Effect of sodium chloride intake on urine volume, urinary urea excretion, and milk urea concentration in lactating dairy cattle

J. W. Spek,^{*}†¹ A. Bannink,† G. Gort,‡ W. H. Hendriks,* and J. Dijkstra*

^{*}Animal Nutrition Group, Wageningen University, PO Box 338, 6700 AH, Wageningen, the Netherlands

†Wageningen UR Livestock Research, PO Box 65, 8200 AB, Lelystad, the Netherlands

‡Biometris, Wageningen University, PO Box 100, 6700 AC, Wageningen, the Netherlands

ABSTRACT

Milk urea nitrogen (MUN; mg of N/dL) has been shown to be related to excretion of urinary urea N (UUN; g of N/d) and total excretion of urinary N (UN; g of N/d) in dairy cows. In the present experiment, it was hypothesized that MUN and the relationship between MUN and UUN or UN is affected by urine volume as a result of dietary sodium chloride intake. Twelve lactating Holstein-Friesian dairy cows (mean $\pm$ SD: milk production 28.1 ± 3.23 kg/d and 190 ± 41 d in milk), of which 4 were fitted with catheters in the urine bladder and jugular vein, were randomly assigned to 4 dietary levels of sodium chloride (3, 9, 14, and 19 g of Na/kg of DM) according to a triple 4×4 Latin square design. Cows were fed at 95% of ad libitum intake, excluding salt addition. Milk was analyzed for MUN and protein content; urine was analyzed for total N, urea, and creatinine content; feces were analyzed for total N and DM content; and blood plasma was analyzed for urea and creatinine content. Creatinine clearance rate (CCR; L/min) and renal urea reabsorption ratio were estimated based on plasma concentrations of urea and creatinine, and total excretion of urea and creatinine in urine. Intake of DM and N, milk production, and milk protein content were (mean $\pm$ SD), on average, 21.4 ± 1.24 kg/d, 522 ± 32.0 g/d, 25.4 ± 2.53 kg/d, and $3.64 \pm 0.186\%$, respectively. A linear relationship was found between Na intake and urine production [urine (kg/d; mean $\pm$ SE) = $7.5 \pm 4.33 + 0.136 \pm 0.0143 \times \text{Na intake (g/d)}$] and between Na intake and MUN [MUN (mg/dL; mean $\pm$ SE) = $13.5 \pm 0.35 - 0.0068 \pm 0.00104 \times \text{Na intake (g/d)}$]. Despite the decrease in MUN with increased Na intake, UN excretion increased linearly with Na intake. Excretion of UUN was not affected by dietary Na content. A linear plateau relationship was observed between CCR and renal urea reabsorption. An increase in CCR coincided with an increase in calculated renal urea reabsorption until a CCR break-

point value (mean $\pm$ SD) of 1.56 ± 0.063 L/min was reached. We conclude that Na intake is negatively related to MUN, whereas UUN is not affected. Variation in mineral intake levels that affect urine volume should, therefore, be taken into account when using MUN as an indicator of UUN in dairy cattle.

Key words: milk urea nitrogen, urinary urea nitrogen excretion, salt, urine production

INTRODUCTION

Negative effects of ammonia emission to the environment are of concern (Draaijers et al., 1989; Howarth et al., 1996). Livestock, in particular cattle, are responsible for the majority of ammonia emission (Pain et al., 1998; Hutchings et al., 2001). In dairy cows, the primary source of ammonia-N from manure is urinary urea N (UUN; g of N/d), which is hydrolyzed to ammonia and carbon dioxide by the activity of microbial urease present in the feces. An increase in plasma urea nitrogen concentration (PUN; mg of N/dL) leads to elevated concentrations of MUN (mg of N/dL) and increased excretion of UUN (Burgos et al., 2007). A positive correlation exists between MUN and UUN, which has led to the development of several predictive models to estimate UUN based on MUN (Burgos et al., 2007; Powell et al., 2011). In addition, models have been developed that predict urinary N (UN; g of N/d) excretion based on MUN alone (Jonker et al., 1998) or with additional parameters included such as BW (Kauffman and St-Pierre, 2001) or daily milk production (Nousiainen et al., 2004). The robustness of these relationships has been challenged, however. Bannink and Hindle (2003) using individual cow data from Dutch N balance trials showed that a significant proportion of the variation (28%) in UN could not be explained by the factor MUN in a wide MUN range of 5 to 30 mg of N/dL. Using a limited MUN range of the data set (5 to 15 mg of N/dL) to represent the relevant range in practice, Bannink and Hindle (2003) showed that the accuracy of predicting UN from MUN decreased, with 77% of the variation remaining unexplained.

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¹Corresponding author: wouter.spek@wur.nl

Under a wide range of dietary conditions, dietary protein content explains most of the variation in MUN (Nousiainen et al., 2004). However, several (interrelated) factors (other than dietary protein content) exist that affect MUN (Spek et al., 2012). One of these factors is the volume of urine produced. Several studies show that water intake has an effect on PUN (Weeth and Haverland, 1961; Weeth and Lesperance, 1965; Utley et al., 1970; Godwin and Williams, 1984) and on MUN (Burgos et al., 2001). The effect of water intake, or urine production, on PUN and MUN might affect the relationship between MUN and UN or UUN. The main objective of this study was to quantify the effect of dietary Na intake on urine production level, PUN, MUN, UN, and UUN, and on the relationship between MUN and UN or UUN. The second objective of this study was to study the effect of dietary Na intake on the volume of blood filtered by the kidneys, as represented by the creatinine clearance rate (CCR; L/min) and renal reabsorption of urea.

MATERIALS AND METHODS

Cows, Housing, and Experimental Design

The experiment was approved by the Institutional Animal Care and Use Committee of the Animal Sciences Group, Wageningen University and Research Centre, Lelystad, the Netherlands. Twelve cows were selected based on BW, parity, DIM, and milk production. At the start of the experiment, the BW of the cows was 655 ± 45 kg, parity 2.8 ± 0.97 , DIM 190 ± 41 d, and milk production 28.1 ± 3.23 kg/d (values expressed as means $\pm$ SD). Eight cows were housed in a freestall barn with cubicles on slatted floors and the other 4 cows were housed in a tiestall to quantitatively collect urine and feces. Groups of 4 cows were randomly assigned to a treatment according to a triple 4×4 Latin square. Treatments consisted of 4 dietary levels of sodium (3, 9, 14, and 19 g of Na/kg of DM), where levels of sodium were established through varying the dietary content of sodium chloride. The range in dietary Na levels was larger than observed in practice to augment variation in urine production, MUN, and renal characteristics. The large range in dietary Na levels is required to detect significant differences between treatments and to clearly show relationships between dietary characteristics, MUN, and urinary excretion of urea. The ingredients and chemical composition of the diets is presented in Table 1. During a 2-wk adaptation period before the commencement of the study, cows were fed the ration with the lowest Na content. During the first 9 d of this adaptation period, cows had ad libitum access to the low-Na content feed to determine

feed intake. During the last 5 d of the adaptation period to the end of the experiment, cows were fed at 95% of ad libitum intake excluding salt addition. After this 2-wk adaptation period, each period in the Latin square design lasted 7 d, where the first 5 d were used for adaptation and d 6 and 7 for sample collection. Cows were milked twice daily at 0500 and 1700 h throughout the experiment. During the noncollection days (d 1–5), cows were fed 2 equal meals, twice daily at 0500 and 1700 h, whereas during collection days (d 6–7), 75% of the daily feed allowance was provided in 8 equal meals every 2 h from 0500 to 1900 h to minimize variation in PUN and MUN caused by variation in feed intake within days. At 2100 h, the remaining 25% of the total daily feed allowance was provided. Daily individual feed intake in the tiestall was determined by subtracting the quantity of orts from the quantity of feed supplied. In the freestall barn, individual feed intake was registered with a computer-controlled HokoFarm roughage intake control (RIC) system (Insentec BV, Marknesse, the Netherlands) and similar to collection days in the tiestall, allowance to feed was in equal-sized meals every 2 h. During the last 2 d of the adaptation period to the end of the experiment the rectal body temperature was measured during the morning and afternoon milking. Cows were considered healthy when feed intake, milk production, and rectal body temperature were equal to the situation at the end of the adaptation period.

Sample Collection

The diets were prepared daily using a paddle mixer (Holaras VDC 1200; Hoopman Machines BV, Aalten, the Netherlands) and a representative sample (~ 700 g) of each TMR was collected daily and stored at -20°C . Samples of each diet for each treatment week were pooled and stored at -20°C pending analyses. During the collection period (d 6–7), milk yield was determined and milk samples (10 mL) were obtained after each milking from the total quantity of milk. Milk samples were stored at 4°C in tubes containing sodium azide and analyzed within 5 d after collection.

The 4 tethered cows were fitted with indwelling Foley urine catheters (Ch 26, 100-cm³ balloon; Bard Benelux N.V., Nieuwegein, the Netherlands) attached to a collection vessel 1 d before each collection period (d 5) and was removed after each collection period. The animals were also fitted with 2-way blood sampling catheters (BD Careflow double lumen; Becton Dickinson BV, Breda, the Netherlands) in the jugular vein 1 d before the first treatment diet was fed, and catheters were kept open by a 33 U of heparin/mL saline solution throughout the experiment. During the collection days (d 6–7) of each treatment period, blood samples (10 mL) were

Table 1. Dietary composition (g/kg of DM, unless otherwise stated) of experimental diets

Composition	Dietary Na content (g/kg of DM)			
	3	9	14	19
Ingredient				
Corn silage ¹	720	709	698	687
Wheat straw, chopped	75	73	72	71
Soybean meal	171	169	166	163
Limestone	9.2	9.1	8.9	8.8
Soybean hulls	7.6	7.5	7.3	7.2
Urea	4.5	4.4	4.4	4.3
Soybean oil	4.1	4.0	4.0	3.9
Feed salt ²	4.1	20.1	35.6	50.6
Mineral premix ³	4.2	4.1	4.1	4.0
Nutrient				
DM (g/kg of feed)	450	458	459	461
CP	155	153	151	151
Ash	71	87	95	113
Crude fat	29	30	29	28
Starch	235	224	216	212
NDF	358	344	343	338
ADF	218	210	209	206
ADL	23	22	23	22
Ca	6.7	6.7	6.4	6.4
K	12.9	13.0	12.3	12.6
Na	2.8	9.2	13.7	19.3
Feeding value parameter				
DVE ⁴	91	90	88	87
OEB ⁵	21	21	20	20
NE _L ⁶ (MJ/kg of DM)	6.76	6.66	6.56	6.47
Rumen-degradable CP	102	100	99	98

¹Corn silage (g/kg of DM, unless specified otherwise): DM, 359 g/kg; CP, 81; starch, 423; NDF, 301; ADF, 162; ADL, 15; ash, 44 [determined with near-infrared spectroscopy (NIRS); BLGG, Oosterbeek, the Netherlands].

²Composition of feed salt: $\geq 99.8\%$ NaCl.

³Contained (per kilogram of mix) 152 g of Ca, 220 g of Mg, 6 g of S, 5,780 mg of Cu, 2,900 mg of Mn, and 5,782 mg of Zn, 290 mg of I, 87 mg of Co, 72 mg of Se, 1,444,000 IU of vitamin A, 346,560 IU of vitamin D₃, and 6,839 IU vitamin E.

⁴Intestinal digestible protein (van Duinkerken et al., 2011).

⁵Rumen-degraded protein balance (van Duinkerken et al., 2011).

⁶Net energy for lactation calculated with the feed unit lactation (VEM) system (Van Es, 1975).

collected in heparin tubes every 2 h from 0500 to 2100 h and at 0100 h. Samples were centrifuged at $1,830 \times g$ for 15 min at room temperature within 2 h and blood plasma separated and collected before being stored at -20°C . Urine and feces were collected quantitatively during collection days (d 6–7) starting at 0500 h. Urine production was recorded every 2 h from 0500 to 2100 h and at 0100 h, urine was weighed and sampled (6 mL), and the urine collection vessel was replaced by an empty vessel containing 40 g of 48% H_2SO_4 from 0500 to 2100 h, whereas at 0100 h, the urine collection vessel was replaced by an empty vessel containing 80 g of 48% H_2SO_4 . After weighing, the urine was added to a collection vessel to collect the urine of the complete collection day. Each time a urine sample was taken, the pH of the collected urine was measured and additional H_2SO_4 was added in case the pH value was above 3.0. At the end of each collection day (at 0500 h), the total quantity of feces and acidified urine of each cow were

weighed, thoroughly mixed, sampled (~ 200 g of feces and 250 mL of urine) and stored at -20°C pending further analysis. Water intake was recorded every 2 h from 0500 to 2100 h for each tethered cow during the collection days using individual water flow meters.

Analytical Procedures

Dry matter content of TMR samples was determined by freeze drying (FTS Systems Dura-Dry MP; SP Scientific, Gardiner, NY), whereafter the freeze-dried samples were ground in a cross beater mill (Peppink, Deventer, the Netherlands) to pass a 1-mm screen and analyzed for DM, ash, N, crude fat, NDF, ADF, and ADL content as described by Abrahamse et al. (2008). Starch was analyzed using enzymatic hydrolysis [International Organization for Standardization (ISO) method 15914; ISO, 2004a]. Calcium, Na, and K contents were determined according to the ISO method

6869 (ISO, 2000) by Pre-Mervo UA (Utrecht, the Netherlands). Milk was analyzed for fat, protein, lactose, and SCC as described by Abrahamse et al. (2008). Milk urea content was determined using the pH difference technique (ISO 14637; ISO, 2004b). Urea concentration in blood plasma and acidified urine was analyzed using the urea LiquiColor test (HUMAN GmbH, Wiesbaden, Germany), which is based on measuring absorbance of light (578 nm) after a modified Berthelot reaction. Urea was analyzed in each blood plasma sample taken. Feces and acidified urine were analyzed for N by the Kjeldahl method with CuSO_4 as the catalyst (ISO 5983; ISO, 1997). Blood plasma and acidified urine samples were analyzed for creatinine by the Veterinary Diagnostic Laboratory of Utrecht University (Utrecht, the Netherlands) by measuring absorbance of light (520 nm) after a modified Jaffé reaction. Creatinine was analyzed in the individual blood plasma samples collected at 0700, 1300, and 1700 h to obtain an average estimate of plasma creatinine over a day. Results of Kokkonen et al. (2001) from dairy goats indicate that these 3 sampling time points will yield a proper average creatinine concentration per day. Creatinine was analyzed as well in acidified urine from a complete collection day per cow. The specific gravity of acidified urine was determined by weighing 1.0 mL of acidified urine using a calibrated pipet from a vortexed sample. The specific gravity of individual acidified urine samples was corrected for the effect of the sulfuric acid on the specific gravity of the urine and used to correct measured urine weight to urine volume.

Calculations

Clearance rate calculations were based on Berne et al. (1998). The CCR (L/min), representing the glomerular filtration rate (**GFR**), or the volume of blood being filtered by the kidneys, was calculated as

$$\frac{\text{Total daily urinary creatinine excretion (mmol/d)}}{\text{Blood plasma creatinine concentration (mmol/d)}} \bigg/ 1,440 \text{ min/d.}$$

The urea clearance rate (UCR, L/min) was calculated as

$$\frac{\text{Total daily urinary urea excretion (mg/d)}}{\text{Blood plasma urea concentration (mg/L)}} \bigg/ 1,440 \text{ min/d.}$$

The renal urea reabsorption ratio (**RRR**) was calculated as $1 - (\text{UCR}/\text{CCR})$. The N balance was calculated as N intake minus N excreted in milk, feces, and urine.

Statistical Analysis

Effects of dietary Na content on DMI, milk yield, milk composition, and MUN for all 12 cows was analyzed using a mixed linear model (using PROC MIXED of SAS version 9.2; SAS Institute Inc., Cary, NC), with period, type of housing, and dietary Na content included as fixed effects. Because of the repeated measurements setup of the experiment, with repeated measurements per cow over periods and per measurement period, we included random effects for cows and for period within cow to allow for correlation between the 2 consecutive collection days per cow in each period, with a compound symmetry covariance structure.

Effects of dietary Na content on N balance characteristics, and on MUN, PUN, urine production, urinary creatinine excretion, blood plasma creatinine concentration, CCR, and RRR, for the 4 tethered cows were determined using the same model as described above, except that the fixed effect of type of housing was removed from the model. For all mixed models, calculation of error degrees of freedom was done by the DDFM = KENWARDROGER method in PROC MIXED and the covariance structure was modeled as compound symmetry. An indication of the proportion of variation explained by dietary Na intake was obtained on some parameters by calculating a coefficient of determination for Na intake (R_{Na}^2) in the mixed linear model according to Edwards et al. (2008). A linear plateau model, adapted from Robbins et al. (2006), was used to model the relationship between CCR and RRR using the PROC NLIN procedure, METHOD = NEWTON. Significance was declared at $P < 0.05$, and tendencies at $0.05 \leq P < 0.10$.

RESULTS

Cows, Diets, Milk Production, and Type of Housing

All cows remained healthy throughout the experiment. The results of the first measurement period were discarded due to an erroneous weighting of the concentrate mixture into the TMR diet for 2 d and analysis of data in this study was based on data from the remaining 3 measurement periods. No feed refusals occurred and N intake across rations did not differ significantly (Table 2). The content of protein and lactose in milk, and SCC did not differ among treatments, whereas milk production and content of fat and urea in milk were affected by dietary Na content. An increase in dietary Na content from 3 to 19 g/kg of DM was accompanied by a tendency ($P = 0.05$) to a decrease in milk production by 4.7%, a linear ($P < 0.01$) decrease in milk fat con-

Table 2. Least squares means for Na intake, DMI, N intake, milk production, and milk composition of lactating dairy cows from both the freestall- and the tiestall-housed animals based on $n = 72$ observations (12 cows $\times$ 3 periods $\times$ 2 d) that were fed diets that differed in Na content

Parameter	Dietary Na content (g/kg of DM)				SE ¹	<i>P</i> -value ²	
	3	9	14	19		L	Q
Na intake (g/d)	69	198	292	417	9.2	<0.01	0.18
DMI (kg/d)	21.0	21.4	21.6	21.6	0.34	0.08	0.74
N intake (g of N/d)	519	527	519	526	9.5	0.78	0.51
Milk yield and composition							
Milk yield (kg/d)	25.7	25.2	25.2	24.5	0.78	0.05	0.59
MUN (mg of N/dL)	13.3	12.1	11.3	10.8	0.32	<0.01	0.36
Fat (%)	4.43	4.40	4.32	4.14	0.135	<0.01	0.11
Protein (%)	3.64	3.64	3.66	3.69	0.059	0.03	0.23
Lactose (%)	4.41	4.36	4.42	4.41	0.049	0.55	0.31
SCC ($\times 1,000$ /mL)	233	314	316	240	98.8	0.99	0.35

¹Standard error of the least squares means.²*P*-values of linear (L) and quadratic (Q) effects of dietary Na content.

tent of 6.5%, and a tendency for an increase ($P = 0.10$) in milk protein content of 1.4% (Table 2). An effect of type of housing ($P = 0.01$) was observed on MUN but not on other parameters such as DMI, milk production, and milk composition. Cows in the freestall barn had a 1.52 mg of N/dL higher MUN than cows in the tiestall.

Variation in MUN

A linear effect was found of dietary Na content on MUN concentration (Table 2). The following relationship between MUN and Na intake presented in Equation 1 was obtained for all animals and intercepts over the 2 housing conditions were averaged, as a significant effect of housing was observed:

$$\text{MUN (mg of N/dL)} = 13.5 \pm 0.35 \\ - 0.0068 \pm 0.00104 \times \text{Na intake (g/d)}$$

[tiestall + freestall barn-housed animals based on $n =$

72 observations (12 cows $\times$ 3 periods $\times$ 2 d),

$$R_{\text{Na}}^2 = 0.60]. \quad [1]$$

Urine production (Equation 2, only tiestall-housed animals) explained slightly more variation of MUN compared with Na intake (Equation 3, only tiestall-housed animals):

$$\text{MUN (mg of N/dL)} = 13.0 \pm 0.47 - 0.046 \pm 0.0104 \\ \times \text{urine production (kg/d)}$$

[tiestall-housed animals based on $n = 24$ observations

(4 cows $\times$ 3 periods $\times$ 2 d), $R_{\text{Na}}^2 = 0.68$]; [2]

$$\text{MUN (mg of N/dL)} = 12.8 \pm 0.44 - 0.0068 \\ \pm 0.00157 \times \text{Na-intake (g/d)}$$

[tiestall-housed animals based on $n = 24$ observations

(12 cows $\times$ 3 periods $\times$ 2 d), $R_{\text{Na}}^2 = 0.64$]. [3]

N Metabolism

The effect of dietary Na content on N metabolism based on observations on the 4 tiestall-housed animals is shown in Table 3. No effect of dietary Na content on daily N intake, N excretion in milk, or UUN was found. Excretion of N in feces showed a small, but significant negative linear relationship with dietary Na content, whereas UN and non-UUN (NUUN; g of N/d) had a small, but significant, positive linear relationship with dietary Na content. The positive linear relationship between NUUN and dietary Na content (Table 3) largely explained the significant effect of dietary Na content on UN, as UUN was not affected by dietary Na content. A positive N balance of, on average, 43 g/d (8% of dietary N intake) was observed.

Urinary N Excretion and MUN

No significant relationship between MUN and UUN ($P = 0.69$) and between MUN and UN ($P = 0.13$) was found (Figure 1). Ratios of UUN:MUN varied from 10.9 to 14.4 at dietary Na contents of 3 and 19 g/kg of DM, respectively. A negative relationship was observed between MUN and NUUN ($P < 0.01$; Figure 1).

Renal Functioning

A positive linear relationship was found between dietary Na content or Na intake and urine production

Table 3. Least squares means of N intake and excretion of N in milk, urine, and feces, and on urea and non-urea N excretion in urine of lactating dairy cows from the tiestall-housed animals based on $n = 24$ observations (4 cows $\times$ 3 periods $\times$ 2 d) that were fed diets that differed in Na content

Parameter (g of N/d)	Dietary Na content (g/kg of DM)				SE ¹	P-value ²	
	3	9	14	19		L	Q
N intake	526	532	518	526	11.4	0.78	0.72
N in milk ³	148	144	141	143	6.1	0.07	0.07
N in feces ⁴	148	143	144	138	6.7	0.04	0.84
Urinary excretion							
Total N	189	185	203	205	5.9	0.03	0.81
Urea N	135	130	138	140	5.1	0.21	0.67
Non-urea N	53	56	65	66	1.4	<0.01	0.61
N balance ⁵	44	51	33	42	11.5	0.65	0.62

¹Standard error of the least squares means.

²P-values of linear (L) and quadratic (Q) effect of dietary Na content.

³Calculated as the sum of daily milk protein divided by 6.38.

⁴Quantity of feces produced multiplied by the N content of fresh feces.

⁵Calculated as N intake minus excretion of N in milk, feces, and urine.

(Figure 2; Table 4) and between dietary Na content and water intake (Table 4). Per extra gram of Na intake, an extra 0.146 ± 0.0172 kg of water was consumed. An increase in Na content resulted in lowered PUN and plasma creatinine concentrations, whereas CCR, RRR, and the quantity of creatinine excreted in urine was unaffected (Table 4). The following linear plateau relationship (adopted from Robbins et al., 2006) between CCR and RRR could be established (Equation 4; Figure 3):

$$\text{RRR} = L + U \times (\text{R} - \text{CCR}), \quad [4]$$

where $(\text{R} - \text{CCR})$ is defined as zero when CCR is larger than R, $L = 0.610 \pm 0.0107$, U (min/L) = -0.423 ± 0.0926 , and R (L/min) = 1.56 ± 0.063 . The number of tiestall housed animals was 24 (adjusted $R^2 = 0.71$).

The prediction of RRR was substantially improved (Equation 5) when the linear factor Na intake was added to the linear plateau model described in Equation 4:

$$\text{RRR} = L + A \times \text{Na intake} + U \times (\text{R} - \text{CCR}), \quad [5]$$

where $(\text{R} - \text{CCR})$ is defined as zero when CCR is larger than R, $L = 0.647 \pm 0.0172$, A (d/g of Na) = -0.00014 ± 0.000055 , U (min/L) = -0.445 ± 0.0807 , and R (L/

Figure 1. Relationship between MUN content (mg of N/dL) affected by dietary Na consumption and excretion of urinary N (UN; g of N/d), urinary urea N (UUN; g of N/d), and non-UUN (NUUN; g of N/d) for the tiestall-housed animals based on $n = 24$ observations (4 cows $\times$ 3 periods $\times$ 2 d). Each regression line is based on simple regression.

Figure 2. Effect of Na intake on urine excretion for the tiestall-housed animals based on $n = 24$ observations (4 cows $\times$ 3 periods $\times$ 2 d). Urine excretion (kg/cow per day) = $7.5 \pm 4.33 + 0.136 \pm 0.0143 \times \text{Na intake [g/cow per day]}$; coefficient of determination for Na intake (R_{Na}^2) = 0.92. Dashed line is urine excretion as predicted by the regression equation derived by Bannink et al. (1999).

Table 4. Least squares means for water intake, production, and specific gravity of urine, creatinine excreted in urine, content of creatinine and urea in blood plasma, urea content of milk, creatinine clearance rate, and renal urea reabsorption of lactating dairy cows from the tiestall-housed animals based on $n = 24$ observations (4 cows $\times$ 3 periods $\times$ 2 d) that were fed diets that differed in Na content

Parameter	Dietary Na content (g/kg of DM)				SE ¹	<i>P</i> -value ²	
	3	9	14	19		L	Q
Water intake (kg/d)	61.7	82.1	90.9	115.7	5.02	<0.01	0.57
Urine (kg/d)	18.2	30.6	46.6	67.7	2.13	<0.01	0.27
Urine specific gravity (g/mL)	1.050	1.039	1.036	1.034	0.0013	<0.01	<0.01
Urine creatinine excretion (mmol/d)	123	114	134	128	13.8	0.65	0.84
Plasma creatinine (μ mol/L)	56.0	53.0	50.3	47.8	1.79	<0.01	0.82
Plasma urea nitrogen (mg of N/dL)	15.2	12.9	13.7	11.8	0.54	<0.01	0.89
MUN (mg of N/dL)	12.5	11.2	10.8	9.9	0.43	<0.01	0.90
Creatinine clearance rate (L/min)	1.49	1.54	1.88	1.89	0.232	0.16	0.76
Renal urea reabsorption ratio	0.58	0.53	0.61	0.54	0.037	0.76	0.62

¹Standard error of the least squares means.

²*P*-values of linear (L) and quadratic (Q) effect of dietary Na content.

min) = 1.56 ± 0.052 . The number of tiestall-housed animals was 24 (adjusted $R^2 = 0.77$).

Condition of Steady State

Water intake tended ($P = 0.10$) to linearly increase from 0500 to 0100 h the next day. Within this period, 2 periods were observed with a numerically higher water intake: one between 0700 and 0900 h and the other between 1300 and 15:00 h, whereas between 0100 and 0500 h, water intake was close to zero (Figure 4). Numerically, 2 peaks in urinary urea nitrogen excretion were observed between 0900 and 1100 h and between 1300 and 1500 h (Figure 4). The level of PUN did not vary substantially during the day (Figure 4). From

1700 to 0500 h the next day, a linear increase ($P < 0.01$) in urine production was observed, which was accompanied by a linear decrease ($P < 0.01$) in urinary urea concentration.

DISCUSSION

Variation in MUN

In various studies, almost invariably, a positive relationship is observed between MUN and UN (Ciszuk and Gebregziabher, 1994; Jonker et al., 1998; Nousiainen et al., 2004) and between MUN and UUN (Burgos et al., 2007; Powell et al., 2011). However, in this study MUN was not positively related to UN and UUN. An increase in Na intake increased UN and decreased MUN significantly, indicating the opposite effect of that observed in other studies. This effect of dietary Na content on the relationship between MUN and UUN or UN shows that Na intake should be considered when MUN is used as an indicator of UN or UUN. The effect of dietary Na content on MUN probably was indirect by its effect on urine production level, which directly affects MUN and the UUN:MUN ratio. Such an effect implies that a change in water intake, or a change in dietary intake of any mineral that stimulates water intake and urine production, will affect MUN as well. Such a direct relationship between water intake and urea level in blood plasma or milk (or both) seems apparent from other studies. Burgos et al. (2001) found that dehydration (50% of ad libitum water intake) resulted in a 58% increase of MUN, whereas Weeth and Lesperance (1965) observed a 14% decrease in PUN in hydrated (150% of ad lib water intake) animals. Urine production may also differ substantially between corn silage-based and grass silage-based rations due to a low and high mineral content of corn silage and grass silage, respectively.

Figure 3. Effect of creatinine clearance rate (CCR; L/min) on renal urea reabsorption ratio (RRR) for the tiestall-housed animals based on $n = 24$ observations (4 cows $\times$ 3 periods $\times$ 2 d). The $RRR = L + U \times (R - CCR)$, where $L = 0.610 \pm 0.0107$, U (min/L) = -0.423 ± 0.0926 , and R (L/min) = 1.56 ± 0.063 ($R^2 = 0.72$). The symbol ● represents the observed value, whereas the symbol ○ represents the residual.

Figure 4. Effect of time of the day on urine urea N excretion (g of N/2 h; ●), urine production (kg/2 h; ◆), water intake (L/2 h; ▲), concentration of plasma urea N (PUN; mg of N/dL; △), and urine urea N (g of N/kg; ○) for the tiestall-housed animals based on $n = 240$ observations (4 cows $\times$ 3 periods $\times$ 2 d $\times$ 10 observations per day), based on least squares means that are corrected for ration and cow.

This was shown by De Campeneere et al. (2006) who observed levels of urine production of 14.4 and 35.0 kg/cow per day for cows fed 100% corn silage- and 100% grass silage-based rations, respectively. According to Equation 2 in the present study, this 20.6 kg/d higher urine production on the grass silage-based ration will lead to a 1.0 mg of N/dL lower MUN. Correcting MUN for the effect of urine production in the study of De Campeneere et al. (2006) reduces the UN:MUN ratio of the grass silage-based diet from 13.9 to 12.4, which is closer to the UN:MUN ratio of 11.1 observed for the corn silage-based ration. In another study, De Campeneere et al. (2009) observed a decrease in MUN from 15.4 to 11.4 mg of N/dL when 500 g/d of NaCl (197 g/d of Na) was added to a corn silage-based ration, which is equivalent to a 2.0 mg of N/dL decrease in MUN per 100 g/d Na supply. This is a much stronger decrease than the decrease of MUN of 0.68 mg of N/dL per 100 g of Na/d established in the present study. The reason for this difference in the size of effect of Na on MUN is not clear, but it is not related to differences in N intake, milk production, and manure N excretion, as these were similar in both studies.

In practice, the variation in dietary Na content is less than that in the present study. The NRC (2001) recommendation of Na for lactating dairy cattle is 2.2 g of Na/kg of DM, but a maximum performance of dairy cows at dietary Na contents of 8.0 g of Na/kg was reported. Assuming these differences in Na levels as the range in dietary Na contents observed in practice, the difference in Na content between 2.2 and 8.0 g of Na/

kg at a DMI of 20 kg/d would result in a calculated MUN difference of around 0.79 mg of N/dL, or 7% of the average level of MUN observed in this study. However, differences in urine volume in practice may be far larger than observed within this limited range (e.g., De Campeneere et al., 2006) and minerals other than Na will affect urine volume.

Type of Housing

Although the effect of dietary Na intake on MUN was similar between cows in the tiestall [$\text{MUN} = -0.0068 \times \text{Na intake (g/d)}$] and cows in the freestall barn [$\text{MUN} = -0.0067 \times \text{Na intake (g/d)}$], a significant difference in MUN of 1.52 mg of N/dL was observed between the 2 types of housing. The reason for this difference in MUN is unknown. No differences in DMI and N excretion in milk were observed between the 2 types of housing. Feed intake patterns were similar for cows in the freestall barn and in the tiestall. One might speculate that cows in the freestall barn had more physical exercise, affecting the overall body metabolism and urea concentration in plasma and milk as well.

Urine Production and Water Intake

In the present study, a significant linear positive relationship was established between Na intake and urine production, suggesting that 0.136 kg of urine is produced per extra gram of Na intake (Figure 2). This value compares reasonably well to the estimate of Ban-

nink et al. (1999) of 0.115 kg of urine per gram of Na intake based on a meta-analysis on trials having a wide range of dietary treatments. Likewise, water intake was positively related to Na intake (Table 4). The estimated slope (0.146 kg of water/g of Na intake) is within the range reported in the literature (0.054 kg of water/g of Na intake; Murphy et al. (1983); 0.406 kg of water/g of Na intake; Meyer et al. (2004).

N Metabolism

The dietary Na content did not affect UUN, whereas NUUN was significantly affected (Table 3). We hypothesize that the highly significant and positive relationship between Na intake and NUUN was the result of an increase in urinary excretion of purine derivatives, originating from absorbed rumen microbial nucleic acids. The increased water consumption with increased dietary Na levels observed in this study (Table 4) may have resulted in an increase in rumen liquid fractional passage rate and, consequently, an increased efficiency in microbial growth rate and outflow of microbial protein to the intestine (Harrison et al., 1975; Dijkstra et al., 1998), which in turn, led to an increase in intestinal absorption of derivatives of microbial nucleic acids, resulting in an increased NUUN. The negative relationship between MUN and NUUN (Figure 1) is in accordance with the aforementioned effect of Na intake on NUUN. In contrast to this hypothesis, however, no effect of mineral intake on urinary allantoin excretion in sheep was found by van Houtert and Leng (1991), indicating that microbial protein synthesis was unaffected by level of salt intake. The positive N balance of 43 g of N/d (8% of N intake), on average, is in line with the average N balance (39 g of N/d) reported by Spanghero and Kowalski (1997) in a review on dairy cattle N balance trials. Spanghero and Kowalski (1997) observed a positive relationship between N balance and dietary N availability. A similar relationship was observed in the present study where 46% of the variation of N balance (ranging from 7 to 74 g of N/d) could be explained by the effect of digestible N intake. In the current study, BW of the cows in the tiestall increased with 15 ± 14.7 kg during the total period of the experimental period of 6 wk, which is unlikely to account for the positive N balance. Unaccounted N losses that result in a positive N balance can be ascribed to a combination of factors such as gaseous N formation (Costa et al., 1968), excretion of N in urine as nitrate that is not detected by the Kjeldahl method (Young et al., 1981), and volatile losses of ammonia from feces and dermal and scurf losses (Spanghero and Kowalski, 1997). However, the possibility of an unaccounted N loss through gaseous

N was challenged by Rasch and Benevenga (2004) who could not account for the loss of 6.7% of infused ^{15}N L-alanine via gaseous N in piglets or via other routes of excretion. Similarly, Spanghero and Kowalski (1997) concluded that volatile N losses from feces and urine, errors in milk N analysis, and the dermal and scurf N losses, together could not fully explain the high N balance observed.

Renal Functioning

A positive linear relationship was observed between Na intake and urine production (Figure 2). The renal mechanism of increased urine excretion after an increase in water intake due to high levels of dietary salt can be explained by (1) an increase in GFR caused by dilation of blood vessels and (2) a decrease in renal reabsorption of water caused by a reduction in release of antidiuretic hormone by the posterior pituitary. The negative correlation between Na content and plasma creatinine concentration (Table 4) shows that at least the GFR (as indicated by CCR) is increased when salt intake increases. Apple et al. (1989) found that the coefficient of determination between GFR, as determined by the inulin clearance rate (gold standard for determination of GFR), and CCR, as measured by a modified-rate Jaffé reaction, was high ($R^2 = 0.92$), indicating that the reliability of CCR as an estimator for GFR is high. The increase in CCR may also have resulted in the decrease in PUN (due to the lower average retention time of urea in the blood) with increasing Na intake, and subsequently may have decreased MUN. However, the potential effect of an increased urea flow to the kidneys on PUN was partially offset by an increase in RRR at the lower range of CCR (Figure 3).

Three possible mechanisms may explain the effect of Na intake on PUN. The first mechanism is that an increase in Na intake will result in an increased GFR and an increase in urine production. This increase in GFR leads to a temporary increase in urea excretion in urine (if renal reabsorption of urea is not changed), resulting in a lower PUN. This lower PUN also lowers renal urea transport and UUN until the previous level of UUN is reached again. The second mechanism is that an increased renal urea excretion in the glomerular filtrate, due to an increase in GFR, is reduced by an increased renal reabsorption of urea from the glomerular filtrate. The third mechanism is related to the function of urea as an osmolyte in the interstitial cells of the inner medulla of the kidney in concentrating urine (Schmidt-Nielsen and Osaki, 1958; Schmidt-Nielsen and O'Dell, 1959; Bagnasco et al., 2000). As more urine is produced, the interstitial concentration of urea

decreases, resulting in less renal reabsorption of water and as a result an increase in urine production. This decrease in the interstitial concentration of urea is possibly caused by a reduction of RRR in the interstitial zone of the kidney and this reduction in RRR possibly affects PUN as well. However, the effect of interstitial urea concentration on PUN has not been tested. The results of this study indicate that renal regulation of urea excretion is a combination of at least the first and second mechanism described here.

CONCLUSIONS

Dietary concentration of sodium chloride or the level of sodium chloride intake linearly increased urine production and total urinary N excretion, and linearly decreased the concentration of milk urea nitrogen, but did not influence daily excretion of urinary urea nitrogen. The level of salt intake should, therefore, be taken into account when milk urea nitrogen is used as an indicator of urinary urea nitrogen excretion by dairy cows. The kidneys adapt to an increase in glomerular filtration rate, caused by elevated levels of Na intake, by increasing renal reabsorption of urea, thereby reducing the effect of salt intake on plasma urea nitrogen concentration.

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