Short communication: Urea hydrolysis in dairy cattle manure under different temperature, urea, and pH conditions

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ABSTRACT

The objective of the study was to quantify the rate of urea hydrolysis in dairy cattle manure under different initial urea concentration, temperature, and pH conditions. In particular, by varying all 3 factors simultaneously, the interactions between them could also be determined. Fresh feces and artificial urine solutions were combined into a slurry to characterize the rate of urea hydrolysis under 2 temperatures (15°C and 35°C), 3 urea concentrations in urine solutions (500, 1,000, and 1,500 mg of urea-N/dL), and 3 pH levels (6, 7, and 8). Urea N concentration in slurry was analyzed at 0.0167, 1, 2, 4, 6, 8, 12, 16, 20, and 24 h after initial mixing. A nonlinear mixed effects model was used to determine the effects of urea concentration, pH, and temperature treatments on the exponential rate of urea hydrolysis and to predict the hydrolysis rate for each treatment combination. We detected a significant interaction between pH and initial urea level. Increasing urea concentration from 1,000 to 1,500 mg of urea-N/dL decreased the rate of urea hydrolysis across all pH levels. Across all pH and initial urea levels, the rate of urea hydrolysis increased with temperature, but the effect of pH was only observed for pH 6 versus pH 8 at the intermediate initial urea concentration. The fast rates of urea hydrolysis indicate that urea was almost completely hydrolyzed within a few hours of urine mixing with feces. The estimated urea hydrolysis rates from this study are likely maximum rates because of the thorough mixing before each sampling. Although considerable mixing of feces and urine occurs on the barn floor of commercial dairy operations from cattle walking through the manure, such mixing may be not as quick and thorough as in this study. Consequently,

the urea hydrolysis rates from this study indicate the maximum loss of urea and should be accounted for in management aimed at mitigating ammonia emissions from dairy cattle manure under similar urea concentration, pH, and temperature conditions reported in this experiment.

Key words: urea hydrolysis, temperature, urea concentration, pH

Short Communication

Ammonia emissions from manure in livestock operations are related to respiratory problems in humans and animals and may contribute to additional air quality and health issues through secondary particulate formation (McCubbin et al., 2002; Behera et al., 2013). Quantifying urea hydrolysis under different conditions is the first step to understanding the factors affecting ammonia production and to identifying potential ammonia mitigation strategies because most of the ammonia in the manure is generated from urea hydrolysis, especially in the short term (Monteny and Erisman, 1998; Dai and Karring, 2014). A previous study from our laboratory predicted ammonia emissions from dairy manure under different N feeding conditions (Burgos et al., 2010), but that experiment was not designed to evaluate the effects of different urea, pH, or temperature conditions. Previous studies have evaluated urease activity in dairy cattle manure under different temperature, urea concentration, or pH conditions but have not evaluated all these factors simultaneously (Muck, 1982; Dai and Karring, 2014). Elzing and Monteny (1997) found that most of the urea was converted to ammonia during the first few hours when urine was applied on fouled slatted floors at 10°C.

The primary pathway for urea hydrolysis in manure begins with the conversion of urinary urea to NH_3 and CO_2 , which is catalyzed by the activity of urease present in feces. Once generated, ammonia in aqueous solution is present as both NH_4^+ and NH_3 , which are coupled in equilibrium by a dissociation reaction. The equilibrium between the volatile NH_3 and nonvolatile NH_4^+ is

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temperature and pH dependent. The volatilization of ammonia occurs via convective mass transfer from the boundary layer of the manure slurry to the air above the surface (Monteny and Erisman, 1998). The mass transfer coefficient is dependent on air velocity, liquid temperature, and air temperature (Arogo et al., 1999).

The optimum pH for maximum urease activity was reported to be from 6.8 to 8 (Muck, 1982; Dai and Karring, 2014). However, none of these studies examined the effects of temperature, urea concentration, pH, and their interactions within the same experiment. The objective of this study was to determine urea hydrolysis rates over time under different temperature, initial urea concentration, and pH conditions.

Fresh feces (3.1% N DM) and artificial urine solutions (urea in distilled deionized water) were combined into a slurry to characterize the rate of urea hydrolysis under 2 temperature conditions (15°C and 35°C), 3 urea concentrations in urine solutions (500, 1,000, and 1,500 mg of urea-N/dL), and 3 pH levels (6, 7, and 8). The choice of temperature, urea concentrations, and pH levels was based on literature values to include relevant treatment levels (Muck, 1982; Burgos et al., 2010; Dai and Karring, 2014). Artificial urine (described below) was used so that initial urea-N concentrations could be created to precisely equal the treatment levels. The feces were obtained from 4 lactating Holstein cows, housed in a tiestall and fitted with indwelling Foley catheters during the last day of the last experimental period in a study described previously (Burgos et al., 2007). The fresh feces used in this experiment were less than 24 h old. The catheters prevented any mixing of feces and urine. A total of 17.5 kg of feces was collected from each cow, resulting in 70 kg of feces, which were thoroughly mixed with a Hobart mixer (Troy, OH). Then, the feces were split into 3 equal 23.3-kg batches. To each feces batch, 21.1 kg of distilled deionized water was added to a final weight of 44.4 kg, such that the resulting slurry contained about 47% added water, which is similar to the proportion of urine to manure found in a previous study (Burgos et al., 2010). A portion (37 kg) of each 44.4-kg batch of slurry was placed into 3 separate containers and the pH of each 37-kg slurry sample adjusted to 6, 7, or 8 using 1 M NaOH and 1 M HCl. Then, the manure from each pH container was transferred into 18 identical clean 2-L plastic bottles, with each bottle containing 1.75 kg of manure slurry. Of these bottles, 9 were placed in a 15°C room and 9 were placed in a 35°C room and allowed to come to thermal equilibrium. This was repeated for each pH container such that there were 27 bottles in each temperature-controlled room. Separately, eighteen 87.5-mL solutions of 500, 1,000, or 1,500 mg of urea-N/dL in deionized distilled water (54 solutions in total) were prepared to be added into

the bottles in each room. The concentration of urea in the artificial urine spans the concentrations of urinary urea-N measured in our previous study (Burgos et al., 2010). For each urea concentration, nine 87.5-mL solutions were placed in each temperature-controlled room and allowed to come to thermal equilibrium.

The experiment was initiated by adding 87.5 mL of each urea concentration to the bottles (and immediately mixed) such that each treatment combination had 3 replicates. The final concentrations for the urea treatments were 250, 500, or 750 mg of urea-N/dL in the slurry, which were considered to be the initial urea concentrations. Bottle contents were mixed before sample collection and a 10-mL sample drawn after 0.0167, 1, 2, 4, 6, 8, 12, 16, 20, and 24 h. Each 10-mL sample was immediately added to a 50-mL tube containing 0.1 mL of 18 M H₂SO₄ and then mixed thoroughly to prevent further urea hydrolysis. The start of the incubations for each treatment condition was staggered by 5 min to ensure that all the bottles had the same sampling schedule. Acidified slurry samples were analyzed for urea N concentration with a diacetyl-monoxime method (Marsh et al., 1957) using a Technicon autoanalyzer (Technicon Instruments Corp., Tarrytown, NY). Total ammoniacal N (**TAN**) in the slurry was measured at 24 h and analyzed using automated colorimetry (Noel and Hambleton, 1976). The initial pH and the slurry pH at 24 h were measured using a Thermo Orion Perpfect pH meter (model no. 320, Thermo Orion, Beverly, MA) with an Accumet pH combination electrode (Fisher Scientific, Pittsburgh, PA). Four observations were removed from the data set because the urea concentration at 0.0167 h was 25% higher than that at time 0 h. These urea concentrations were considered sampling errors due to inadequate mixing because urea concentration should decrease with time as a result of hydrolysis.

A nonlinear mixed-effects model was used to describe the effects of temperature, initial urea concentration, and pH on urea hydrolysis over time. In particular, an exponential function was used to model the data with respect to time:

$$y_{ij} = \phi_{1,i} \exp(-\phi_{2,i} \text{time}_{ij}) + \varepsilon_{ij}, \quad [1]$$

where y_{ij} is the urea concentration in the i th bottle ($i = 1, \dots, 54$) at sampling time j ($j = 1, \dots, n_i$), time_{ij} is the corresponding sampling time, and ε_{ij} is the error. Because of initial indication of heteroscedasticity for the errors, they were assumed to be normally distributed with mean zero and $\text{Var}(\varepsilon_{ij}) = \sigma^2 \exp(2\delta_i \text{time}_{ij})$. In this framework, the variance is an exponential function of time and the parameter δ_i is unrestricted, allowing the variance to increase or decrease with time

(Pinheiro and Bates, 2000). The indexing i on δ_i allows it to depend on a factor describing the experimental treatments. We used the urea treatment to accommodate further errors' heterogeneities across initial urea levels: $\delta_i = \varphi_1 z_{1,i} + \varphi_2 z_{2,i} + \varphi_3 z_{3,i}$, where $z_{1,i}$, $z_{2,i}$, and $z_{3,i}$ are binary dummy variables relating δ_i to the urea-N level treatment (500, 1,000, or 1,500 mg of urea-N/dL); and φ_1 , φ_2 , and φ_3 are the parameters of the variance function describing changes in the errors variance with time for the 3 initial urea level treatments.

The parameters to be estimated in Eq. [1] are $\phi_{1,i}$ and $\phi_{2,i}$, which represent the estimated urea concentration at time zero and the exponential rate of decay or urea hydrolysis rate. In the nonlinear mixed effects framework, treatment effects are incorporated directly into the model parameters:

$$\begin{bmatrix} \phi_{1,i} \\ \phi_{2,i} \end{bmatrix} = \begin{bmatrix} \alpha_1 x_{1,i} + \alpha_2 x_{2,i} + \cdots + \alpha_{17} x_{17,i} + \alpha_{18} x_{18,i} \\ \beta_1 x_{1,i} + \beta_2 x_{2,i} + \cdots + \beta_{17} x_{17,i} + \beta_{18} x_{18,i} \end{bmatrix} + \begin{bmatrix} b_{1,i} \\ b_{2,i} \end{bmatrix}, \quad [2]$$

where $x_{1,i}$, ..., $x_{18,i}$ are binary dummy variables representing the effects of the temperature (15°C and 35°C), pH (6, 7 and 8), and initial urea concentration in urea solution (500, 1,000, or 1,500 mg of urea-N/dL) treatments on the model parameters. Eighteen binary dummy variables were needed to represent main effects, 2-way and 3-way interactions. In this notation, the fixed effects determining treatment effects on the intercept and rate parameter are given by $(\alpha_1, \dots, \alpha_{18})$ and $(\beta_1, \dots, \beta_{18})$, respectively. Multiple records were collected longitudinally on each bottle; therefore, observations originated from the same bottle are not independent and an intrinsic contribution of the manure sample in each bottle for the total variation in the data is present. Specific levels for these bottle effects are, however, not of interest in this study, and the bottle effects ($b_{1,i}$ and $b_{2,i}$) were therefore assumed to be random. In short, the random effects represent shifts in the parameters of the exponential model for each bottle (from the average parameters in its treatment group), which are, on average, zero. It was assumed that observations corresponding to different bottles were independent and that the errors ε_{ij} were independent of the $b_{1,i}$ and $b_{2,i}$. Further, random effects were assumed to be independent and distributed as

$$\begin{bmatrix} b_{1,i} \\ b_{2,i} \end{bmatrix} \sim N \left(\begin{bmatrix} 0 \\ 0 \end{bmatrix}, \begin{bmatrix} \sigma_1^2 & \sigma_{12} \\ \sigma_{12} & \sigma_2^2 \end{bmatrix} \right), \quad [3]$$

where σ_1^2 , σ_2^2 , and σ_{12} are the variance components associated with the bottles' random effects. The model

was fitted using maximum likelihood in the nlme package in R (Pinheiro et al., 2016). Hypothesis testing for fixed effects used conditional F-tests based on the conditional estimate of the variance (Pinheiro and Bates, 2000). The exponential decay rates were predicted using the fixed effects of the full model described in Eq. [2]. Subsequently, a stepwise approach was used to reduce the model and determine appropriate multiple comparisons for the urea hydrolysis rate parameters. First, the significance of the 3-way and 2-way interactions was sequentially examined. If the 3-way interaction was not different from zero (at the given 0.05 statistical significance level), it was removed from the model and a model containing only 2-way interactions was then fitted. Next, if 2-way interaction terms were not significantly different than zero, the main effects were compared directly. If any interaction term was significant for 2 factors, one factor was compared within each level of the other factor. Multiple comparisons were conducted with the multcomp package in R (Hothorn et al., 2008) for which the single-step method was used to adjust the significance level and construct 95% family-wise confidence intervals. A similar model reduction approach was used to describe the effects of temperature, urea concentration and pH on final TAN or final pH except a linear model was used and TAN was transformed as a natural log to ensure normally distributed and homoscedastic residuals.

Table 1 shows the estimate of the individual rates (ϕ_2) of the full model Eq. [2] before model reduction. The estimates of the rates of hydrolysis, as well as the associated standard errors, are given for the 18 possible combinations of temperature, urea, and pH. The fast rates of urea hydrolysis are particularly high, especially at the higher temperature, and even some rates at 15°C were relatively fast. These results show that the fast rates of urea hydrolysis require careful experimental designs to capture the urea hydrolysis and subsequent changes in NH_3 and NH_4^+ concentrations in the slurry and ammonia emitted. Careful mass balance experiments may be able to characterize this reaction more precisely.

Sequential model reductions resulted in a model with the rate parameter with main effect of initial urea concentration, pH, and temperature as well as a 2-way interaction of initial urea level and pH treatments. Figure 1 shows that the reduced model fit was overall very good across all treatment combinations and replicates. Figure 2 shows the differences between the rates of urea hydrolysis of the main effect of temperature and the selected contrasts between urea level and pH combinations of the reduced model. The higher temperature showed faster rates of urea hydrolysis compared with the lower temperature ($P < 0.0001$). This effect of tem-

perature is supported by previous observations (Muck, 1982).

Because the initial urea level and pH interaction was statistically different than zero (at the 0.05 significance level), 18 contrasts were determined comparing hydrolysis rates for the initial urea level within pH levels and vice versa (Figure 2). At the intermediate urea concentration added (1,000 mg of urea-N/dL), the urea hydrolysis rate was higher for the pH 6 treatment than for the pH 8 treatment ($P = 0.002$). The finding of pH 6 showed that the highest hydrolysis rate was slightly lower than that found by Muck (1982), which was between pH 6.8 and 7.6 and lower by 2 pH units compared with a more recent study (Dai and Karring, 2014; pH 8). These differences could be due to the experimental conditions, such as temperature and pH, between studies and also because of the shift of pH in the current study toward a median pH across all treatments to pH 8.25. The current experiment did not buffer the slurries so pH changes were possible from initial to final measurements. All other contrasts comparing hydrolysis rates of treatments with varying pH, within initial urea concentration levels, were not significantly different than zero (Figure 2). The rate of urea hydrolysis was higher for the intermediate initial urea level (1,000 mg of urea-N/dL) than the treatment of higher initial urea level (1,500 mg of urea-N/dL) across pH. In particular, the rate of urea hydrolysis at the intermediate initial urea level was greater than that at the highest initial urea at pH 6 ($P < 0.001$), pH 7 ($P = 0.049$), and pH

8 ($P = 0.014$). These differences imply that urease activity may have been limiting as the urea concentration levels increased in the final slurry from 500 to 750 mg of urea-N/dL. The other contrasts describing the change in urea hydrolysis rate with varying initial urea concentration within pH were not statistically different from zero. As mentioned previously, the final median pH was 8.25 across all treatments. All 2-way and 3-way interactions were significant in the linear model describing treatment effects on the final pH, making any meaningful comparisons difficult. Essentially, the pH in all treatment combinations shifted toward 8.25 with a first quartile of 7.84 and the third quartile of 8.68.

The TAN linear model only had significant main effects for temperature and urea. The pH main effect and all interactions were not significant. The estimated TAN marginal means, with associated standard errors in parentheses, for the urea initial levels were 186 (5.20), 331 (5.02), and 468 (4.87) mg of N/dL for low, medium, and high initial urea concentration levels, respectively. If no NH_3 were emitted, these levels, assuming all urea was hydrolyzed, should be 250, 500, and 750 mg of N/dL. This implies that about 26, 34, and 38% of the urea-N hydrolyzed was emitted for the low, medium, and high urea concentration levels. The reason for the low levels of emission could be because of pH, surface area, or air flow. Future studies should measure these emissions to ensure mass balance of the N. As expected, the TAN levels were significantly higher for high temperature compared with low temperature. In particular,

Table 1. Predicted urea hydrolysis rates (ϕ_2 , /h) and associated standard errors (SE) using the exponential model of urea hydrolysis over time¹

Urea level ² (mg of N/dL)	pH	Temperature (°C)	Estimate (/h)	SE
500	6	15	1.04	0.43
		35	56.01	29.01
	7	15	1.20	0.41
		35	21.14	28.29
	8	15	0.34	0.07
		35	2.43	1.91
1,000	6	15	0.31	0.03
		35	1.02	0.22
	7	15	0.22	0.01
		35	1.34	0.30
	8	15	0.19	0.01
		35	1.09	0.21
1,500	6	15	0.17	0.01
		35	1.26	0.16
	7	15	0.18	0.01
		35	1.05	0.14
	8	15	0.16	0.01
		35	0.73	0.09

¹Rates were dependent on pH, temperature, and urea level and were computed using the fixed effects of the full model described in Eq. [2].

²In initial urine solution.

Figure 1. Urea-N concentration in slurry versus time. Dots represent the observations and the lines are the estimated fitted curve in the reduced model specific to each bottle (mixed predictions using random and fixed effects). The panel titles describe temperature, pH, initial urea level and replicate of each slurry (i.e., 35.6.500.1 in the first panel refers to temperature 35°C, pH 6, initial urea level in urine solution of 500 mg/dL, and replicate 1, respectively).

the estimated marginal means (and standard errors) were 311 (4.24) and 345 (3.98) mg of N/dL for the 15°C and 35°C temperature treatments, respectively.

The results from this study likely represent maximum rates of urea hydrolysis because of the thorough mixing

of the slurry before each sampling. In commercial dairy operations with freestall barns, considerable mixing of urine and feces on the barn floor is expected from cattle walking through the manure, although such mixing may be not as rapid or as thorough as in this study.

The urea hydrolysis rates determined in this study for different initial urea concentration, temperature, and pH conditions are useful for estimating the ammoniacal N produced from urea. In conclusion, initial urea concentration and temperature are important factors affecting urea hydrolysis. Higher urea concentrations were associated with lower rates of urea hydrolysis. Urea hydrolysis rates were higher at 35°C than at 15°C irrespective of pH and initial urea levels. The effect of pH on urea hydrolysis was only observed between pH 6 and pH 8 at the intermediate initial urea level in urine solution (1,000 mg of urea-N/dL). Any pH lower than 6 or greater than 8 may elicit a different response. The urea hydrolysis rates for all conditions tested in this study, as determined with an exponential model, demonstrated that the majority of urea is hydrolyzed within the first few hours after urine and feces mixing. The treatment combination with the fastest rate of urea hydrolysis was 35°C, pH 6, and initial urea level in urine solution of 500 mg of N/dL. Conversely, the treatment combination with the slowest rate of urea hy-

drolysis was 15°C, pH 8, and urea level in urine solution of 1,500 mg of N/dL. The interrelationship between initial urea concentration, temperature and pH levels could inform management strategies aimed at reducing the amount of urea hydrolysis and subsequent ammonia levels from dairy cattle manure.

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Figure 2. Multiple comparisons for the rate parameter (ϕ_2 , /h) of the exponential reduced model describing urea hydrolysis. The main treatments are initial urea concentration in urine solution (1,500, 1,000, and 500 mg of urea-N/dL), temperature (Temp; 15°C and 35°C), and initial pH (6, 7, and 8). Rows correspond to the comparison being made (i.e., $a - b$ is the rate of level a minus the rate of level b , and $c : d - e$ is the rate of level d minus the rate of level e within the level c of the other factor). Note: If interaction terms were not significantly different than zero, the main effects were compared directly. If interaction terms were significantly different than zero for any 2 factors, one factor was compared within each level of the other factor. The single-step method (Hothorn et al., 2008) was used to adjust the significance level and construct 95% family-wise confidence intervals.

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