Effect of dietary crude protein modification on ammonia and nitrous oxide concentration on a tie-stall dairy barn floor

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ABSTRACT

Dietary crude protein (CP) reduction is considered a useful strategy to minimize cow N excretion and NH₃ and N₂O emissions. The aim of the current work was to relate dietary CP modification to whole-animal N balance and subsequent NH₃ and N₂O concentrations on a tie-stall barn floor. The effect of temperature on NH₃ and N₂O concentration was also studied. Three Holstein mid to late lactating cows were confined in separate tie-stalls and randomly assigned to 3 diets with varying CP content [low CP (LCP): 14.1%; moderate CP (MCP): 15.9%; high CP (HCP): 16.9%]. Increasing N intake (from 438.6 to 522.8 g of N/d) improved milk yield (from 22.1 to 24.2 kg/d). However, N use efficiency tended to decrease with increasing dietary CP, as shown by milk N use efficiency (from 23.9 to 22.6%), milk urea N (from 15.4 to 18.7 mg/dL), and excreted N per milk yield unit (from 14.7 to 16.4 g of N/kg of milk). Because of higher N excretion, NH₃ concentration on the dairy barn floor increased (LCP: 7.1 mg of NH₃/m³; MCP: 10.4 mg of NH₃/m³; HCP: 10.8 mg of NH₃/m³). In contrast, N₂O concentration did not respond to dietary manipulation (mean 1.1 mg of N₂O/m³). Temperature, which ranged between 12.6 and 18.0°C, did not affect NH₃ and N₂O concentrations at the stall level. However, when fecal and urinary samples were incubated at 4, 19, and 29°C in the laboratory, ammonia concentration increased for all diets, especially for the MCP and HCP diets, as the temperature increased. In contrast, N₂O concentration was negatively related to increasing temperature. In conclusion, data from the current trial demonstrate that lowering dietary CP minimizes NH₃ concentration on dairy stall floors although temperature controls the rate of NH₃ volatilization. On the other hand, N₂O concentration is not affected by dietary treatments on tie-stall floors.

Key words: dietary crude protein, nitrogen excretion, tie-stall, ammonia emission

INTRODUCTION

Animal husbandry results in considerable N losses to the atmosphere in terms of ammonia (NH₃) and nitrous oxide (N₂O) emissions (Amon et al., 2001, 2006). Overall, animal husbandry contributes about 40% of global NH₃ emissions (van Aardenne et al., 2001) with dairy farming activity being the largest source of NH₃ emission (Bussink and Oenema, 1998). On the other hand, 63% of global N₂O emissions can be attributed to agricultural production, where dairy production systems are the main contributors (Weiske et al., 2006). At the dairy farm level, the main sources of NH₃ and N₂O fluxes are the dairy barn, the manure stored in slurry pits, and the manure applied on grasslands (Amon et al., 2001). Although less studied than NH₃ fluxes from grasslands, the large contribution of dairy stalls to NH₃ emissions (around 20%; Frank et al., 2002) has promoted several studies (Monteny and Erisman, 1998; Powell et al., 2008). However, there are fewer studies on N₂O fluxes from dairy stalls (Amon et al., 2001; Külling et al., 2003).

According to the Gothenburg (1999) and Kyoto (1997) Protocols, European countries are required to adopt practices that contribute to minimizing national NH₃ and N₂O emissions from different activities, including animal husbandry. Many strategies have been already tested to affect NH₃ fluxes from barn floors (e.g., animal management, animal nutrition, bedding, manure management, ventilation; Monteny and Erisman, 1998). Among these strategies, dietary manipulation is considered an effective and easy strategy to minimize the effect of NH₃ fluxes in stalls (de Boer et al., 2002; Frank et al., 2002). In contrast, little information is available about the effect of dietary modification on N₂O emission (Cárdenas et al., 2007). Dietary composition of livestock has an effect on total N, ammoniacal-N, and the soluble organic fraction of manure (Velthof et

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Table 1. Composition of the diets used in the experiment

Item	Experimental diet ¹		
	LCP	MCP	HCP
Ingredient, kg of DM/d			
Corn silage	5.27	5.42	5.41
Alfalfa hay	5.33	4.60	3.75
Beet pulp	0.92	0.89	0.89
Barley grain	4.52	2.73	1.84
Corn grain	0.00	0.91	1.84
Beans	0.84	1.07	0.45
Soybean meal	0.00	1.18	2.73
Cottonseed	1.05	1.01	1.03
Tallow	1.05	1.01	1.03
Dicalcium phosphate	0.10	0.09	0.08
Limestone	0.18	0.17	0.16
Sodium bicarbonate	0.10	0.09	0.08
Vitamin-mineral mix	0.10	0.09	0.08
Chemical composition, % of DM			
CP	14.1	15.9	16.9
NDF	37.7	37.3	36.8
ADF	20.6	20.8	21.1
Fat	3.7	3.9	3.9
NFC	34.6	33.5	34.3
Ash	9.9	9.4	7.9

¹Three diets with low, moderate, and high CP content (LCP: 14.1%; MCP: 15.9%; HCP: 16.9%).

al., 2005), and therefore modifications on N₂O emission might be expected from diet manipulation.

The aim of the current work was to study the effect of CP content in the ration on the N balance of lactating dairy cows and to relate it to the accumulation of NH₃ and N₂O on a barn floor. A second objective was to study the effect of temperature on NH₃ and N₂O concentrations at different CP contents in the ration.

MATERIALS AND METHODS

Animals, Diets, and Experimental Design

Three first-lactation Holstein cows (210 ± 12 d in milk, 644 ± 8 kg of BW, and 27 kg of milk per day) were selected from the experimental dairy herd of Instituto de Educación Secundaria “La Granja” (Cantabria, Spain). Cows were randomly assigned to 1 of 3 diets in a 3 × 3 Latin square design. The duration of each experimental period was 15 d, with 11 d for diet adaptation and 4 d for data collection. Cows were held in separated metabolic tie-stalls to control the individual feed intake and collect whole fecal excreta. Cows had free access to drinking water and mineral supplements.

The experimental diets were formulated to contain 3 levels of CP on a DM basis: low CP (**LCP**, 14%), moderate CP (**MCP**, 16%), and high CP (**HCP**, 17%), being isoenergetic and with a forage:concentrate ratio of 52:48. Corn silage and alfalfa hay were used as forage sources and concentrates were formulated with varying amounts of soybean meal to fit diet CP content (Table

1). Diets were fed twice daily as a TMR at 0900 and 1700 h, and provided at a level to produce at least 10% refusals. Total refusals were weighed the next morning to determine the daily DMI for each cow. Samples of individual feedstuffs were taken for laboratory analyses twice weekly during the adaptation period and during 2 d after finishing the experimental period. Feedstuffs were analyzed for DM content at 105°C (AOAC, 1990), ash (AOAC, 1990), OM (AOAC, 1990), fat (Soxtec System HT6, Foss Tecator, Eden Prairie, MN), CP (Kjeldahl N method by Kjeltec 2300 Autoanalyzer, Foss Tecator AB, Hoganas, Sweden), NDF, and ADF (van Soest et al., 1991); NFC was estimated as 100 – (fat + CP + ash + NDF).

Daily deposited whole fecal samples were collected from each cow after the morning milking and weighed for their quantification. A subsample (300 g) was taken for laboratory analyses and incubations, and remaining was flushed with water into the slurry pits located behind each metabolic stall under a slatted floor (0.51 m³). Samples were stored at –20°C until analysis. Samples were oven-dried at 105°C in a forced-air oven for DM determination, and at 55°C for N and C analysis (Leco CNS 2000 analyzer, Leco Corp., St. Joseph, MI). Fecal pH was also measured (Schott CG-843 pH meter, Schott, Stafford, UK). Apparent digestibility of DM was calculated as the amount of digested DM (ingested DM – excreted DM in feces) per unit of ingested DM. Daily fecal N excretion was calculated multiplying the whole fecal amount and N content in each subsample.

Spot urine samples were collected from each cow by using a noninvasive method with buckets. Individual urine samples (2 or 3 per cow) were composited to provide one urine sample per day (0.5–2.0 L/d). A 200-mL subsample was acidified with 10% H₂SO₄ solution to analyze for N (Kjeldahl N method), urea N (**UUN**; Douglas and Bremner, 1970), purine derivatives, and creatinine (Balcells et al., 1992). Nonacidified urine subsamples (100 mL) were separated for laboratory incubations and were analyzed for pH (Schott CG-843 pH meter), N, and urea using the same methods. Urine volume was computed using creatinine as a marker assuming a daily creatinine excretion of 22.6 mg/kg of BW (Gonda et al., 1996). Daily urinary N excretion was calculated multiplying computed urine volume and N content in each sample. Data from purine derivatives (allantoin, uric acid, xanthine, and hypoxanthine) were used for estimating the ruminal microbial protein synthesis (**MPS**; Chen and Gomes, 1992).

Cows were milked twice daily at 0800 and 1830 h and milk weights were recorded at each milking. Individual milk samples (a.m./p.m. composite) were placed in a sealed container and preserved with 2-bromo-2-nitropropane-1,3-diol. Milk samples were shipped to the Milk Institute of Santander (Cantabria, Spain) and analyzed for CP and fat (MilkoScan 4000, Foss Analytical, Hillerød, Denmark), and MUN (MilkoScan FT6000). Nitrogen use efficiency (**NUE**) was calculated as N in true milk protein (conversion factor of 6.38) multiplied by the daily milk yield and expressed as a percentage of total N intake.

On-Farm Trace Gas Measurements

Concentrations of NH₃ and N₂O were measured using a photoacoustic infrared gas analyzer (Brüel and Kjaer 1302 Multi-Gas Monitor; detection limit 0.2 ppm for NH₃ and 0.03 ppm for N₂O; Brüel and Kjaer, Naerum, Denmark) equipped with an internal pump. Gas measurements were always carried out between 1000 and 1330 h to minimize the effects of diurnal variation (Conrad et al., 1983) and began 3 h after cleaning the excreta accumulated the day before. Gas measurements were always conducted on freshly deposited feces and urine. Measurements were carried out from the LCP to the HCP diet to prevent any carryover effect between treatments. Gases were measured in each stall through a Teflon tube at 15-cm distance over the barn floor; the tube was continuously repositioned 30 to 50 cm behind each cow until the steady-state value was reached for NH₃ (approximately 20 min). Gas sampling from nearby slurry pits was avoided if possible. Atmospheric temperature (HI 935005 thermometer, Hanna Instruments, Bedfordshire, UK) was recorded at the

beginning and at the end of each sampling. One control point was established next to the farm (200 m) but free of dairy cow influences to record NH₃ and N₂O at atmospheric concentrations.

The stall (30 m²) was naturally ventilated, with air flow passing from an air opening on the western wall (2 m²) to the stall access door (5 m²) located on the eastern wall. The door was always closed when gases were measured to avoid lateral air flow through metabolic tie-stalls, which might promote carryover effects among treatments. Tie-stalls were physically separated through 1-m-high cemented walls to prevent lateral air contamination.

Laboratory Incubations

A slurry incubation trial was carried out in the laboratory at 3 temperatures (4, 19, and 29°C) to simulate the effect of temperature on NH₃ and N₂O concentration from different CP diet-derived slurries. The minimum temperature represented the mean temperature in winter in the Basque country (northern Spain), 19°C was the mean temperature in summer, and 29°C represented a typical hot, summer day in the territory. Incubations were conducted inside a plant growth chamber (Sanyo MLR-351H, Sanyo, Osaka, Japan), whose temperature variability was 4 ± 1.3°C, 19 ± 0.2°C, and 29 ± 0.2°C. Slurry was created by mixing 60 g of fresh fecal samples and 30 mL of nonacidified urine samples (2:1 ratio) in a 1-L glass jar. This ratio was established according to the fecal and urinary excretion data set obtained previously in commercial dairy farms from the Basque country (Arriaga et al., 2009). The jars were sealed with lids fitted with rubber stoppers in which one Teflon tube was inserted and connected to a photoacoustic infrared gas analyzer (Brüel and Kjaer 1302 Multi-Gas Monitor). A polyethylene tube was used to transport the outflow gas from the photoacoustic analyzer into the jars, thus avoiding the disturbance of jar atmosphere. Before starting measurements, jars and slurries were adapted to the corresponding temperature. Measurements were carried out for 40 min to consider the concentration within the linear increase of the gas. Nonincubated slurry subsamples were dried at 105°C for DM analysis (AOAC, 1990) and at 55°C for N and C analysis (Leco CNS 2000 analyzer, Leco Corp.).

Statistical Analysis

On-farm and laboratory data were analyzed by using SAS 8.0 software (1999; SAS Inst. Inc., Cary, NC), using PROC MIXED. Data were previously analyzed for the normality test of Shapiro-Wilk to comply with the “equal variances” assumption. Data from manure incu-

bation were base-10 log-transformed to fit the normality test. Two model sets were used for statistical analysis. First, a model was carried out to test the effect of the diets on NUE, N excretion, milk parameters, and on-farm NH_3 and N_2O concentrations. In this model, diet was the fixed effect and animal was used as a random effect. The interaction of diet and day was studied. The second model was used for laboratory NH_3 and N_2O concentrations; it included diet and temperature as fixed effects and animal as a random effect. The interaction between diet and temperature was also analyzed. Differences were analyzed by least squares means, and when differences were relevant, the LSD test was used to determine significant differences among treatments. Univariate and multivariate regressions were carried out to test the effect of nutritional parameters on NH_3 and N_2O concentrations on the dairy barn floor and to study the effect of manure source on NH_3 and N_2O accumulation in laboratory incubations. Collinearity tests were carried out in multivariate regressions. Regression models for the on-farm data set included N intake, dietary CP content, fecal N content, urinary N content, UUN, MUN, and milk yield. The regression model for incubations included fecal N, pH, and N content, urinary pH, N, and UUN content, and slurry OM and N content. Temperature was introduced in this model to test its effect on NH_3 and N_2O accumulation. Differences among treatments were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Feeding Trial

Table 1 shows the ration and chemical composition of the diets. Feed intake did not differ among treatments and averaged 19.4 kg of DM/d ($P > 0.05$). Forage:concentrate ratio was established around 50:50 for all diets. As expected, CP levels were different between treatments ($P < 0.05$; Table 1). This range of dietary CP concentrations was selected to represent target CP contents in lactating dairy cow nutrition. Thus, the HCP diet represented dietary CP recommended for high-yielding dairy cows (Ipharraguerre and Clark, 2005; Olmos-Colmenero and Broderick, 2006). The MCP diet represented a diet consistent with studies indicating that 16.0% CP did not compromise the performance of mid lactation cows (Castillo et al., 2001). Finally, the LCP diet represented dietary CP content able to maintain milk productivity in mid and late lactation cows (Frank et al., 2002).

Milk Yield and Composition

Milk yield and MUN differed because of dietary treatment ($P < 0.05$), whereas FCM, milk CP, fat con-

tents, and feed efficiency were not affected ($P > 0.05$; Table 2). Milk yield increased with higher CP content of the rations and averaged 24.2 kg/d in HCP, 23.7 kg/d in MCP, and 22.1 kg/d in LCP. When milk yields were corrected by fat content (4% FCM), data did not show any difference between treatments because milk fat percentage tended to increase with the lower CP ($P < 0.10$; Table 2). Feed efficiency, defined as FCM/DMI, was not affected by dietary treatment ($P > 0.05$) and averaged 1.14 kg of FCM/kg of DMI. Milk protein content was slightly higher in the HCP diet compared with the MCP and LCP diets (Table 2). Similarly, MPS increased with higher CP rations (Hoover and Stokes, 1991), differing between the LCP diet (1.17 kg of MPS) and the MCP and HCP diets, which averaged 1.48 and 1.52 kg of MPS, respectively ($P < 0.05$). Milk urea N increased with higher N intake treatments ($P < 0.05$) and the LCP diet had the lowest MUN value (15.4 mg/dL) compared with MCP (18.7 mg/dL) and HCP (17.8 mg/dL).

Cow N Balance

As expected, daily N intake varied among treatments ($P < 0.05$) and ranged from 522.8 g/d in the HCP treatment to 490.8 g/d for MCP and 438.6 g/d for LCP. These N intake levels have been reported as being capable of maintaining recorded milk yields without compromising any physiological feature (Castillo et al., 2001). Milk NUE values did not differ between treatments ($P > 0.05$) and averaged 23.9% in LCP, 22.9% in MCP, and 22.6% in HCP. Recorded NUE values were similar to those observed by Castillo et al. (2001) for mid lactating cows fed similar N intakes. Efficiency values also coincided with NUE data collected in commercial farms from the Basque country within the tested N intake range (25.1%, SD 3.1%; data not published). Data showed that decreasing N intake for mid to late lactating cows might increase NUE because the lactation period may better limit milk responses from high-protein diets (Huhtanen and Hristov, 2009).

As Yan et al. (2006) reported from an extensive review, around 70 to 75% of the N consumed by dairy cows was excreted in feces and urine (Table 2). Nutritional CP manipulation is known to be more effective in controlling urinary N excretion than fecal N output, as the urinary pathway is reported to be the main N excretion source above 400 g of N ingested (Kebreab et al., 2001). Urinary N output accounted for 46.0, 52.4, and 48.3% of N intake in LCP, MCP, and HCP diets, respectively ($P > 0.05$). These percentages might be questioned because urinary N excretion was estimated through creatinine concentration in spot urine samples, which have been reported to show diurnal variation (Gonda

Table 2. Effect of dietary CP content on N metabolism

Item	Experimental diet ¹			SE	P-value
	LCP	MCP	HCP		
N intake, g/d	438.6	490.8	522.8	7.5	<0.05
Milk output					
Milk yield, kg/d	22.1	23.7	24.2	0.5	<0.05
4% FCM, kg/d	21.9	22.6	22.5	0.6	0.66
Milk CP, %	3.01	3.02	3.10	0.03	0.15
Milk fat, %	3.95	3.70	3.60	0.1	0.06
MUN, mg/dL	15.4	18.7	17.8	0.4	<0.05
N use efficiency, %	23.9	22.9	22.6	0.6	0.24
Urinary excretion					
Urinary N excretion, g/d	197.8	255.0	255.2	18.1	0.05
Urine volume, kg/d	25.0	34.3	32.6	2.9	0.08
Urine N, % of N intake	46.0	52.4	48.3	4.0	0.51
Urea N, g/L	5.77	6.22	7.31	0.5	0.08
Microbial CP, kg/d	1.17	1.48	1.52	0.1	<0.05
Fecal excretion					
Fecal N excretion, g/d	117.6	129.6	112.0	3.7	<0.05
Fecal DM, kg/d	4.53	3.75	3.79	0.1	<0.05
Fecal N, % of N intake	29.0	22.9	22.5	0.7	<0.05
Total N excretion					
Total N excretion, % of N intake	71.5	75.3	71.0	3.9	0.64
N excretion per milk, g of N/kg of milk	14.7	15.5	16.4	1.4	0.09

¹Three diets with low, moderate, and high CP content (LCP: 14.1%; MCP: 15.9%; HCP: 16.9%).

et al., 1996). However, a recent publication confirmed constant creatinine excretion (0.212 mmol/kg of BW) throughout the day in Holstein dairy cows (Chizzotti et al., 2008). In addition, if urinary N excretion had been calculated as the difference between ingested N and the sum of milk and feces N, results would have been overestimated by 3.2% (Table 2). Increasing CP intake has been related to higher amounts of UUN, which is the main N compound in urinary samples (Bristow et al., 1992). Urinary urea N and excreted urinary volume tended to increase with higher CP diets ($P < 0.10$; Table 2). Nitrogen excreted per unit of milk yield did not differ between treatments ($P > 0.05$; Table 2).

On-Farm NH_3 and N_2O Measurements

Ammonia. On-farm NH_3 concentrations showed that minimizing the dietary CP content resulted in lower NH_3 concentrations on tie-stall floors ($P < 0.05$). When decreasing the CP content from 16.9 to 14.1%, mean NH_3 concentration was reduced by 36.5% from 10.8 mg of NH_3/m^3 in HCP to 7.1 mg NH_3/m^3 in LCP. This result meant that NH_3 concentration would be reduced by 13% per unit reduction in dietary CP content. Such a reduction rate agreed with previous data published in the literature (Külling et al., 2001; Frank et al., 2002).

Ammonia emission begins in dairy stalls when fecal urease rapidly converts UUN to NH_3 on the barn floor (Powell et al., 2008). Ammonia is in an equilibrium state with NH_4^+ ion as the result of chemical and (micro)

biological features of manure (pH, DM, urease activity; Monteny et al., 2002) or physical parameters such as air temperature (van der Stelt et al., 2007). Fecal pH (7.4–8.5), DM content (16.5–22.4%), and urinary pH (8.2–8.8) did not show any response to nutritional treatments ($P > 0.05$); therefore, differences in NH_3 concentration were not related to these parameters. In contrast, UUN showed an increasing trend from LCP to HCP ($P < 0.1$; Table 2), even though diurnal patterns in the concentration of urea in urine of lactating dairy cows were previously observed (Burgos et al., 2007). The same authors reported that UUN responded linearly to dietary CP content in different stages of lactation. Besides, they confirmed that UUN was significantly related to MUN in mid to late lactating cows ($R^2 > 0.95$). In this sense, MUN was the main diet-derived parameter related to NH_3 concentrations through univariate and multivariate regressions ($P < 0.05$; $R^2 = 0.20$). Milk urea N ranged between 15.4 and 18.7 mg/dL in LCP and MCP, respectively. These data were within the range established by the linear relationship determined between dietary CP and MUN by Nousiainen et al. (2004).

Although different authors have reported the influence of temperature on NH_3 emission (van der Stelt et al., 2007; Powell et al., 2008), no relationship was established between air temperature and NH_3 concentration in the current study ($P > 0.05$). Mean temperature in the stall was 14.6°C, ranging from 12.6 to 18.0°C. According to Braam et al. (1997), temperature did not

limit urease activity on the floor. Thus, we suggest that the narrow range of temperatures recorded during the sampling period might have hidden the effect of temperature on NH_3 concentration.

Nitrous Oxide. On-farm N_2O concentrations did not respond to different dietary CP levels ($P > 0.05$) in this trial. The LCP treatment averaged 1.21 mg of $\text{N}_2\text{O}/\text{m}^3$, whereas MCP and HCP showed concentrations around 1.10 mg of $\text{N}_2\text{O}/\text{m}^3$. Recorded concentrations were slightly higher than those estimated by Jungbluth et al. (2001) in dairy tie-stalls, with measured N_2O emissions between 1.4 and 10.0 g/h. Although some authors concluded that N_2O emission rate may not be significantly different from zero in dairy barns (Sneath et al., 1997), our data supported that N_2O emission may be detected on-farm, because the control N_2O concentration was 0.55 mg of $\text{N}_2\text{O}/\text{m}^3$. These data suggest that more on-farm research will be necessary considering that more detailed data are required to associate default emission factors in defined livestock classes to reduce uncertainties in national N_2O inventories (Gac et al., 2007).

Limited published studies exist but suggest that N_2O emissions may be reduced by lowering the dietary CP content (Külling et al., 2001). However, our results did not show the same trend concerning dietary CP content and N_2O concentration. Although reducing the CP of diets has an effect on total N and NH_4^+ -N content of the manure (Velthof et al., 2005), high dietary CP content may be associated with increased digestibility of the diet, particularly of fiber (Külling et al., 2001). This fact is, to some extent, negatively related to manure fiber content and the subsequent surface crust formation, which enables N_2O synthesis (Sommer et al., 2000; Külling et al., 2003). Külling et al. (2003) reported that N_2O formation was related to the thick surface crust formed on liquid manure because of the relatively high fiber content of forage-based rations. This effect might be possible in our trial because N_2O measurements might be partially affected by slurry accumulated in storage, which was located close to the air sampling area in the dairy barn. Apparent digestibility of our rations decreased by reducing dietary CP content as fecal daily excretion increased ($P < 0.05$). Fecal daily excretion was 4.5 kg of DM/d in LCP diet and averaged 3.7 kg of DM/d in MCP and HCP diets. In addition, fecal samples differed in their C:N ratio ($P < 0.05$), ranging from 12.6 in HCP to 14.1 in LCP. The higher alfalfa hay intake (1.5 kg of DM/d) together with the lower daily N intake (84 g/d) in LCP treatment explained the differing C:N between diets. In the present study, the contrasting effects of lower CP content of the diet and lower digestibility may have resulted in no net difference in N_2O concentration.

Table 3. Effect of temperature on NH_3 concentration (mg/ m^3) in incubated jars

Temperature, °C	Experimental diet ¹		
	LCP	MCP	HCP
4	15.2 ^a	14.2 ^a	19.4 ^a
19	39.6 ^b	48.9 ^b	53.3 ^b
29	82.7 ^c	116.1 ^c	121.0 ^c

^{a-c}Values with different superscripts within the same column indicate significant differences ($P < 0.05$).

¹Three diets with low, moderate, and high CP content (LCP: 14.1%; MCP: 15.9%; HCP: 16.9%).

Effect of Temperature on NH_3 and N_2O

Ammonia. Ammonia concentration increased with higher temperatures in the incubation jars for all dietary treatments ($P < 0.05$; Table 3). Ammonia accumulation rate increases with higher temperatures because increased temperature results in an increase in the $\text{NH}_3/\text{NH}_4^+$ ratio, a decrease in the solubility of NH_3 in the liquid phase of slurry, and an increase in the activity of microorganisms present in slurry (van der Stelt et al., 2007). Incubation data showed that NH_3 concentration increased 2.7 mg of NH_3/m^3 per degree in LCP diet, and the increase was 4.1 mg NH_3/m^3 in MCP and HCP diets. Similarly, van der Stelt et al. (2007) demonstrated that when the temperature was increased from 4 to 20°C, NH_3 volatilization increased 2.6 to 5.8 times, whereas when the temperature was increased to 35°C, NH_3 concentration increased a further 3.0 to 6.0 times. In the present study, we assumed that NH_3 concentration might have increased further with a longer incubation period, especially for higher CP and higher incubation temperatures.

Dietary treatments did not affect NH_3 concentration at 4 and 19°C, but concentration was significantly greater for MCP and HCP than for LCP at 29°C ($P < 0.05$), with an increase of 13.7 mg/ m^3 per increased CP unit. Direct comparison of NH_3 data between incubation and on-farm fluxes may not be appropriate because NH_3 emission includes both diffusion and convection processes (van der Stelt et al., 2008). The small size of the incubation jar facilitates the high NH_3 concentration and may alter the diffusion process because of NH_3 saturation. A stepwise regression with NH_3 concentration as the independent variable showed that those parameters related to nutritional features, such as slurry OM, urinary pH, and UUN, explained 22.8% of the variance. Some reports related the increasing dietary CP with higher manure pH values (Misselbrook et al., 2005), probably related to higher urinary pH. Similarly, several reports recorded the effect of increasing UUN on NH_3 concentration (Monteny et al., 2002; Burgos

Table 4. Effect of temperature on N₂O concentration (mg/m³) in incubated jars

Temperature, °C	Experimental diet ¹		
	LCP	MCP	HCP
4	1.13 ^b	1.17 ^b	1.04 ^b
19	0.88 ^a	0.86 ^a	0.84 ^a
29	0.85 ^a	1.10 ^b	0.87 ^a

^{a,b}Values with different superscripts within the same column indicate significant differences ($P < 0.05$).

¹Three diets with low, moderate, and high CP content (LCP: 14.1%; MCP: 15.9%; HCP: 16.9%).

et al., 2007). When temperature was included in the regression model, the coefficient of determination of the model increased to 89%. Thus, in accordance with previous results (Amon et al., 2001; van der Stelt et al., 2007), NH₃ flux was positively related to temperature.

Nitrous Oxide. As Table 4 shows, temperature influenced N₂O concentration ($P < 0.05$) although this relationship was in contrast to NH₃ concentration in incubated jars. Samples incubated at 4°C showed the highest N₂O concentration of all dietary treatments, averaging 1.11 mg of N₂O/m³. Slurry incubated at higher temperatures averaged 0.86 mg of N₂O/m³. Nevertheless, such concentrations were low according to data reported by other authors (Amon et al., 2001; Jungbluth et al., 2001; Külling et al., 2003). Similar to on-farm data, dietary treatments did not differ in N₂O concentration at different temperatures ($P > 0.05$). A small effect of dietary CP on N₂O accumulation was observed when the multivariate regression (N₂O concentration as dependent variable) included urinary and fecal pH in the model, explaining just 6.7% of variance. In contrast, Cárdenas et al. (2007) reported a significant role of livestock diet in slurry composition and consequently in the emissions of N₂O from slurry amended incubated soils. They reported that reducing the NH₄⁺ and soluble organic C added with the slurry mitigated the N₂O emission. However, the lack of nitrifier and denitrifier microbial genera in dairy fecal samples (Dowd et al., 2008) might explain the meager effect of dietary CP on N₂O concentration in our results. Therefore, unless allowing the physical contact of excreted N and nitrification and denitrification microbial sources (soil pollution, bedding materials, feedstuffs), N₂O accumulation from barns may be limited (Sneath et al., 1997).

CONCLUSIONS

Reducing dietary CP content from 17 to 14% DM in mid to late lactating cows minimized N excretion and subsequent NH₃ concentration on tie-stall floors. In contrast, tie-stall floor N₂O concentration was not affected

by dietary CP manipulation. Different CP diet-derived slurries incubated in the laboratory demonstrated that NH₃ concentration increased with temperature, especially in high CP diets. In contrast, N₂O concentration did not show the same positive relationship with increasing temperature. We conclude that limiting dietary CP content minimizes NH₃ concentration on dairy stall floors, although temperature will control the rate of NH₃ volatilization. On the other hand, the N₂O concentration on tie-stall floors is low and is not affected by dietary CP manipulation.

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