Urea Kinetics in Wethers Exposed to Different Ambient Temperatures at Three Dietary Levels of Crude Protein*

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ABSTRACT : Eighteen Suffolk wether lambs (BW = 24 kg) were chronically exposed to temperatures of cold (2°C) or warm (22°C). The experimental design consisted of a 2×3 factorial with a single crossover of environment treatment. The sheep were closely shorn and were housed in individual metabolic crates in controlled environment rooms. Sheep consumed pelleted diets *ad libitum*, which consisted of mainly barley grain and brome grass, and diets contained 7, 11 or 14% crude protein (CP). Animals were catheterized via one jugular vein with a PVC catheter and received a single injection of 60-65 Ci of [^{14}C]urea. Plasma urea-N (PUN), urinary urea (UU), and carbon specific radioactivity were measured. Urea metabolism was not affected by environment. Percent urea recycling and urea space clearance were highest ($p<0.05$) on the low nitrogen diet. Urea pool was increased ($p<0.10$) for the 14% CP diet. Both UU and PUN concentration were positively related ($p<0.01$) with diet CP content. Therefore, dietary CP content significantly influenced urea metabolism, however, cold exposure did not alter those parameters. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 6 : 795-801)***

Key Words : Urea Recycling, Urea Flux, Crude Protein, Cold, Sheep

INTRODUCTION

Nitrogen (N) metabolism in the rumino-reticulum may vary considerably due to dietary and environmental influences. Many studies with ruminants have demonstrated an increase in urea recycling under conditions of low dietary N intake, or have examined relationships between urea recycling and growth in circumstances of low protein intake (Bunting et al., 1989). The rumen has been assumed to be the principal site of appearance of recycled urea into the digestive tract (Nolan and Leng, 1972); however, urea is also recycled to other parts of the digestive system (Nolan and Stachiw, 1979). Urea is not the only small N-containing molecule returned to the tract in this way, but it is by far the most significant, quantitatively. The gastrointestinal tract plays an important role in the degradation of various other endogenous nitrogenous substances, including enzyme secretions, mucous, amino acids and epithelial cells desquamating from the digestive tract mucosa. The recycling of urea to the digestive tract of ruminants and its degradation to provide ammonia for microbial metabolism has been proposed as a mechanism whereby ruminants conserve N during periods of protein deprivation (Christopherson, 1985).

The amount of endogenous urea which passes into the gastrointestinal lumen is considerable and represents a large percentage (40-60%) of the total daily amount of urea synthesized in the ruminant body (Christopherson, 1985). Ammonia concentrations in rumen fluid have been inversely correlated with urea recycling to the rumen. High proportional degradation of dietary N is usually associated with a low rate of urea recycling, low blood urea concentration and low N intake (Ford and Milligan, 1970). The amount of urea degraded in the rumen increases with increasing PUN concentration and increasing rate of urea production. In domestic ruminants given a N deficient ration, urea excretion in the urine is reduced with the urea being preferentially transferred to the digestive tract and converted into microbial protein (Huntington, 1987). The recycling of urea to the digestive tract of ruminants and its degradation to provide ammonia for microbial metabolism has been proposed as a mechanism whereby ruminants conserve dietary nitrogen during periods of protein deprivation (McBride and Christopherson, 1984). In one study more endogenous urea was shown to enter the rumen of cold-exposed sheep compared to that of warm exposed sheep (Kennedy and Milligan, 1978a,b). Therefore, animals may be better able to conserve N in a cold environment and be less constrained by a low nitrogen diet than animals in a warm environment. The present study was designed to examine this question by measuring urea metabolism at different dietary CP levels in growing lambs exposed to two ambient temperatures.

MATERIALS AND METHODS

Animals

Eighteen Suffolk wethers were chronically exposed to temperatures of cold (C: 2°C) or warm (W: 22°C) at three

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diet levels of CP (7, 11 and 14%). Sheep were fed pelleted diets containing 7, 11 or 14% CP *ad libitum*. Diets contained barley grain (*Hordeum vulgare*) and brome grass (*Bromus inermis Leyss*) which contained 8.4 and 4.8% CP, respectively. The vitamin, mineral, and energy contents of the diets were calculated to approximate the requirements of the sheep, based on BW at the start of the experiment. Water and cobalt-iodized salt blocks were also available *ad libitum*. Feed was weighed immediately prior to feeding and offered once daily at 10:00 at a rate of 10-15% in excess of the voluntary feed consumption of the previous day. Feed not consumed was collected and weighed back daily during the course of the experimental period in order to determine daily feed intakes.

Treatments were designed as W7, W11, W14, C7, C11 and C14, respectively. During the temperature adaptation periods, sheep were accustomed to the urine collection funnel which was attached to the abdomen. One day prior to the radioisotope injection, urine collection funnels were fitted to all sheep. The metabolic crates were designed for sheep involved in nutrient experiments and *in vivo* isotope studies. Easily cleaned, chemically resistant and nonabsorbent molded fiberglass reinforced plastic was used for the walls and back, the feed and water containers, and the excreta collector funnels of the crates.

Catheterization and isotope injection

Animals were catheterized via one jugular vein with a PVC catheter (1.01 mm ID, 1.67 mm OD) inserted through a 14 gauge needle and held in place by a suture around the catheter and through the skin. The catheter was then connected to a PVC tubing extension. Prior to the injection the radioisotope, 200 ml of an isosmotic saline solution was prepared containing 6.25 Ci and 0.1 mg urea per ml. For practical reasons of scheduling the frequent blood and urine sample collections, ^{14}C-urea was given to animals in the cold environment on day 29 and to animals in the warm environment on day 30. Each sheep within an environment treatment received a single injection of 60-65 Ci of [^{14}C] urea (50 mCi/mmole, >99 atom %, ICN Radiochemicals Inc.) via the jugular catheter. After injection, the injection syringe and catheter were immediately flushed with 20 ml sterile physiological saline solution (9 g NaCl/l).

Sampling procedure

Following [^{14}C]-urea injections, blood and urine samples were collected for determining ^{14}C, urea-N, and ammonia-N. Blood samples were collected, sterile, heparinized tubes from jugular catheters into syringes and transferred just before [^{14}C]-urea injection and at 1, 7, 26, and 47 h after injection. Plasma was immediately separated by centrifugation at 1,500×g for 10 minutes. Nine urine samples were collected from each wether in containers without acid surrounded by ice (to prevent microbial action) at 3, 6, 9, 12, 15, 18, 24, 36 and 48 h after injection. Urine was directed into the collection bottles by using a rubber funnel which was attached snugly to the abdomen below the prepuce of the lambs with elastic straps.

Analytical procedures

The radioactivity of ^{14}C was analyzed on a Liquid Scintillation Counter (Mark III, Searle Analytic Inc.). Counting efficiency was determined by the channels ratio method and counts for radioactivity were corrected for quenching from an external standard. Plasma was deproteinized and acidified with 6% $HClO_4$ (1:1, v/v), which also removed any $^{14}CO_2$ and the supernatant was saved for counting ^{14}C radioactivity. In preparation for counting, samples (1 ml urine or 400 µl plasma supernatant) were placed in a 20 ml scintillation vial together with 15 ml aquasol counting solution (NEN Co). Urine samples were thoroughly mixed prior to pipetting. Samples were refrigerated for 24 h to allow for chemiluminescence decay. The samples were counted for 20 min or until the counting errors were 0.25% or less. The total DPM were determined for each sample.

PUN and UU were determined according to colorimetric assay. Buffered urease solution was prepared using 200 Sumner units (= 30 mg) of special purified urease (70 mmole Units, one µmole unit will liberate one µmole of NH_3 from urea per min at pH 7.0 at 25°C; Sigma) per 100 ml of 1% solution of the buffer which was mixed with 6 g of KH_2PO_4 and 2 g of Na_2HPO_4 in 1.0 L. Plasma and urine samples were diluted by an appropriate amount (1:100 and 1:1,000, respectively) prior to analysis. One ml of buffered urease solution was added to each tube and incubated in a 37°C water-bath for five minutes. Free ammonia was measured by the same procedure as that described, substituting water for the urease solution. After incubation, the following solutions were added immediately after each other to each tube with automatic pipettes: 2 ml of sodium phenate, 3 ml of 0.01% sodium nitroprusside, and 3 ml of sodium hypochlorite. Each solution was mixed thoroughly with a vortex mixer and placed in the dark at 18°C for 30 minutes to allow maximum color development. Optical density (PC800 Colorimeter) was measured at 630 m and urea-N was estimated from the standard calibration curve after subtraction of the readings of free ammonia and blank.

Mathematical computation

Sample DPM was derived by urea concentration to compute the specific radioactivity of urea-C in plasma or urine. The size of the urea pool was calculated as the injected dose of isotope divided by specific radioactivity of the urea at zero time. Zero time specific radioactivity as estimated by extrapolation of the plot of the natural logarithm of urea specific radioactivity against to zero time. Urea flux was estimated as urea pool times the rate of

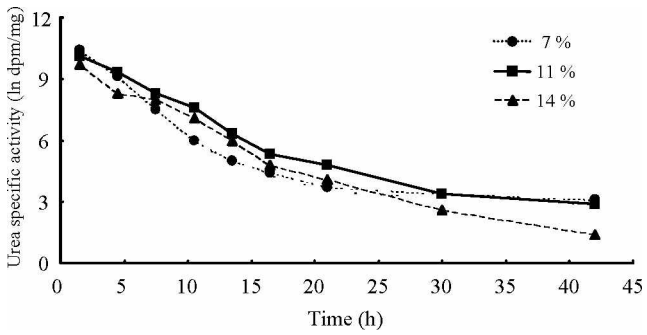

Figure 1. The relationship between urinary urea specific radioactivity (ln dpm/mg) and time (h) for 42 h in the Cold environment.

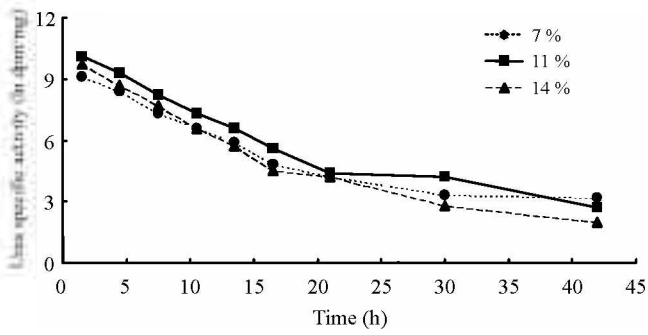

Figure 2. The relationship between urinary urea specific radioactivity (ln dpm/mg) and time (h) for 42 h in the Warm environment.

Table 1. The relation between the natural logarithm of urine urea specific radioactivity (dpm/mg, Y) and time (h, X) for 18 h after injection using first order kinetics determinations on sheep maintained in warm or cold environment

	Warm			Cold		
	Equation	r^2	SE[1]	Equation	r^2	SE[1]
7	Y = 10.667-0.352X	0.756	0.949	Y = 10.620-0.330X	0.953	0.418
11	Y = 10.567-0.314X	0.700	0.877	Y = 10.721-0.351X	0.746	0.985
14	Y = 10.392-0.311X	0.825	0.705	Y = 10.189-0.329X	0.941	0.365

* Standard error of estimate of Y-axis intercept at T_0.

Table 2. Urea metabolism by the first order kinetics in the young lambs fed three different dietary crude protein levels in the warm and cold environment[1]

Diet CP (%)	Warm			Cold		
	7	11	14	7	11	14
Urea pool size (g)	3.258^{a}	3.601^{ab}	4.292^{bc}	3.386^{a}	3.085^{a}	5.256^{c}
Urea pool size (g/kg$^{0.75}$)	0.289^{ab}	0.298^{ab}	0.358^{c}	0.311^{b}	0.238^{a}	0.435^{c}
Urea space (l)	12.30	7.43	7.99	13.20	6.41	9.62
Urea space (l/kg$^{0.75}$)	0.486	0.268	0.292	0.548	0.253	0.347
Half-life (h)	1.970	2.210	2.232	2.101	1.973	2.106
Turnover time (h)	2.837	3.182	3.211	3.025	2.843	3.033
Clearance (l/d)	233.3^{c}	125.0^{a}	125.6^{a}	221.6^{c}	113.8^{a}	165.3^{b}

[1] Means (n = 6) in the same row that do not have a common superscript differ ($p<0.05$).

decline (k fraction) of the plot of the natural logarithm of specific radioactivity with time. Urea space was estimated by dividing the total urea pool size by the concentration of plasma urea. The biological half-life of urea, expressed as t½, was calculated as 0.693 divided by k. The turnover time of urea was calculated as 1.44 times half-life. Urea space clearance was estimated from urea flux divided by plasma urea concentration. Urea recycled was calculated as urea flux minus urea excreted. Urea recycling was calculated as a proportion of urea flux multiplied 100. Urea carbon irreversible lost into the gastrointestinal tract (GIT) is equal to the total plasma urea flux minus the urea that is excreted in urine. Least-square regression analysis was used to obtain equations describing values of Ln specific radioactivity vs. time (Steel and Torrie, 1980).

RESULTS

The decline in ^{14}C-urea specific radioactivity over 42 h after injection is shown in Figure 1 and 2. Urea specific radioactivity decreased exponentially after injection and appeared to follow first order kinetics for the first 18 h. From 18 to 48 h there was a second exponential phase. Exponential equations for urea flux and kinetic parameters based on regression for the first 18 hours after injection are shown in Table 1. There were no significant diet or temperature effects on the regression coefficients (Table 1).

Urea pool, urea space, urea half-life, turnover time and urea space clearance are shown in Table 2. The urea pool size was positively related to the dietary CP content except for C11 treatment, which was lowest (3.085 g) among all treatments. The urea pool size was markedly increased by the 14% CP diet in both environments. There was no significant difference between the warm and cold environments. Urea space was reduced by increasing diet CP content. The values were 12.30, 7.43 and 7.99 in the warm, and 13.20, 6.41 and 9.62 in the cold for the 7, 11, and 14% diets. For the 7% CP diet, urea space appeared high in both environments. There were no significant differences due to environment treatments.

Table 3. Urea flux, urea recycling, urea excreted and plasma urea-N concentration in the young lambs fed three different crude protein levels diet in the warm and cold environment*

Diet CP (%)	Warm			Cold		
	7	11	14	7	11	14
Urea flux (g/d)	27.5	27.1	32.0	26.8	26.0	41.5
Urea flux (g/kg$^{0.75}$)	11.27	11.89	11.98	10.89	12.94	12.06
Urea excreted (g/d)	3.47	10.21	15.91	5.74	11.01	16.83
Urea excreted (g/kg$^{0.75}$)	0.31	0.86	1.33	0.53	0.85	1.40
Urea recycle (g/d)	24.03	16.88	16.08	21.06	14.99	24.67
Urea recycle (g/kg$^{0.75}$)	2.13	1.42	1.34	1.93	1.16	2.05
Urea recycling (%)	87.4^{d}	62.3^{b}	50.3^{a}	78.6^{c}	57.7^{b}	59.4^{b}
PUN (mg/dl)	11.8^{a}	21.6^{b}	25.5^{c}	12.1^{a}	23.0^{bc}	25.1^{c}

[1] Means (n = 6) in the same row that do not have a common superscript differ ($p<0.05$).
Determined on 18hrs urine after injection of ^{14}C-urea by first-order kinetics.

The mean biological half-lives of urea were 2.04, 2.09 and 2.17 h for the 7, 11 and 14% CP diets, respectively, in both environments (Table 2). There was no significant difference between the warm and cold environments. Urea turnover times were 2.94, 3.01 and 3.13 h in both environments when sheep were fed 7, 11 and 14% CP diets. There were no significant differences due to diets or temperature treatments. There was a significant ($p<0.05$) diet effect on urea space clearance rate. Significantly higher rates of urea space clearance were observed on the low protein diet in the both environments. However, cold exposure did not affect urea clearance rate.

Urea flux, urea excreted, urea recycled, PUN and UU are shown in Table 3. There were no significant differences in the urea flux, urea excreted, and urea recycled, but there were significant differences in the percentage of urea recycled, PUN and UU due to diet CP. Cold exposure did not significantly change urea metabolism in sheep although the highest value for urea flux (41.5 g/d) was appeared in the C14 treatment. The relationship between urea flux and dietary crude protein level was generally positive in both environments. The urea flux was similar between 7 and 11% CP diet in both temperature treatments, but it increased markedly in the W14 and C14 treatments. However, there was no significant difference between environments as shown by the analysis of variance.

The percentage of urea recycled was negatively ($p<0.01$) related to the N intake in both environments (Table 3). The urea recycling percentages were 87.4, 62.3 and 50.3 in the warm, and 78.6, 57.7 and 59.4 in the cold, for the 7, 11 and 14% CP diets, respectively. The diet effect was significant ($p<0.01$) but there was no significant difference between the warm and cold environment. PUN was increased ($p<0.01$) from 11.8 to 21.6 and 25.5 in the warm, and from 12.1 to 23.0 and 25.1 in the cold, as diet CP increased from 7 to 11 and 14% CP, respectively. These positive ($p<0.01$) relationships were similar in both environments.

DISCUSSION

A two-compartment open model was used initially to describe the exponential decline in ^{14}C-urea specific radioactivity with the lapse of time over 42 h (Figure 1) after injection. The second and slower component had very little effect on the estimation of urea kinetics. Therefore, regression lines were fitted for Ln ^{14}C-urea specific radioactivity vs. time for the first 18 h (Table 1). The sheep were in a steady-state during the 18 h period of estimation of urea flux, as indicated by determination of the urinary urea excretion rate per unit time over 24 h, even though feed was replenished once daily. This may have been because the sheep were fed *ad libitum* and therefore had feed available at all times.

Urea flux was increased 17.2 and 57.2% in the W14 and C14 treatment compared with results for the 7 and 11% CP diets, respectively, in association with the increasing N intake. Although there was about a 30% higher urea flux and urea recycling rate in the cold compared to the warm environment for the 14% CP diet, this was not significant. This differs from the increased urea transfer to the rumen during cold exposure reported by Kennedy et al. (1986). In the latter study, endogenous urea provided 29% of N available from dietary and endogenous urea sources in sheep in the warm, compared to 41% for cold-exposed sheep given equal intakes of brome grass pellets (Dixon and Milligan, 1984). In the present experiment the contribution of recycled urea represented about 50% of dietary and endogenous urea sources, although it is not clear how much of this entered the rumen. The irreversible loss of urea was 6.7 and 10.5 g/d in roughage fed sheep consuming 21 and 33 g N/d, respectively in a study by Egan and Ulyatt (1980). These are 30 to 45% of the flux values in the present study. Very low values of irreversible loss of urea of 0.33 and 0.78 g/d were also reported in calves when intakes were 12 and 21 g N/d, respectively (Bunting et al., 1987). Also, Moon et al. (2004) reported that nitrogen intake was different in summer and winter.

The positive relationship between urea excretion and N

intake is consistent with other reports in the literature. However, when sheep were fed roughage diets providing 21 and 33 g N/d intake, more than double the amount of urea was excreted (16.3 and 20.1 g/d) in the urine compared to the present results (Egan and Ulyatt, 1980). Nguyen et al. (2004) reported that N intake is balanced to fecal N, urinary N, and retained N. This may have been due to a lower fermentable OM supply to the rumen microbes which limited their ability to trap recycled urea compared to the situation in the present study. Therefore, urea excretion was relatively small in the concentrate diet-fed sheep compared with roughage-fed sheep. Also urea excretion was not affected by the environment treatment but dietary N intake had a major effect.

The absolute amount of urea recycled was similar for all intakes of dietary N in the warm environment. The present results support Kennedy and Milligan (1980) study, in which transfer of plasma urea via the rumen wall to the rumen digesta was high (6.2-9.8 g/d) in sheep given brome grass pellets. Total urea transfer from blood to rumen has been reported to range from 0.6 to 2.3 g N/d for sheep given lucerne or low quality diets (Nolan and Stachiw, 1979) but these are considerably lower than recycling rates in sheep given high intakes of a pelleted brome-grass diet (7.3-9.6 g N/d) (Kennedy and Milligan, 1978b). On the other hand, somewhat lower amounts of urea were recycled (3.8 and 7.8 g/d) in calves fed low-quality roughage at intakes of 12 and 21 g N/d, respectively (Bunting et al., 1987). In the present study the rates of urea excretion and the amount of urea recycling were positively related to PUN since transfer of urea from blood to rumen occurs along a concentration gradient.

High proportional rates of recycling (83%) were observed in the 7% CP diets in both environments. This result agrees with many other similar studies in sheep (Mousa et al., 1983), and in the tammar wallaby (Kennedy and Hume, 1978) when low protein diets were fed. Mousa et al. (1983) observed that percent urea recycling increased from approximately 77 to 94% with decreases in N balance in sheep and goats. Kennedy and Hume (1978) found urea recycling values of 84 and 79%, respectively, on low protein diets in the tammar. In other studies with ruminants on a low N intake, up to 90% of the urea entering the body urea pool was recycled into the digestive tract (Mugerwa and Conrad, 1971). These studies generally agreed that there is an inverse relationship between dietary N content and percent urea recycling.

Urea metabolism in domestic ruminants seems to vary greatly with the amount of N ingested and the serum urea level. Increased protein intake is generally associated with increased PUN concentrations in ruminants. In the present study, the PUN concentration was increased from 12.0 to 25.3 mg/dl when the intake of sheep was increased from 13.8 to 31.9 g N/d. This result is consistent with many previous studies with increased N intake in which PUN increased to reach a plateau at approximately 30 mg/dl (Obara and Shimbayashi, 1980). Godwin and Williams (1984) reported that when N input was raised from 7.6 to 17.6 g/d, PUN increased to approximately 30 mg/dl but remained relatively constant when N intake was increased further to 23.3 g. However, PUN may increase above this level when sheep are given N supplements on a low quality roughage diet. Therefore, there is a complicated regulation of PUN involving influences of dietary N content, diet type, fermentable OM, rumen ammonia concentration and urea recycling.

Urea pool size was positively related to the dietary CP content except for the 11% CP diet in the cold environment where pool size was lower than that at 7% CP. The reason for this result is not clear, but was not expected since N intake was approximately double that of the lambs on the 7% CP diet in the cold. The general increase with diet CP agrees with several recent studies (Bunting et al., 1987). Urea pool size was 4.9 and 6.5 g in the roughage fed sheep consuming 21 and 33 g N/d, respectively (Egan and Ulyatt, 1980). Slightly smaller urea pool sizes of 0.421 and 1.767 g were observed in calves consuming 12 and 21 g N/d, respectively (Bunting et al., 1987). On the other hand, the urea pool size markedly increased for the 14% CP diet in both environments in the present experiment. These values may have been affected not only by the high N intake and large amount of soybean meal but also by the large intake of readily fermentable carbohydrate.

In this study, urea space did not change significantly with diet CP. However, Cocimano and Leng (1967) reported that urea space decreased from 55 to 25% of body weight in sheep as the protein content of the diet decreased from 27% to 4% CP. In the present experiment, the urea space tended to decreased from 60 to 30% of body weight in young lambs when diet CP increased from 7 to 11 and 14%. On the other hand, urea space (0.474 and 0.467 $l/kg^{0.75}$) was not affected by level of N intake (12 and 21 g/d) in studies in calves (Bunting et al., 1987). Also urea space was 21.0 and 27.4 l in the roughage fed sheep (21 and 33 g N/d), respectively (Egan and Ulyatt, 1980). There is obviously tremendous variation in estimates of urea space reported in the literature. Theoretically, urea space can never be higher than total body water and usually is about 60-75% of body weight in regularly fed ruminants (FAO, 1985) but may differ depending on diet CP content and sources.

In conclusion, diet CP content has a large effect on the overall urea metabolism in the young lamb and the proportion of urea flux and recycled urea may play a significant role in maintaining rumen function when diets low in CP are consumed. However, the present study did not provide evidence for increased urea recycling in the

cold nor did urea recycling explain the sustained feed intake and growth of lambs on the 11% CP diet in the cold as reported by Sun and Christopherson (2001).

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