

## Effect of intraruminal osmolality changes arising from administration of sodium chloride on transfer of blood urea to rumen in sheep

(Kesan perubahan osmolaliti intraruminal lanjutan daripada pemberian natrium klorida terhadap pemindahan urea darah ke dalam rumen biri-biri)

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Key words: blood urea, rumen bicarbonate, rumen osmolality, urea transfer

### Abstrak

Suntikan tunggal 15 g NaCl ke dalam rumen telah meningkatkan tekanan osmotik cecair rumen daripada 369 kepada 474 mOsmol/kg. Kesan ini juga mengurangkan pemindahan urea darah ke dalam cecair rumen daripada 2.3 kepada 1.6 g N setiap hari pada biri-biri yang diberi makan 50 g rumput kering oat cencang setiap jam. Dirumuskan bahawa pengurangan pemindahan urea adalah disebabkan oleh pengurangan input urea melalui air liur. Dinding rumen didapati tidak telap kepada urea sepanjang tempoh kajian.

### Abstract

A single injection of 15 g NaCl into the rumen, which increased the osmotic pressure of rumen fluid from 369 to 474 mOsmol/kg, resulted in a decrease in the transfer of blood urea into rumen fluid from 2.3 to 1.6 g N/day in sheep fed 50 g of chaffed oaten hay every hour. It is concluded that the reduced urea transfer was the result of a reduced urea input via saliva. The rumen wall remained virtually impermeable to urea throughout the study.

### Introduction

The osmolality of rumen contents has not received much attention as a potential factor influencing urea transfers to the rumen (Egan et al. 1986). The osmolality of rumen contents is quite variable, being influenced by factors such as the type of feed and time of feeding (Warner and Stacy 1965). Net water flux out of the rumen changes with rumen osmolality (Warner and Stacy 1972) and saliva production decreases with increased rumen osmotic pressure (Warner and Stacy 1977) and presence of salts in the

drinking water. After a ruminant has eaten, the release of salts and catabolism of polymers increase the rumen concentration of osmotically active compounds and the rumen contents become hypertonic. Under these conditions, water tends to diffuse into the rumen and urea which is also diffusible, may enter together with the inflow water. The permeability coefficients for passive diffusion of urea and water across lipid bilayer membranes, i.e.  $5 \times 10^{-2}$  and  $7 \times 10^{-4}$  cm/s, respectively, are highly relative to many other compounds. It is thus possible

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that urea recycling to the ruminoreticulum is influenced by the osmotic pressure of rumen contents through the effect of osmolarity on water kinetics, although the effects on urea transfer might be expected to be less than for water itself.

The aim of this study was to examine the effect of a sudden increase in rumen osmolality caused by a single injection of sodium chloride (NaCl) on water kinetics and on urea N transfer to the rumen of sheep.

## **Materials and methods**

### ***Sheep and experimental diets***

Four mature Merino crossbred wethers (35.9 kg  $\pm$  1.2 S.E.) were given a daily ration consisting of 1 200 g oaten chaff dry matter (DM) which contained 902 g/kg organic matter and 18.6 g/kg nitrogen in equal hourly portions. Sheep were adapted to the basal diet for 35 days and being held in a metabolism crate for 7 days before measurements were taken.

### ***Experimental procedure***

Both jugular veins of the sheep were catheterized at approximately 1500 h on Day 1 of trial 1 and a continuous infusion (0.2 mL/min) of  $^{14}\text{C}$ -urea (50 mg urea N, 0.3  $\mu\text{Ci}/\text{mL}$  in isotonic saline) was maintained into one of the catheters from 2240 h on Day 1 until 2040 h on Day 2. At 1000 h on Day 2, drinking water was removed and a single injection of chromium-EDTA (15 mL, 60 mg approx.) was administered into the rumen of each sheep to determine the outflow rate (Downes and McDonald 1964). After 930 min of infusion (1430 h), 15 g of NaCl in 50 mL water was administered intraruminally through a tube attached to the syringe. In trial 2, a continuous intraruminal infusion (1.0 mL/min) of  $^{14}\text{C}$ -bicarbonate (50 mg sodium bicarbonate, 0.015  $\mu\text{Ci}/\text{mL}$ ) was maintained between 1000 h and 1900 h on the day of the trial. Sodium chloride (15 g, 50 mL) was administered intraruminally in a single dose at 1430 h (250 min after the start of infusion).

Samples of rumen fluid and blood were collected before infusions and at frequent intervals during the infusions. Carbon dioxide from blood and rumen fluid was obtained from approx. 5 mL of fluid according to the procedure of Leng and Leonard (1965). The osmolality of rumen fluid was determined after centrifugation (20 000  $\times$  g) by the freezing point depression method on a Fiske osmometer according to the procedure of Leng and Leonard (1965). Other chemical and radioactivity analyses were carried out as described by Dixon and Nolan (1986).

### ***Calculations and statistical analyses***

All specific activities of carbon were normalized to a daily infusion rate of 100  $\mu\text{Ci}$  isotope. The rates of irreversible loss of plasma urea and rumen bicarbonate (g C/d) were calculated as infusion rate divided by plateau specific activity ( $\mu\text{Ci}/\text{g}$  carbon). The plateau specific activities of blood urea, and rumen bicarbonate carbon and plasma urea N concentrations were determined using three samples taken 120 min before injection of NaCl. The values were then compared with three samples taken in the same period 60–160 min after administration of NaCl. Isotope kinetic analysis was conducted as described by Dixon and Nolan (1986).

The figures giving the natural logarithm of chromium (Cr) concentration with time were obtained by adjusting the information on individual animal to the mean value. Data obtained before and after intraruminal NaCl administration were examined by analysis of variance where sheep (d.f. = 3) and time relative (d.f. = 3) were sources of variation. The difference between urea transfer into the rumen in the four sheep before and after NaCl administration was tested using the paired t-test.

## **Results and discussion**

The effects of administration of 15 g NaCl on the osmolality of rumen contents during an intravenous infusion of  $^{14}\text{C}$ -urea and on

intraruminal infusion of  $^{14}\text{C}$ -bicarbonate are given in *Table 1*. The osmolality of 350–380 mOsmol/kg obtained before NaCl administration is between the values of approximately 260–280 in fasted animals and 400 in fed animals reported by Warner and Stacy (1965), but are higher than values of 278–291 mOsmol/kg reported by Norton, Mackintosh et al. (1982) in continuously fed sheep. The mean increase in rumen osmolality obtained in three samples taken 31–101 min after NaCl administration was 101–113 mOsmol/kg which, assuming a 5 L rumen fluid volume, is close to the expected increase of 95 mOsmol/kg.

#### ***Fractional outflow rate from the ruminoreticulum***

Infusion of NaCl in steady-state experiments has increased the fractional outflow rate of rumen fluid (Harrison and McAllan 1980). In this experiment where a single dose of NaCl was administered, the overall fractional outflow rates of fluid from the rumen were 9.4% (SE = 2.8) and 8.8% (SE = 1.7) per hour in the trials when  $^{14}\text{C}$  was infused into the blood and rumen, respectively. Hypertonic conditions did not promote water flux into the rumen. On the contrary, inflow rate was reduced to essentially zero (–2.4% per hour; SE = 9.7) during the 31–96 min after NaCl was administered into the rumen in trial 1 and to 3.9% (SE = 10.4) per hour, in 41–101 min following NaCl administration in trial 2. No information on outflow rate was obtained from the Cr data as loss of water from the

rumen per second has no effect on Cr concentration.

Bailey and Balch (1961) reported that saliva production was decreased from approx. 70 to 20 mL/min following ruminal dosing of 500 g NaCl in cows. Similarly, Warner and Stacy (1977) found that saliva production decreased from approx. 0.35 to 0.1 L in 13 h when rumen osmolality was increased from 250 to 500 mOsmol/kg. Thus, it appeared that the inhibition of salivary secretion following intraruminal administration of a large dose of NaCl was a more important factor influencing net water entry into the rumen than an increase in water diffusion across the rumen wall in response to the resulting increase in ruminal osmolality (Warner and Stacy 1972).

#### ***Urea and bicarbonate metabolism***

As NaCl was administered simultaneously to all sheep, there was a possibility of changes in urea metabolism due to diurnal variations. However, as the animals were in a steady-state condition and rumen fraction outflow rates and osmolality were immediately influenced in a similar manner by NaCl administration in both trials, it can be concluded that differences obtained were related to NaCl administration rather than to time of sampling.

Data concerning urea and bicarbonate metabolism (pre and post-NaCl administration) are given in *Table 2*. Plasma urea concentrations were marginally higher (by 4%,  $p = 0.04$ ) after NaCl administration (*Table 2*). In contrast, Dixon and Milligan

Table 1. Effects of single intraruminal dose of 15 g NaCl on rumen pH and osmotic pressure

Measurement	Pre-NaCl	Post-NaCl	SEM	Sig. difference
Rumen fluid pH	6.21	6.01	0.24	0.087
Osmolality (mOsmol/kg)				
$^{14}\text{C}$ -urea	379	479	20.4	0.004
$^{14}\text{C}$ -bicarbonate	359	472	23.9	0.011

Pre-NaCl data were obtained 120 min before NaCl administration

Post-NaCl data were obtained 60–160 and 31–101 min after NaCl administration for rumen osmolality and pH, respectively

SEM is based on 8 observations per mean except for rumen osmolality (n=11)

(1983) found no effect on plasma urea concentrations after adding 1% (w/v) NaCl to the drinking water of steers. The rate of irreversible loss of blood urea carbon was decreased ( $p = 0.005$ ) by 4.4% following administrations of NaCl (Table 2). In contrast, Godwin and Williams (1984) found that extracellular fluid volumes increased when NaCl was continuously infused intraruminally.

The irreversible loss of 5.8 g daily for blood urea C is within accepted ranges (e.g. Nolan and Leng 1972; Norton, Janes et al. 1982; Norton, Mackintosh et al. 1982) for sheep given a diet supplying 20 g N/day but is higher than the 2.3 g daily obtained by Nolan and Stachiw (1979) when a wheaten chaff-based diet supplying 6.2 g N/day was given. The 4% decrease ( $p = 0.003$ ) in rate of irreversible loss after administration of NaCl (Table 2) may be related to the observed decrease ( $p = 0.087$ ) in rumen pH because less ammonia is absorbed from the rumen when pH is reduced (Hoge 1964).

Intraruminal administration of NaCl which increased rumen fluid osmolality, resulted in a decrease ( $p > 0.00$ ) in the proportion of rumen bicarbonate carbon arising from blood urea (Table 2). The irreversible loss of bicarbonate carbon in the rumen (Table 2) was not affected significantly by the administration of NaCl,

but was greater than could be accounted for by the amounts produced from rumen fermentation. This latter result is similar to that reported by numerous workers (e.g. Norton, Mackintosh et al. 1982) and indicates that rumen bicarbonate arise from sources other than fermentation of the feed.

#### ***Urea recycling to the rumen***

There was a 32% decrease ( $p = 0.01$ ) in urea N transfer to the rumen in the 60–160 min period following NaCl administration. Clearance of urea to the rumen from the plasma was also reduced ( $p = 0.03$ ) from 75 to 49 L/day (Table 2). Dixon and Milligan (1983) reported that the inclusion of NaCl (0.94 g/kg liveweight) in drinking water had no influence on irreversible loss of plasma urea N or urea transfer into the rumen. However, the difference in osmotic gradient between the blood and rumen for animals with and without salt in their drinking water was only 13.5 mOsmol/kg which is a very small increment compared with that under normal conditions (Warner and Stacy 1965). Under certain circumstances, a considerable amount of water drunk bypasses the rumen (Woodford et al. 1985; Garza and Owens 1989). Thus inclusion of salt in drinking water might not have influenced the rumen as much as expected.

Table 2. Indices of urea metabolism before and after a single intraruminal dose of 15 g NaCl

Measurement	Pre-NaCl	Post-NaCl	SEM	Sig. difference
<b>Blood urea</b>				
Concentration (mg/L)	32.7	34.0	0.32	0.014
Irreversible loss (g N/day)	13.6	13.0	0.12	0.003
Transfer quotients (%)				
Rumen bicarbonate	1.46	1.13	0.029	0.000
Blood bicarbonate	0.94	0.94	0.019	0.844
Transfer to rumen (g N/day)	2.32	2.32	0.061	0.014
Clearance (L/day)	74.8	49.0	6.28	0.026
<b>Rumen bicarbonate</b>				
Irreversible loss (g C/day)	67.4	57.5	5.81	0.248
Transfer to blood urea (g/C)	0.51	0.77	0.65	0.71

Pre-NaCl data were obtained 120 min before NaCl administration

Post-NaCl data were obtained 60–160 and 31–101 min after NaCl administration for urea metabolism

SEM is based on 12 observations per mean

The reduction in blood urea clearance rate, coupled with a reduced rumen liquid dilution rate following NaCl administration indicated that the reduced transfer of urea to the rumen in our experiment was caused by a reduction in transfer of body fluids to the rumen. As salivary secretion has been found to account for 0.4–2.2 g of urea N daily entering the rumen (Kennedy and Milligan 1978; Norton, Mackintosh et al. 1982), saliva production probably accounted for most of the urea recycled to the ruminoreticulum in this experiment. A large reduction in salivary secretion and hence N transfer to the rumen via saliva would be expected because salivary secretion is reduced by the administration of NaCl (Bailey and Balch 1961) and the resulting increases in osmotic pressure in the rumen (Warner and Stacy 1977). These results, thus, suggest that the reduction in urea transfer into the rumen following NaCl administration was due to a reduction in the salivary secretion rate rather than a change in the permeability of the rumen wall to urea. Whether the reduction in salivary secretion is a direct effect of the increased osmolality is not known.

In conclusion, the results of this experiment indicate that the amount of endogenous urea transferred to the rumen is reduced when there is a sudden increase in the osmolality of rumen contents brought about by a single intraruminal administration of NaCl. It seems likely that a similar decrease in urea recycling may occur in animals immediately after eating which tends to increase osmolality. This reduction in urea transfer appears to be related to a decrease in the volume of saliva entering the rumen rather than to specific changes in the permeability of the rumen wall.

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