

Influence of milk urea concentration on fractional urea disappearance rate from milk to blood plasma in dairy cows

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ABSTRACT

The relationship between milk urea nitrogen (MUN; mg of N/dL) and urinary N excretion is affected, among others, by diurnal dynamics in MUN, which in turn is largely influenced by feed intake pattern and characteristics of urea transfer from blood plasma to milk and vice versa. This study aimed to obtain insight in urea transfer characteristics within the mammary gland and from the mammary gland to blood plasma in dairy cows at various concentrations of plasma urea nitrogen (PUN; mg of N/dL) and MUN. Urea transfer from milk to blood plasma and urea transfer within the mammary gland itself was evaluated in a 4×4 Latin square design using 4 lactating multiparous Holstein-Friesian cows (milk production of $39.8 \pm 4.70 \text{ kg/d}$ and 90 ± 3.9 d in milk). Treatments consisted of 4 primed continuous intravenous urea infusions of 0, 5, 10, and 15 g of urea/h. Boluses of [15N15N]urea were injected in cistern milk at 20, 60, and 100 min before the 1700 h milking. Milk was collected in portions of approximately 2 L at the 1700 h milking. Milk samples were analyzed for urea and enrichment of ¹⁵N-urea. Results from one cow were discarded because of leakage of milk from the teats after injection of boluses of [15N15N]urea. Increasing urea infusion rate linearly increased PUN from 11.4 (0 g of urea/h) to 25.9 mg/dL (15 g of urea/h) and MUN from 10.3 (0 g of urea/h) to 23.5 (15 g of urea/h) mg of N/dL. The percentage of injected [¹⁵N¹⁵N]urea recovered from milk at the time of injection was not affected by urea infusion rate and varied between 65.1 and 73.0%, indicating that a substantial portion of injected [¹⁵N¹⁵N]urea was not accounted for by collected milk. The estimated fractional disappearance rate of 15 N-urea from milk to blood (K_{urea} ; per hour) linearly increased from 0.429 (0 g of urea/h) to 0.641 per hour (15 g of urea/h). Cistern injected [15N15N]urea diffused within 20 min after injection toward alveoli milk. Calculations with the average K_{urea} estimated in this study show that 89% of an initial difference between PUN and MUN will have disappeared after 4 h. In conclusion, urea disappearance from milk in the mammary gland is substantial, as well as the intramammary urea exchange between cistern, duct, and alveoli milk. However, results have to be interpreted with caution given the lack of full recovery of dosed 15 N urea at time of injection. Information on $K_{\rm urea}$ is useful to quantify the effects of diurnal variation in PUN on MUN, which enhances the utility of MUN as an indicator for N excretion in urine.

Key words: dairy cow, urea, urea transfer, milk, blood plasma

INTRODUCTION

A positive relationship exists between the concentrations of MUN (mg of N/dL) and blood plasma (**PUN**; mg of N/dL) and the excretion of N in urine (Ciszuk and Gebregziabher, 1994). Because of this positive relationship, MUN is proposed in several studies as an indicator of urinary N excretion and ammonia emission (Nousiainen et al., 2004; Burgos et al., 2007; Van Duinkerken et al., 2011b). However, the relationship between MUN and urinary N excretion is affected by several factors (reviewed by Spek et al., 2013), among others by diurnal dynamics in MUN, which in turn is largely influenced by feed intake pattern (Gustafsson and Palmquist, 1993) and characteristics of urea transfer between blood and milk (Spek et al., 2012a). A substantial number of studies have measured the effect of feed or N intake on diurnal patterns of rumen ammonia (e.g., Gustafsson and Palmquist, 1993; Boucher et al., 2007; Agle et al., 2010) and PUN (e.g., Piccione et al., 2006; Cummins et al., 2009; Røjen et al., 2011). Fewer studies have focused on diurnal variation in PUN and its effect on variation in diurnal MUN. Some studies have measured disappearance of injected labeled urea in the mammary gland of milk goats (Linzell and Peaker, 1971) and of dairy cattle (Spek et al., 2012a). However, these were small-scale studies with a low number of animals, and more information on fractional rates of urea transfer from milk to blood and vice versa are desired

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to predict diurnal variation in MUN based on diurnal variation in PUN. Furthermore, we hypothesized that fractional rates of transfer of urea from the mammary gland to blood and vice versa might be affected by the concentration of MUN or PUN. The aim of this study was to determine urea fractional disappearance rates from the mammary gland and urea transfer characteristics within the mammary gland at various concentrations of PUN and MUN by means of continuous intravenous infusions of urea and by intramammary injected pulse doses of [¹⁵N¹⁵N]urea at various times before milking.

MATERIALS AND METHODS

The study was approved by the Institutional Animal Care and Use Committee of the Animal Sciences Group, Wageningen University and Research Centre, Lelystad, the Netherlands.

Cows, Housing, and Experimental Design

Four lactating, multiparous Holstein-Friesian cows were selected based on DIM and milk production. At the start of the experiment, cows had an average BW of 636 \pm 42.7 kg, parity of 2.5 \pm 1.00, DIM of 90 \pm 3.9 d, and milk production of $39.8 \pm 4.70 \text{ kg/d}$ (values expressed as means \pm SD). Cows were housed in a tiestall barn on rubber mats and wood shavings. Cows were randomly assigned to a treatment according to a 4×4 Latin square design that was orthogonal for carryover effects. Treatments consisted of 4 rates of continuous intravenous urea infusion (0, 5, 10, and 15 g of urea/h) dissolved in a saline solution administered during sample collection days. The experiment lasted 7 wk and consisted of 3 adaptation weeks to a simultaneous change in housing condition (from free stall to tie stall) and a change from a high protein diet (~160 g of CP/kg of DM) to the low protein TMR of 135 g of CP/kg of DM (Table 1), followed by 4 experimental periods. Each experimental period of 1 wk consisted of 3 sample collection days (Monday, Wednesday, and Friday). The days in between sample collection days were included as washout days for infused urea and injected boluses of labeled urea. Cows had ad libitum access to feed during the first 16 d of the 3-wk adaptation period, and subsequently, feed intake was restricted to 95\% of ad libitum feed intake of each individual cow during the rest of the experiment. Cows were milked twice daily at 0500 h and 1700 h throughout the experiment. During the noncollection days, cows were fed equal meals twice daily at 0500 and 1700 h, whereas during collection days, 50% of the daily feed allowance was provided in 6 equal meals every 2 h from 0500 until

Table 1. Dietary composition (g/kg of DM unless otherwise stated) of the TMR

Item	TMR
Ingredient	
Corn silage ¹	668
Wheat straw, chopped	26
Rapeseed meal	160
Soybean hulls	90
Palm fatty acids	20
Limestone	10
Sodium carbonate	10
Molasses	6.9
Urea	2.8
Mineral premix ²	2.3
Feed salt ³	1.9
Magnesium sulfate	1.8
Magnesium oxide	0.6
Nutrients	
DM (g/kg of feed)	491
CP	135
Ash	67
Crude fat	46
Starch	235
NDF	368
ADF	236
Ca	7.9
K	9.6
Na	3.8
Feeding value	
$NE_{L}^{4}(MJ/kg \text{ of DM})$	6.69
DVE_{\cdot}^{5}	63
OEB^6	10
Rumen degradable CP ⁷	85

 $^1\mathrm{Corn}$ silage (g/kg of DM unless specified otherwise): DM, 416 g/kg; CP, 69; starch, 381; NDF, 353; ADF, 196; ADL, 20; ash, 42 (determined with near infrared spectrometry; Blgg, Wageningen, the Netherlands). Calculated intestinal digestible protein (DVE), 50 g/kg of DM; rumen degraded protein balance (OEB), -41 g/kg of DM; NE_L, 6.54 MJ/kg of DM.

 $^2\mathrm{Contained}$ per kilogram of mix 108 g of Ca; 240 g of Mg; 4,960 mg of Cu; 9,624 mg of Mn; 13,440 mg of Zn; 696 mg of I; 520 mg of Co; 102 mg of Se; 2,000,000 IU of vitamin A; 440,000 IU of vitamin D₃; and 6,000 IU of vitamin E.

³Composition of feed salt: ≥99.8% NaCl.

⁴Net energy for lactation calculated with the Dutch VEM (feed unit lactation) system (Van Es, 1975).

⁵Intestinal digestible protein (Van Duinkerken et al., 2011a).

⁶Rumen degraded protein balance (Van Duinkerken et al., 2011a).

 $^7\mathrm{Based}$ on the Dutch protein evaluation system (Van Duinkerken et al., 2011a).

1700 h to minimize variation in PUN and MUN caused by variation in feed intake during that day. At 1700 h the remaining 50% of the total daily feed allowance was provided. Daily individual feed intake in the tie stall was determined by subtracting the fresh weight of orts from the weight of fresh feed supplied.

Infusions and Sample Collection

Three batches of the TMR were made during the experiment, and each batch was immediately stored at -20° C. Two days before feeding, the quantity of feed

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