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# Relationship between urinary energy and urinary nitrogen or carbon excretion in lactating Jersey cows

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## ABSTRACT

Measurement of urinary energy (UE) excretion is essential to determine metabolizable energy (ME) supply. Our objectives were to evaluate the accuracy of using urinary N (UN) or C (UC) to estimate UE and ultimately improve the accuracy of estimating ME. Individual animal data (n = 433) were used from 11 studies with Jersey cows at the University of Nebraska–Lincoln, where samples were analyzed after drying (n = 299) or on an as-is basis (n = 134). Dried samples resulted in greater estimated error variance compared with as-is samples, and thus only as-is samples were used for final models. The as-is data set included a range (min to max) in dry matter intake (11.6–24.6 kg/d), N intake (282–642 g/d), UE excretion (1,390–3,160 kcal/d), UN excretion (85–220 g/d or 20.6–59.5% of N intake), and UC excretion (130–273 g/d). As indicated by a bias in residuals between observed and predicted ME as dietary crude protein (CP; range of 14.9–19.1%) increased, the National Research Council dairy model did not accurately predict ME of diets, as dietary CP varied. The relationship between UE (kcal/d) and UN (g/d) excretion was linear and had an intercept of 880 ± 140 kcal. Because an intercept of 880 is biologically unlikely, the intercept was forced through 0, resulting in linear and quadratic relationships. The regressions of UE (kcal/d) on UN (g/d) excretion were UE = 14.6 ± 0.32 × UN, and UE = 20.9 ± 1.0 × UN − 0.0357 ± 0.0056 × UN$^2$. In the quadratic regression, UE increased, but at a diminishing rate as UN excretion increased. As UC increased, UE linearly and quadratically increased. However, error variance was greater for regression with UC compared with UN as explanatory variables (8.42 vs. 7.42% of mean UE). The use of the quadratic regression between UN and UE excretion to predict ME resulted in a slope bias in ME predictions as dietary CP increased. The linear regression between UE and UN excretion removed slope bias between predicted ME and CP, and thus may be more appropriate for predicting UE across a wider range of dietary CP. Using equations to predict UE from UN should improve our ability to predict diet ME in Jersey cows compared with calculating ME directly from digestible energy.
**Key words:** metabolizable energy, bomb calorimetry, energy metabolism, regression

## INTRODUCTION

Accurate estimation of dietary energy supply is essential to predict performance of lactating dairy cows. Considerable work has been completed regarding estimation of digestible energy (**DE**) of diets by estimating the digestibility of individual nutrients (Weiss and Tebbe, 2019). Work has also been completed regarding the estimation of $CH_4$ production (Appuhamy et al., 2016). However, minimal work has been completed to estimate urinary energy (**UE**) loss in dairy cows, which is needed to calculate ME. Historically, empirical equations have been used to estimate ME from DE. The National Research Council (**NRC**) calculates dietary ME content (Mcal/kg of DM) as 1.01 × DE (Mcal/kg of DM) − 0.45, with a slight correction for fat to account for a 100% efficiency in conversion of DE to ME (NRC, 2001). For typical dairy diets, the efficiency of converting DE to ME averages about 85%. Urinary energy represents 5 to 7% of DE supply or up to 50% of the energy difference between DE and ME (Drehmel et al., 2018; Judy et al., 2018; Reynolds et al., 2019). Because increasing dietary CP increases UE excretion (Hynes et al., 2016), the efficiency of converting DE to ME for a diet with excess CP can be lower than a diet in which CP is closer to requirements. In many current nutrition models, variation in dietary CP does not contribute to variability in efficiency of converting DE to ME.

Estimating UE from urinary N (**UN**) would be useful because UN is more commonly measured than UE

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in a research setting, and UE is needed to accurately estimate ME. In addition to UN, urinary C (**UC**) concentration is correlated with UE concentration (Blaxter, 1989). The relationship between UN or UC and UE is a function of the relationship between the heat of combustion of the energy-containing molecules and the respective N or C contents. Variation in the heat of combustion per unit of N is considerably greater than that of C, and thus UC is thought to be a better predictor of UE (Blaxter et al., 1966). The heat of combustion (kcal/g of N) of major energy-containing molecules in urine (calculated from NIST, 2020) are as follows: urea (5.4), allantoin (7.3), hippuric acid (71.7), creatinine (13.2), creatine (13.2), uric acid (8.2), xanthine (9.2), hypoxanthine (10.3), and AA (average 35.3). Historical estimates suggest that on average UE equaled 14.3 kcal/g of N (Blaxter, 1989). Because urea and hippuric acid have the lowest and greatest UE-to-UN ratios, respectively, the UE-to-UN ratio of urine is sensitive to their excretions. Urinary excretion of hippuric acid, which is formed from the conjugation of dietary derived benzoic acid with glycine (Martin, 1982), is correlated with DMI (Blaxter et al., 1966). The proportion of UN that is excreted as urea increases with increasing UN excretion by dairy cattle (Spek et al., 2013b); consequently, increasing proportion of UN as urea should decrease the heat of combustion of urine per gram of N. For the same molecules listed above, UE-to-UC ratios are between 7 and 13 kcal/g of C and averaged 10 kcal/g of C in cattle and sheep (Blaxter et al., 1966). Additionally, in cattle, average mean squared error of regressions between UE and UN or UC were 16.4 and 5.3% of mean UE, respectively (Blaxter et al., 1966). Furthermore, urine is often dried before analysis of N and energy, which may lead to N and energy loss due to potential N volatilization (Jacobs et al., 2011). Therefore, the objectives of the current work were to develop an equation to describe the relationship between UN or UC and UE concentration and excretion. We hypothesized that accounting for UE when predicting ME can improve our ability to predict dietary ME compared with predicting ME from DE, and that residual variation would be lower when using UC compared with UN to explain UE. A secondary objective of this work was to determine if drying urine samples before analysis affected equation precision.

## MATERIALS AND METHODS

### Data Collection

Individual cow data were sought because urinary excretion and N are measured on an individual animal basis, and UE is predicted on an individual animal basis that is not on a treatment basis. Additionally, data with required variables were limited, and by using individual animal data, number of observations was increased. We identified 11 studies conducted at the University of Nebraska–Lincoln's Dairy Metabolism Unit (Lincoln, NE) from 2013 to 2019 where Jersey cows were used. In total, 433 observations (cow-periods) were assembled from 91 cows from experiments that used 2 to 4 periods.

Across all 11 studies, urine output was measured for 4 d via total collection using a size 30 French Foley bladder catheter. Two distinct methods were used to quantify UE and UN. For studies 1 to 7 (n = 299), acidified urine samples were boiled in a hot water bath to remove moisture, and the resulting paste was freeze dried to remove most of the remaining moisture (Drehmel et al., 2018). Dried samples were then analyzed for gross energy content using an isoperibol bomb calorimeter (Parr 6400 Calorimeter) and N content (FlashSmart N/Protein Analyzer, CE Elantech Inc.; AOAC International, 2000, method 990.03). For studies 8 to 11 (n = 134), urine samples were not boiled and freeze-dried before analysis. Gross energy content was determined after drying approximately 4 g of sample in a bomb capsule at 60°C until dry (~4 h). This method was similar to the method used by Jacobs et al. (2011), who determined UE of swine urine on undried or dried samples. To determine N concentration of urine in studies 8 to 11, samples were submitted to Cumberland Valley Analytical Services Inc. (Waynesboro, PA) for analysis on an as-is basis using a combustion method (Leco FP-528 N Combustion Analyzer; Leco Corp.). Urine samples for studies 8 to 11 were analyzed for C using a combustion method (Flash 2000; Thermo Fisher Scientific) following the procedure of urine processing described in Morris et al. (2019). The method of urine acidification differed between studies 1 to 7 and 8 to 11. In studies 1 to 7, 50 to 100 mL of 12 *N* HCl was added to urine collection containers 1 to 2 times per day. For studies 8 to 11, 6 *N* HCl (approximately 800–1,200 mL) was added to the urine collection container at the beginning of the daily collection. Urine pH was measured at the end of each day, and quantity of acid used was adjusted to maintain a pH <5.0. The sample collection and handling method (dried for 7 or as-is for 4 studies) was coded into the data and set as a categorical variable. The as-is data set contained 134 observations from 32 cows across 14 periods.

In the full data set, DE and ME were measured via total collection of feces and urinary excretion; bomb calorimetry of feeds, feces, and urine; and quantification of $CH_4$ production via headbox-style indirect

calorimetry (Drehmel et al., 2018; Judy et al., 2018; Reynolds et al., 2019).

### Model Derivation and Evaluation

Dietary DE and ME are calculated in nutrition models on a diet basis (NRC, 2001), and thus evaluation of methods to predict ME should be evaluated on a diet basis. In the current data set, treatments represented unique diets. To create an evaluation data set, means for individual treatments (n = 45) were derived from the full data set. To evaluate the NRC calculation of ME, residuals between observed ME and ME calculated from DE using NRC equation 2-10 (NRC, 2001) were calculated and regressed against dietary CP. Intercept, slope biases, and residual mean squared error as a percent of mean were evaluated using the lm function of R (3.6.2, https://www.r-project.org/).

Models were fitted using R with the lmerTest function (Kuznetsova et al., 2017). Models included UE as a response variable and either UN or UC as explanatory variables. The fixed effect of method (dried or as-is) and interaction of method with UN were included in initial models. To account for the variation associated with using individual animal observations, all models included the random effects of cow, period within study, and study. Study effect was generally equal to 0 and was removed from final models.

Rather than reporting root mean squared error, we chose to report the square root of the estimated variance associated with cow, period within study, and error. The units for all variance estimates are the same units as the response variable. Variance estimates were expressed as a percent of the mean of the response variable. Slope bias was evaluated by regressing residual against predicted values and comparing the slope coefficient to 0.

To evaluate application of the derived models to improve our ability to estimate ME, the same method described above to evaluate the NRC (2001) model was used except ME was predicted by subtracting estimated UE and $CH_4$ energy from measured DE. Urinary energy was estimated using each equation we derived, and $CH_4$ energy for each treatment was estimated as 0.294 × DMI (kg/d) − 0.347 × dietary crude fat (% DM) + 0.0409 × NDF digestibility (%; Nielsen et al., 2013).

## RESULTS AND DISCUSSION

### Evaluation of NRC Equation to Predict ME

Dietary ME content (Mcal/kg of DM) in NRC (2001) is calculated from DE with a slight correction for dietary fat to account for 100% efficiency in conversion of DE to ME for fat energy. This approach does not account for variation in CP intake, which is known to be positively correlated with UE (Hynes et al., 2016). The NRC (2001) model underpredicted ($P < 0.01$) ME content by 0.070 Mcal/kg of DM (2.7% of mean ME) on average (Figure 1). As dietary CP increased, residual ME decreased (slope $P = 0.01$), which occurred because CP was positively correlated with UE (r = 0.57; $P < 0.01$; data not shown). Therefore, the positive relationship between dietary CP and UE should be accounted for when predicting ME.

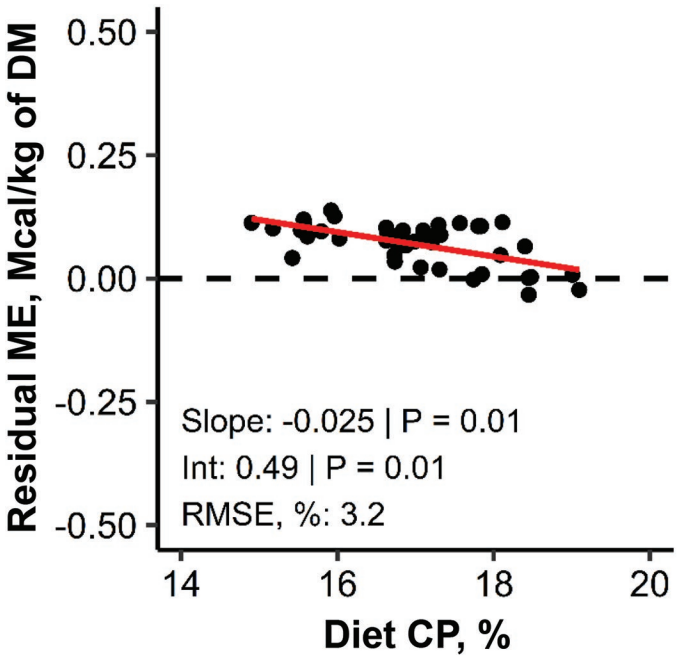


**Figure 1.** Residual (observed − predicted) ME (Mcal/kg of DM) versus dietary CP (%). Predicted ME was calculated from observed digestible energy using NRC (2001) equation 2-10. Each data point represents a treatment mean from the full data set (n = 45). Slope and intercept (int) estimate as well as corresponding *P*-values for the linear regression (red line) are listed at the bottom of the figure. RMSE = residual mean squared error expressed as a percent of mean observed ME.

### Effect of Method of Urine Collection and Processing

Intercepts and slope for regressions between UE and UN differed by method ($P < 0.02$; data not shown). Additionally, variance estimates, in general, were at least 2-fold greater for the full data set compared with when the same regressions were generated using the as-is data set (i.e., samples were acidified to pH <5.0 during collection, and analysis was completed on

**Table 1.** Descriptive statistics of the data used for determining the relationship between urinary energy and N excretion in lactating Jersey cows when urine was analyzed as-is (n = 131–134)[1]

| Item | Mean | SD | Minimum | Maximum |
|---|---|---|---|---|
| Animal description | | | | |
| DIM, d | 209 | 63 | 88 | 346 |
| Parity | 3.04 | 1.14 | 2.00 | 6.00 |
| BW, kg | 461 | 54 | 363 | 606 |
| BCS[2] | 3.24 | 0.40 | 2.00 | 4.13 |
| DMI, kg/d | 18.3 | 2.4 | 11.6 | 24.6 |
| Milk yield, kg/d | 21.5 | 4.8 | 10.8 | 34.3 |
| ECM,[3] kg/d | 29.4 | 5.6 | 14.8 | 48.2 |
| Fat yield, kg/d | 1.26 | 0.25 | 0.61 | 2.28 |
| Protein yield, kd/d | 0.80 | 0.15 | 0.46 | 1.21 |
| Urine | | | | |
| Excretion, kg/d | 23.8 | 5.3 | 15.3 | 46.1 |
| N, % | 0.686 | 0.136 | 0.203 | 0.977 |
| C, % | 0.869 | 0.187 | 0.334 | 1.25 |
| Energy, kcal/g | 0.104 | 0.021 | 0.035 | 0.155 |
| Energy, kcal/d | 2,381 | 314 | 1,390 | 3,160 |
| N utilization, g/d | | | | |
| Intake | 486 | 66 | 285 | 642 |
| Feces | 176 | 29 | 111 | 249 |
| Milk | 141 | 27 | 80 | 217 |
| Urine | 158 | 24 | 85 | 220 |
| Urine, % of N intake | 32.7 | 4.61 | 20.6 | 59.5 |
| Balance[4] | 12 | 30 | −98 | 88 |
| Urine C excretion, g/d | 199 | 24.5 | 130 | 273 |
| Dietary composition | | | | |
| DE, Mcal/kg DM | 2.91 | 0.14 | 2.49 | 3.29 |
| ME, Mcal/kg DM | 2.57 | 0.14 | 2.17 | 2.95 |
| CP, % DM | 16.6 | 0.74 | 14.8 | 17.6 |
| NDF, % DM | 32.1 | 3.8 | 21.4 | 43.0 |
| Starch, % DM | 26.4 | 3.7 | 16.2 | 35.7 |
| Crude fat, % DM | 4.4 | 1.0 | 2.2 | 6.9 |
| S, % DM | 0.28 | 0.01 | 0.26 | 0.30 |

[1]n = number of total observations from all 4 studies in which samples were acidified to pH <5.0 during collection and analysis was completed on samples that were not dried.

[2]On a 1 to 5 scale.

[3]ECM = 0.327 × milk yield (kg) + 12.95 × fat (kg) + 7.20 × true protein (kg; Tyrrell and Reid, 1965).

[4]Calculated as N intake − fecal N − milk N − urinary N.

samples that were not dried). Boiling and drying might have increased N or energy loss (or both) from samples before analysis, and the additional step of the sample drying process might have induced additional variation into UE and UN measurements. Adequate acidification is essential to prevent N loss (Spanghero and Kowalski, 1997). Samples collected and analyzed via the dried method may not have been adequately acidified to prevent N loss; pH was not measured on these samples. Therefore, we only used data collected and analyzed via the as-is method, where samples were acidified to a pH <5.0 during collection. Descriptive statistics for this data set are reported in Table 1.

### Relationship Between UE and N or C

Urinary N and C are associated with energy-containing compounds and are correlated with UE (Street et al., 1964; Blaxter et al., 1966). Our objective was to evaluate the relationship between UE and UN or UC in lactating Jersey cows and generate equations to estimate the error associated with estimating UE from UN or UC. Additionally, we were interested in deriving equations that could predict UE with adequate precision and accuracy. A strong relationship was observed between UE and UN concentration; UE (kcal/g) = 0.127 ± 0.0074 UN (g/100 g) + 0.0165 ± 0.0054 (equation 1; Figure 2). From 402 data points from growing and lactating cattle and sheep, Street et al. (1964) reported that UE (kcal/g) = 0.117 UN (g/100 g) + 0.026. Across the range of our data set, these 2 equations closely agreed in terms of estimating UE from UN.

Estimating UE from UN is useful because UN is more commonly measured than UE. When regressing UE (kcal/d) on UN (g/d) excretion with an intercept (equation 2), the quadratic term was not different from 0 ($P = 0.27$) and was thus removed; the intercept was 880 ± 139 kcal/d (Figure 2). The observed intercept of 880 g/d in UN equation 2 is biologically unlikely because the quantity of organic compounds in urine

that do not contain N is extremely small. The large intercept value for UN equation 2 is likely due to assuming linearity all the way to 0 g/d of N excretion. The minimum UN excretion in this data set was 80 g/d. Some UE can originate from N-free molecules such as hydrogen sulfide and ketones; however, in general, quantity of S and ketones excreted in urine is small (Huhtanen et al., 1993; Morris et al., 2018). Further regressions were fitted by assuming an intercept of 0. In a linear regression without an intercept term (equation 3), the relationship between UE and UN excretion was 14.6 ± 0.32 kcal/g of N. A similar value of 14.3 kcal/g of N was reported for ruminants, although species was not specified (Blaxter, 1989). In residual analysis of UN equation 3, UE was underpredicted below the mean UN and overpredicted above the mean ($P = 0.07$; Figure 3), which likely occurred because the regression was forced through 0. When the intercept was forced through 0, the quadratic term was different from 0 (equation 4; $P < 0.01$; Figure 2). With increasing UN in equation 4, UE increased but at a diminishing rate. The first derivatives of equation 4 at 100, 150, and 200 g of UN were 13.8, 10.2, and 6.6 kcal/g if N, respectively. Slope bias was not different ($P = 0.12$) from 0 in equation 4 (Figure 3).

The quadratic relationship between UE and UN excretion suggests the UE per unit of UN decreases with increasing UN excretion. To better understand this relationship, we then fit additional regressions with UE (expressed as kcal/g of N) as the response variable and UN as the explanatory variable (Figure 4). As UN excretion (g/d) increased, UE per gram of N decreased (equation 5). The predicted ratio of UE to UN at 100, 150, and 200 g of UN excretion was 17.7, 15.6, and 13.6 kcal/g of N, respectively. A decreasing UE-to-UN ratio with increasing UN excretion supports the notion that, as UN excretion increases, compounds with high enthalpies per gram of N (i.e., hippuric acid, free AA,

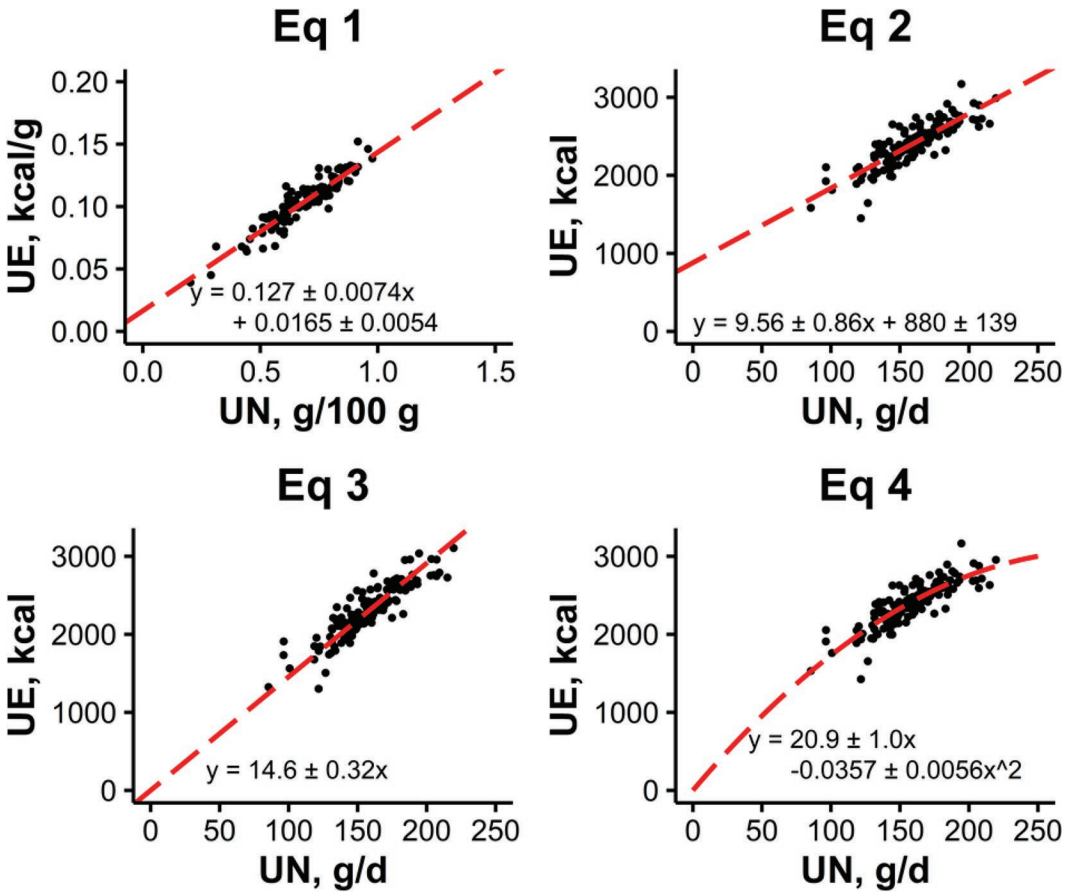


**Figure 2.** Relationship between urinary energy (UE) concentration or excretion and urinary N (UN) concentration or excretion for lactating Jersey cows for equations 1, 2, 3, and 4 (n = 134). Intercept was forced through 0 in equations 3 and 4. All linear and quadratic coefficients were different from 0 ($P < 0.01$). Standard errors are reported as the ± value. Refer to Table 2 for equation fit statistics. All data are reported as original data corrected for the random effects of cow and period within study.

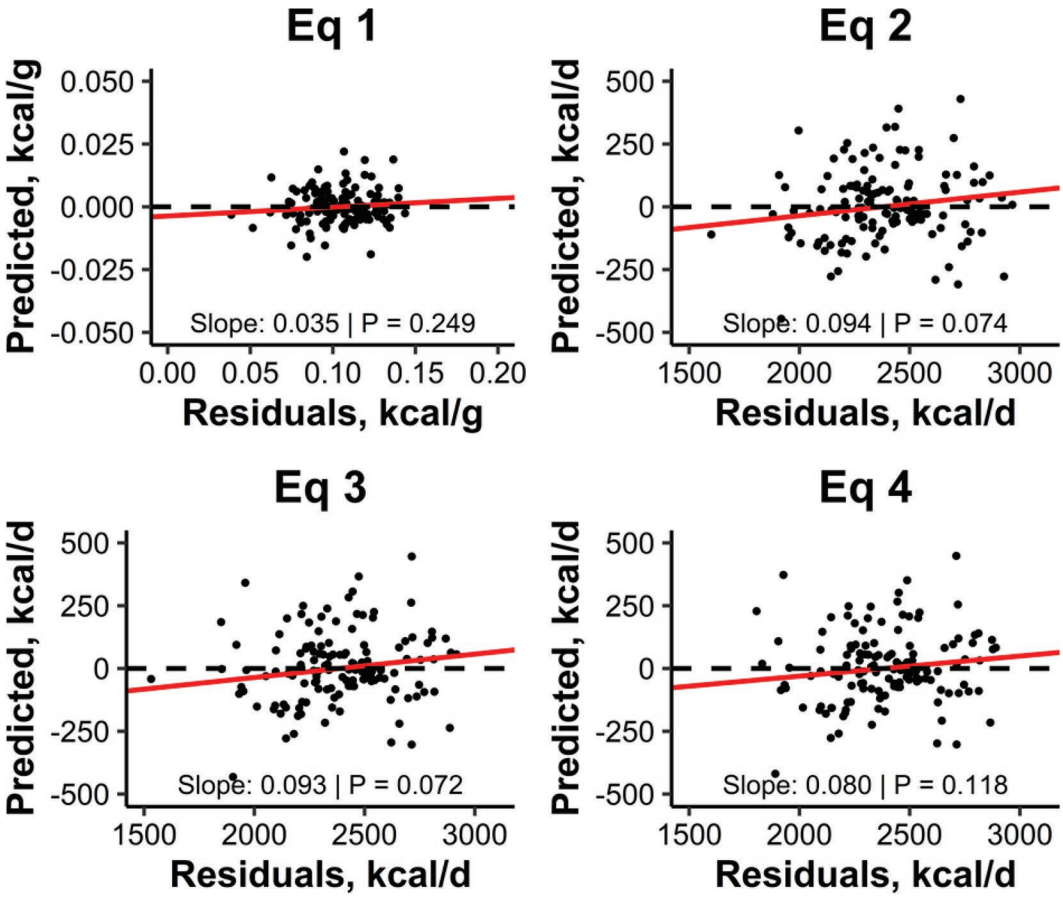


**Figure 3.** Residuals (observed − predicted) versus predicted urinary energy concentration or excretion for relationship with urinary N concentration or excretion for equations 1, 2, 3, and 4 (Figure 2; n = 134). Slope estimate and *P*-values for the slope of the linear relationship are listed at the bottom of the figure. All data are reported as original data corrected for the random effects of cow and period within study.

creatinine, and creatine) are diluted by urea, which has a heat of combustion of 5.4 kcal/g of N. In a meta-regression from which dairy breed was not specified, when urea N excretion was regressed on UN excretion, the intercept term, which represents the quantity of daily nonurea N excretion, was 51.9 ± 4.42 g/d (Spek et al., 2013a). However, nonurea N excretion is likely related to DMI. Increased DMI is likely correlated with urinary excretion of purine derivatives and hippuric acid via increased microbial protein synthesis and absorption of hippuric acid precursors, respectively (Spek et al., 2013a). Furthermore, UN and urea N excretion are linearly related (Spek et al., 2013b), and urea N excretion increases with dietary CP supply. Therefore, at similar DMI, as UN excretion increases, the proportion of UN that is urea, in theory, increases asymptotically toward 100%. Thus, the heat of combustion of urine per gram of N will decrease toward that of urea. Bristow et al. (1992) measured the major constituents of urine from 10 Holstein dairy cows and reported that the nonurea N portion was on average 24.4 ± 6.3% hippuric acid, 28.7 ± 14.5% allantoin, 5.1 ± 1.5% uric acid, 2.0 ± 0.7% xanthine and hypoxanthine, 15.1 ± 4.5% creatinine, 10.7 ± 4.5% creatine, 3.5 ± 5.0% AA, and 10.4 ± 13.3% ammonia. The nonurea N proportion has an average heat of combustion of combustion of 24.8 ± 5.4 kcal/g of N, which is much larger than the that of urea (5.4 kcal/g; calculated from Bristow et al., 1992 and NIST, 2020). Urinary urea excretion was not measured in the current study. In future work, measuring total N and urea N may improve our understanding of the relationship between UE and UN excretion.

Excretion of some nonurea N compounds in urine is likely a function of BW and DMI. The total mass of urinary excretion of creatinine is derived from muscle turnover and is linearly associated with BW (Brody, 1945). However, the heat of combustion of creatinine is 13.2 kcal/g of N, which is similar to the average of our data (14.6 from equation 3). Thus, changes in excretion of creatinine likely has little effect on heat of combustion of urine per gram of N. Urinary purine derivatives (e.g., allantoin, uric acid, xanthine, and

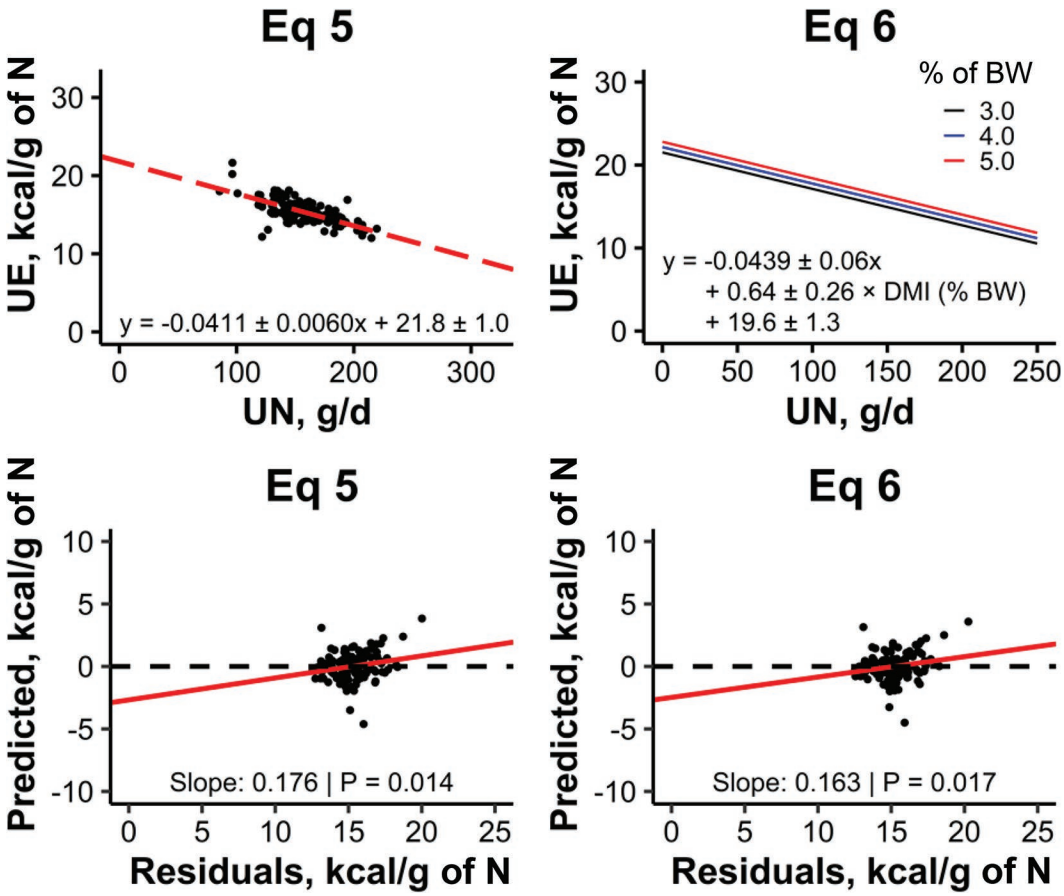


**Figure 4.** Relationship between urinary energy (UE) per gram of N and urinary (UN) excretion or concentration for lactating Jersey cows for equations 5 and 6, and residual (observed − predicted) versus predicted values (n = 134). All coefficients were different from 0 ($P < 0.01$). Coefficient SE are reported as the ± value. Slope estimate and *P*-values for the slope of the linear relationship for residuals versus predicted plots are listed at the bottom of the figure. All data are reported as original data corrected for the random effects of cow and period within study.

hypoxanthine) are derived from absorbed microbial protein (Gonzalez-Ronquillo et al., 2003), which, along with dietary chemical composition, is driven by DMI (Roman-Garcia et al., 2016). As energy retention and DMI increase, percentage of UN as hippuric acid and kilocalorie per gram of N for urine increased in sheep (Blaxter et al., 1966). We tested the effects of DMI and BW on UE (kcal/g of N) by adding DMI (% of BW). The addition of DMI decreased $\hat{\sigma}_e$ (7.81 vs. 7.98% of mean; Table 2). Substantially increasing DMI (1% of BW) only increased UE by 0.64 ± 0.28 kcal/g of N (equation 6; Figure 4). Therefore, the effect of DMI is generally small. At the average UN excretion (158 g/d), increasing DMI from 4 to 5% of BW increased UE by 101 kcal/d or 0.2% of average DE intake for the data set.

Historically, UE has been estimated from UC (Blaxter et al., 1966). In the current data set, UE and UC concentration were linearly associated with a regression that was UE (kcal/g) = 0.0959 ± 0.0050 UC (g/100 g) + 0.0211 ± 0.0046 (Figure 5; equation 7). When regressing UE (kcal/d) on UC (g/d) excretion with an intercept (equation 8), the quadratic term was not different from 0 ($P = 0.17$) and was thus removed. The intercept was 1,180 ± 182 g/d. Similar to equation 2, a large positive intercept is not biologically founded, and thus further models were fitted by assuming an intercept of 0. When regressing UE (kcal/d) on UC (g/d) excretion without an intercept term (equation 9), the relationship between UE and UC was 11.9 ± 0.22 kcal/g of C. This value is in the range (7–13 kcal/g of C) of UE-to-UC ratio of urinary compounds, but slightly greater than the value of 10 kcal/g of C reported for cattle and sheep (Blaxter et al., 1966). When using the urinary compound concentration reported by Bristow et al. (1992), the calculated ratio between UE and UC was 10.5 kcal/g of C. A quadratic relationship was also observed when regressing UE on UC without an intercept (equation 10); however, unlike when regressing UE on UN, this quadratic relationship for UE versus UC is

**Table 2.** Fit statistics[1] for models to explain the relationship between urinary energy (UE) and urinary N (UN) or urinary C (UC) concentration and excretion

| Equation[2] | AICc | $\hat{\sigma}_c$ | $\hat{\sigma}_c$, % of mean | $\hat{\sigma}_{p(s)}$ | $\hat{\sigma}_{p(s)}$, % of mean | $\hat{\sigma}_e$ | $\hat{\sigma}_e$, % of mean |
|---|---|---|---|---|---|---|---|
| Urinary N | | | | | | | |
| 1 | −835 | 0.0061 | 5.84 | 0.0054 | 5.21 | 0.0078 | 7.50 |
| 2 | 1,806 | 119 | 5.01 | 95 | 3.98 | 171 | 7.21 |
| 3 | 1,848 | 121 | 5.10 | 161 | 6.75 | 189 | 7.93 |
| 4 | 1,826 | 124 | 5.22 | 92 | 3.88 | 170 | 7.14 |
| 5 | 499 | 0.954 | 6.27 | 0.497 | 3.27 | 1.21 | 7.98 |
| 6 | 497 | 0.912 | 5.99 | 0.569 | 3.74 | 1.19 | 7.81 |
| Urinary C | | | | | | | |
| 7 | −806 | 0.0032 | 3.04 | 0.0052 | 4.99 | 0.0089 | 8.52 |
| 8 | 1,819 | 149 | 6.23 | 100 | 4.21 | 186 | 7.80 |
| 9 | 1,859 | 30.9 | 1.30 | 150 | 6.28 | 232 | 9.73 |
| 10 | 1,855 | 157 | 6.57 | 100 | 4.19 | 184 | 7.73 |

[1]Number of observations = 134; AICc = corrected Akaike information criterion, $\hat{\sigma}_c$ = square root of the estimated variance associated with cow, $\hat{\sigma}_{p(s)}$ = square root of the estimated variance associated with period within study, $\hat{\sigma}_e$ = square root of the estimated residual variance.
[2]Equation 1 = linear regression between UE (kcal/g) and UN or UC (g/100 g); equation 2 = linear regression between UE (kcal/d) and UN or UC (kcal/d); equation 3 = linear regression between UE (kcal/d) and UN or UC (kcal/d) with a 0 intercept; equation 4 = quadratic regression between UE (kcal/d) and UN or UC (kcal/d) with a 0 intercept; equation 5 = linear regression between UE (kcal/g of N) and UN (kcal/d); equation 6 = linear regression between UE (kcal/g of N) and UN (kcal/d) plus DMI (% of BW).

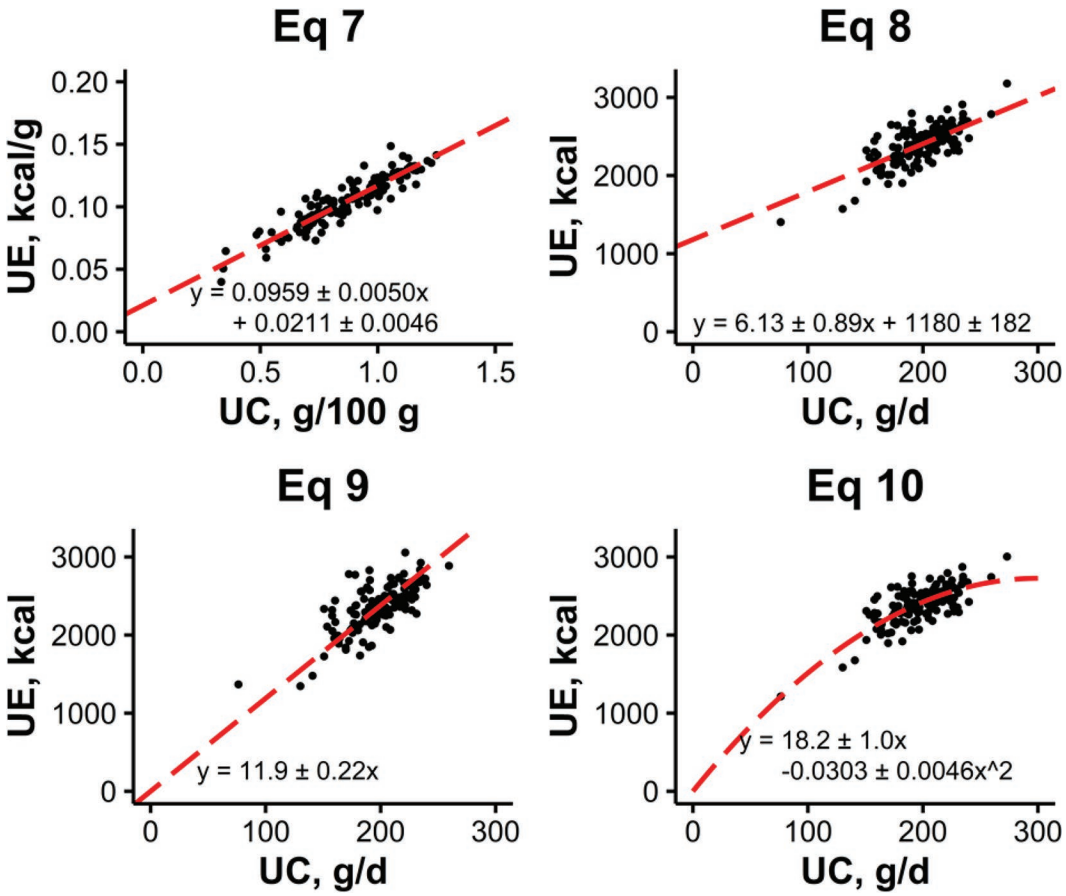


**Figure 5.** Relationship between urinary energy (UE) concentration or excretion and urinary C (UC) concentration or excretion for lactating Jersey cows for models 1, 2, 3, and 4 (n = 134). Intercept was forced through 0 in equations 3 and 4. All linear and quadratic coefficients were different from 0 ($P < 0.01$). Coefficient SE are reported as the ± 28 value. All data are reported as original data corrected for the random effects of cow and period within study.

not biologically found. As discussed for equations based on UN, excretion of urinary urea is more variable than the nonurea fraction (Bristow et al., 1992; Spek et al., 2013b), and the heat of combustion of urea is greater than the heat of combustion of the nonurea fractions of urine (12.7 vs. 9.5 kcal/g of C). Therefore, as UC and urea excretion increase, UE should increase at an increasing rate and a positive quadratic term would be expected. However, urea account for 33% of urinary C excretion on average (Calculated from Bristow et al., 1992). Because urea only contains 1 C, increasing urea excretion will have a small effect on the proportion of UC that is from urea. A linear relationship between UE and UC is most logical.

Based on the work of Blaxter et al. (1966), we expected $\hat{\sigma}_e$ to be less when regressing UE on UC compared with UN. However, $\hat{\sigma}_e$ for equations 2 to 4 was on average 1.0 percentage units lower compared with equations 8 to 10 (7.43 vs. 8.42% of the mean; Table 2). Blaxter et al. (1966) reported that predicted variance for estimating UE with UC for cattle and sheep was 3.9% of mean and did not predict variance for UN because the relationship was nonsignificant. The precision in estimating UE from UN is likely much improved compared with the work of Blaxter et al. (1966) because methods of determining N have been improved considerably since the 1960s. In the data set used by Blaxter et al. (1966), UN was determined by the Kjeldahl method, which has been recently shown to have a more than 3-fold greater analytical variation resulting from incomplete recovery of some N-containing compounds compared with modern combustion methods that were similar to those used in the current data set (Bremner and Mulvaney, 1982; Morris et al., 2019). Additionally, variation was likely inflated in the Blaxter et al. (1966) experiment when UN was used because sheep were fed diets that contained as much as 35% CP. The slope bias was observed for equations 8 and 9 (Figure 6), which further supports the use of UN rather than UC to estimate UE.

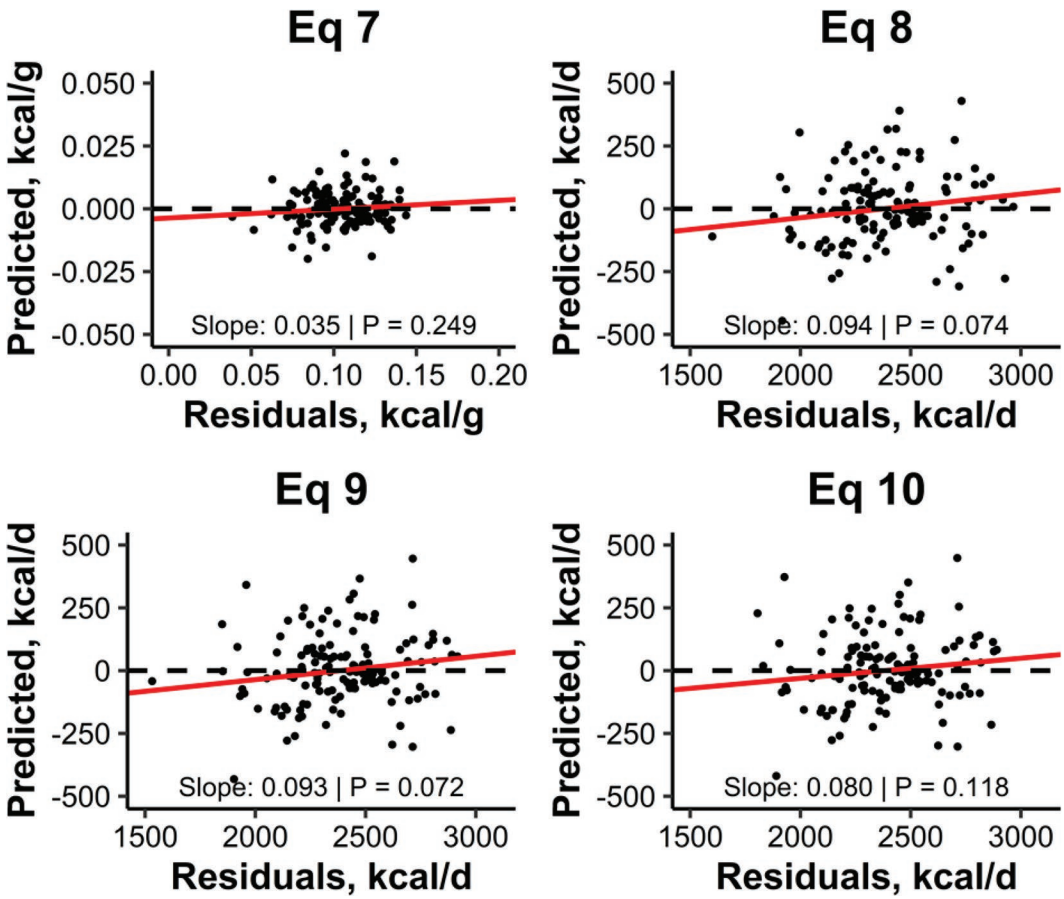


**Figure 6.** Residuals (observed − predicted) versus predicted urine energy concentration or excretion for relationship with urinary C concentration or excretion for models 1, 2, 3, and 4 (Figure 4; n = 134). Slope estimate and *P*-values for the slope of the linear relationship are listed at the bottom of the figure. All data are reported as original data corrected for the random effects of cow and period within study.

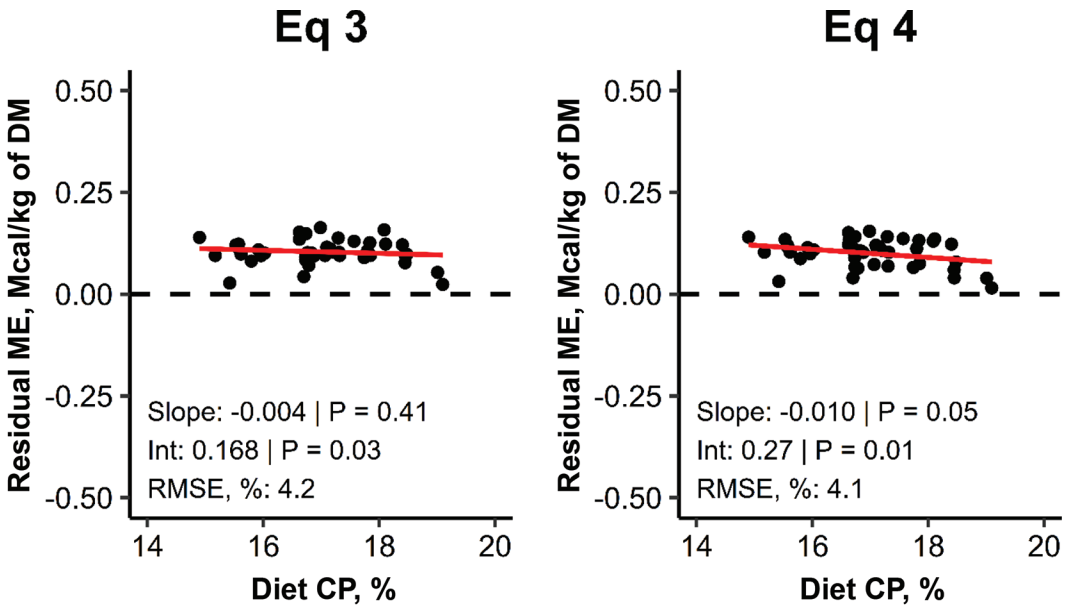


**Figure 7.** Residual (observed − predicted) ME (Mcal/kg of DM) versus dietary CP (%). Predicted ME was calculated from observed digestible energy using urinary N equations 3 or 4 (Figure 2) to estimate urinary energy, and $CH_4$ energy estimated as 0.294 × DMI (kg/d) − 0.347 × dietary crude fat (% DM) + 0.0409 × 38 NDF digestibility (%; Nielsen et al., 2013). Each data point represents a treatment mean from the full data set (n = 45). Slope and intercept (int) estimate as well as corresponding *P*-values are listed at the bottom of each figure. RMSE = residual mean squared error expressed as a percent of mean observed ME.

### *Evaluation of Using Estimated UE to Predict ME*

By using the regressions generated in the current study to estimate UE excretion (equation 3 or 4; Figure 2) and previously published equations to estimate $CH_4$ energy, ME can be predicted. Because dietary energy values in nutritional models (NRC, 2001) and the $CH_4$ prediction were generated on a dietary basis, treatment means were used (n = 45). Ideally, a completely independent data set would be used to evaluation models derived in the current publication; however, data to do so are limited. When regressing residual ME (observed − predicted ME) on dietary CP, an underprediction of 0.10 Mcal/kg of DM (3.9% of ME) was observed ($P < 0.01$; Figure 7). This underprediction of ME occurred because $CH_4$ energy was overpredicted by 0.11 Mcal/kg of DM (data not shown). When UN equation 3 was used to estimate UE, slope bias was not observed ($P = 0.41$) between residual ME and diet CP. However, when UN equation 4 was used to estimate UE, a slope bias was observed ($P = 0.05$) because UE was underpredicted at high UN. In the data set used for evaluation, 13.3% of observations had greater UN excretion than the maximum value in the data set used to derive models. This demonstrates that equation 3 is better for determining values outside the range of data used for derivation and should be used to predict UE rather than equation 4, whereas equation 4 may be more appropriate for predicting values within the range of the current study.

The inference space for the current work is past-peak lactation Jersey cows, and these equations may not apply to other breeds or fresh cows. Compared UN excretion expressed as a function of N intake as well as UE expressed as a function of energy intake are similar across breeds (Kauffman and St-Pierre, 2001; Uddin et al., 2020). The relationship between UE and UN is similar between cattle and sheep (Street et al., 1964; Blaxter, 1989). Therefore, we speculate that the linear relationship between UE and UN (14.6 ± 0.32 kcal/g of N) might be similar among breeds, but this should be experimentally confirmed. Urinary N excretion does not differ with DIM (Spek et al., 2013b), and thus the relationship between UE and UN excretion is likely similar as stage of lactation changes. Work from the current study should be expanded upon by conducting studies with different breeds and over different stages in lactation.

## CONCLUSIONS

Current equations used by the NRC to predict ME do not account for the positive correlation between diet CP and UE excretion. For urine samples that were dried and underacidified before analysis for N and energy, error variance was 2-fold greater. As UN excretion (g/d) increased, UE excretion (kcal/d) increased quadratically such that, as N excretion increased, the

rate of increase in energy excretion diminished. This quadratic relationship occurred probably because the heat of combustion per gram of N for urea is lower than nonurea N in urine (5.4 vs. 23.9 kcal/g of N), and the proportion of urinary N from urea likely increases as UN increases. Additionally, a linear increase in UE was observed as UN increased. A linear and quadratic increase in UE excretion was observed as UC excretion increased. However, error variance was greater for regression with UC compared with UN as explanatory variables. In Jersey cows, when predicting ME using the quadratic UN equation, a slope bias was observed as CP increased. However, no slope bias was observed with the linear UN equation, and this equation was able to account for the negative relationship between ME and diet CP that is not currently accounted for in the NRC equation. Although the curvilinear relationship between UE and UN derived in the current study is biologically based, the curvilinear equation underestimated UE when datapoints were greater than those used for derivation. A linear equation to predict UE excretion from UN excretion may be more appropriate for a wider range of dietary CP.

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