

## *Ruminant nutrition and metabolism*

### **Effects on dairy cow urine volume and N metabolism at different K intake levels**

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#### **Introduction**

Dairy cow rations may vary considerably in their concentration of several macrominerals. This is due both to ration composition and to variation within a certain feed type. Within the grass-legume forages, there is a large variation in especially potassium content. For the 20000 Swedish farm samples of grass-clover silage (Feed code 165) contained in the current Norfor Feedstuff table, K concentrations range from about 10 to 33 g/kg DM, when expressed as mean  $\pm$  2 standard deviations. These variations have implications for the metabolism of dairy cows, as they affect the liquid turnover and could be expected to give rise to secondary effects because of increased water intake and urine volume. Increased urine volume is associated with decreased milk urea concentration (De Campenere et al., 2006; Spek et al., 2012).

The objective of the experiment presented here was to assess the effects of incremental amounts of  $\text{KHCO}_3$ , with respect to urine volume, water intake and N metabolism measures such as rumen ammonia, milk urea, urinary urea and total urinary N.

#### **Materials and Methods**

Six ruminally cannulated lactating dairy cows of the Swedish Red breed (BW  $618 \pm 38$  kg, parity  $2.0 \pm 0.0$ , DIM  $86 \pm 15$  at experimental onset) were used in a 3 x 3 Latin square experiment. Period length was 14 days, with sampling days 10-14. The cows were kept in individual tie stalls and milked there at 06:00 and 17:00 h. The same basal ration was fed to all cows at individually fixed levels throughout the experiment to result in no refusals. The ration consisted of silage, a commercial concentrate (SOLID 620, Lantmännen, Stockholm, Sweden) and urea (AB Johan Hansson, Uppsala, Sweden) in the proportions 39.3:60.0:0.7 on a dry matter basis. The silage was a second cut from a timothy dominated grass-clover ley grown on an unfertilized organic soil and round baled at 56% DM with 375 g sodium benzoate/tonne fresh weight. Each bale was thoroughly cut and mixed in a TMR wagon, before feeding. Silage was fed in the manger with two equal meals at 05:45 and 16:45 h, respectively, and concentrates were fed in a separate trough with four equal meals at 06:00, 09:00, 13:00 and 17:00 h, respectively. The two first concentrate meals were fortified with urea, manually mixed into the concentrates at feeding. Separate small amounts of silage and concentrateorts were collected immediately before silage feeding, weighed and sub-sampled once daily.

Experimental treatments were either basal ration only (Treatment L) or basal ration with food grade potassium bicarbonate (Univar Europe, Rotterdam, Netherlands) added to provide double (M) or threefold (H) daily potassium intake compared to the basal ration. Bicarbonate was manually mixed into each individual silage portion prior to feeding.

The cows were test milked for three consecutive days, and strip milking samples were obtained each second hour day 12, from 06:00 to 24:00 h. Samples from test milking were analyzed by

standard infrared methods for fat, protein, lactose and DM. Test milking samples as well as strip milking samples were also analyzed colorimetrically for urea by a standard diacetyl-monoxime procedure (Technicon, 1974). All further analyzes on the different sample categories collected were performed by the standard wet chemistry methods described by Eriksson et al. (2012) if not stated otherwise. Fecal spot samples were obtained twice daily during four days for analysis of acid insoluble ash (AIA). Quantitative urine collection was performed for three days and samples were analysed for urea, creatinine and Kjeldahl N. Ruminant liquid samples obtained at 19 different hours during four days were analyzed for pH, NH<sub>3</sub>-N,  $\alpha$ -amino-N and volatile fatty acids (VFA). Further, feeds and fecal samples were analyzed for DM, ash, NDF, Kjeldahl N and soluble N (feeds only). Feeds, refusals, feces and urine samples were analyzed for mineral elements by inductively coupled plasma-atomic emission spectroscopy (Spectro flame, SPECTRO Analytical Instruments, Kleve, Germany) after digestion with nitric acid.

Data were analyzed with Procedure Mixed of SAS 9.2 (SAS Institute Inc., Cary, NC, USA) with treatment as fixed factor and cow and period as random factors after initial tests for effects from previous treatment and interactions. Results are presented as least square means with single degree of freedom linear and quadratic contrasts. Probabilities for different least square means when reported are Tukey adjusted. Time series data were analyzed by a similar Procedure Mixed model that included time and the interaction time  $\times$  treatment.

## **Results and Discussion**

Intake of basal ration and of all constituents except for K was the same for all treatments in spite of some refusals (Table 1). Digestibility of DM, OM and NDF increased with K intake, which most likely could be attributed to the ruminal buffering from KHCO<sub>3</sub>. Apparent digestibility of K increased, consistent with previous findings of a negative intercept for K digestibility (Fisher et al., 1994; Bannink et al, 1999). Ruminal pH increased linearly with KHCO<sub>3</sub> addition, accompanied by a shift from propionate to acetate in rumen VFA pattern. All shifts in VFA pattern occurred between treatments L and M, whereas the numerical pH increase continued also in treatment H. Ruminal NH<sub>3</sub>-N concentration decreased linearly but  $\alpha$ -amino-N levels were unaffected by K level.

Milk production and concentrations of milk components were not affected by treatment except for a linear decrease in milk urea concentration (Table 2). The magnitude of the decrease in milk urea concentration was 0.7 mM, similar to what Spek et al. (2012) found for increasing dietary Na content. Milk urea concentration was almost identical for the two experiments when plotted against the sum of daily K and Na intake on a molar basis (Figure 1). This was probably to a large extent due to very similar N intakes and production levels. However, the reduction in milk urea concentration was considerably less than the 1.4 mM that De Campenere et al. (2006) reported. Their experiments compared maize and ryegrass silages, respectively, and the outcome may have been influenced by factors other than ration mineral content. Morning diurnal milk urea pattern relative to feeding (Figure 2) was similar to Gustafsson and Palmquist (1993), with urea peaks about four hours post feeding. The same peak levels were not reached in the afternoon. This may, at least partially, be explained by silage, urea and concentrates being fed in the first morning meal, whereas the first afternoon meal was without silage and, hence, provided less total N.

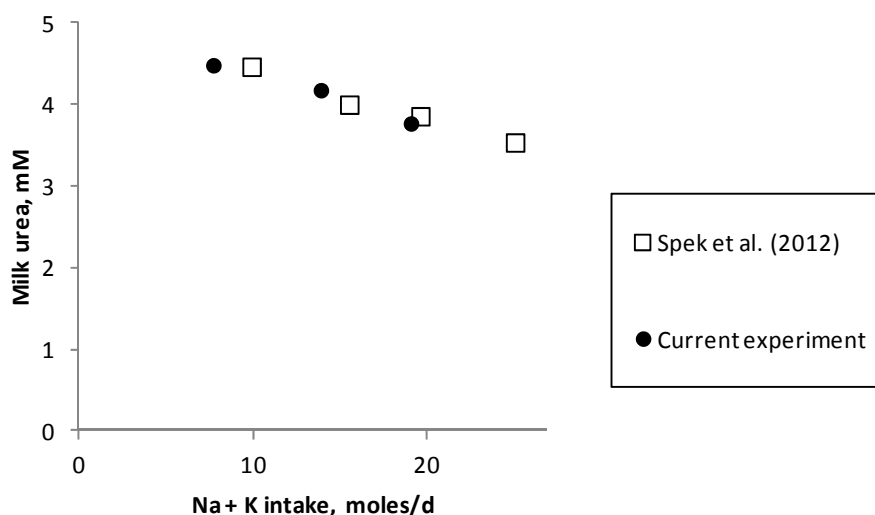
There was a large linear effect on drinking water intake and urinary output with increased K intake (Table 2), similar to the experiment with KCl addition by Fisher et al. (1994). The slope for urinary output (kg/d) against K intake (g/d) was 0.058, the same coefficient that Bannink et al. (1999) reported and close to the 0.053 previously obtained for Swedish growing cattle and dairy cows (Eriksson, 2011). The correlation between K intake and urinary output has been ascribed to the maintenance of plasma or urinary osmolality (Bannink et al., 1999; Kume et al., 2008). Kume et al. (2008) showed that urinary K concentration increased with K intake to an asymptote of about 13 g/L. Above this level, urinary K excretion would occur entirely by increased urine volume. Treatment L in the current experiment had lowest urinary K concentration ( $P = 0.01$ ), while the other treatments appeared to have reached the asymptotic level for urinary K concentration. The range of urinary K concentration within a dietary K level narrowed down with increasing K intake, so that the between cow standard deviation was 1.6, 1.0 and 0.5 g/kg for treatments L, M and H, respectively.

**Table 1** Intake, digestibility and rumen liquid characteristics for mid lactating cows fed incremental amounts of  $\text{KHCO}_3$  on top of a common basal ration

	K level			SED	P for contrast	
	Low	Medium	High		Linear	Quadratic
<b>Intake</b>						
Basal ration, kg DM/d	20.2	20.3	20.2	0.13	0.84	0.45
$\text{KHCO}_3$ , g/d	0	616	1142	63	-	-
Total DMI, kg/d	20.2	20.9	21.3	0.18	<0.001	0.17
N, g/d	533	535	532	2.9	0.85	0.25
Soluble N, g/d	165	167	165	1.75	0.80	0.22
NDF, g/d	7103	7121	7060	73.5	0.59	0.59
K, g/d	240	483	686	27.5	-	-
Na, g/d	29.9	30.1	30.1	0.1	0.06	0.39
<b>Digestibility</b>						
DM	0.71	0.73	0.74	0.003	<0.001	0.10
OM	0.72	0.73	0.74	0.003	<0.001	0.25
NDF	0.59	0.62	0.64	0.010	<0.001	0.16
N	0.71	0.71	0.71	0.001	0.85	0.41
K	0.89	0.93	0.95	0.009	<0.001	0.14
<b>Rumen liquid values</b>						
pH	5.93	6.04	6.11	0.06	0.01	0.41
VFA, mM	121.5	120.3	119.9	2.73	0.55	0.83
Acetate proportion	0.649	0.672	0.672	0.009	0.04	0.13
Propionate proportion	0.204	0.176	0.178	0.012	0.06	0.14
Butyrate proportion	0.112	0.114	0.114	0.005	0.63	0.76
$\text{NH}_3$ -N, mg/dL	6.65	6.51	5.84	0.35	0.05	0.55
$\alpha$ -amino-N, mg/dL	5.87	6.10	6.22	0.30	0.26	0.74

**Table 2** Milk production, milk and urinary N compounds and liquid turnover for mid lactating cows fed incremental amounts of  $\text{KHCO}_3$  on top of a common basal ration

	K level			SED	P for contrast	
	Low	Medium	High		Linear	Quadratic
Milk, kg/d	27.4	28.0	27.9	0.59	0.35	0.50
ECM, kg/d	28.6	29.3	29.3	0.70	0.36	0.58
Milk protein, g/d	944	958	950	22.1	0.75	0.57
Milk urea, mM	4.48	4.18	3.77	0.13	0.001	0.92
Urinary urea N, g/d	108	105	101	2.48	0.02	0.90
Total urinary N, g/d	132	143	134	3.26	0.55	0.01
Urine, kg/d	14.0	27.4	39.9	2.18	<0.001	0.18
Creatinine, g/d	15.5	15.5	14.9	0.32	0.12	0.49
Urinary K, g/kg	10.5	12.4	12.4	0.43	0.002	0.02
Drinking water int. kg/d	82.3	98.7	113.1	2.58	<0.001	0.14
Water balance, kg/d	20.7	21.9	25.7	1.31	0.006	0.47

**Figure 1** Milk urea concentration as a function of daily Na + K intake for mid-lactating cows consuming 519-533 g N/d and producing 25-28 kg milk/d. Data from the current experiment (incremental K amounts) and from Spek et al. (2012) (incremental dietary Na proportion).

Water balance (Table 2; Drinking water + feed water – milk water – urinary water – fecal water) was highest with the H diet ( $P < 0.05$ ). Increasing water balance with increasing K intake was also reported by Fisher et al. (1994), but was unexplained also in their study. Creatinine excretion (Table 2) was similar to previous results at our laboratory (24.2 – 25.1 g/kg LW) and did not differ between treatments, indicating successful urinary collection.

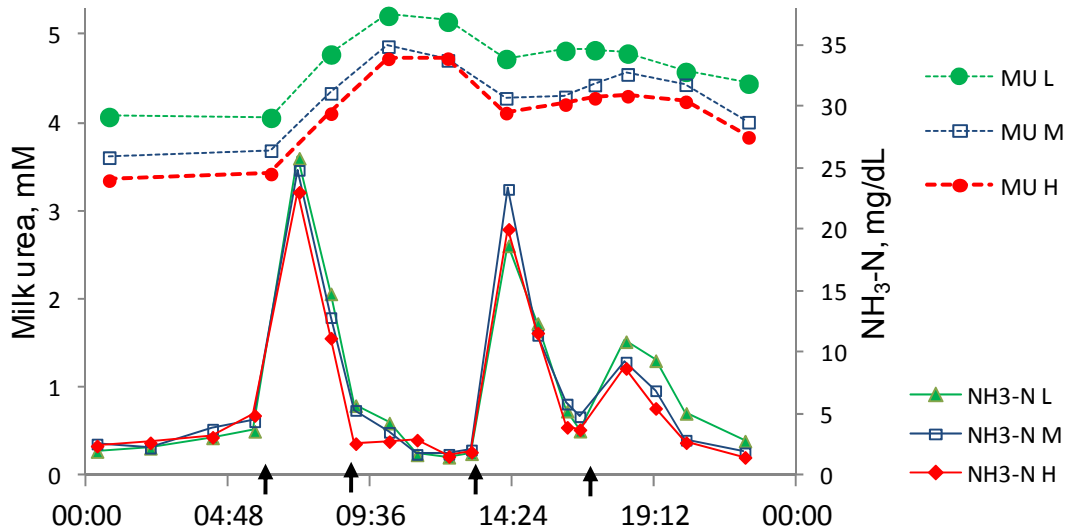
Spek et al. (2012) found a linear increase in total urinary N excretion but no effect for urinary urea N excretion with incremental dietary Na proportion for dairy cows. In the experiment reported here, it was a curvilinear effect on total urinary N, with highest excretion for treatment M and a small, linear decrease in urinary urea N excretion for increased K intake (Table 2).

**Conclusions**

Dietary sodium and potassium reduce milk urea to a similar extent on a molar basis through increased drinking water intake and urinary output. This should be taken into account when interpreting milk urea results.

**Acknowledgements**

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**Figure 2** Diurnal concentrations of milk urea (MU, mM) and rumen NH<sub>3</sub>-N in mid-lactating cows consuming low (L, 240 g/d), intermediate (M, 483 g/d) or high (H, 686 g/d) amounts of K. Arrows indicate meals (silage: 05:45 and 16:45 h; urea fortified concentrates 06:00 and 13:00 h; concentrates 09:00 and 17:00 h). Largest standard error of difference is 0.19 mM of milk urea and 2.0 mg NH<sub>3</sub>-N/dL, respectively.

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