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ORIGINAL ARTICLE

The rate and pattern of urea infusion into the rumen of wethers alters nitrogen balance and plasma ammonia

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Summary

Changes in N balance, urinary excretion of purine derivative (PD), urea, creatinine and ammonia and plasma ammonia, glucose, urea, insulin and IGF-1 were examined in four wethers (37 ± 2.6 kg BW). The animals were fitted with permanent ruminal catheters, fed lucerne hay (9.4 MJ/day; 23 g N/day; 7 g soluble N/day, 6 equal meals/day) and treated with contrasting rates of urea infusion into the rumen: first, a continuous infusion (CT), at 3.2 mg urea-N/min for 10 days and then a discontinuous infusion (DT) at 156 mg urea-N/min for 4 min; in 6 daily doses with the meals for 7 days. N balance was calculated from pooled samples of faeces and urine. Jugular blood samples were collected before and 1.5 h after the morning meal (M1) on days CT10, DT2, DT4 and DT6. N retention decreased during DT ($p = 0.01$) due to a significant increase of N excretion in urine (4 g/day; $p = 0.009$) and faeces (1 g/day; $p = 0.02$). Dry matter ($p < 0.001$) and N digestibility *in vivo* ($p = 0.01$) decreased significantly during DT. Urinary urea and PD excretion were not altered by treatment. Significant linear ($p = 0.004$) and quadratic ($p = 0.001$) effects were observed for plasma ammonia in M1 (from 170 CT10 to 235 μM DT2 and returned to 120 μM DT6). No changes were observed in plasma glucose, urea, insulin and IGF-1. Results indicate that changes from CT to DT reduced N retention in sheep due to enhanced urinary N excretion, but it was not associated with changes in urinary urea or PD excretion; or plasma concentrations of insulin and IGF-1. As the dry matter (DM) an N digestibility could account a 0.23 of the decrease in N retention; the largest fraction of the reduction in N retention remained unexplained by the results.

Keywords urea, rumen, nitrogen balance, plasma ammonia, sheep

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Introduction

In ruminants, the intake of pastures fertilized with nitrogen, pasture silage or supplements with high contents of urea, where NPN represents a high percentage of the total N of the diet, is frequently associated to a low efficiency of N utilization. This has been manifested with a lower daily body weight gain (Elizalde and Santini, 1992; Enrique, 1998), a reduction of gastrointestinal organ mass (Enrique, 1998), a lower N retention (Ragland-Gray et al., 1997; Enrique, 1998) and a reduction of the *Longissimus dorsi* area (Milton et al., 1997).

These diets present an unbalance between the amount of energy (E) and nitrogen (N) available in the rumen (Elizalde and Santini, 1992) and/or an asynchrony of N and E ruminal release for microbial protein (MP) synthesis, which provoke a marked increase in the ammonia concentration of the ruminal fluid (Elizalde and Santini, 1992; Henning et al., 1993) and a lower MP flow to the duodenum than expected from calculations based on ruminal degradable N and organic matter (OM; Sinclair et al., 1993). From the point of view of the contribution of substrates for tissue metabolism, ruminants fed on these diets have a high portal flow and hepatic uptake of ammonia and also a high rate of urea synthesis (Maltby et al., 1991; De Visser et al., 1997) as well as a tendency to a low net flow of amino acids from the splachnic tissues to those tissues of productive interest (Maltby et al., 1991). Moreover, in experiments where peripheral ammonia concentrations were increased by infusion of urea or NH_4^+, it was observed a reduction in plasma insulin concentration (sheep; Fernández et al., 1988; dairy cows, Choung and Chamberlain, 1995), a hormone that participates in the regulation of protein deposition.

The purpose of this experiment was to quantify the effect of a change in the pattern and rate of NPN ruminal release on N balance, urinary excretion of purine derivative (PD), urea, creatinine and ammonia, and plasma concentrations of ammonia, urea, glucose, insulin and IGF-1.

Materials and methods

The current experiment followed the principles and guidelines for handling and care of wild and domestic animals for teaching and research, as defined in the Animal Welfare Act (Act No 087/02), approved by the Academic Board of the Faculty of Veterinary Sciences, UNCPBA, Tandil, on August 5, 2002.

Animals and diet

Four Corriedale wethers (37 ± 2.6 kg BW) surgically fitted with permanent PVC catheters (outer diameter, 1.20 mm; inner diameter, 0.80 mm) in the rumen, were housed in individual pens (2.6 m^2), under continuous lighting and daily fed 850 g of dry matter (DM) of lucerne hay (9.4 MJ ME/day; 23 g N/day; 7 g soluble N/day), in 6 equal meals (2.00, 6.00, 10.00, 14.00, 17.30 and 22.00 h) throughout the experiment and for 30 days before the first experimental period, using automatic feeders. Water was freely available up to a maximum of 3.0 l/day. (i.e., 1.3 times the daily water requirement, NRC, 1985).

Infusions and design

Urea was infused into the rumen with a peristaltic pump (Gilson Microplus®, Gilson S.A.S., Villiers le Bel, France) on two consecutive experimental periods, first continuously (CT; 9.5 g urea/day; 3.2 mg N/min) during 10 days, and then discontinuously (DT; 8.1 g urea/day in 6 doses/day at 156 mg N/min, for 4 min, with each meal) during 7 days.

Sample collection and analysis

A total collection of faeces and urine was performed daily from day 7 to 10 of CT and from day 2 to 7 of DT. Faeces were collected into a bag fitted to the perineum and around the annum with straps tied to a chest piece. Urine was collected into a flexible, V-shaped container, made of PVC laminated fabric strapped over the back of the animal, and was continuously transferred to large (5 l), dark-coloured bottle containing 250 ml of 10% SO_4H_2 (v/v) through a PVC tubing by suction with vacuums pumps. Urine pH was ≤2.5 at all times during collection. Faeces and urine were weighed, thoroughly mixed and subsamples were stored at −20 °C until analysis. Hay samples were obtained on the same experimental days. Subsamples of hay and faeces were oven dried at 60 °C and aliquots analyzed for DM (oven drying at 100 °C) and total-N content (Kjeldahl). Aliquots of urine samples were analyzed for total-N (Kjeldalhl), allantoin and uric acid (Chen et al., 1990), urea (Milano et al., 2000), creatinine (Wiener®, Wiener Laboratorios S.A.I.C., Rosario, Argentina) and ammonia (Milano et al., 2000). N balance and PD, urea, creatinine and ammonia excretion were calculated from data pooled across 4 days in CT (day 7–10) and across 2-day periods (days 2–3, day 4–5 and day 6–7) in DT. On days 10 of CT and 2, 4 and 6 of DT, jugular blood samples were collected into heparinised syringes 1.5 h after the 14.00-h meal, mixed and immediately centrifuged at 4 °C. An aliquot of plasma was analysed immediately for ammonia concentration and the rest was stored at −20 °C until analysis for urea, glucose (Milano et al., 2000), insulin (Díaz-Torga et al., 2001) and IGF-1 (Lacau-Mengido et al., 2000).

Statistical analysis

Results were analyzed using the PROC MIXED procedure of SAS statistical software package version 9.2 (SAS Institute, Cary, NC, USA), with variants treated as repeated measurements within animals. Linear (L) and quadratic (Q) effects were estimated for temporal changes in all variants during DT. For the analysis, period CT was considered as day 0 of DT. Results were declared not significant when p value >0.05.

Results

N Balance

Feed intake and total N input to the gut (N intake from lucerne hay plus urea-N infused into the rumen) were not different between treatments (Table 1). Whole body N retention was reduced by 5 g during DT ($p = 0.006$) due to an increase in both urinary ($p = 0.009$) and faecal N losses ($p = 0.02$; Table 1). Efficiency of N retention (N retention/N intake) decreased significantly from 38% in CT to 16% on day 6 of DT ($p = 0.007$; Table 1). N retention and efficiency of N retention showed a significant positive quadratic effect that suggests a possible stabilization or reversal of the decrease after 1 week of discontinuous infusion of urea.

Table 1 Dry matter (DM) intake, N balance, *in vivo* digestibility of N (NDIV) and DM (DMDIV), efficiency of N retention (ENR, N retained/total N input) and faecal DM excretion during continuous (CT: 3.2 mg N/min) and discontinuous (DT2-6: 156 mg N/min for 4 min, six times a day) ruminal infusion of urea in wethers (Mean ± SD, n = 4. Regression coefficients: L; Q)

		DT (days)			p		
	CT*	2 †	4 ‡	6 §	L	Q	RMSE
DM intake/g/day)	929.5 ± 47	884.8 ± 69	928.9 ± 47	928.9 ± 47	NS	NS	7.29
N balance (g/day)							
Intake	25.9 ± 1.1	24.9 ± 1.2	25.1 ± 1.2	25.2 ± 1.3	NS	NS	1.09
Faeces	3.9 ± 0.7	4.5 ± 0.5	4. 7 ± 0.7	5.0 ± 0.9	0.02	NS	0.65
Digestible	22.1 ± 1.3	20.5 ± 1.1	20.5 ± 1.4	20.1 ± 1.4	0.05	NS	1.19
Urine	12.1 ± 3.1	15.0 ± 1.4	16.6 ± 2.0	15.9 ± 2.3	0.009	NS	2.19
ENR (%)	38.4 ± 9.6	21.6 ± 7.0	14.9 ± 12.1	16.2 ± 10.6	0.007	0.04	8.7
DMDIV (%)	70.7 ± 4.7	67.1 ± 4.1	64.5 ± 4.4	58.8 ± 2.2	0.0005	NS	3.5
NDIV (%)	84.9 ± 2.6	81.9 ± 1.8	81.4 ± 2.9	80.1 ± 3.7	0.01	NS	2.6
Faeces (g MS/day)	272 ± 42	291 ± 37	329 ± 37	382 ± 17	0.0003	NS	31

CT, continuous infusion; RMSE, root mean square error; NS, not significant; L, linear; Q, quadratic.
*CT7 to CT10.
†DT2 to DT3.
‡DT4 to DT5.
§DT6 to DT7.

DM and N digestibility *in vivo* (Table 1)

In vivo DM digestibility decreased significantly from 70.7% to 58.8% (p = 0.0005) during DT, and this was accompanied by a 40% increase in faeces DM excretion (p = 0.0003). *In vivo* N digestibility and digestible N intake decreased significantly by 6% and 10%, respectively, during DT (p = 0.05).

Urinary parameters (Table 2)

Urea N and PD excretion in urine did not change during DT. Urinary ammonia excretion in urine showed significant linear (p = 0.04) and quadratic (p = 0.05) effects and, overall, was lower in DT than CT. Urinary creatinine-N excretion increased by 38% during DT (p = 0.04).

Plasma

The change from a continuous to a discontinuous rate of urea-N infusion into the rumen caused an initial increase in plasma ammonia concentration (235 μm in DT2 and DT4) followed by a marked decrease to 121 μm in DT6, a value lower than that found in CT (Table 3). This was well described by a model with significant lineal (p = 0.004) and quadratic (p = 0.001) coefficients. Additionally, the increment M1-M0 (y, μm) showed a curvilinear response of as a function of time in DT (x, d; R^2 = 0.43; MSE = 2242; p = 0.001: $y = -210.62$ (SE = 72.7; p = 0.009) + 134.11 (SE = 35.4; p = 0.001) x − 14.97 (SE = 3.88; p = 0.001) x^2. Clinical signs of hyperammonemia were not observed during the experiment. The change of the rate of urea infusion into the rumen during the

Table 2 Urinary excretion of urea, purine derivatives (PD), creatinine, ammonia and urine volume during continuous (CT: 3.2 mg N/min) and discontinuous (DT2-6: 156 mg N/min for 4 min, six times a day) ruminal infusion of urea in wethers (Mean ± SD; n = 4; Regression coefficients: L; Q)

		DT (days)			p		
Urinary parameters	CT*	2 †	4 ‡	6 §	L	Q	RMSE
Urea (g N/day)	12.3 ± 4.5	11.7 ± 2.6	10.6 ± 2.1	10.9 ± 1.6	NS	NS	2.6
PD (mg N/day)	337 ± 106	411 ± 91	350 ± 88	363 ± 24	NS	NS	77.2
Creatinine (mg N/day)	312 ± 110	395 ± 119	407 ± 25	429 ± 55	0.04	NS	77.3
Urine (ml/day)	1418 ± 382	1273 ± 382	1470 ± 579	1428 ± 513	0.002	0.001	20.1

CT, continuous infusion; RMSE, root mean square error; NS, not significant; L, linear; Q, quadratic.
*CT7 to CT10.
†DT2 to DT3.
‡DT4 to DT5.
§DT6 to DT7.

Table 3 Changes in ammonia, urea, glucose, insulin and IGF-1 plasma concentration during continuous (CT: 3.2 mg N/min) and discontinuous (DT2-6: 156 mg N/min for 4 min, six times a day) ruminal infusion of urea in wethers (Mean ± SD, $n = 4$; p value of Regression coefficients L; Q)

		DT (days)			p		
Plasma	CT*	2†	4‡	6§	L	Q	RMSE
Ammonia (μM)	170 ± 98	235 ± 88	235 ± 90	121 ± 54	0.004	0.001	41.6
δ ammonia (μM)	16 ± 78	−2 ± 90	85 ± 104	20 ± 64	0.001	0.001	47.3
Urea (mM)	13.5 ± 2.0	12.4 ± 1.4	12.0 ± 2.6	14.2 ± 2.0	NS	NS	3.5
Glucose (mM)	3.14 ± 0.44	3.42 ± 0.28	3.23 ± 0.18	3.54 ± 0.14	NS	NS	61.2
Insulin (nM)	0.35 ± 0.14	0.33 ± 0.06	0.39 ± 0.08	0.33 ± 0.08	NS	NS	0.10
IGF-1* (nM)	246 ± 64	340 ± 106	373 ± 164	268 ± 64	NS	NS	85.5

CT, continuous infusion; RMSE, root mean square error; NS, not significant; L, linear; Q, quadratic.
*CT7 to CT10.
†DT2 to DT3.
‡DT4 to DT5.
§DT6 to DT7.

experiment had no effect on urea (mean ± SE; 13.0 ± 2.0 mM), glucose (3.33 ± 0.30 mM), insulin (0.35 ± 0.09 ng/ml) and IGF-1 (306 ± 81 ng/ml) plasma concentrations (Table 3).

Discussion

The rate of urea infusion in DT (156 mg N/min) was chosen to achieve a hyperammonemia in the first 2 h after feeding, similar to that observed in ruminants consuming diets with high content in ruminal available N (300 μM in plasma obtained from jugular vein; Sinclair et al., 2000). Although the criteria used to define the discontinuous rate of urea infusion was the increase in plasma ammonia concentration, this rate of infusion is close to the maximum potential intake rate of soluble N (134 mg N/min) in sheep grazing forages with high content of soluble N in our region (Pampa *Húmeda Argentina*), based on the maximum intake rate reported for sheep (7 g DM/min; Kenney and Black, 1983) and the maximum content of soluble N in early vegetative stage of *Avena Sativa* (19.2 mg soluble N/g DM; Martínez et al., 2005).

The change of infusion rate of urea-N into the rumen increased the N excretion in urine and faeces by 4 and 1 g, respectively, during DT and caused a significant reduction in whole body N retention (38% lower than CT). The efficiency of N retention (retained N/intake N) was diminished from 38 in CT to 13% in DT6. These results indicate that the wethers made a more efficient use of the same amount of urea-N for body N retention when this was infused into the rumen in a continuous form.

Most of the findings in the literature (Houston et al., 1974; Prior, 1976; Henning et al., 1993) are consistent with the results of the current experiment which indicate that when the N is rapidly and discontinuously incorporated into the ruminant diets, the N retention in the short term will be lower than that on a diet with the same quantity of N, but added in a continuous form and at a low rate. However, it is very difficult to find similar patterns between experiments regarding the factors that impact the N retention, as even with differences in the final N content of the diet, with or without urea and/or with different energy input in the diet, the response of N retention to N input seems to be very complex and, therefore, so is the interpretation of results.

One finding that surprised us, and we do not yet understand, was that the *in vivo* digestibility of dry matter (DMIVD) decreased significantly by 12% during DT. This situation reduced the metabolizable energy intake by approximately 2.49 MJ ME/day. On the assumption of 0.62 g N is retained in growing lambs per MJ ME when N is not limiting (AFRC, 1993), this reduction in DMIVD could explain only 20% of the difference in N retention. Prior (1976) also noted a drop in the DMIVD in lambs from 61% to 41% when changed from 12 meals/day to 2 meals/day, although this was mainly due to a decrease in feed intake.

In the same way, the decrease in digestible N intake could have altered N retention. Assuming that the decrease of 2 g/day in digestible N during DT includes only metabolizable protein and that it was used for tissue deposition with an efficiency of 59% (AFRC, 1993), the decrease in digestible N could account for 20% of the observed reduction of N retention in DT.

Thus, as a result of changing the pattern of urea-N input to the rumen, the decreased digestibility of DM and N could explain a maximum of 40% of the decrease in N retention in DT. Most of the decrease in

the N retention seems to depend on other mechanisms.

The main cause of the diminution in the N retention during DT was the increase of urinary N excretion. However, the increase in urea-N elimination in urine reported in experiments with increasing amounts of urea infused into the rumen (growing steers; Awawdeh et al., 2004) or added to the diet (heifers; Marini and Van Amburgh, 2003; lambs; Marini et al., 2004) was not observed in the current experiment. Moreover, the ratio N-urea/total N in urine decreased from 1.09 to 0.68 during DT, suggesting that other nitrogenous constituents of the urine increased during DT. This is similar to the results reported by Enrique (1998), who found that the addition of urea to the diet of lambs at 2% DM increased three times the excretion of urea-N but also doubled the elimination of nitrogenous constituents of the urine. Among the N compound analyzed in urine in the current experiment, only creatinine-N excretion was significantly increased from 271 in CT to 400 mg N/day in DT6; however, in relative terms, this represents only 3% of the difference in urinary N excretion observed between CT and DT. Other N compounds which were not determined in the present experiment, such as orotic acid, hippuric acid, amino acids and peptides or proteins, could have been responsible for the increase in urinary N excretion in DT. Their contribution to the increase in urinary N excretion could be the subject of future investigations.

There were no significant differences between treatments in the excretion of microbial purine derivatives (MPD). Consequently, one can assume that the microbial amino acid absorption in the duodenum, an estimated 5.2 g N microbial/day (assuming a digestibility of 83% purine, a purine N content of 70 mg N/mmol, an N-purine: total N DMDR 11.6:100 and fractional excretion in urine of 84%; Chen and Gomes, 1992), was similar in both treatments. Consequently, the decrease in digestible N and N retention were not a result of a lower microbial N supply to the duodenum.

When ruminal infusion of urea-N was changed from continuous to discontinuous form, plasma ammonia concentration increased to 220 μM in DT2 and DT4, followed by a decrease in DT6 similar to those found in CT, suggesting that after the first 4 days of DT there was an increase in ammonia uptake by the liver or by the peripheral tissues, subsequent to an escape of ammonia to peripheral plasma. Although there were no signs of ammonia toxicity in the current experiment, works carried out in different species showed plasma concentrations close to 300 μM, much lower than those reported to cause clinical signs in ruminants (800–900 μM; Symonds et al., 1981), ammonia could reduce plasma insulin concentration (sheep; Fernández et al., 1988; dairy cows, Choung and Chamberlain, 1995), inhibit insulin secretion and reduce uptake of glucose by peripheral tissues (rat; Schlienger et al., 1975). These changes could be related to the decrease in body protein retention. Other studies indicate that ammonia could also stimulate hepatic glucose production (Barej et al., 1987; Fernández et al., 1990; and Milano and Lobley, 2001) increase plasma glucose concentration (Ortolani and Antonelli, 2004). However, no significant changes between treatments were found in plasma concentrations of urea, glucose, insulin and IGF-1 that might have explained the decrease in N retention observed in the current experiment.

Implications

The results indicate that for a similar daily input of urea N to the rumen, the change from continuous to discontinuous infusion with the meals, reduced body N retention from 10 to 4 g/day during the first week of treatment. This reduction of body N retention was associated with a decrease in apparent DM and N digestibility, with an increase in N urinary excretion and a transient increase in plasma ammonia concentration, but was not associated with changes in urinary excretion of PD or plasma concentrations of insulin and IGF-1. Urinary urea excretion was not affected by the treatments; the increase of N urinary excretion was due to an increase in the excretion of unidentified nitrogenous compounds. More research is needed to identify which is the mechanism that altered N retention during discontinuous urea-N infusion, how the hormonal status of the ruminant was affected and how the body N mass was compromised.

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