Milk urea concentration as an indicator of ammonia emission from dairy cow barn under restricted grazing

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ABSTRACT

Bulk milk urea concentration was evaluated to assess its potential as an indicator of ammonia emission from a dairy cow barn in a situation with restricted grazing. An experiment was carried out with a herd of, on average, 52 Holstein-Friesian dairy cows. The cows were housed in a naturally ventilated barn with cubicles and a slatted floor, were fed ensiled forages and feed supplements, and each day were allowed 8.5 h of grazing. The experiment was a balanced randomized block design, replicated 3 times. The experimental factor was the bulk milk urea level, which was adjusted to levels of 15, 35, and 55 mg of urea per 100 g of milk, respectively, by changing the level of nitrogen fertilization of the pasture, the herbage mass and grass regrowth age, and the level and type of feed supplement. Ammonia emission from the barn was measured using sulfur hexafluoride as the tracer gas. Ammonia emission generally increased upon an increase in adjusted milk urea levels. A dynamic regression model was used to predict ammonia emission from bulk milk urea concentration, temperature, and a slurry mixing index. This model accounted for 66% of the total variance in ammonia emission and showed that emission increases exponentially with increasing milk urea concentration. At levels of 20 and 30 mg of urea per 100 g of milk, ammonia emission increased by about 2.5 and 3.5%, respectively, when milk urea concentration increased by 1 mg/100 g. Furthermore, emissions from the barn increased 2.6% when temperature increased by 1°C. The study showed that bulk milk urea concentration is a useful indicator for ammonia emissions from a dairy cow barn in a situation with restricted grazing.

Key words: ammonia emission, milk urea, restricted grazing

INTRODUCTION

In the National Emission Ceilings Directive of the European Union (European Union, 2001), maximum emission levels for 2010 are set for the individual countries of the European Union. These maxima concern sulfur dioxide, nitrogen oxides, nonmethane volatile organic components, and ammonia (NH₃). The Dutch maximum NH₃ emission level is 128 kt/yr, with a maximum contribution from the agricultural sector of 96 kt/yr. This target has only a 50% chance of being achieved with unchanged policy, and additional efforts in the livestock production sector are necessary (VROM, 2007).

Emissions of NH₃ from livestock originate largely from urea excreted with urine (Bussink and Oenema, 1998). Urea hydrolysis is catalyzed by the enzyme urease, which is produced by microorganisms that are abundantly present in feces and thus also upon fouled floors and in slurry pits. Urinary urea concentration and temperature are the main factors influencing the rate of NH₃ volatilization (Monteny, 2000). Previous experiments and modeling approaches have shown that the urinary urea concentration and urea-nitrogen excretion in grams per day are important predictors of NH₃ emission from manure (James et al., 1999) or dairy barns (Smits et al., 1995; Monteny et al., 1998; Cassel et al., 2005). Smits et al. (1997) stated that nutritional measures related to reduction of NH₃ emission should focus on reduction of urinary urea concentration by lowering the N concentration in the diet rather than by increasing the urine volume.

Frank and Swensson (2002) stated that NH₃ release from cow manure was reduced when the protein level in the diet was lowered and that the concentration of MUN was positively correlated with protein level in the diet and NH₃ emission. In that study, a ventilated chamber and the analytical technique of Andersson (1994) were used to determine NH₃ release from feces and urine.

Several studies did not directly assess the effect of diet on ammonia emission but did demonstrate that reducing dietary CP content resulted in less total N

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excretion and less urinary N excretion (Kebreab et al., 2002; Nousiainen et al., 2004; Misselbrook et al., 2005), with MUN being positively correlated to urinary N excretion (Kebreab et al., 2002; Nousiainen et al., 2004; Burgos et al., 2007). However, the use of MUN to predict urinary N excretion has been questioned by others (Kauffman and St-Pierre, 2001; Tas et al., 2006), and the robustness of these relationships is debatable. Some studies indicate that MUN alone is not a good indicator for urinary N excretion and that additional parameters such as BW (Kauffman and St-Pierre, 2001; Kohn et al., 2002) or diet characteristics (De Campeneere et al., 2006) are important.

Misselbrook et al. (2005) reported some shortcomings of laboratory techniques for NH_3 emission measurements, although other studies successfully used laboratory techniques (Lascano et al., 2008) or experimental chambers (Powell et al., 2008b) in estimating the effects of diet on NH_3 emission. As most dairy cow barns are naturally ventilated, the relevance of (dietary) measures to reduce nitrogen losses by NH_3 emission of the dairy barn should best be evaluated making use of measuring techniques for naturally ventilated barns, such as a concentration ratio method using a tracer gas (Monteny and Erisman, 1998; Mosquera Losada, 2007; Schrader et al., 2007). van Duinkerken et al. (2005) used sulfur hexafluoride (SF_6) as a tracer gas and demonstrated that NH_3 emission from a naturally ventilated dairy barn could be significantly reduced by altering the diet. Furthermore, they showed that bulk milk urea concentration is a useful indicator to monitor and control NH_3 emission reduction. The study of van Duinkerken et al. (2005) was performed in a situation where cows were confined to the barn 24 h per day. A remaining question is whether NH_3 emission levels from dairy barn can be monitored, controlled, and reduced by using milk urea concentration as an indicator, when farms apply restricted grazing with cows being outdoors for several hours a day. On most Dutch dairy farms, pasturing is applied during the grazing season and Kroodsma et al. (1993) showed that emission rate decreases exponentially when cows have left the house for grazing. Furthermore, it has been demonstrated that NH_3 emission is usually rather high during the grazing season (Kroodsma et al., 1993; Powell et al., 2008a), mainly because NH_3 emissions are positively correlated with temperature (Monteny, 2000; van Duinkerken et al., 2005; Sommer et al., 2006). Furthermore, the fresh pasture grass that is ingested during grazing is usually rich in N, enhancing the urinary N excretion.

The aim of the present study was to evaluate the potential of milk urea concentration as an indicator for NH_3 emission reduction from a dairy cow barn in a situation with restricted grazing.

MATERIALS AND METHODS

Experimental Design

Design and Treatments. The experiment had a balanced randomized block design and was repeated 3 times. There was replication within herd. In this particular experiment, replicate was not a group of animals but one series (= block) of 3 subperiods of 3 wk each. The 3 treatments were randomized over 3 subperiods within each series. The randomized block design was followed to eliminate series (= block) effects, which improves the power of the experiment. Randomly creating a series of diets over the whole investigation period has the risk of confounding and is less efficient than the approach followed in the current experiment.

The experimental factor was the bulk milk urea level, which was adjusted to 3 levels of 15, 35, and 55 mg of urea per 100 g of milk, respectively (treatments **U15**, **U35**, and **U55**), corresponding to levels of 7.2, 16.8, and 26.4 mg of MUN/100 mL, respectively. Each level was maintained for 3 wk, with periods randomly distributed over the whole research period. The actual sequence of treatments was U35, U15, U55, U35, U55, U15, U15, U35, and U55. The final period of the 9 treatment periods was shortened to 2 wk because adverse weather and grazing conditions (wet and weak pasture surface) prevented cows from grazing. The total duration of the experiment was therefore 26 wk (April 24 to October 22).

Animals and Housing. The experiment was performed at a research farm in Lelystad, the Netherlands. The cubicle house was naturally ventilated with space boarding in one sidewall and no ventilation openings in the other sidewall. The lying area was arranged in 2 rows of 34 and 31 cubicles, respectively, on either side of a central feeding alley. Slurry was stored beneath the slatted floor and the cubicles. The barn is described in more detail by van Duinkerken et al. (2005).

During the experiment, 52 lactating Holstein Friesian dairy cows were present, on average, in the barn, with the exception of a daily 8.5-h grazing period. No young-stock or dry cows were housed in the barn, except for animals in the last 10 d before their expected calving date, which were added to the lactating herd.

Strategy to Adjust Milk Urea Levels. The prediction model of Schepers and Meijer (1998) was used to predict the milk urea level based on diet composition and to adjust the diet. In this model, the RDP balance according to the Dutch standard ["Onbestendig Eiwit Balans" (**OEB**); Tamminga et al., 1994] is the predominant factor influencing milk urea concentration.

An area of 15.6 ha was used for grazing and divided into 13 plots of 1.2 ha each. The daily average grazing

Table 1. Actual amount of feed supplied in the barn (averaged per cow) per treatment replicate

Treatment-replicate ¹	Grass silage (kg of DM/d)	Corn silage (kg of DM/d)	Extracted soybean meal (kg/d)	Extracted rapeseed meal (kg/d)	MgO (g/d)	Urea (g/d)	Compound concentrate (kg/d)
U35-1	5.0	—	—	—	40	—	4.6
U15-1	—	7.0	0.5	—	20	—	4.3
U55-1	4.0	—	—	—	40	50	4.4
U35-2	5.0	—	—	—	40	—	4.3
U55-2	6.0	—	—	—	60	100	4.1
U15-2	—	7.0	—	—	—	—	3.7
U15-3	—	7.0	—	—	—	—	4.0
U35-3	6.0	—	—	1.0	50	—	3.7
U55-3	7.5	—	—	—	50	—	4.1

¹Treatments U15, U35, and U55 included 3 levels of 15, 35, and 55 mg of urea per 100 g of milk, respectively.

time was 8.5 h and started around 0730 h. A rotational grazing system was used with an average stay of the herd of 3 sequential days per plot. The sward was a stand of predominantly perennial ryegrass.

A combination of tools was used by a 2-person expert team to control diet composition (thereby influencing milk urea levels) in each treatment period: 1) the level and type of indoor supplemented feed, 2) the level of N fertilization on individual pasture plots, and 3) the herbage mass and regrowth age of the grass on the pasture plots. Diets were formulated based on the expert team's estimation of feed intake and pasture grass composition, combined with the feed analyses of ensiled forages and concentrates. The composition of diets, but also of compound concentrates, was adjusted for each treatment, taking into account the NE_L requirements according to the Dutch standard [1 **VEM** ("Voeder Eenheid Melk") = 6.9 kJ of NE_L; van Es, 1975, 1978) and true protein digested in the small intestine ["Darm-verteerbaar Eiwit" (**DVE**)] according to the Dutch standard (Tamminga et al., 1994). In the barn, cows were fed (a mixture of) corn and (or) grass silage and occasionally extracted formaldehyde-treated soybean meal or extracted rapeseed meal to increase the level of DVE. In treatment periods U55-1 and U55-2 (the first and second periods in which U55 was applied, or periods 3 and 5 chronologically), respectively, 50 and 100 g of urea/animal per day was added to the forage mixture to increase the OEB of the diet. The forages and supplements were mixed using a feed mixer wagon and were fed at levels shown in Table 1. Finally, compound concentrate was fed according the NE_L requirement (CVB, 2002) for each individual cow, using 2 computer-controlled concentrate dispensers and including the allocation of 0.5 kg of compound concentrate in the milking parlor at every milking for each cow.

Fertilizer was adjusted per individual plot according to recommendations for fertilization of grassland and fodder crops of the Dutch Commission on Fertilization of Grassland and Fodder crops (CBGV, 1998) and tak-

ing into account the objectives for milk urea levels per treatment. Every week, an expert team visited all plots to judge grass quality and grazing conditions, to set up the grazing strategy for the next week, and to decide on the harvesting and fertilization moments of certain plots to obtain suitable pasture plots for the upcoming weeks.

Measurements and Data

Feed Intake and Feed Sampling. Intake of pasture grass was not measured. Grass composition and feeding value were analyzed for each of the 9 main periods. Of each parcel used for grazing in the main period, a representative sample was taken at turning into pasture by cutting 25 subsamples equidistantly divided over 3 diagonal lines across the parcel (CVB, 2003). Samples were cut at a height of 6 cm above the surface and pooled for that main period. Grass samples were preserved by drying during 36 h at 70°C in a forced air oven. Next, they were stored in a dry and dark storage room at room temperature. At the end of the experiment, all 9 pooled grass samples were analyzed by the ALNN laboratory (Wargea, the Netherlands) for DM, inorganic matter (ash), CP (N × 6.25), crude fiber, sugars, NO₃, Na, and K (all according to CVB, 2003) and in vitro digestibility of organic matter (**dOM**) according to Tilley and Terry (1963).

In the barn, individual intake of the basal feed mixture was registered with a Hokofarm RIC system (Insentec, Marknesse, the Netherlands), consisting of 40 computer-controlled weighing troughs. Supplemental compound concentrate was fed with 2 automated concentrate dispensers, registering individual concentrate intake per day. Details of sampling of diet, storage of samples, and chemical analyses are given by van Duinkerken et al. (2005).

The actual amounts of feed supplied in the barn are given in Table 1 and feed refusals were negligible. The composition of pasture grass, feed mixture components,

Table 2. Composition and feeding value [digestibility of OM (dOM) in % of OM, VEM per kg of DM, others in g/kg of DM] of pasture grass

Treatment–replicate ¹	CP	Crude fiber	OM	dOM	Nitrate	Sugar	K	Na	VEM ²	DVE ³	OEB ⁴
U35–1	241	203	894	79.7	8.1	37	37.7	0.8	971	100	73
U15–1	233	195	894	81.1	3.3	41	30.8	1.2	987	102	63
U55–1	211	194	895	81.4	1.9	152	32.6	0.6	980	100	41
U35–2	221	210	897	79.9	2.4	127	30.0	1.3	967	100	49
U55–2	255	203	896	79.1	6.7	93	32.9	1.3	971	102	80
U15–2	228	205	891	78.4	4.4	103	31.7	1.4	942	98	54
U15–3	267	219	894	76.2	2.2	38	31.7	1.4	933	100	91
U35–3	282	204	893	76.9	4.8	55	34.7	1.3	950	102	104
U55–3	308	202	886	77.4	1.1	78	34.8	1.1	964	103	130

¹Treatments U15, U35, and U55 included 3 levels of 15, 35, and 55 mg of urea per 100 g of milk, respectively.

²VEM = NE_L according to Dutch standards.

³DVE = true protein digested in the small intestine according to Dutch standards.

⁴OEB = RDP balance according to Dutch standards.

and compound concentrates are presented in Tables 2, 3, and 4, respectively.

Milk Yield and Composition, Including Milk Urea. Cows were milked twice daily at approximately 0600 and 1700 h. Individual milk yield was recorded at each milking. Every week, during the Wednesday p.m. and Thursday a.m. milkings, individual milk samples were taken, pooled per cow, and analyzed for fat and protein content by the “Melk Controle Station” milk-testing laboratory (Zutphen, the Netherlands) according to EN ISO/IEC standard 17025 [International Organization for Standardization (ISO), 2000].

A sample of the bulk milk was taken every 6 milkings (i.e., 3 a.m. and 3 p.m. milkings), just before emptying of the tank. Bulk milk samples were analyzed for milk urea concentration with the Bran and Luebbe Traacs 800 auto-analyzer by the milk-testing laboratory (Zutphen, the Netherlands) according to de Jong et al. (1992). In addition, at every milking, a representative milk sample was taken of the mixed milk of all cows in the herd, by using a needle that was implanted in the central milk pipe of the milking parlor toward the bulk milk tank. Through this needle, a proportional amount of milk was drained from the herd milk and collected in a tube. Before the experiment started, we determined that this needle sampling method gave exactly the same milk urea concentration as sampling of the milk from one herd milking gathered in the bulk tank; the method was validated on 3 randomly selected days. Urea concentration of each herd milk sample was analyzed as described previously.

Urine Composition. On d 1, 3, and 5 of the third week of every 3-wk period, except in period 9, urine was sampled. On each sampling day, 2 sampling moments occurred (the first was just before the a.m. milking and the second was before the p.m. milking, when cows just entered the barn after grazing). On these occasions, as many cows as possible were randomly sampled for urine

on a voluntary basis (no stimulation). On each occasion, at least 12 cows were sampled using a urine collecting device on a long stick, and urine of each cow was stored in a 60-mL tube, labeled, and cooled immediately in a box with frozen cooling elements. Immediately after the last sample was collected, urine samples were placed in a freezer and later transported frozen to the laboratory. In this laboratory, a pooled sample was made per a.m. or p.m. sample and analyzed for total N, urea-N, NH₄-N, Na, K, and pH.

Slurry Sampling. On the first day of every treatment period, the slurry level in the pit was reduced to a remaining level of 75 cm of slurry to eliminate any possible effect of slurry level on NH₃ emission. At the end of a 3-wk treatment period, about 20% of the slurry volume in the pit originated from the current treatment and about 80% from previous treatments. The slurry level was reduced by mixing the slurry followed by pumping the surplus to slurry storage on another location at the farm. Mixing and pumping of slurry was restricted to these times only, such that the composition of the top layer of the slurry in the pit was predominantly influenced by the diet and negligibly by the older, underlying slurry. During mixing, all barn doors were opened to maximize the ventilation rate and to minimize the risk of accumulation of toxic gases.

Per treatment period (except in period 9), 2 sample types were collected in the third week of each treatment period: (1) a sample of the top layer of the slurry and (2) a sample of the mixed slurry. For both sample types, slurry was sampled at 10 evenly distributed locations through the slats. For this purpose, a special sampling device (cup shaped; 100 mL) was attached to a long stick. The samples were collected in a jar, stored in a cooler, and transported at 4°C to the laboratory. Samples of one sampling moment were mixed, and a pooled sample was analyzed for total N, NH₄-N, and pH. In addition, for mixed slurry, DM, Na, and K were analyzed.

Table 3. Composition and feeding value [DM in g/kg, digestibility of OM (dOM) in % of OM, NH₃ as % of CP, VEM per kg of DM, others in g/kg of DM] of supplemented feedstuffs

Treatment–replicate ¹	DM	CP	Crude fiber	OM	Crude fat	dOM	NH ₃	Starch	Sugar	K	Na	VEM ²	DVE ³	OEB ⁴
Grass silage														
U35–1	627	126	268	899	—	76.3	10	—	139	32.1	0.8	877	78	–6
U55–1	582	205	231	896	—	78.6	5	—	112	32.3	1.8	933	93	1
U35–2	584	139	276	892	—	75.2	8	—	98	35.5	0.8	854	76	6
U55–2	573	185	250	892	—	76.9	5	—	87	33.5	1.4	897	86	39
U35–3	477	139	259	885	—	78.1	7	—	110	27.8	1.5	889	73	6
U55–3	406	196	243	882	—	76.6	16	—	17	39.4	1.0	884	73	95
Corn silage														
U15–1	330	74	175	953	—	76.2	—	388	—	10.3	0.0	966	46	–27
U15–2	310	69	204	947	—	74.3	—	309	—	11.2	0.0	932	46	–35
U15–3	293	77	220	942	—	71.4	—	274	—	11.6	0.0	883	44	–28
Soybeans, extracted formaldehyde treated ⁵														
U15–1	884	485	58	929	29	—	—	—	114	24.5	0.2	1,152	429	15
Rape seed, extracted														
U35–3	866	375	132	922	49	—	—	—	108	14.4	0.0	1,016	146	152

¹Treatments U15, U35, and U55 included 3 levels of 15, 35, and 55 mg of urea per 100 g of milk, respectively.²VEM = NE_L according to Dutch standards.³DVE = true protein digested in the small intestine according to Dutch standards.⁴OEB = RDP balance according to Dutch standards.⁵Mervobest (Premervo, Utrecht, the Netherlands).

Ammonia Emission Measurements. Emission of NH₃ was measured continuously (24 h/d) with a concentration ratio method using sulfur hexafluoride (SF₆) as a tracer gas. Approximately 14 mL of SF₆ per min was injected with the aid of a mass flow controller, and SF₆ was diluted in air (5 L/min). This air amount was controlled with the aid of another mass flow controller. Tracer gas injection points were installed below a comfort plate at the end of the cubicles near the slatted floor, as illustrated in the cross-section in Figure 1, to ensure optimal distribution of the gas near the source of NH₃ emission. There were 30 injection points in total and air was equally distributed over these points by orifices installed at each point. The tracer gas injection was continuously performed during 24 h/d over the whole research period. The principle of the constant release tracer gas has also been applied by Demmers et al. (2000; 2001) and Marik and Levin (1996). In a study in a forced-ventilated cow building, the method was validated by Scholtens et al. (2004). Methods for measuring airflow rates were reviewed by Phillips et al. (2001).

Air in the top of the building was continuously sampled using a collecting tube. At 3 points in the top of the building, orifices were installed that allowed a fixed airflow of 500 mL/min each (total of 1,500 mL/min). The collecting tube was split in 2 parts after a pump. One branch went to a NH₃ converter and a nitrogen oxides (NO_x) monitor. Collected air was taken to this NO_x monitor and a new cycle was started every 6 min. Per 6 min, the NH₃ concentration was determined (simultaneously, the background air NH₃ concentration in the side wall was determined by using a collecting point in the side wall). The other branch went to a gas chromatograph and an air sample was analyzed for SF₆ concentration every 12 min. Six minutes later, a background SF₆ concentration was measured (also every 12 min). The whole setup operated continuously and automatically.

The source strength of NH₃, being the NH₃ emission, was calculated by using the following equation, assuming perfect mixing of NH₃ and SF₆:

$$\text{MFSF}_6:\text{MFNH}_3 = \text{CTSF}_6:\text{CTNH}_3, \quad [1]$$

where MFSF₆ = mass flux of SF₆ injected near the floor (g/h); MFNH₃ = mass flux of NH₃ (from floor and pit; g/h); CTSF₆ = concentration of the SF₆ in the exhaust air (mg/m³); and CTNH₃ = concentration of NH₃ in the exhaust air (mg/m³).

Statistical Analysis of Urine and Slurry Composition

The model used to analyze the data on urine composition was

Table 4. Chemical composition and feeding value (VEM per kg, others in g/kg) of compound concentrates¹

Treatment–replicate ²	DM	OM	CP	Crude fat	Crude fiber	Starch	Sugar	VEM ³	DVE ⁴	OEB ⁵
U35–1	891	922	192	45	109	88	86	940	90	50
U15–1	890	926	145	46	143	39	131	940	105	–15
U55–1	892	914	192	54	110	93	69	940	90	50
U35–2	894	912	192	53	105	84	75	940	90	50
U55–2	896	910	191	54	113	89	67	940	90	50
U15–2	898	926	155	44	152	51	114	940	110	–10
U15–3	890	924	155	46	145	43	124	940	110	–10
U35–3	899	915	182	53	112	81	79	940	90	40
U55–3	894	916	196	54	115	87	67	940	90	55

¹Values provided by the feed manufacturer (Agrifirm, Meppel, the Netherlands).
²Treatments U15, U35, and U55 included 3 levels of 15, 35, and 55 mg of urea per 100 g of milk, respectively.
³VEM = NE_L according to Dutch standards.
⁴DVE = true protein digested in the small intestine according to Dutch standards.
⁵OEB = RDP balance according to Dutch standards.

$$Y = \mu + \beta + \tau + \beta \cdot \tau + b + b \cdot p + b \cdot p \cdot d + b \cdot p \cdot d \cdot t, \quad [2]$$

where Y is the response variable (total N, urea-N, pH, Na, and K, respectively), with the fixed terms μ (constant), β (treatment effect; U15, U35, and U55, respectively), τ (time effect, a.m. or p.m.), $\beta \cdot \tau$ (treatment $\times$ time interaction) and the random terms b (block = replicate), $b \cdot p$ (period within block), $b \cdot p \cdot d$ (day within period within block, correlated autoregression, order 1), $b \cdot p \cdot d \cdot t$ (time within day within period within block; final residual error term).

The model used to analyze the data on both mixed slurry composition and composition of the top layer of the slurry was

$$Y = \mu + \beta + b + b \cdot p, \quad [3]$$

where Y is the response variable (total N, NH₄-N, and pH, respectively), with the fixed terms μ (constant) and β (treatment effect; U15, U35, and U55, respectively) and random terms b (block = replicate) and $b \cdot p$ (period within block; final residual error term).

The parameters of models [2] and [3] were estimated with REML in Genstat (Genstat 5 Committee, 1993), and the effects of treatment and time were tested using the Wald test.

Dynamic Regression Analyses: Modeling

The factors affecting NH₃ emission from the barn were estimated using a dynamic regression model according to Pankratz (1991). The method used is largely similar to the modeling technique used by van Duinkerken et al. (2005). Statistical analyses were performed with emission data after logarithmic transformation. This transformation was based on general assumptions,

which were checked retrospectively by an analysis of residues, as described by van Duinkerken et al. (2005). The first general assumption was that, by definition, concentrations have a positive value and are not normally distributed. Furthermore, with observations distributed over a large range, the observations at a relatively high level are more spread than observations at lower levels. A lognormal distribution is appropriate for such situations and data are normally distributed after log transformation. Furthermore, after log transformation, additive effects on a log scale become multiplicative on the original scale. A retrospective analysis of residues showed that a normal distribution of data on a log scale was valid and that a lognormal distribution on the original scale was appropriate.

In the study of van Duinkerken et al. (2005), the effect of bulk milk urea concentration on day t was taken into account, as was the effect of bulk milk urea concentration on previous days. In the transfer functions, data on previous days were weighted according to an exponential decay model (Pankratz, 1991). Furthermore, it was assumed that repeated observations of the response variate were correlated and that this correlation can well be described by an autoregressive process of first order [AR(1)].

The model used in the current study is written as

$$Y_t = C + \omega_1 T_t + \frac{\omega_{21}}{1 - \delta_1 B} U_t + \frac{\omega_{22}}{1 - \delta_1 B} U_t^2 + \frac{\omega_3}{1 - \delta_2 B} I_t + \frac{1}{1 - \phi B} a_t, \quad [4]$$

where $Y_t = \ln(\text{NH}_{3,t})$, natural logarithm of NH₃ emission on day t , with emission in kilograms of NH₃ per cow per 175 d (with 175 d being the average length of a grazing season in the Netherlands); $t = 1, \dots, 182$; C = constant; T_t = outdoor temperature minus 15°C, on

Figure 1. Cross section of the dairy cow barn and location of the tracer gas injection points. Color version available in the online PDF.

day t ; U_t = milk urea concentration on day t , in mg/100 g; I_t = index for slurry mixing on day t , where $I_t = 0$ for no mixing and 1 for mixing; ω = regression parameters for the size of effects; δ = decay parameters; ϕ = autocorrelation coefficient for residual effects; $a_t \sim N(0, \sigma_a^2)$ = random innovation effect with variance equal to σ_a^2 ; B = backshift operator: $BX_t = X_{t-1}$, where $X_t = U_t, U_t^2, I_t$, or a_t .

Furthermore, the total variance of residual effects (σ_{res}^2) was

$$\sigma_{res}^2 = \frac{\sigma_a^2}{1 - \phi^2},$$

and the percentage variance accounted for by the model is

$$100 \left(1 - \frac{\sigma_{res}^2}{\sigma_{tot}^2} \right),$$

with σ_{tot}^2 being the total variance of the logarithmically transformed NH_3 emission Y_t . Model [4] is fitted to the observed emission for the urea concentration in bulk milk.

RESULTS

Milk Yield and Milk Fat and Protein

In Table 5, milk yield and milk fat and protein percentages are given, all averaged for the last week of each treatment period. Also included in this table is the number of animals involved in milk recordings and milk sampling. The weighted averaged milk production level over the whole trial was 25.0 kg/d with 4.49% fat and 3.33% protein.

Milk Urea Content

The development of the expected and actual urea concentrations in bulk milk during the course of the experiment is illustrated in Figure 2. The intended dispersion in bulk milk urea level (15 to 55 mg of urea per 100 g of milk) was achieved; some samples contained less than 15 mg of urea per 100 g of milk. The highest level of 55 mg/100 g was only achieved at one sampling moment and, especially in U55-1 and U55-2, actual milk urea levels were lower than targeted. The course of the actual milk urea data was more irregular than the expected course, which was not surprising because of the variable grazing conditions (i.e., composition and intake of pasture grass) and because of random variance of MUN determination (Broderick, 2003; Kohn et al., 2004).

Table 5. Milk yield and milk composition in the last week per treatment replicate and number of animals involved in milk recordings and milk sampling

Treatment–replicate ¹	Animals ²	Milk (kg/d)	Fat (%)	Protein (%)
U35–1	49	26.2	4.48	3.21
U15–1	50	28.4	4.31	3.37
U55–1	45	25.9	4.33	3.22
U35–2	46	25.6	4.52	3.31
U55–2	48	25.6	4.37	3.28
U15–2	51	23.1	4.44	3.51
U15–3	51	23.4	4.56	3.43
U35–3	47	22.5	4.46	3.36
U55–3	46	22.1	4.63	3.33

¹Treatments U15, U35, and U55 included 3 levels of 15, 35, and 55 mg of urea per 100 g of milk, respectively.
²Number of animals involved in the milk recording and milk sampling; the whole treatment group generally included some more animals (e.g., heavily pregnant animals).

Herd milk urea concentration per milking is displayed in Figure 3, with a.m. and p.m. milkings shown separately. Average urea concentration in bulk milk was 31.4 ± 11.4 mg/100 g of milk for the whole experiment. Average urea concentrations in herd milk for the whole experiment were 33.6 ± 11.5 and 30.3 ± 13.4 mg/100 g for p.m. and a.m. milkings, respectively. The general development in herd milk urea concentration in time resembled that in bulk milk urea concentration, but the variation in herd milk urea concentration was higher than that in bulk milk urea concentration (Figure 3).

Urine and Slurry Composition

Data on urine composition were averaged per treatment per sampling time, as shown in Table 6. The concentrations of total N, urea-N, and Na in urine increased with increasing adjusted milk urea levels. Furthermore, levels of total N, urea-N, and K in urine were higher in a.m. than in p.m. sampling times, whereas Na concentration in urine was higher for p.m. sampling than for a.m. sampling.

The composition of the top layer of the slurry in the pit in the third week of each treatment was averaged per

Figure 2. Development of the actual and expected bulk milk urea concentration (mg/100 g) during the course of the experiment.

treatment and is presented in Table 7. Total N, $\text{NH}_4\text{-N}$, and pH in the top layer of the slurry were higher with higher levels of adjusted milk urea. The data on mixed slurry composition in the third week of each treatment were averaged per treatment and are presented in Table 8. Concentration of $\text{NH}_4\text{-N}$ was higher with higher levels of adjusted milk urea, whereas DM content of the mixed slurry was lower with higher levels of adjusted milk urea. The concentration of K in mixed slurry was highest for U55.

Ammonia Emission

Figure 4 shows the development of daily averaged NH_3 emission, barn temperature, and outdoor temperature; considerable day-to-day variation in NH_3 emission was observed. Some NH_3 emission values could not be collected during the last 2 wk of September because of technical malfunctioning of the emission measurement equipment. The observed NH_3 emission was correlated with temperature.

Emission Model

A dynamic regression model for NH_3 emission from the barn was derived based on outdoor temperature and bulk milk urea concentration. First, the general dynamic regression model [4] was adjusted to the logarithm of the NH_3 emission data. Genstat (Genstat 5 Committee, 1993) was used to calculate the auto-correlation function and the partial auto-correlation function for the residuals of the AR(1) model.

The parameter estimates and standard error per parameter of the fitted emission model are given in Table 9. The model accounted for 66% of the variance in NH_3 emission. The emission model showed that NH_3 emission increased 2.6% when outdoor temperature increased by 1°C. Furthermore, emission increased exponentially with increasing milk urea concentration. At a level of 20 mg of urea per 100 g of milk, NH_3 emission increased 2.5% when milk urea concentration increased by 1 mg/100 g. At a level of 30 mg of urea per 100 g of milk, NH_3 emission increased 3.5% when milk urea concentration increased by 1 mg/100 g. Finally, it appears that mixing of the slurry immediately followed by pumping it to another slurry storage outside the barn increased NH_3 emission by 11% on the day of mixing. This effect disappeared rapidly in the next few days.

Figure 5 illustrates the calculated median NH_3 emission, the lower and upper limits of the 95% confidence interval for the expected emission, and the 95% confidence interval for the individual emission data.

DISCUSSION

Emission Models

Several NH_3 emission models for dairy farms have been developed, especially during the last decade. Some models have attempted to describe emissions on the farm level, including animal housing, slurry storage, slurry application, and grazing (Pinder et al., 2004; Schils et al., 2007), whereas others focused on emissions from naturally ventilated dairy barns (Monteny et al., 2002; Wang et al., 2005) or tie-stall chambers (Powell et al., 2008b). Sommer et al. (2006) reviewed the literature focusing on the NH_3 emissions from buildings housing livestock, impermeable yard areas, and manure storage. They modeled both the processes of NH_3 release from manure and the transport of NH_3 in animal houses.

In this study, the applicability of bulk milk urea as an indicator for NH_3 emission from the dairy barn was assessed, because this parameter is available as a management tool in common practice.

Emission Model Parameters

This study showed that NH_3 emission from a dairy barn could be estimated with 66% of variance accounted for using a model with bulk milk urea content, outdoor temperature, and a slurry mixing parameter. To model the correlation between milk urea concentration and NH_3 emission, several treatments were formulated to create a wide range of milk urea data. To increase the power of the emission model, some additional variables were incorporated into the model. Temperature and slurry mixing and handling had a significant and large effect on NH_3 emissions and therefore were included in the emission model.

The effects of temperature and slurry handling correspond with the effects found by van Duinkerken et al. (2005) in the same barn with cows kept indoors for 24 h/d. As it is not common practice to mix and pump slurry every 3 wk during the grazing season, it is assumed that the slurry mixing parameter $I_t = 0$ under practical conditions.

Variability in Milk Urea Levels

For modeling purposes, milk urea concentration was forced to levels varying between approximately 15 and 55 mg/100 g of milk. However, to develop an emission model based on a dynamic regression approach, it is not necessary to achieve the exact milk urea levels that were expected based on diet composition. This discrepancy was taken into account when the experiment was

Table 6. Urine composition, averaged per treatment per sampling time

Item	Period	Treatment ¹			SED ²		P-value ³		
		U15	U35	U55	Treatment	Time	Treatment	Time	Treatment × time
Total N (g/kg)	a.m.	6.3	7.5	9.1	0.43	0.83	0.002	<0.001	0.254
	p.m.	4.7	6.7	7.5					
Urea-N (g/L)	a.m.	3.5	5.7	7.9	0.29	0.65	<0.001	<0.001	0.014
	p.m.	2.5	4.9	5.8					
pH	a.m.	8.3	8.4	8.4	0.03	0.12	0.467	0.020	0.345
	p.m.	8.2	8.3	8.4					
K (g/L)	a.m.	13.7	13.9	12.4	0.78	0.78	0.017	<0.001	0.427
	p.m.	10.5	11.8	11.3					
Na (g/L)	a.m.	0.14	0.23	0.39	0.08	0.08	0.163	<0.001	0.192
	p.m.	0.39	0.40	0.49					

¹Treatments U15, U35, and U55 included 3 levels of 15, 35, and 55 mg of urea per 100 g of milk, respectively.

²Standard error of differences for the same level of factor, respectively, for the fixed terms treatment and time.

³P-value (chi probability) based on Wald test for the fixed terms treatment and time.

designed. It was assumed a priori that large day-to-day variability in MUN would occur (Rajala-Schultz and Saville, 2003), particularly because of fluctuations in pasture grass composition, feed intake, and grazing conditions. Furthermore, deviations between expected and actual milk urea concentration (Figures 2 and 3) were anticipated. The targeted range of 15 to 55 mg of urea per 100 g of milk was achieved in the experiment.

In common practice, farmers have only bulk milk urea concentration available as management information; therefore, bulk milk is the most practical base for the development of a management tool. In this experiment, as in common Dutch dairying practice, the milk bulk tank was emptied every 3 d (generally after 6 milkings). Clear milking-to-milking variation in milk urea concentration was observed within one milk delivery period of 3 d (Figure 3). In this study, the p.m. milking

Figure 3. Development of actual and expected milk urea concentration (mg/100 g) per a.m. and p.m. milking during the course of the experiment.

Figure 4. Averaged daily ammonia emission (g/h) from the barn, barn temperature (°C) and outdoor temperature (°C).

generally showed a higher milk urea concentration than the a.m. milking. This is common in situations with restricted grazing when N-rich pasture grass is grazed during daytime and cows are supplemented in the barn between the p.m. and a.m. milking with feedstuffs that have a smaller N content than grass.

Urine Composition

Data on urine composition (Table 6) illustrate that the concentrations of both total N and urea-N increased with increasing adjusted milk urea levels. This positive correlation corresponds with the physiological process of urea, formed in the liver and secreted in blood plasma, diffusing to other body fluid pools such as milk (Gustafsson and Palmquist, 1993), saliva, and rumen liquid (Huntington and Archibeque, 1999) and being (actively) excreted in the urine via the kidneys (Burgos et al., 2007). Therefore, it is generally assumed that an

increase in plasma urea nitrogen (**PUN**) concentration leads to higher levels of MUN and increased excretion of urinary urea nitrogen (**UUN**).

From the data on urine composition in Table 6, it appears that diurnal variation in total N, urea-N, K, and Na concentrations exists. Total N, urea-N, and K concentrations were higher at the a.m. than at the p.m. sampling time, whereas Na concentration in urine was higher for p.m. sampling than for a.m. sampling. Gustafsson and Palmquist (1993) also found clear diurnal variations in MUN and PUN concentrations, with MUN concentrations reaching peak values around 4 h after a meal. Furthermore, the study of Broderick and Clayton (1997) demonstrated that sampling time influences the MUN–UUN relationship. In their study, different relationships were found between BUN and MUN when assessed from MUN in milk collected at either the a.m. or p.m. milking; BUN was more highly correlated with mean daily MUN concentration. In general, level

Table 7. Composition of the top layer in the slurry pit in the third week of each treatment period, averaged per treatment

Item	Treatment ¹			SED ²	P-value ³
	U15	U35	U55		
Total-N (g/kg)	4.23	4.69	4.94	0.115	<0.001
NH ₄ -N (g/kg)	1.44	1.76	1.96	0.100	<0.001
pH	6.9	7.1	7.1	0.04	<0.001

¹Treatments U15, U35, and U55 included 3 levels of 15, 35, and 55 mg of urea per 100 g of milk, respectively.

²Standard error of the difference.

³P-value (chi probability) based on Wald test.

Table 8. Composition of the mixed slurry in the third week of each treatment period, averaged per treatment

Item	Treatment ¹			SED ²	P-value ³
	U15	U35	U55		
Total-N (g/kg)	3.80	3.83	3.86	0.045	0.41
NH ₄ -N (g/kg)	2.03	2.11	2.21	0.020	<0.001
pH	7.6	7.5	7.7	0.07	0.24
DM (g/kg)	68.4	65.4	61.8	1.74	0.001
Na (g/kg)	0.55	0.62	0.59	0.073	0.58
K (g/kg)	4.83	4.82	5.01	0.026	<0.001

¹Treatments U15, U35, and U55 included 3 levels of 15, 35, and 55 mg of urea per 100 g of milk, respectively.

²Standard error of the difference.

³P-value (chi probability) based on Wald test.

of feed intake, feed composition, feed intake pattern, milking frequency, and urinating behavior are major factors affecting diurnal variations in PUN, MUN, and UUN and their interrelations.

Data in Table 6 also indicate that the ratio between urea-N and total N was affected by treatment. This ratio was approximately 55% for U15 and it increased as adjusted milk urea levels were higher. The highest ratio (approximately 87%) was achieved at the a.m. sampling time for U55.

Slurry Composition

Differences in composition of mixed slurry between treatments were rather limited (Table 8). The concentration of NH₄-N in the slurry was lowest for U15 and highest for U55, although differences were rather small. It should be taken into account that at the end of a 3-wk treatment period about 80% of the slurry volume in the pit originates from previous treatments. This indicates that treatment effects on mixed slurry composition are leveled out, to a great extent. However, total N and urea-N levels in urine (Table 6) are strongly influenced by diet (treatment), thus affecting total N and NH₄-N concentration in the top layer of the slurry in the pit (Table 7). It should be noted that the top layer of slurry is not a homogeneous fraction because of differences in gravity between urine and solid feces components. van der Stelt et al. (2008) studied the effects of dietary protein and energy levels on cow manure excretion and calculated ammonia volatilization. In their experiment, they had no interference of older manure in the slurry pit, enabling them to examine thoroughly the correlation between diet and manure characteristics. van der Stelt et al. (2008) found that increasing the CP content of the feed (108 to 190 g/kg of DM) resulted in an average increase in total N content of the slurries of 56%. Total ammonia nitrogen amounted to 52 to 77% of the total N content present in manure slurries. A low CP

content of diets reduced total ammonia N concentrations in the slurries by 43%. In agreement with the calculated aqueous NH₃ content, the total amount of NH₃ volatilized from manure slurries was much greater (on average 10 times greater) when the cows were fed greater levels of CP.

Emission Reduction Potential

The similarity of results between the study of van Duinkerken et al. (2005) (summer feeding experiment) and the present study (restricted grazing experiment) confirms that feeding measures have high potential to reduce NH₃ emission and that bulk milk urea is a useful indicator for NH₃ emission reduction. These conclusions are supported by several studies in which positive relationships were found between (1) dietary CP concentration, MUN, and urinary N excretion (Kebreab et al., 2002; Nousiainen et al., 2004; Burgos et al., 2007);

Table 9. Estimates and SE for the parameters of the emission model [4] and variance components of the model

Parameter ¹	Estimate	SE
C	1.25	0.20
ω_1	0.026	0.006
δ_1	0.85	0.05
ω_{21}	-0.0013 ²	0.0022
ω_{22}	0.000088	0.000042
δ_2	0.45	0.49
ω_3	0.11	0.06
ϕ	0.72	0.06
σ_a^2	0.0216	—
σ_{tot}^2	0.044	—
σ^2	0.130	—

¹ C = constant; ω = regression parameter for the size of effects; δ = decay parameter; ϕ = autocorrelation coefficient for residual effects;

σ_a^2 = variance of random innovation effect; σ_{tot}^2 = the total variance of the logarithmical transformed NH₃ emission; σ^2 = the total variance of residual effects.

²Parameter estimate not different from zero, based on a t -test.

Figure 5. Median ammonia emission (NH_3), 95% CI for individual emission (Conf), and 95% CI (Pred) for the expected emission based on model [4] (NH_3 emission in kg/animal per 175 d; bulk milk urea in mg/100 g), standardized for outdoor temperature of 15°C and $I_t = 0$ (I_t = index for slurry mixing on day t , where $I_t = 0$ for no mixing and 1 for mixing).

(2) urinary N excretion and NH_3 emission (Smits et al., 1995; Monteny et al., 1998; Cassel et al., 2005); and (3) MUN and NH_3 emission (Frank and Swensson, 2002; Powell et al., 2008a).

In the Netherlands, the national average milk urea concentration decreased from 30 to 25 mg/100 g of milk over the period 1998 to 2001 (van Duinkerken et al., 2005), corresponding with a decrease of MUN from 14.4 to 12.0 mg/100 mL. This reduction in MUN indicates that farmers were able to reduce NH_3 emissions from dairy barns with about 12.5% on average over this period (van Duinkerken et al., 2005). In various other European studies with varying circumstances (Cottrill et al., 2002; González Rodríguez and Vázquez Yáñez, 2002; Nousiainen et al., 2004), milk urea levels were reported that are clearly above 18 mg/100 g milk (= 8.6 mg of MUN/100 mL of milk). This level is the benchmark for optimal bulk milk urea concentration when the results of Schepers and Meijer (1998) are evaluated for a situation with feeding according energy and protein requirements and an RDP balance of 0 g/d. Taking into account the levels of milk urea in various European Union regions, high potential exists for further reduction of NH_3 emission by nutritional measures.

CONCLUSIONS

The emission of NH_3 from a naturally ventilated dairy cow barn in a situation with restricted grazing

was strongly influenced by both temperature and diet. Reduction of NH_3 emission was predicted adequately (66% of variance accounted for) by a dynamic regression model using bulk milk urea concentration, outdoor temperature, and a slurry mixing parameter as explanatory variates. This study demonstrates that, in common dairy farming practice, NH_3 emission reduction can be monitored and controlled using milk urea concentration as an indicator. Ammonia emission increased exponentially with increasing milk urea concentration. At a level of 20 or 30 mg of urea per 100 g of milk, NH_3 emission increased 2.5 and 3.5%, respectively, when milk urea concentration increased by 1 mg/100 g. In this study daily averaged outdoor temperature ranged from 8 to 25°C and ammonia emission from the barn increased with approximately 2.6% when outdoor temperature increased with 1°C. Taking into account the current European levels of milk urea, there is a rather high potential for further reduction of NH_3 emission by feeding measures.

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