.001)	
Butyrate, % molar			0.024 (0.047)				0.022 (0.073)	
Fit statistics								
n	115	115	58	66	71	115	58	115
Mean (model)	0.602	0.602	0.741	0.752	0.764	0.602	0.741	0.602
$\hat{\sigma}_s$	0.446	0.436	0.527	0.453	0.442	0.377	0.418	0.424
$\hat{\sigma}_e$	0.281	0.206	0.295	0.245	0.216	0.206	0.296	0.195
$\hat{\sigma}_s/\hat{\sigma}_e$	1.59	2.12	1.78	1.85	2.05	1.83	1.41	2.18
$\hat{\sigma}_s$/mean	74.1	72.4	71.1	60.2	57.8	62.6	56.5	70.4
$\hat{\sigma}_e$/mean	46.6	34.2	39.9	32.6	28.3	34.2	40.0	32.3
AICc	21.9	17.1	26.2	24.2	39.9	17.8	24.7	3.83
CCC	0.313	0.347	0.121	0.294	0.430	0.520	0.339	0.400

[1]There was no adjustment for beef cattle species, OM, CP, ADF, DM digestibility, pH, VFA total, acetate, propionate, and acetate:propionate ratio; $\hat{\sigma}_s$ = SD for study; $\hat{\sigma}_e$ = SD for error; AICc = corrected Akaike information criterion; CCC = concordance correlation coefficient obtained in the Monte Carlo analysis.

[2]There was no adjustment for nonlactating heifers.

[3]There was no adjustment for any ruminant species.

Urea N Utilized for Anabolism

The UUA reflects the portion of urea recycled to the GIT that is used for anabolic functions (Lobley et al., 2000). Although sometimes UUA is interpreted as the amount of urea N used for microbial protein synthesis, it is important to note that UUA measurement also includes a portion of the labeled N in unmeasured forms that are excreted in feces and urine. One might expect UUA to reflect the amino acids from the labeled rumen microbial protein retained in body proteins, but there are other physiological processes related to the nitrogen metabolism that could contribute to UUA (Wickersham et al., 2008). Therefore, UUA is not an accurate measure to be used when predicting RDP supply. Inclusion of RS in models 6 and 7 enabled explanation of additional variation (CCC = 0.423 and 0.350, respectively) in UUA (g/d) compared with models 2 and 3 (CCC = 0.030 and 0.151), where the species terms were omitted (Table 9). The improvement in CCC associated with the categorical species effect suggests differences among species in terms of their baseline UUA. However, there were few or null improvements when the response variables were expressed on a metabolic BW basis (Table 10), which indicates that the different RS evaluated have a similar physiological response to modulate the amount of UUA under variations in feeding management. Including EV such as DMI (model 5; CCC = 0.392) or RS (model 6; CCC = 0.423) in the UUA models increased the ability of the models to explain the variation in UUA (g/d) compared with the DC model (Table 9; model 2; CCC = 0.030). Ruminal ammonia concentration was significant or tended to be ($P \leq 0.06$) in 3 out of 4 models when the variation in the metabolic weight was accounted (Table 10). Total digestible nutrients content tended ($P = 0.09$) to be significant in 2 out of 5 models and positively affected the amount of UUA ($g/kg^{0.75}$), suggesting that energy supply partially drives the fates of urea in the body toward anabolic functions. According to Reynolds and Kristensen (2008) the amount of UUA is probably controlled by rumen microbial requirements. Therefore, we hypothesized that the ammonia and TDN have an indirect effect on UUA by increasing the rumen microbial growth in the rumen and the utilization of the recycled urea for microbial protein synthesis, despite the limitations of these measures.

Gastrointestinal Entry Rate Relative to Urea N Entry Rate (GER:UER)

The ratio GER:UER can be defined as the proportion (%) of UER that returns to the GIT (Lobley et al., 2000). Inclusion of RS in model 6 enabled explanation of additional variation (CCC = 0.521) in GER:UER compared with model 2 (CCC = 0.366), where only dietary characteristics were considered (Table 11). The improvement in CCC associated with the categorical species effect suggests variation exists among species in terms of their capacity to recycle urea. However, there is a variation in diet composition across species (Table 2), which makes difficult the interpretation of these results. Whether the variation in GER:UER ratio across species is a function of diet composition differences or particularities in N metabolism intrinsic to each species remains unclear. In regards to ruminal and dietary factors affecting GER:UER ratio, increasing CP and ruminal ammonia concentration consistently decreased ($P \leq 0.05$) the proportion of UER that returns to the GIT (Table 11), which was expected due to negative effects of ammonia on urea transport across the ruminal epithelium (Kennedy and Milligan, 1980; Reynolds and Kristensen, 2008). In this study, increasing the ratio of energy to protein (TDN/RDP) increased the GER:UER ratio. In fact, Huntington (1989) fed a 12% CP high-starch diet and observed an increase in the amount of urea transferred directly to the rumen from blood, which may be due to an increased energy:protein ratio in the diet. In our work, TDN content was significant ($P < 0.001$) in 3 out of 5 models, but the signal directionality was different across models, which makes difficult to understand if TDN is increasing or decreasing GER:UER ratio. Ruminal pH was significant ($P = 0.002$) in 3 out of 4 models that included ruminal factors with a positive effect on GER:UER ratio (Table 11). According to Abdoun et al. (2006) when the intraruminal pH is lowered, the permeability of the rumen wall for ammonia absorption is depressed (Abdoun et al., 2006). Therefore, increasing the ruminal pH within physiological limits could increase ammonia absorption from the rumen reducing its concentration and perhaps increasing urea recycling to sustain microbial protein synthesis.

Urea N Returned to Ornithine Cycle Relative to Gastrointestinal Entry Rate of Urea N (ROC:GER)

The ratio ROC:GER represents the proportion of recycled urea that returned to ornithine cycle and will largely represent ammonia derived from urea metabolism in the gut (Reynolds and Kristensen, 2008). Inclusion of RS in model 6 enabled explanation of additional variation (CCC = 0.402) in ROC:GER ratio compared with model 2 (CCC = 0.304), which omitted the species terms (Table 12). The improvement in CCC associated with the categorical species effect suggests variation exists among species in ROC:GER ratio. As discussed previously, this could be a response in the

Table 9. Parameter estimates in the UUA (urea N used for anabolism, g/d) models using ruminant species (RS), dietary characteristics (DC), ruminal factors (RF), and experimental variables (EV) data sets individually and in combination (*P*-values are given in parentheses)

Item[1]	RS	DC	RF	DC+RF	DC+EV	DC+RS	RS+RF[2]	RS+DC+RF+EV[3]
Model	1	2	3	4	5	6	7	8
Intercept	28.2 (0.002)	22.3 (<0.001)	−5.63 (0.732)	−458.2 (0.014)	2.67 (0.718)	20.0 (0.041)	20.5 (0.124)	2.67 (0.718)
Sheep	−21.9 (0.125)					−22.3 (0.117)	−19.9 (0.481)	
Nonlactating heifers	−7.73 (0.706)					−10.2 (0.617)		
Lactating dairy cows	40.1 (0.005)					36.3 (0.009)	41.4 (0.041)	
DMI, kg/d					3.12 (<0.001)			3.12 (<0.001)
OM, % DM				3.43 (0.033)				
CP, % DM		0.924 (0.039)				0.745 (0.092)		
NDF, % DM				−0.645 (0.032)				
Starch, % DM				−1.05 (0.051)				
ME, Mcal/kg				21.9 (0.102)				
pH				22.0 (0.095)				
N-NH_3, mg/dL							0.841 (0.073)	
VFA total, m*M*			0.418 (0.014)	0.429 (0.034)				
Fit statistics								
n	104	104	55	51	104	104	63	104
Mean (model)	37.5	37.5	33.3	34.5	37.5	37.5	48.6	37.5
$\hat{\sigma}_s$	24.6	31.3	18.8	15.0	24.3	24.5	31.4	24.3
$\hat{\sigma}_e$	11.1	11.0	7.03	7.28	10.1	11.0	13.5	10.1
$\hat{\sigma}_s/\hat{\sigma}_e$	2.21	2.83	2.67	2.06	2.41	2.23	2.33	2.41
$\hat{\sigma}_s$/mean	65.6	83.6	56.4	43.6	64.8	65.4	64.6	64.8
$\hat{\sigma}_e$/mean	29.7	29.5	21.1	21.1	26.9	29.3	27.7	26.9
AICc	901.8	930.3	432.6	395.3	903.8	901.0	553.7	903.8
CCC	0.429	0.030	0.151	0.332	0.392	0.423	0.350	0.418

[1]There was no adjustment for beef cattle species RDP, ADF, lignin, TDN/RDP ratio, TDN, DM digestibility, OM digestibility, acetate, propionate, butyrate, and acetate:propionate ratio; $\hat{\sigma}_s$ = SD for study; $\hat{\sigma}_e$ = SD for error; AICc = corrected Akaike information criterion; CCC = concordance correlation coefficient obtained in the Monte Carlo analysis.

[2]There was no adjustment for nonlactating heifers.

[3]There was no adjustment for any ruminant species.

Table 10. Parameter estimates in the UUA (urea N used for anabolism, g/kg$^{0.75}$) models using ruminant species (RS), dietary characteristics (DC), ruminal factors (RF), and experimental variables (EV) data sets individually and in combination (*P*-values are given in parentheses)

Item[1]	RS	DC	RF	DC+RF	DC+EV	DC+RS[2]	RS+RF[2]	RS+DC+RF+EV
Model	1	2	3	4	5	6	7	8
Intercept	0.407 (<0.001)	0.185 (0.442)	0.341 (0.001)	0.782 (0.003)	1.93 (0.003)	0.185 (0.442)	0.341 (0.001)	−0.116 (0.326)
Sheep	−0.049 (0.694)							−0.003 (0.979)
Nonlactating heifers	−0.069 (0.710)							−0.233 (0.184)
Lactating dairy cows	0.111 (0.348)							−0.408 (0.009)
DMI, g/kg$^{0.75}$					0.003 (0.005)			0.005 (<0.001)
CP, % DM								0.012 (0.003)
RDP, % CP basis					−0.009 (0.021)			
ADF, % DM					−0.032 (0.001)			
TDN/RDP ratio, g/g		−0.018 (0.001)				−0.018 (0.001)		
TDN, % DM		0.006 (0.090)				0.006 (0.090)		
DM digestibility, %					−0.012 (0.059)			
N-NH_3, mg/dL			0.010 (0.063)	0.011 (0.047)			0.010 (0.063)	
Fit statistics								
n	104	104	63	63	104	104	63	104
Mean (model)	0.448	0.448	0.482	0.482	0.410	0.448	0.482	0.448
$\hat{\sigma}_s$	0.223	0.208	0.255	0.251	0.068	0.208	0.255	0.203
$\hat{\sigma}_e$	0.107	0.102	0.116	0.114	0.081	0.102	0.116	0.094
$\hat{\sigma}_s/\hat{\sigma}_e$	2.08	2.05	2.19	2.21	0.838	2.05	2.19	2.16
$\hat{\sigma}_s$/mean	49.8	46.4	52.9	52.1	16.5	46.4	52.9	45.3
$\hat{\sigma}_e$/mean	24.0	22.7	24.1	23.6	19.7	22.7	24.1	20.9
AICc	−32.3	−34.0	−16.6	−13.9	0.102	−34.0	−16.6	−34.1
CCC	0.063	0.137	−0.007	0.008	0.295	0.144	0.001	0.199

[1]There was no adjustment for beef cattle species, OM, NDF, lignin, starch, ME, OM digestibility, pH, VFA total, acetate, propionate, butyrate, and acetate:propionate ratio; $\hat{\sigma}_s$ = SD for study; $\hat{\sigma}_e$ = SD for error; AICc = corrected Akaike information criterion; CCC = concordance correlation coefficient obtained in the Monte Carlo analysis.

[2]There was no adjustment for any ruminant species.

differences found in the diet composition across species (Supplemental Table S15; http://hdl.handle.net/10919/101353). In agreement with Batista et al. (2017), increasing CP and ruminal ammonia levels increased the ROC:GER ratio. Variables associated with energy supply had substantial effects on the ROC:GER ratio (Table 12). Lignin was significant ($P \leq 0.05$) and positively affected the ROC:GER ratio in all models that included dietary characteristics. This relationship indicates that in low-quality diets a greater portion of the urea recycled would be directed to the ornithine cycle instead of anabolic functions due to low ruminal microbial protein synthesis. Increasing the ratio of energy to protein (TDN/RDP) decreased ($P \leq 0.03$) the ROC:GER ratio, which could indicate that GER was directed to anabolic pathways. Increasing ruminal pH led or tended to lead ($P \leq 0.06$) to lower ROC:GER in 3 out of 4 models that included ruminal variables (Table 12). This relationship suggests a greater portion of GER might have been used for anabolic functions when rumen pH was high. Rumen microbial growth is sensitive to low ruminal pH (Russell and Wilson, 1996). A possible explanation for the lower ROC:GER under higher ruminal pH would be greater microbial protein synthesis as an outcome, which could have increased capture of recycled urea. However, to our knowledge, no studies have explicitly evaluated the role of ruminal pH on urea kinetics in ruminants.

Urea N Utilized for Anabolic Purposes Relative to Gastrointestinal Entry Rate (UUA:GER)

The ratio UUA:GER is usually increased as CP intake is reduced (Reynolds and Kristensen, 2008). Inclusion of RS in model 6 improved the ability of the model to explain the variation (CCC = 0.223) in UUA:GER ratio compared with model 2 (CCC = 0.179), where we did not use the species terms for model derivation (Table 13). The improvement in CCC associated with the categorical species effect suggests that variation among species in UUA:GER should be considered in future predictive models of urea kinetics. As discussed above, this could also be a response to the differences found in the diet composition across species (Supplemental Table S15, http://hdl.handle.net/10919/101353). Increasing CP decreased UUA:GER (models 5 and 8; Table 13). In agreement with our findings, Reynolds and Kristensen (2008) showed that in cattle fed low-CP diets (<12%), the fraction of urea entry used for anabolic purposes was greater (28–72%) than for higher-protein diets (17–26%). In that study, the authors stated that other dietary factors also might influence the fate of urea N in the gut lumen. We found RDP content was significant ($P \leq 0.03$) and correlated positively to the UUA:GER ratio in 3 out of 5 models that included dietary characteristics, which could be related to an increase in rumen microbial protein synthesis in those diets (Reynolds and Kristensen, 2008). Besides the variation across RS and CP level, we also identified energy supply and ruminal factors as other variables influencing UUA:GER. Increasing TDN/RDP ratio increased or tended to increase ($P \leq 0.07$) the UUA:GER in 4 out of 5 models that included dietary characteristics, showing the importance energy supply to optimize the use of urea recycled toward anabolic functions rather than excretion pathways, despite the opposite effect observed for TDN content, which requires further investigation. In regards with the reduction in the UUA:GER ratio by an increase in the acetate molar proportion (models 4 and 8; Table 13), one could hypothesize that this could be related to a reduction in the propionate proportion, which is positively associated to non-fibrous carbohydrate intake in ruminants. In agreement with the ROC:GER results previously discussed, increasing ruminal pH increased ($P \leq 0.05$) the UUA:GER ratio (models 3, 4, 7, and 8; Table 13), which could be associated with greater microbial protein synthesis in the rumen and capture of recycled urea.

Incorporation of Recycled Urea N into Microbial N Relative to Gastrointestinal Entry Rate of Urea N (INC:GER)

The INC (g/d) is a much more accurate measure of the recycled urea used for microbial protein synthesis compared with UUA and should preferably be used in models to predict RDP supply. Unfortunately, there are few studies evaluating the incorporation of urea recycled using the approach described by Wickersham et al. (2008). Due to the low number of observations only 2 INC:GER models were derived from our database. Despite the great importance of CP levels for the utilization of recycled urea for microbial protein synthesis in low-N diets (Batista et al., 2017), the INC:GER model derived using dietary characteristics included only TDN:RDP ratio as a significant term ($P < 0.001$). Increasing TDN:RDP increased the INC: GER ratio. The preference for TDN:RDP over CP suggests that information on energy supply is essential to optimize the incorporation of urea recycled into rumen microbial protein. In the INC:GER model derived with ruminal factors only ammonia was significant ($P < 0.001$). The contribution of recycled urea to microbial protein synthesis has more importance in situations when ruminants are fed with low-N diets, likely because of a limitation in rumen ammonia N (Firkins et al., 2007). In our study, increasing rumen ammonia N decreased the incorporation of recycled urea, which might be due

Table 11. Parameter estimates in the GER:UER (gastrointestinal entry rate relative to urea N entry rate, %) models using ruminant species (RS), dietary characteristics (DC), ruminal factors (RF) and experimental variables (EV) data sets individually and in combination (*P*-values are given in parentheses)[1]

Item[2]	RS	DC	RF	DC+RF	DC+EV	DC+RS	RS+RF[3]	RS+DC+RF+EV[4]
Model	1	2	3	4	5	6	7	8
Intercept	74.2 (<0.001)	145.4 (<0.001)	−9.92 (0.734)	−9.92 (0.734)	161.7 (<0.001)	180.5 (<0.001)	−5.40 (0.851)	92.4 (<0.001)
Beef cattle								
Sheep	−13.9 (0.057)					−7.29 (0.186)	−16.7 (0.037)	
Nonlactating heifers	−30.2 (0.019)					−18.6 (0.052)		
Lactating dairy cows	−7.31 (0.327)					8.50 (0.166)	5.05 (0.386)	
DMI, g/kg$^{0.75}$					0.119 (0.003)			0.156 (<0.001)
CP, % DM		−0.827 (0.046)			−0.964 (0.019)	−1.08 (0.009)		
NDF, % DM					−0.455 (0.010)	−0.535 (0.003)		
ADF, % DM		−0.508 (0.070)						
Starch, % DM								−0.605 (<0.001)
TDN/RDP ratio, g/g		1.56 (<0.001)			1.82 (<0.001)	1.67 (<0.001)		
TDN, % DM		−1.09 (<0.001)			−1.43 (<0.001)	1.42 (<0.001)		
pH			14.8 (0.002)	14.8 (0.002)			14.2 (0.002)	
N-NH_3, mg/dL			−1.54 (<0.001)	−1.54 (<0.001)			−1.54 (<0.001)	−1.72 (<0.001)
Butyrate, % molar								−1.19 (0.001)
Fit statistics								
n	124	124	65	65	124	124	65	61
Mean (model)	68.2	68.2	67.3	67.3	68.2	68.2	67.3	68.6
$\hat{\sigma}_s$	15.1	13.6	12.0	12.0	11.3	11.2	9.53	1.75
$\hat{\sigma}_e$	6.70	5.00	4.07	4.07	4.94	4.95	4.07	5.26
$\hat{\sigma}_s/\hat{\sigma}_e$	2.25	2.71	2.95	2.95	2.28	2.26	2.34	0.332
$\hat{\sigma}_s$/mean	22.1	19.9	17.8	17.8	16.6	16.4	14.2	2.55
$\hat{\sigma}_e$/mean	9.82	7.33	6.05	6.05	7.25	7.26	6.05	7.67
AICc	965.7	926.9	449.8	449.8	921.9	903.1	435.6	427.3
CCC	0.101	0.366	0.511	0.481	0.483	0.521	0.009	0.798

[1]UER = urea N entry rate; GER = gastrointestinal entry rate.

[2]There was no adjustment for beef cattle species, OM, RDP, lignin, ME, DM digestibility, OM digestibility, VFA total, acetate, propionate, acetate:propionate ratio; $\hat{\sigma}_s$ = SD for study; $\hat{\sigma}_e$ = SD for error; AICc = corrected Akaike information criterion; CCC = concordance correlation coefficient obtained in the Monte Carlo analysis.

[3]There was no adjustment for nonlactating heifers.

[4]There was no adjustment for any ruminant species.

Table 12. Parameter estimates in the ROC:GER (urea N returned to ornithine cycle relative to gastrointestinal entry rate of urea N, %) models using ruminant species (RS), dietary characteristics (DC), ruminal factors (RF), and experimental variables (EV) data sets individually and in combination (*P*-values are given in parentheses)[1]

Item[2]	RS	DC	RF	DC+RF	DC+EV	DC+RS	RS+RF[3]	RS+DC+RF+EV[3]
Model	1	2	3	4	5	6	7	8
Intercept	44.9 (<0.001)	−28.9 (0.172)	121.3 (0.003)	110.0 (0.029)	−6.59 (0.836)	13.7 (0.526)	121.3 (0.003)	−65.9 (0.025)
Beef cattle								
Sheep	5.86 (0.499)					−7.47 (0.328)		
Nonlactating heifers	−18.1 (0.163)					−40.4 (0.001)		
Lactating dairy cows	15.0 (0.065)					−9.13 (0.245)		
CP, % DM		0.964 (0.012)			2.89 (<0.001)			1.80 (<0.001)
RDP, % CP basis				−0.594 (0.017)				
ADF, % DM						−1.35 (0.011)		
Lignin, % DM		6.74 (0.003)		6.26 (0.039)	4.77 (0.053)	11.2 (0.004)		10.5 (0.003)
Starch, % DM				−0.516 (0.073)		−0.641 (0.008)		
TDN/RDP ratio, g/g		−0.915 (0.029)		−2.19 (<0.001)		−1.52 (<0.001)		
TDN, % DM		0.774 (0.003)		0.974 (0.015)		1.00 (0.001)		0.833 (0.008)
DM digestibility, %					2.52 (0.005)			
OM digestibility, %					−2.30 (0.013)			
pH			−12.0 (0.062)	−12.1 (0.029)			−12.0 (0.062)	
N-NH_3, mg/dL			0.831 (<0.001)				0.831 (<0.001)	0.407 (0.112)
Fit statistics								
n	113	113	62	62	41	113	62	66
Mean (model)	48.7	48.7	54.8	54.8	60.7	48.7	54.8	53.3
$\hat{\sigma}_s$	16.0	15.4	15.7	16.2	20.6	13.1	15.7	14.2
$\hat{\sigma}_e$	3.30	2.52	2.81	2.02	1.23	2.53	2.81	2.77
$\hat{\sigma}_s/\hat{\sigma}_e$	4.83	6.10	5.58	8.05	16.8	5.17	5.58	5.14
$\hat{\sigma}_s$/mean	32.8	31.6	28.6	29.7	34.0	26.9	28.6	26.7
$\hat{\sigma}_e$/mean	6.79	5.18	5.12	3.68	2.02	5.19	5.12	5.20
AICc	859.4	829.3	454.5	429.6	262.9	810.7	454.5	483.4
CCC	0.132	0.304	0.167	0.225	0.203	0.402	0.175	0.382

[1]ROC = urea N returned to ornithine cycle; GER = gastrointestinal entry rate.

[2]There was no adjustment for beef cattle specie, DMI, OM, NDF, ME, VFA total, acetate, propionate, butyrate, acetate:propionate ratio; $\hat{\sigma}_s$ = SD for study; $\hat{\sigma}_e$ = SD for error; AICc = corrected Akaike information criterion; CCC = concordance correlation coefficient obtained in the Monte Carlo analysis.

[3]There was no adjustment for any ruminant species.

Table 13. Parameter estimates in the UUA:GER (urea N utilized for anabolic purposes relative to gastrointestinal entry rate of urea N, %) models using ruminant species (RS), dietary characteristics (DC), ruminal factors (RF), and experimental variables (EV) data sets individually and in combination (*P*-values are given in parentheses)[1]

Item[2]	RS	DC	RF	DC+RF	DC+EV	DC+RS	RS+RF[3]	RS+DC+RF+EV[3]
Model	1	2	3	4	5	6	7	8
Intercept	47.1 (<0.001)	25.7 (0.203)	−28.8 (0.440)	−25.3 (0.539)	83.3 (0.006)	2.68 (0.840)	−28.8 (0.440)	−85.2 (0.321)
Sheep	−2.73 (0.710)					−3.17 (0.672)		
Nonlactating heifers	15.8 (0.154)					18.9 (0.096)		
Lactating dairy cows	−17.9 (0.017)					−14.7 (0.051)		
OM, % DM								1.36 (0.054)
CP, % DM					−2.57 (<0.001)			−1.34 (0.011)
RDP, % CP basis		0.439 (0.031)		0.599 (0.011)		0.504 (0.013)		
ADF, % DM					2.33 (0.006)			
Lignin, % DM					−13.8 (0.001)			
Starch, % DM				0.778 (0.004)				
TDN/RDP ratio, g/g		1.50 (<0.001)		2.24 (<0.001)		1.40 (<0.001)		0.877 (0.070)
TDN, % DM		−0.369 (0.107)		−1.11 (0.001)				−0.699 (0.021)
DM digestibility, %					−2.60 (0.002)			
OM digestibility, %					2.43 (0.005)			
pH			12.0 (0.047)	20.9 (<0.001)			12.0 (0.047)	17.5 (0.007)
N-NH_3, mg/dL			−0.751 (<0.001)				−0.751 (<0.001)	
Acetate, % molar				−1.47 (<0.007)				−0.910 (0.005)
A:P ratio				7.16 (0.017)				
Fit statistics								
n	102	102	59	51	41	102	59	51
Mean (model)	43.7	43.7	39.5	36.4	34.7	43.7	39.5	36.4
$\hat{\sigma}_s$	13.3	15.0	14.4	12.1	22.9	13.6	14.4	13.0
$\hat{\sigma}_e$	3.08	2.57	2.67	1.78	1.17	2.57	2.67	2.07
$\hat{\sigma}_s/\hat{\sigma}_e$	4.31	5.85	5.41	6.79	19.6	5.29	5.41	6.27
$\hat{\sigma}_s$/mean	30.4	34.4	36.6	33.1	66.1	31.0	36.6	35.7
$\hat{\sigma}_e$/mean	7.05	5.88	6.77	4.88	3.37	5.87	6.77	5.69
AICc	739.0	737.0	416.5	339.2	257.3	715.9	416.5	349.7
CCC	0.204	0.179	0.123	0.382	0.070	0.223	0.137	0.377

[1]UUA = urea N used for anabolism; GER = gastrointestinal entry rate.

[2]There was no adjustment for beef cattle species, DMI, NDF, ME, VFA total, propionate, and butyrate; A:P ratio = acetate:propionate ratio; $\hat{\sigma}_s$ = SD for study; $\hat{\sigma}_e$ = SD for error; AICc = corrected Akaike information criterion; CCC = concordance correlation coefficient obtained in the Monte Carlo analysis.

[3]There was no adjustment for any ruminant species.

Table 14. Parameter estimates in the INC:GER (incorporation of recycled urea N into microbial N relative to gastrointestinal entry rate of urea N, %) models using dietary characteristics (DC) and ruminal factors (RF); *P*-values are given in parentheses[1]

Item[2]	DC	RF
Model	1	2
Intercept	15.6 (0.018)	44.1 (<0.001)
TDN/RDP ratio, g/g	1.42 (<0.001)	
N-NH_3, mg/dL		−1.35 (<0.001)
Fit statistics		
n	43	24
Mean (model)	28.5	34.7
$\hat{\sigma}_s$	15.5	14.8
$\hat{\sigma}_e$	3.02	2.06
$\hat{\sigma}_s/\hat{\sigma}_e$	5.12	7.14
$\hat{\sigma}_s$/mean	54.3	42.5
$\hat{\sigma}_e$/mean	10.6	5.95
AICc	332.2	173.8
CCC	0.151	0.187

[1]INC = incorporation of recycled urea N into microbial N; GER = gastrointestinal entry rate.

[2]There was no adjustment for OM, CP, RDP, NDF, ADF, lignin, starch, TDN, ME, pH, VFA total, acetate, propionate, butyrate, and acetate:propionate ratio; $\hat{\sigma}_s$ = SD for study; $\hat{\sigma}_e$ = SD for error; AICc = corrected Akaike information criterion; CCC = concordance correlation coefficient obtained in the Monte Carlo analysis.

to a reduction in the probability of utilization of endogenous N relative to the ammonia supply from dietary protein degradation (Reynolds and Kristensen, 2008).

Implications and Limitations

In this study, we provided evidence for differences in the fate of urea in the body across RS. However, whether these differences are related to the intrinsic N metabolism of each species or due to the diet variation remains unclear. Understanding these differences could lead to improvements in N use efficiency in ruminant diets by formulating more accurate and precise low-N diets considering the particularities for each species. Apart from the variation across species, we provided evidence that dietary and ruminal factors other than protein and ammonia play an important role in the production and utilization of urea recycled in the body, with particularly important evidence supporting relationships for energy supply and ruminal pH. Our study provided a better understanding of the factors affecting urea kinetics and microbial usage of recycled which is useful to improve the estimation of N supply and N requirements by future protein models in ruminants that accounted for urea recycling.

Despite efforts to derive comprehensive models explaining physiological variation in urea kinetics, there are several limitations in this study that should be considered before results are used out of context. No evaluation of model predictive capacity was undertaken and models were not designed to be used in ration formulation exercises or other predictive capacities. Although the results of cross-validation are presented in the supplemental materials, cross-validation is an imperfect means of model evaluation for predictive purposes and should not be considered as ratification of any models derived in this work as acceptable for naïve prediction. Additionally, the models herein used the data available, which has limitations in terms of complete factorial overlap in variables, making assessment of interactions among factors challenging. Further experimental work, assessing dietary interactions in particular, is needed to confirm some conflicting and confounding relationships identified in this work. Finally, we evaluated only linear relationships due to the number of candidate models derived. Future work with additional experimental data should consider nonlinearity in responses and specifically evaluate how dietary, ruminal, species, and experimental parameters influence the shape of nonlinear relationships (e.g., between urea kinetics and CP supply). Unfortunately, such analysis is outside the scope of the present study and the available data.

The urea kinetics leveraged were obtained exclusively from studies using the doubly labeled urea technique and are reflective only of measurements obtained from that technique. There are several experimental strategies for measuring these urea kinetics and other strategies may result in different revealed dynamics. The effect of measurement method on the relationships revealed herein is a subject of future work.

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