Effect of water intake on the nitrogen balance of sheep fed a low or a medium protein diet

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Mutton merino wethers (n = 16) fed either a low nitrogen (LN, with a crude protein [CP] content = 5.4%) or a medium nitrogen (MN, with CP = 10.3%) diet were subjected to two water treatments; either *ad libitum* or half *ad libitum* access to water, in a two-period, two-treatment cross-over design. Sheep were adapted to the diets for at least three weeks before the start of the trial, and were kept on each water treatment for one week. A digestibility trial was carried out during the last five days of that week, and concomitant jugular blood samples were taken daily. The group fed the LN diet consumed 820 ± 128, 44.6 ± 6.9 and 2828 ± 524 g/day organic matter (OM), CP and water respectively, when allowed free access to water. Restricting water intake to 1463 ± 330 g/day reduced the intake of OM and CP to 697 ± 183 and 37.9 ± 9.9 g/day respectively. In the group fed the MN diet, reducing the water intake from 2937 ± 372 to 1406 ± 301 g/day reduced the OM and CP intakes from 936 ± 64 and 96.6 ± 6.6 to 665 ± 92 and 68.6 ± 9.4 g/day respectively. As a result, urine output decreased from 944 ± 327 and 772 ± 150 ml/day to 505 ± 60 and 509 ± 276 ml/day for sheep fed the LN or MN diets respectively. Water restriction in the MN group decreased the amount of nitrogen retained from 45.9 to 9.4 g/day, largely as a result of the decreased intake of nitrogen (about 30 g/day). Although the intake of OM also decreased proportionately, the amount lost in the faeces remained the same, suggesting that OM digestibility was decreased (from 68 to 52%). In the LN group, restricting the water intake improved nitrogen retention from 1.1 to 9.2 g/day, despite the depressed intake of feed CP. This was due to a large decrease in the amount of nitrogen lost via the faeces. There was also a concomitant increase in OM digestibility (54 to 71%). Although the amount of nitrogen lost via the faeces was found to be relatively constant (about 4.5 gN/day), this was reduced when the amount of water lost via this route declined below 500 g/day. In sheep fed the MN diet, restricting water intake reduced CP intake. In sheep fed the LN diet, the same restriction resulted in a reduced nitrogen excretion via the faeces, rather than a reduced CP intake. Nitrogen loss via the urine was not affected by restricting water intake in the sheep fed the LN diet, in contrast to the MN-fed group, in which CP intake was drastically reduced, while not materially affecting the amount of nitrogen excreted in the urine. Restricting water intake halved urine production in both groups of sheep. The amount of water lost via the faeces was only halved in the sheep fed the LN diet, while those fed the MN diet showed no change. Neither diet nor water treatment affected the glomerular filtration rate (GFR). When sheep were fed a diet containing adequate CP, restricting water intake severely reduced the amount of nitrogen retained. However, when sheep were fed a diet low in CP, the same restriction appeared to increase the amount of nitrogen retained. The effect

of infrequent watering may therefore appear to ameliorate the often low-protein grazing associated with arid areas.

Keywords: Nitrogen balance, restricted water intake, water balance, sheep

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Introduction

As a result of recurrent drought in Southern Africa, ruminant livestock frequently have to manage with inadequate water supplies. It is common practice on many farms in arid areas to only water sheep every second or even third day. Early studies showed that sheep may withstand prolonged periods of restricted watering, i.e. every second day, without adversely affecting feed intake, or growth (Clark & Quin, 1949a; Clark & Quin, 1949b). When cows fed 11–12% crude protein (CP) were restricted to 60% of their *ad libitum* water intake, the water content of their urine and faeces declined to about 66% of normal, without affecting nitrogen balance (Balch *et al.*, 1953). Since the organic matter (OM) and CP digestibilities were unaffected, these authors concluded that there was no benefit to be gained from restricting water intake. Restricting the CP content of the diet fed to cattle in East Africa reduced the amount of nitrogen excreted in the urine as urea (Livingston *et al.*, 1962). Furthermore, restricting the water intake of these cows further reduced the loss of nitrogen. As a result of this and similar work by others (Asplund & Pfander, 1972; Thornton & Yates, 1968), it was suggested that the apparent reduction in nitrogen excretion may lead to an improved nitrogen retention. However, conflicting results, some positive (More & Sahni, 1981) and some negative (Singh *et al.*, 1976; Bohra & Ghosh, 1977), leave the matter unresolved.

It is our hypothesis that the nitrogen content of the diet is the factor determining the outcome of this research. Lack of response to water deprivation is often associated with diets containing above maintenance levels of CP, while trials carried out in Africa or India often used feeds with a CP content of less than 5%. It was therefore decided to investigate the interaction between the intakes of nitrogen and water under conditions of low protein concentration in the diet fed to sheep that are moderately adapted to arid conditions (South African Mutton Merino).

Methods

Animals and diets

A group of 16 SA Mutton Merino wethers with a mean body mass of 24.7 ± 1.9 kg was housed individually in metabolic crates. The sheep were randomly allocated to two groups. Group 1 were fed a low nitrogen diet (LN) based on milled oats (*Avena sativa*) hay, with a metabolizable energy (ME) content (estimated) of 9.53 MJ/kg and a measured crude protein (CP) content of 5.4%, to which was added molasses powder, limestone and salt to improve palatability and calcium content. The ration was balanced according to NRC standards (NRC, 1985), using a Lotus 123 spreadsheet. Group 2 were given a medium nitrogen diet (MN), made by supplementing the LN diet with urea to raise the CP content to 10.4%. The ME content of this diet therefore remained similar to the LN diet. Both groups were allowed *ad libitum* access to feed, and were given three weeks to adapt to their diets. During the last week of this period, *ad libitum* intake of water was determined for each sheep. During the first experimental period, four sheep in each group were restricted for seven days to half their previous *ad libitum* water intake. During the last four days of this period, intakes of

water and feed and outputs of faeces and urine were measured. Suitable subsamples (10% of the total) of feed, faeces and urine were taken and stored at -20°C for later analysis. On the last day of this period, blood samples were drawn from a *v. jugularis* at 08:00. After taking a small subsample for analysis of haematocrit, the plasma was separated by centrifugation, and stored at -20°C for later analysis.

Water treatments were then reversed, so that those sheep that were receiving water *ad libitum* were restricted, and those that had been restricted in their water intake now received water *ad libitum*. After allowing seven days for adaptation to the altered water regime, the experiment was repeated.

Analyses

Feed, faeces, urine

Random grab samples (n = 5) of feed were taken at weekly intervals during the trial, stored in sealed plastic bags and pooled before analysis. The pooled sample was analysed in triplicate for moisture, OM and CP according to the AOAC (1980) and acid-detergent and neutral-detergent fibre (ADF and NDF; Van Soest & Wine, 1967). Faecal samples were analysed for moisture, OM, CP, acid- and neutral-detergent fibre content (AOAC, 1980). The urea and creatinine concentrations in the urine were determined using commercially available kits (Boehringer Mannheim, Germany). Total nitrogen in the urine was determined according to the AOAC (1980).

Blood

Subsamples were immediately withdrawn from all blood samples for the determination of haematocrit values, following which the plasma fraction was separated by centrifugation. After measuring plasma sodium concentrations by means of an ion-specific electrode (Instrumentation Laboratory, System 501, Milano), the remainder of the plasma was stored at -20°C until the concentrations of urea and creatinine (Boehringer Mannheim, Germany) could be determined.

Calculations

Metabolic water was calculated from the relevant, apparently digestible fractions of the feed intake using the factors given by Schmidt-Nielsen (1964). Insensible water loss was taken as the difference between total intake (water drunk + feed moisture + metabolic water) and water lost via the faeces and urine.

Glomerular filtration rate (GFR) was estimated from the plasma clearance rate of endogenous creatinine (Bastl *et al.*, 1985), on the assumption that in the sheep, creatinine is neither reabsorbed nor excreted by the nephron tubule (Nawaz & Shah, 1984).

$$GFR = \frac{[creatinine]_{ur} \cdot Vol_{ur}}{[creatinine]_{pl}} \quad (1)$$

where $[creatinine]_{ur}$ = concentration of creatinine in the urine (µmol/l), Vol_{ur} = urine volume (*l*/d) and $[creatinine]_{pl}$ = concentration of creatinine in the plasma (µmol/l)

The plasma clearance of urea was calculated as follows (Bastl *et al.*, 1985):

$$Plasma\ clearance\ of\ urea\ (l/d) = \frac{[urea]_{ur} \cdot Vol_{ur}}{[urea]_{pl}} \quad (2)$$

where $[urea]_{ur}$ = concentration of urea in the urine (µmol/*l*), Vol_{ur} = urine volume (l/d) and $[urea]_{pl}$ = concentration of urea in the plasma (µmol/l).

The fractional excretion of urea, i.e. that fraction of filtered urea which is excreted in the urine (expressed as a percentage), was calculated as follows (Bastl *et al.*, 1985):

$$Fractional\ excretion\ of\ urea = \frac{[urea]_{ur} \,.\, Vol_{ur} \,.\, 100}{GFR \,.\, [urea]_{pl}} \quad (3)$$

Statistics

Data was subjected to an analysis of variance for a two-period, two-treatment cross-over design, according to the methods outlined by Grizzle (1965; 1974) and Hills & Armitage (1979), using the SAS generalized linear model (SAS, 1992).

Results

The composition of the diets fed to the sheep is shown in Table 1. The addition of urea raised the N content of the LN diet (largely oaten hay) from 5.4% to 10.2% in the MN diet, without materially affecting either the ADF or NDF fractions. The diets were essentially identical, except for the addition of the urea.

Growth rates during the experimental period for sheep fed either the LN or the MN diets are shown in Figure 1. Growth rates appeared to increase sharply when either of the two groups were changed from the half *ad libitum* water regime to *ad libitum* access. This increase probably represented an increase in body water reserves. The MN group of sheep gained body mass during the experimental period, while the sheep fed the LN diet maintained a constant body mass, which had a tendency to decline over the entire experimental period (statistically insignificant).

The *ad libitum* intake of water, about 2.9 litres per day per sheep, was not affected by the nitrogen level of the two diets (see Table 2a). Halving the intake of drinking water halved the output of urine in both groups of sheep. The group fed the LN diet (5.4% CP) further reduced water loss when water intake was halved by extracting more water from the digesta, as shown by the reduced water content of the faeces (705 ± 223 and 331 ± 53 g/day on *ad libitum* and half *ad libitum* water intake, respectively). This response was not found in the group fed the MN diet. Both groups of sheep appeared to be able to reduce insensible loss of water by more than 60% when water intake was restricted.

Although the amount of CP taken in by the sheep fed the MN diet was about twice that of the LN sheep (15.46 ± 1.06 vs 7.14 ± 1.10 gN/day), the amount of OM consumed did not differ significantly. In both groups, restricting water intake significantly ($p < 0.05$) decreased intake of both OM and CP, although the effect was more pronounced in the MN group (30% vs 15%). While the OM content of the faeces was reduced by restricting water intake in those sheep fed the LN diet, there was surprisingly little effect in the MN group. Faecal nitrogen followed the same pattern, showing a

Table 1 Mean (±SD) proximate analysis of the low (5.4% = LN) or medium (10.3% = MN) crude protein diet

Diet	Component	Mean (%)	SD
LN	Ash	6.6	0.63
	OM	93.4	0.63
	N	10.2	0.13
	ADF	34.2	2.35
	NDF	58.1	2.47
MN	Ash	9.8	1.78
	OM	90.2	1.78
	N	5.4	0.07
	ADF	34.3	1.44
	NDF	54.7	2.15

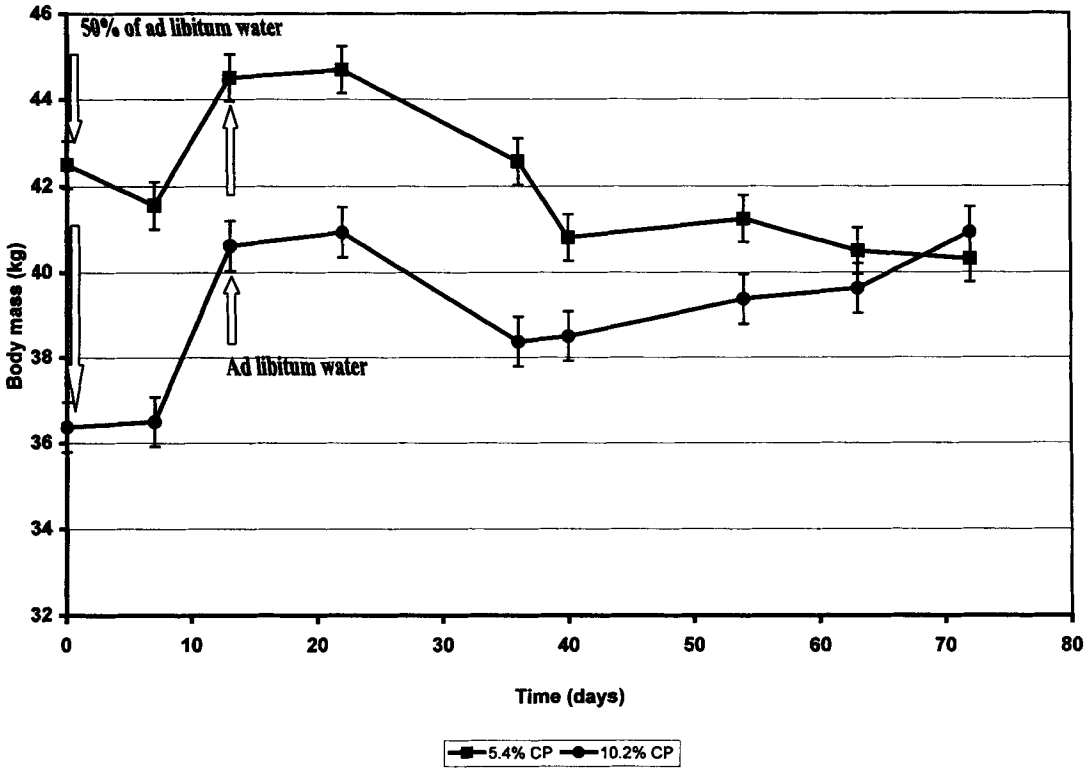

Figure 1 Change in body mass of 2 groups of sheep (n = 2 × 8), one group fed the LN diet (5.4% CP, solid squares) and the other fed the MN diet (10.2% CP, solid circles) during the experimental period. After determining *ad libitum* intake of water and feed, the water intake of each group was initially restricted (half previous intake) for 10–14 days, after which free access to water was once again allowed

remarkably constant outflow (4.05 to 5.14 gN/day). By contrast, the LN group of sheep, given half their normal intake of water, appeared to reduce their outflow of nitrogen in their faeces (2.85 gN/day).

The amount of N lost via the urine was largely determined by the amount taken in from the diet, and was not affected by restricting the water intake. On the other hand, the group of sheep fed the LN diet removed more N from the digesta when their water intake was halved. These factors combined to reduce N-retention in the MN group when water was restricted (from 7.3 ± 1.4 to 1.5 ± 1.0 gN/day), largely as a result of the decreased intake. However, the N-retention of the LN group did not decrease under the same circumstances, despite a diminished intake, mainly due to an increased uptake from the digestive tract. Indeed, the amount retained appeared to show a slight positive trend, albeit statistically insignificant (0.18 ± 1.41 to 1.47 ± 1.15 gN/day).

The ANOVA of the N-retention data suggested that the *Nitrogen* × *Water* interaction was highly significant (Table 3a), despite the apparent lack of significant differences between the respective nitrogen retention values (Table 3b). However, closer examination of this data, when plotted as individual data points, then did show some positive correlation between water deprivation and nitrogen retention. For example, when the amount of N taken in is correlated against the amount of

Table 2a Mean (±SD) water balance (g/day) in sheep fed either a low (5.4% = LN) or a medium (10.3% = MN) crude protein diet, and offered water *ad libitum* or half *ad libitum*

Diet	Water intake			Water output				
	Drinking	Feed	Metabolic	Total	Faeces	Urine	Total	Insensible
LN	2828[a]	57[a]	255[a]	3140[a]	705[a]	948[a]	1653[a]	1487[a]
	(524)	(9)	(73)	(543)	(223)	(324)	(562)	(506)
	1463b	57[a]	285[a]	1805[b]	331[b]	505[b]	836[b]	969[b]
	(330)	(9)	(86)	(451)	(53)	(60)	(109)	(126)
MN	2937a	61[a]	383[b]	3381[a]	562[a]	773[a]	1335[a]	2046[a]
	(372)	(7)	(39)	(428)	(228)	(150)	(401)	(614)
	1406b	60[a]	210[a]	1676[b]	586[a]	366[b]	952[b]	724[b]
	(301)	(9)	(54)	(369)	(219)	(98)	(314)	(239)

[a,b,c,d]Values in the same column with different superscripts differ significantly at the $p < 0.05$ level

Table 2b Mean (±SD) intake of OM and N, as well as OM and N outputs via faeces and urine (all expressed in g/day) in sheep fed a low (5.4% = LN) or a medium (10.3% = MN) crude protein diet, with *ad libitum* or half *ad libitum* access to water. N retention is also given for these groups

Diet	Water access	Feed intake		Faeces output		Urine output	% Digestibility		Nitrogen retention	
		OM	N	OM	N	N	OM	N	g/day	%
LN	ad lib	820[a]	7.14[a]	370[a]	5.14[a]	1.81[a]	54[a]	27[a]	0.18[a]	1.6[a]
		(128)	(1.10)	(98)	(1.52)	(0.40)	(11)	(21)	(1.41)	(20.7)
	half ad lib	697[b]	6.06[b]	200[b]	2.85[b]	1.74[a]	71[b]	53[a]	1.47[a]	21.5[a]
		(183)	(1.58)	(52)	(0.78)	(0.35)	(6)	(10)	(1.15)	(11.3)
MN	ad lib	936[a]	15.46[c]	299[a]	4.05[a]	6.30[b]	68[b]	74[b]	7.34[b]	47.4[b]
		(64)	(1.06)	(55)	(0.61)	(0.94)	(5)	(4)	(1.42)	(8.2)
	half ad lib	665[b]	10.98[d]	316[a]	4.40[a]	5.07[c]	52[a]	9.4[a]	1.50[a]	13.4
		(92)	(1.50)	(56)	(0.85)	(0.88)	(9)	(8)	(1.01)	(8.4)

[a,b,c,d]Values in the same column with different superscripts differ significantly at the $p < 0.05$ level

N excreted in the faeces of individual sheep, then the differences between the two groups of sheep may be clearly seen. The amount of N lost via the faeces in the group fed the MN diet remained

Table 3a Anova table describing the errors associated with factors influencing the retention of nitrogen by sheep fed either a low (7% = LN) or a medium (11% = MN) crude protein diet, with *ad libitum* or half *ad libitum* access to water

Source of variation		df	MS	SL
Between subjects				
	Nitrogen	1	4054.5012	0.0000
	Group (or carry-over)	1	0.0162	0.9845
	Nitrogen by Group	1	47.2392	0.3038
	Sheep (in Nitrogen and Group)	12	40.9303	
Within subjects				
	Period	1	3.4980	
	Nitrogen × Period	1	16.6176	
	Water	1	1615.6770	
	Nitrogen × Water	1	3982.3350	0.0001
	Residual	1	120.0286	

Table 3b Analysis of the differences between the amounts of nitrogen retained in sheep fed either a low (5.4% = LN) or a medium (10.3% = MN) crude protein diet, with *ad libitum* or half *ad libitum* access to water

Diet	Nitrogen retained when on			
	Half ad lib water	Ad lib water	Difference (Δ)	SL (H_0:Δ=0)
LN	1.475	0.179	1.296 ± 0.877	0.1650
MN	1.507	7.35	-36.52 ± 0.877	0.0000

constant when water was restricted, despite the lower intake. However, restricting water intake in the LN group did not shift the intake but did appear to decrease the amount of N lost via the faeces, i.e. increased its uptake from the digestive tract (See Figure 2).

When the amount of water in the faeces is correlated against the amount of N in the faeces (Figure 3), it becomes clear that the reduction in water content of the faeces in response to water restriction is reflected by a similar reduction in the N content of the faeces. This would seem to suggest that the increased absorption of water was associated with the increased uptake of N from the tract.

The loss of CP via the urine displayed a totally different pattern to that lost via the faeces (Figure 4). The graph shows that this amount was clearly affected by the CP intake of each individual

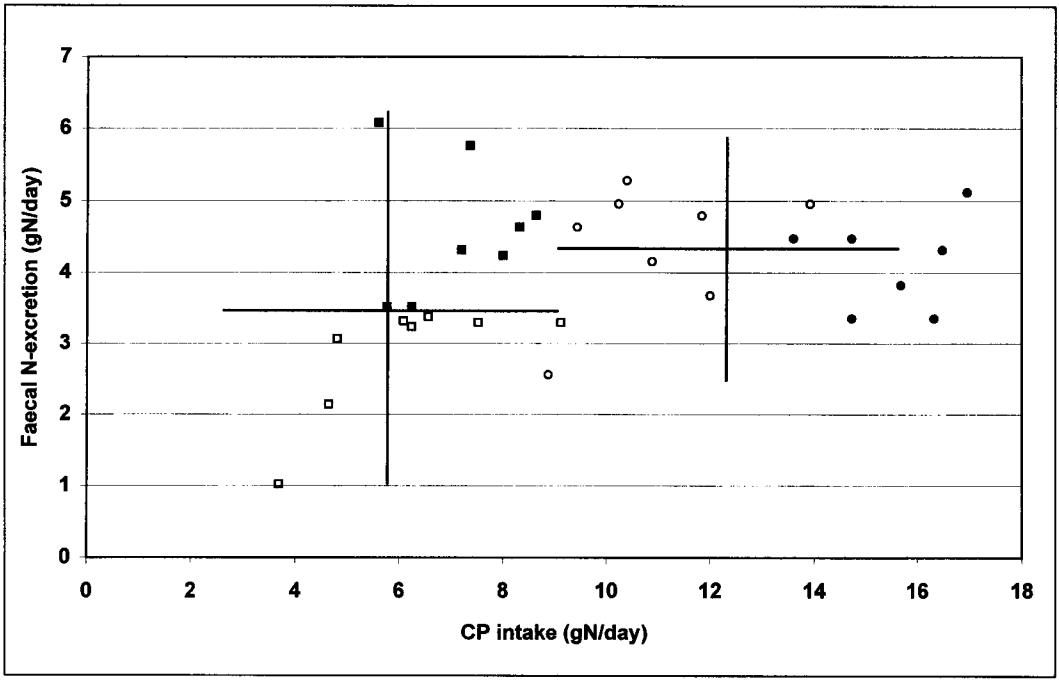

Figure 2 Relationship between the CP intake and the amount of nitrogen excreted via the faeces from sheep fed either the LN (squares) or MN (circles) diet, and with free (solid symbols) or restricted (open symbols) access to water

sheep, although this was not apparent from the summary data of the means in Table 2b. The relationship between the urea concentration in the urine and CP intake is well known and is clearly shown in Figure 4. The amount lost via the urine increased linearly with the amount N ingested at levels of intake above 1 gN/day. Below this value, the amount of N excreted via the urine remained constant, contributing to the negative N-balance at low N intakes. However, restricting water intake in the MN group appeared to decrease not only the intake of N, but also to increase the relative amount lost via the urine.

Kidney function was examined in detail, in order to quantify the loss of nitrogen via this route. The data are presented in Table 4. While glomerular filtration rate remained remarkably constant, unaffected by N intake or water availability, the volume of urine halved when water intake was restricted (see also Table 2a). The concentration of urea in plasma reflected the intake of N, and increased by between 50% and 80% when water intake was halved. Sheep fed the MN diet excreted about 4–5 times more urea than did those on the LN diet, both in terms of concentration as well as amount. Restricting water intake appeared to slightly increase the urea concentration in both the plasma and the urine, without changing the amount excreted.

Discussion

The proximate analysis of the two diets used in this experiment clearly shows the difference between them in terms of their nitrogen content. The intake of either diet was sufficient to ensure growth of the sheep during the experimental period, with no obvious effects of poor palatability, or

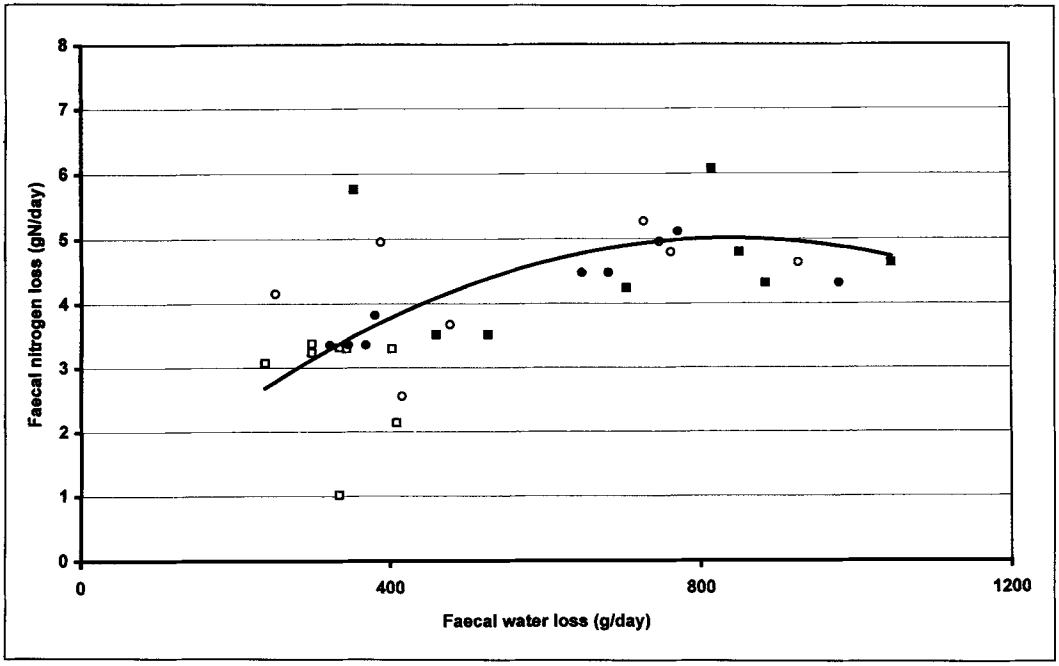

Figure 3 Relationship between the amount of water and nitrogen excreted via the faeces from skeep fed either the LN (squares) or MN (circles) diet, and with free (solid symbols) or restricted (open symbols) access to water

of insufficient nitrogen. In fact, addition of urea to the LN diet was associated with an apparent increase rather than a decrease in intake (Tolkamp *et al.*, 1998). The temporary hiatus caused by the imposition of the water restriction period was rapidly made up when water was again freely offered, leading to a return to the normal growth curve of each group. From this observation, it is likely that the 1 kg differences observed during these restriction phases were due to water loss from the digestive tract, rather than actual body mass changes.

While the primary goal of this investigation was not to examine water balance *per se*, the data suggest that the movement of nitrogen closely paralleled that of water retention in the digestive tract, rather than that of the kidney. For this reason, it is necessary to discuss the water balance of these sheep before examining their ability to retain nitrogen.

Both groups halved water loss via the kidneys when their water intake was halved. However, it was only the LN group that showed a similar response via the digestive tract. This observation strongly suggests that the mechanism responsible for this additional extraction of water from the digestive tract is not simply due to the dehydration, i.e. a simple vasopressin and/or aldosterone response, but is linked in some way to the level of nitrogen in the diet. It is not clear from our data what this connection may be, but it is possible to speculate. Urea is not only excreted by the kidney, but contributes at least half of the osmotic gradient in the medulla that is responsible for the concentration of the filtrate (Gowrishankar *et al.*, 1998). While the recycling of urea in this region is complex (De Rouffignac, 1990), it is largely controlled by the load filtered (i.e. proportional to the plasma concentration of urea) and the effect of vasopressin (increases the permeability of the

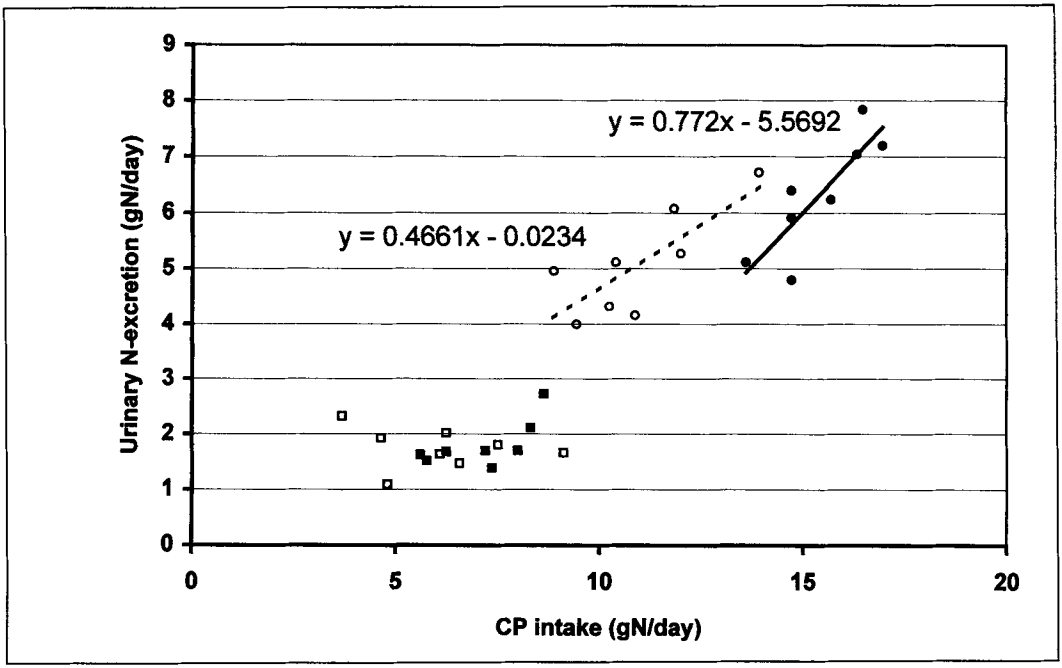

Figure 4 Relationship between CP intake and the amount of nitrogen excreted via the urine from sheep fed either LN (squares) or MN (circles) diet, and with free (solid symbols) or restricted (open symbols) access to water

medullary collecting tubule to urea) (Knepper & Nielsen, 1993; Nielsen & Knepper, 1993; Trinh & Bankir, 1998). At low levels of nitrogen intake, the plasma urea concentrations will decline to below the normal range (2.5–7.0 mMol), and thus adversely affect the ability of the kidney to concentrate the urine. Plasma urea concentrations in the LN group of sheep declined to 1.7 mMol, thus contributing to the slightly higher output of urine by this group, irrespective of water regime. The actual effect of the vasopressin response to the water restriction regime was not measured, but may be inferred from the approximately 50% reduction in urine volumes noted in both groups. On the other hand, vasopressin is not known to play a major role in the increased uptake of water by the digestive tract. The hormone that contributes significantly to this aspect is aldosterone, which acts to retain sodium, and therefore indirectly water. The fact that no increased uptake of water from the digestive tract was found in the MN group, suggests that it is aldosterone rather than vasopressin that is responsible for the increase in water uptake from the digestive tract.

In both groups, large differences in the amount lost via the insensible routes were found, pointing to the importance of these mechanisms. In all cases, the amount of water lost via the insensible route was as much as, if not more than, all other routes put together, and was quantitatively as important as the kidney. Similar data have been reported for sheep and goats, in which the loss via respiratory routes was more than via sweating (Robertshaw, 1968). However, since Robertshaw (1968) has found that the respiratory rate of sheep does not respond to dehydration, it is likely that most of the accommodation found in the present experiment (about 500 and 1200 ml water retained via this route in the LN and MN groups respectively) is largely due to variation in the sweating rate.

Table 4 Mean (±SD) values for glomerular filtration rate (GFR), specific GFR, urea concentrations in plasma ($[Urea]_{pl}$) and in urine (($[Urea]_{ur}$), urine volume (Vol_{ur}), sodium concentration in plasma ($[Na]_{pl}$), daily urea excretion via urine ($Ur_{ext\ ur}$), plasma clearance of urea (Pl.cl.urea) and fractional excretion of urea (Fr.ex. urea) in sheep fed either the low nitrogen or medium nitrogen diet and given *ad libitum* or half *ad libitum* access to water intake

	Diet			
	Low Nitrogen		Medium Nitrogen	
	ad lib water	half ad lib water	ad lib water	half ad lib water
GFR	35.80[a]	35.43[a]	38.7[a]	36.13[a]
l/d	(13.41)	(7.19)	(12.62)	(12.69)
Specific GFR	1.59[a]	1.56[a]	1.68[a]	1.57[a]
l/kg per d	(0.59)	(0.28)	(0.48)	(0.58)
$[Urea]_{pl}$	1.7[a]	3.1[b]	5.0[c]	7.2[d]
Mmol/l	(0.4)	(0.9)	(0.9)	(1.0)
$[Urea]_{ur}$	36.3[a]	48[a]	222[b]	324[c]
Mmol/l	(7)	(26)	(68)	(83)
Vol_{ur}	0.95[a]	0.5[b]	0.77[a]	0.37[b]
l/d	(0.33)	(0.06)	(0.15)	(0.10)
$[Na]_{pl}$	140[a]	148[b]	140[a]	146[b]
Mmol/l	(7)	(5)	(4)	(7)
$Ur_{ext\ ur}$	32[a]	28[a]	146[b]	105[b]
Mmol/d	(9)	(14)	(33)	(35)
Pl.cl.urea	19.43[a]	9.60[b]	30.79[c]	14.94[ab]
l/d	(6.02)	(6.03)	(9.11)	(9.11)
Fr.ex. urea	47.6[a]	27.1[a]	65.7[c]	41.3[a]
%	(15.8)	(14.5)	(4.6)	(4.6)

[a,b,c,d]Values in the same row with different superscripts differ significantly at the $P < 0.05$ level

This route may lead to a maximum loss of about 900 ml/day in these sheep, which corresponds well with the recorded data. In addition, Maloiy & Taylor (1971) found that dehydration did not change respiration rate in either sheep or goats, which they interpreted as evidence that these animals did

not have the physiological mechanisms for adapting insensible water loss to hot, arid conditions. While it appears likely that sweating is indeed responsible for most of the insensible loss in the present experiment, it is likely that respiration must have contributed towards the control of this water loss.

Halving the water intake reduced the feed intake of both groups, albeit by more in the MN group (-30% vs -15% in the MN vs LN groups, respectively). As a result, the nitrogen intake of the LN group suffered less than that of the MN group (7.14 ± 1.10 vs 15.46 ± 1.06 gN/day for the LN and MN groups, respectively). Both nitrogen and organic matter digestibilities were increased in the LN group when the water intake was halved. The opposite effect was observed in the MN group when subjected to the same treatment. Taken together with the water retention data, these results suggest that the LN group, when water restricted, controlled water and nitrogen retention by increasing digesta retention time, while the MN group remained unaffected.

While the analysis of variance suggested that the retention of the LN group as a whole was not significantly improved by reducing the water intake (Table 3a and b), the data from individual sheep provided more detail regarding the movement of water and nitrogen. When the data from the MN group is examined on an individual basis, it is clear that their response to water restriction followed the expected pattern, i.e. DM intake (and therefore CP intake) was reduced, without reducing the amount lost via the faeces (Singh *et al.*, 1976). However, when the LN group was subjected to the same water restriction, their intake was not as drastically reduced, whereas the amount of nitrogen lost via the faeces was halved (Figure 2). When taken in conjunction with the data in Figure 3, which shows that the amount of nitrogen lost via the faeces declines sharply when faecal water loss is less than about 500 ml/day, this data suggests that the observed improvement in nitrogen retention in the LN group is intimately linked to the retention of water in the digestive tract. Our data do not allow us to determine where in the digestive tract this mechanism is operating. However, it is tempting to speculate that the amount of urea that entered the rumen (indirectly via saliva and directly through the rumen wall, Egan *et al.*, 1986), increased when water intake was restricted, in parallel to the plasma concentration of urea that increased under these circumstances. This would provide an increased supply of nitrogen for microbial protein synthesis. Furthermore, if this was coupled to a decrease in the rate of passage of digesta, this would have led to an improvement in OM digestion (Asplund & Pfander, 1972), thereby providing the carbon skeletons required for this to occur. In fact, the LN group appeared to show considerable improvements in both OM and CP digestibilities (Table 2b), thereby further contributing to protein digestion and amino acid uptake.

The role of the kidney appeared to lie in restricting the loss of urea while ensuring that the optimum amount of water was retained in sheep with restricted access to water. In order to do this, it is necessary that the function of the kidney be critically controlled. Factors that may have influenced this control were determined, and their significance examined.

Glomerular filtration rate (GFR) was unaffected by the protein content of the diet. Although it is generally accepted that GFR is an important determinant of the concentrations of non-protein-nitrogen substances in the plasma (English *et al.*, 1980), in one trial it was suggested that plasma urea concentrations, in fact, feed back to influence GFR (Choshniak & Arnon, 1985). However, water restriction in the current trial had no effect on GFR. Hydrostatic pressure in the glomerulus, and therefore GFR, is kept constant when mean arterial blood pressures lie between 75 and 180 mm Hg (Navar, 1978). In this experiment it is unlikely that systemic arterial pressure fell below this minimum value. Even when sheep are completely denied access to water for two days, GFR remains unaffected (Meintjes, R.A. 1999). It has been suggested that antidiuretic hormone (ADH) may increase GFR in sheep (Yesberg *et al.*, 1973). Although the mechanism whereby this is achieved is

not clear (Davis & Schermann, 1971; Hassid *et al.*, 1986), it is possible that ADH maintains normal GFR values in the dehydrated sheep.

The higher plasma urea concentrations obtained in animals with restricted access to water, regardless of diet, was not due to haemoconcentration, as plasma sodium concentrations increased approximately 5% compared to a 40–80% increase in urea concentration. Because GFR values were similar, differences in the amount of filtered urea (GFR. $[urea]_{pl}$) could only be attributed to differences in the plasma concentrations of urea in sheep exposed to different treatments. It is proposed that the elevated plasma urea concentrations obtained in the water-restricted sheep were due to a change in the tubular re-absorption of urea in sheep. In this experiment, only 27% and 41% of filtered urea was excreted in the LN and MN groups respectively when their water intake was restricted, compared to 48% and 66% in the corresponding groups given free access to water. ADH promotes urea re-absorption, along with water re-absorption, from the collecting duct (Jamison, 1983). It is therefore probable that the differences in the fractional excretion of urea may be explained by the expected higher concentrations of ADH in sheep when water intake is restricted. When animals on similar water intakes but on different diets were compared, the fractional excretion of sodium in sheep on the higher protein intake significantly exceeded that of sheep on the lower protein intake. The mechanism whereby dietary protein and plasma urea concentrations affect tubular re-absorption of urea remains unknown.

Factors, other than plasma ADH concentrations, known to affect urea re-absorption across the renal tubular cells include the diffusion gradient of urea from filtrate to plasma and the rate of filtrate flow (Lote, 1992). The latter is a function of GFR. In general, between 50% and 70% of filtered urea is excreted at normal GFR, and this may reduce to 30% to 50% when urine flow rates are reduced (Kaplan & Kohn, 1992; Livingston *et al.*, 1962; Chasis & Smith, 1938). In the present study, the plasma clearance rates of urea varied in direct proportion to the fractional excretion of urea. This is not surprising, as the only difference in calculating these two parameters lies in the inclusion of GFR in the divisor of plasma clearance rate, and the GFR did not differ significantly between sheep on different treatments.

These mechanisms all serve to maintain a basal rate of urea excretion when nitrogen intake levels are below the minimum required for nitrogen balance (about 8 gN/day), and allow any excess to requirement to be excreted when above this amount (see Figure 4; and Egan *et al.*, 1986). It is when the nitrogen intake is below maintenance level, and when the kidney is obliged to excrete a minimum amount of urea to remain functional that the water and nitrogen retention mechanism in the digestive tract appears to be activated. While it may be possible to explain some of this improvement in nitrogen retention in terms of digesta flow (retention time) when water intake is restricted, it does not fully explain the different responses of the sheep fed the medium- and low-nitrogen diets.

The results do not contradict the hypothesis that restricting water intake improves nitrogen retention when the intake of CP is at or below the minimum required for nitrogen balance. The role of the kidney has been shown to be relatively less important than that of the gastrointestinal tract, although the exact mechanism/s responsible for effecting nitrogen retention remain unclear.

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