Diurnal urinary urea concentration patterns in lactating cows, dry cows and heifers at different dietary crude protein concentrations

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Introduction

Ruminants have evolved a system of N circulation to maintain rumen function in spite of temporary imbalance between ruminally available protein and fermentable carbohydrates in the diet. A surplus of ammonia from ruminally degraded protein enters the plasma urea pool together with ammonia stemming from amino acids oxidized by the liver or gut wall (Lapierre et al., 2005). It may then be recirculated to the rumen via the rumen wall or through saliva and be utilized for microbial growth.The N status can be monitored by milk urea or experimentally by rumen ammonia measurements. Diurnal pattern of rumen ammonia concentration is affected by the properties of the ration, but also by feeding routines. Dairy cows fed a total mixed ration (TMR) once daily (Colmenero & Broderick, 2006) showed an increase within 1 h to 15-20 mg NH_3-N/dL, where the maximum was depending on ration crude protein (CP) concentration. About 10 h later, rumen NH3-N concentration had returned to the baseline, which was approximately 3-10 mg NH_3-N/dL, also depending on ration CP concentration. With different feeding routines, different ammonia patterns will occur. It is common in Sweden that forage (*i.e.* silage) is fed separately from concentrates at two or more occassions per day. In a series of feeding trials at SLU, grass-legume silage was fed at ad libitum or at semi-restricted levels three to four times daily to mid-lactating cows (Eriksson et al., 2004; Eriksson, 2010; Eriksson et al., 2012). This has resulted in typical patterns with a peak two hours after feeding and complete return to a baseline of 5-10 mg NH_3-N/dL four hours post feeding.

Lefcourt et al. (1999) demonstrated a diurnal rhythm with a zenit at 10:30 h for blood urea in dairy cows fed TMR once daily at 09:00 h. The concentration began to rise long before the daily feeding in their study, which suggests that the rhythm was not only caused by the feed itself. Urea from the plasma pool that is not recirculated is excreted in urine. There is often a slope close to 1 for amount of urinary urea vs. total urinary N, *i.e.* the entire urinary N increase is as urea. Also urinary urea may exhibit diurnal patterns similar to blood urea.

The objective of the study reported here was to investigate if such a common diurnal pattern exists for concentration and total excretion of urinary urea N.

Materials and Methods

Experiment 1 (Eriksson, 2003) involved six dairy cows in mid to late lactation (DIM = 205, ECM = 22 kg/d) fed rations with alfalfa-perennial ryegrass silage, rapeseed cake, fodder beets and potatoes at 18.7 kg dry matter (DM)/d. Ration CP concentration was 154 g/kg DM. Total 48-h urinary collection was performed during 48 h with a harness and funnel device (Eriksson et al., 2004). Analysis of urea N and creatinine was made on a Technicon Autoanalyzer.

In Experiment 2 (Eriksson et al., 2009), 18 cows in mid to late lactation (DIM = 217, ECM = 22 kg/d at spot sampling) were fed incremental amounts of fodder beets and potatoes for a 21-d test period, whereafter individual rations were fixed at 17.0 ± 1.7 kg DM/d. The same feeds as in Experiment 1 were used and ration CP concentration was the same, 154 g/kg DM. Urinary spot-sampling was made during 48 h, yielding a total of 290 samples analyzed as described for Experiment 1.

Experiment 3 (Pelve et al., 2012) was a 3 x 3 Latin square with six dry cows (weighing 563 ±46 kg) and six heifers (weighing 309 ±34 kg) in periods of 21 d. Experimental treatments were three different forages harvested from seminatural grasslands as haylage (DM>770 g/kg) or as hay, all with measured crude protein (CP) concentrations and in vitro organic matter digestibilities (OMD). From vegetation characteristics, growing areas were classified as periodically inundated shore meadow (118 g CP/kg DM, OMD 0.57), naturalised cultivated grassland (76 g CP/kg DM, OMD 0.61) or species-rich naturalised cultivated grassland (80 g CP/kg DM, OMD 0.65). The forages were provided at 15 g/kg BW for cows and 20 g/kg BW for heifers. Urinary spot-sampling was performed during the last 5 days of each period, yielding a total of 597 samples, analyzed as in Experiments 1 and 2.

Data from spot samples in Experiments 2 and 3 were assigned to the 2-h interval when respective sample had been taken. Data were then analyzed by procedure Mixed of SAS 9.2 (SAS Institute, Inc., Cary, North Carolina, USA) with models that included as a minimum: sampling time as a fixed variable and animal as a random variable. If significant ($P<0.05$), sampling day (Exp. 1 and 2) and period and treatment (Exp. 3) were included as fixed variables and their interactions with individual as random variables. Least square means were then calculated for the time intervals.

Results and Discussion

The urinary urea N (UN) concentration in Experiment 1 was constant throughout the day (Figure 1). However, urinary volume increased after the first measurement points in the morning so that total amount of UN peaked 10 hours after first feeding, at 15:00 h (*e.g*. the collection interval from 13:00 h to 17:00 h). The ratio UN/creatinine N followed the curve well. In Experiment 2, UN concentration peaked at 10:00 h, but the ratio UN/creatinine N that should describe total excretion of UN reached its peak value at 20:00 h (Figure 2).

The two forages with lowest CP concentration in Experiment 3 resulted in very low urinary urea concentrations (Figure 3). The ration with 80 g CP/kg DM and highest OMD gave a daily UN excretion of 2.7 g with heifers and 10 g with cows (Pelve et al., 2012). With the forage containing 118 g, UN concentration was similar to Experiments 1 and 2. For heifers, there was a peak with this forage 6 hours after first feeding (14:00 h) for both UN concentration and the ratio UN/creatinine N. For cows on that forage, as well as for both heifers and cows on the diets of lower CP concentration, a single peak was much less obvious or totally absent.

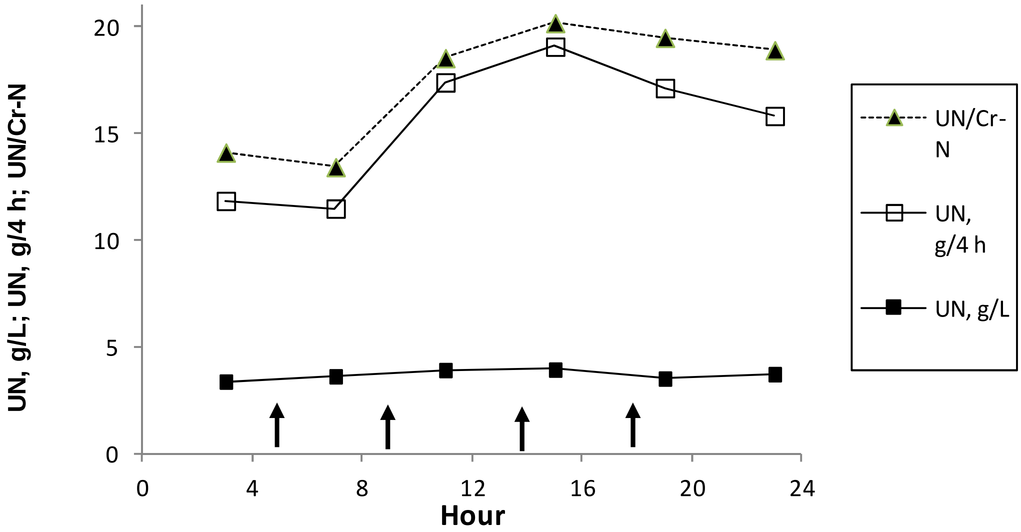

Figure 1 Total excretion and concentration of urinary urea N from quantitative collection on 6 mid-lactating cows. UN/Cr-N = ratio urinary urea N/urinary creatinine N. Arrows indicate meals.

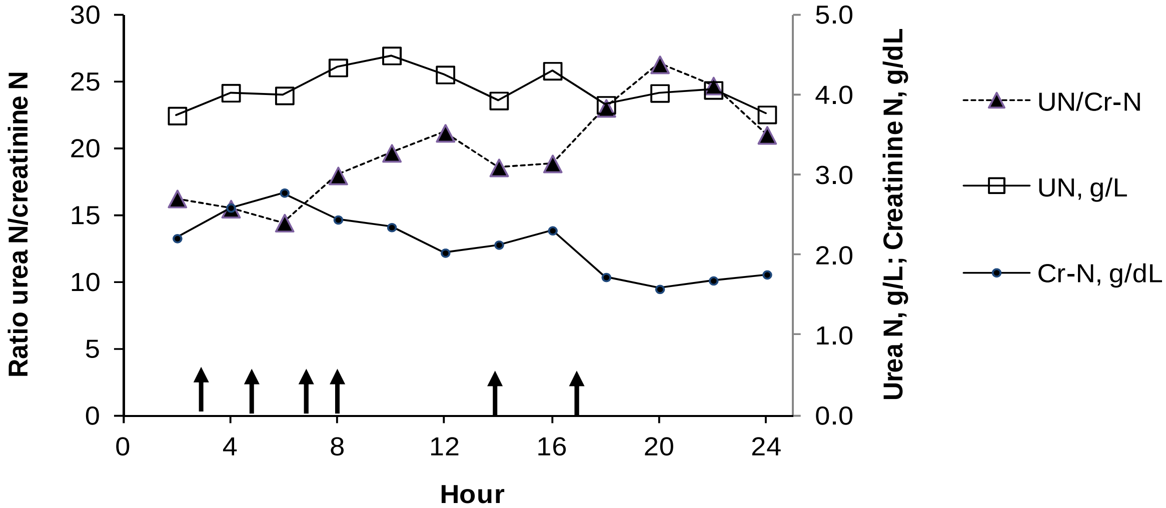

Figure 2 Concentration of urinary urea N (g/L) and urinary creatinine N (g/dL) in spotsamples from 18 mid-lactating cows (N=290). UN/Cr-N = ratio urinary urea N/urinary creatinine N. Arrows indicate meals.

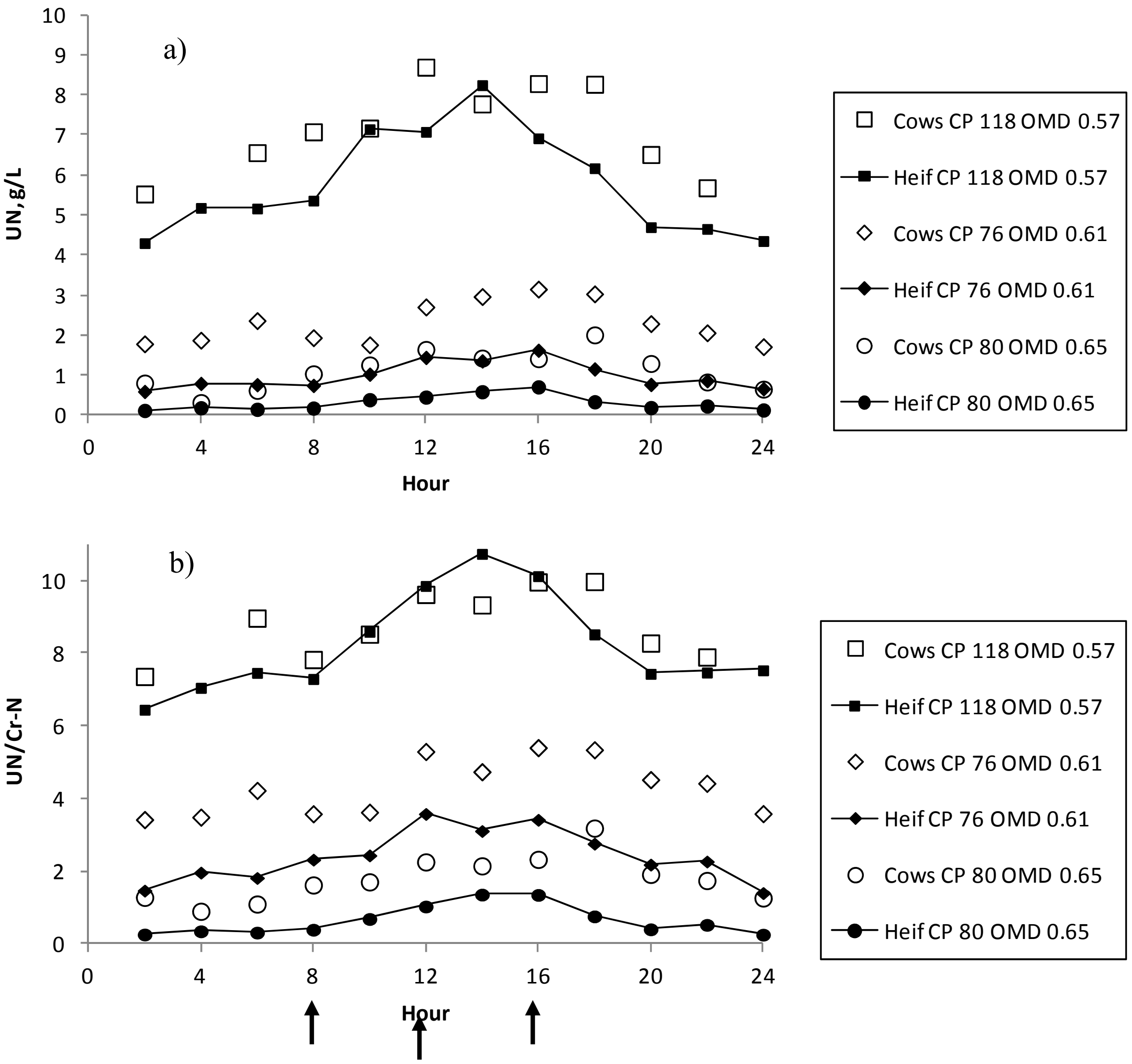

Figure 3 Urinary urea N (UN) concentration (a) and ratio urinary urea N/urinary creatinine N (b) in spot samples (N = 597) from dry cows and heifers stall-fed forages of varying crude protein concentration (CP as g/kg DM) and *in vitro* organic dry matter digestibility (OMD). Arrows indicate meals.

Conclusions

Peak values for urinary urea N concentration occur sometimes and sometimes not in lactating cows. Total urinary urea N excretion from 2-4 h intervals in lactating cows peak but at very different times of the day. Both concentration and total excretion of urinary urea N may peak in growing heifers if ration crude protein concentration allows that.

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