THE EFFECTS OF 24H WATER DEPRIVATION WHEN ASSOCIATED WITH SOME ASPECTS OF TRANSPORTATION ON THE BEHAVIOUR AND BLOOD CHEMISTRY OF SHEEP

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Abstract *Animal Welfare 1999, **8:** 229-241*

When sheep are transported they are potentially exposed to a number of factors, including water and feed deprivation, low space allowance and elevated environmental temperature, that are not related to vehicle motion but could result in animal welfare problems, either on their own or in combination. In a 2x2 factorial experiment, groups of sheep (n = 6) were moved from individual pens where they had access to hay and water to environmental chambers kept at either 14°C or 21°C. Within each chamber, half the sheep had access to water but they were all kept at a space allowance of 0·41m^2 sheep^{-1} without feed. After 24h they were returned to their individual pens and offered hay and water. Behaviour and a range of biochemical measurements of dehydration and feed restriction were recorded before, during and after the treatment period. During the treatment period there was no evidence of dehydration, and sheep with access to water drank less than they did before the treatment. The plasma concentration of free fatty acids increased during fasting and, post-treatment, the intake of hay was greater than before treatment. The rapid post-treatment intake of dry feed was associated with some evidence of dehydration, as indicated by increased plasma osmolality and plasma vasopressin concentration. This suggests that following provision and consumption of large quantities of feed after a period without access to feed and water during transportation, sheep must be allowed sufficient time to drink before a subsequent journey is undertaken.

Keywords: *animal welfare, behaviour, blood biochemistry, dehydration, sheep, transport*

Introduction

The behavioural and/or physiological responses of sheep during road journeys have recently been reported by Knowles *et al* (1993; 1994; 1995; 1996), Bradshaw *et al* (1996), Broom *et al* (1996), Cockram *et al* (1996; 1997) and Parrott *et al* (1998). The framework provided by the Farm Animal Welfare Council's 'five freedoms' (FAWC 1994) can be used to evaluate the welfare of sheep and provide a basis for the interpretation of their behavioural and physiological responses during and after transport (Cockram *et al* 1996). When sheep are transported, they are potentially exposed to a number of factors not related to vehicle motion which could result in animal welfare problems either on their own or in combination. These factors include hunger and thirst (due to feed and water restriction) and thermal and physical discomfort (due to inadequate ventilation and space). The effect of water restriction is likely to be greater at those environmental temperatures at which sheep lose heat by latent heat of vaporization. Parrott *et al* (1996) showed that sheep can be kept for 48h without feed and water at a temperature of 35°C without increases in either plasma osmolality or plasma cortisol concentration. However, it is possible that the sheep could have made considerable physiological and behavioural adjustments in response to water loss during the 48h period, and possibly have experienced thirst and discomfort, while remaining able to maintain a relatively constant plasma osmolality. Although Parrott *et al* (1996) noted that some sheep were observed to pant during the period of water deprivation at high temperature, the behavioural responses of the sheep were not recorded in their study. The low space allowances frequently associated with transport might impair the ability of sheep to lose heat and any effects of water restriction might, therefore, be greater in sheep transported at a lower space allowance than those found in non-transported sheep kept at a higher space allowance. Feed restriction for 24h can result in increased plasma concentrations of metabolites in sheep (Warriss *et al* 1989) and the immediate response of sheep after a journey of 24h is to eat large amounts of dry feed (Cockram *et al* 1997). This rapid feeding can result in temporary dehydration due to salivation and movement of water from the circulation into the rumen due to increased ruminal osmolality (Ternouth 1968); it is possible that the effects of this dehydration could be increased by previous water restriction.

This paper examines the effects of 24h of water deprivation on the behaviour and physiology of sheep when they were confined without feed at a reduced space allowance to simulate some of the conditions associated with commercial transportation. The effects of temperature and the provision of water during the treatment period on behaviour and a range of biochemical measurements of dehydration and feed restriction were investigated, both during the treatment period (when no food was available) and the post-treatment period (when food and water were available).

Materials and methods

Animals and management

Our 24 subjects were 13-month-old Scottish Blackface ewes with a mean (± SEM) liveweight of 30 ± 0.2 kg and a fleece length of about 90mm. They had been overwintered on grass with hay and sugar beet pulp supplement. One week before the start of the treatment, the sheep were individually housed in pens with slatted floors at a space allowance of 2·81m^2 $sheep^{-1}$. They were provided with 2kg^{-1} day chopped hay (915g dry matter [DM] kg^{-1}; acid-detergent fibre 419g kg^{-1} DM, crude protein 82g kg^{-1} DM, ash 79g kg^{-1} DM and organic matter 921g kg^{-1} DM) at 0900h, and water *ad libitum*.

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Procedures

All procedures were conducted under the appropriate Home Office licences. At 0900h on the day of treatment, 12 sheep were moved into an environmental chamber at a mean (± SEM) thermoneutral temperature of 14 ± 0.09 °C and 12 sheep were moved into a similar chamber at an elevated temperature of 21 ± 0.17 °C for 24h. Within each chamber, the sheep were penned without feed in two groups of six on wood shavings at a space allowance of 0·41m^2 $sheep^{-1}$, with each group visually isolated. The space allowance used was within the range of space allowances included in the European Council Directive concerning the protection of animals during transport (European Council 1995) and within that used by hauliers to transport sheep in Scotland (Jarvis & Cockram 1994). One group in each chamber had access to water provided in a water trough, and the other group had an empty covered trough of the same surface area. After 24h, the sheep were returned to their home pens with access to hay and water.

Blood sampling

The sheep were prepared with jugular cannulae 2 days before the start of the treatment (Cockram *et al* 1996). Blood (3ml samples) was collected into Sarstedt monovette tubes (Sarstedt Ltd, Leicester, UK) containing lithium heparin at 3h intervals between 0900h and 2100h on the day before the start of treatment; at 2h and 1h before the start of treatment; at 2h intervals during the treatment; and at 3h, 6h, 9h, 12 h, 18h, 24 h, 30h and 36h after the end of the treatment. From each heparinized blood sample, 0.5ml (subsequently used for glucose analysis) was immediately extracted and mixed in a tube containing fluoride. At 2h before the start of treatment, 5ml of additional blood (subsequently used for vasopressin analysis) was collected after each heparinized sample, into Sarstedt monovette tubes containing 500µl of 225mmol l^{-1} EDTA, 0·005mol l^{-1} 1,10 phenanthroline and 500 kallikrein inhibitor (KI) units of aprotinin (Sigma, Dorset, UK). The tubes were stored in iced water during blood sampling and were then centrifuged at 5°C and the plasma removed and stored at -20°C.

Behavioural observations

Direct observations of behaviour were made by scan sampling the sheep at 10min intervals between 0900h and 2100h on the day before the start of the treatment and for 12h immediately after the treatment. The behaviour was recorded on a Psion Organiser LZ64, and analysed using Observer©, version 2, behavioural observational software (Noldus 1990; Noldus & Potting 1990). The following behaviours were recorded: standing (stationary posture); moving (upright posture involving a change in location with one or more feet off the ground); lying (recumbent posture with the body in contact with the floor and the legs flexed either underneath the body or alongside the body); eating (ingestion of food followed by jaw movements and swallowing); ruminating (regurgitation, jaw movements and swallowing not immediately preceded by eating); drinking (muzzle immersed in water for more than 5s); panting (rapid breathing with open mouth); oral (any other oral activity); and idling (no apparent behaviour). The proportion of scans spent in each of the above behaviours was calculated for each 1h period of the observations. During the treatment period, the behaviour was recorded using time-lapse video equipment and subsequently analysed as described above for direct observations. In addition, the frequency of drinking was recorded continuously during the 24h treatment period.

Other measurements

Rectal temperatures were recorded at 1500h on the day before the start of treatment and at 1500h during the treatment period, using a clinical thermometer. The hay intake per sheep (between 0900h and 0900h) for the 24h before treatment and for the first 24h post-treatment was recorded as the difference between the weight of hay offered and the weight of hay remaining in the rack. The water intake per group during the treatment period and the water intake per sheep (between 0900h and 0900h) for the 24h before treatment and for the first 24h post-treatment were recorded as the difference between the volume of water offered and the volume of water remaining in the container. The air temperatures in the environmental chambers and in the sheep pens before and after treatment were recorded at 5min intervals using Tinytalk Data Loggers (Orion Components Ltd, Chichester, UK). The mean (± SEM) temperatures in the sheep pens during the 12h before and after the treatment period were 11 ± 0.1 °C and 9 ± 0.1 °C, respectively.

Laboratory analyses

Packed cell volume was measured and the plasma samples analysed for osmolality and plasma concentrations of cortisol, vasopressin, β-hydroxybutyrate, sodium, potassium and chloride using the methods described by Cockram *et al* (1996). Total plasma protein concentration was measured by the biuret method (Gornall *et al* 1949). The plasma concentrations of free fatty acids (Randox Laboratories Kit FA/115S; Randox Laboratories, Crumlin, Co Antrim, UK) and glucose (Randox Laboratories Kit GL 586), were measured on a Bayer Diagnostics RA-2000 random access chemistry analyser (Bayer Diagnostics, Basingstoke, UK) at 37°C.

Statistical analysis

The effects of temperature, water (presence of water) and time (for biochemical data = blood sampling time; for behavioural data = 1h observation periods) on each variable were analysed using a repeated measures 2x2 factor analysis of variance (Laird & Ware 1982) and the mixed procedure within SAS, version 6 (Statistical Analysis Systems Institute Inc, Cary, North Carolina, USA). Where there were significant interactions between temperature, presence of water and time, the differences between least square means were examined by comparing both within and between pre-treatment, treatment and post-treatment periods. No statistical analysis of the behaviour of the sheep during the treatment period is reported as an individual's behaviour within a group may not have been independent of the other group members. However, post-treatment results from individually penned sheep were regarded as independent and subjected to statistical analysis.

Results

Hay and water intake

Mean (± SEM) hay intake post-treatment (1·3 ± 0.3 kg) was greater than that pre-treatment (1·2 ± 0.03 kg), $P < 0{\cdot}05$. The total water intake during the treatment period was 3l for the group kept at 14°C and 4l for the group kept at 21°C. Mean (± SEM) water intake post-treatment (3·4 ± 0·12 l) was greater than that pre-treatment (2·4 ± 0·17 l), $P < 0{\cdot}001$. However, there was no effect of temperature or provision of water during the treatment period on either hay or water intake post-treatment.

Rectal temperature

The mean (± SEM) rectal temperature during the treatment (39·2 ± 0.06 °C) was significantly lower than that before treatment (39·6 ± 0.07 °C), $P < 0{\cdot}0001$.

Behaviour

During the first 12h of the treatment period, there was no apparent effect of temperature on drinking (at 14°C, 16 drinking events per group; at 21°C, 18 drinking events per group). During the second 12h of the treatment period, there were five drinking events in sheep kept at 21°C, but in those kept at 14°C there was only one drinking event. No panting was observed during the treatment period. During the first 12h of the treatment period, sheep without access to water were observed lying down for 10 per cent (mean value) of the scans, but among those with access to water the figure was 38 per cent (Figure 1). The amount of

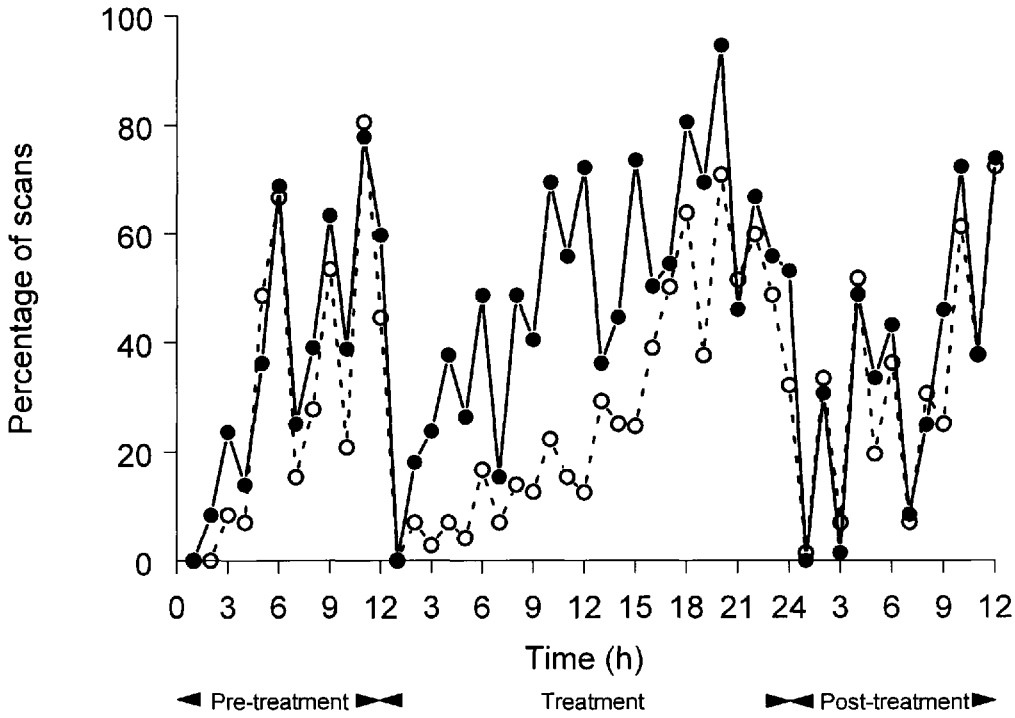

Figure 1 **Effect of water provision during 24h without feed (treatment) on the mean percentage of scans during each 1h period in which sheep were observed lying down: with access to water (—●—); without access to water (- - ○ - -).**

lying increased over the second 12h treatment period, with sheep with access to water continuing to spend more time lying (observed in 59% of scans) than those without access to water (44% of scans). There was no apparent effect of temperature on lying behaviour. There was, apparently, less rumination during the first 12h of the treatment period than during the pre-treatment period (Figure 2). During the first 12h of the treatment period, sheep kept at 14°C ruminated less (2% of scans) than those kept at 21°C (9% of scans), but during the second 12h of the treatment period, similar amounts of rumination occurred (7% of scans).

There was an increase in the proportion of scans spent moving at the beginning of the 24h treatment period and sheep held at 14°C spent a greater proportion of scans idling than those kept at 21°C.

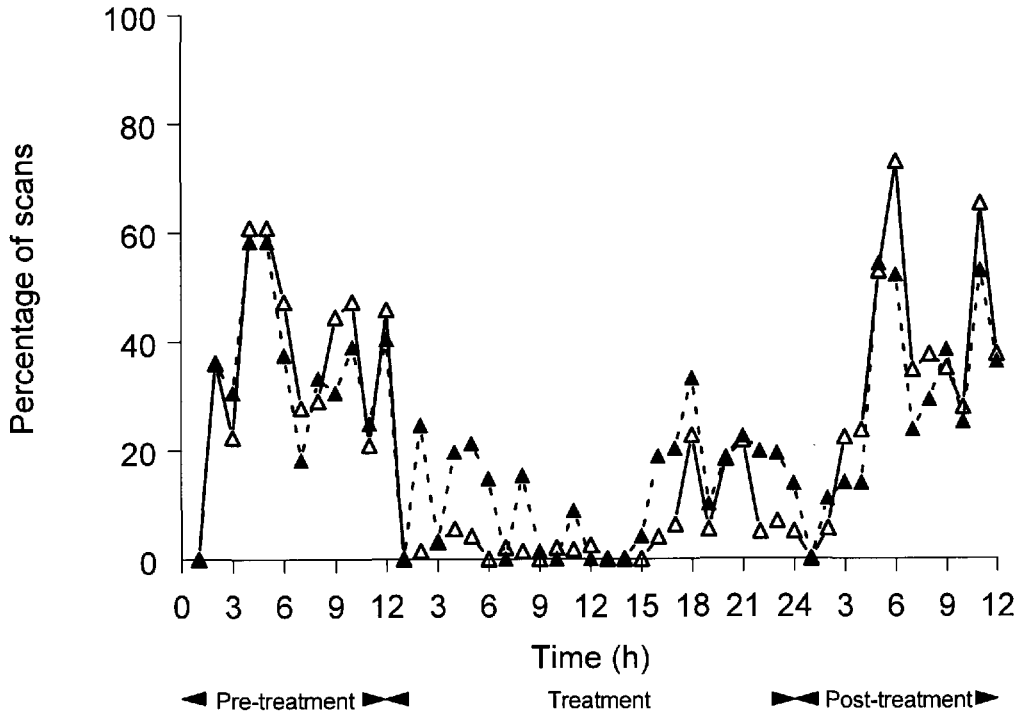

Figure 2 **Effect of temperature during 24h without feed (treatment) on the mean percentage of scans during each 1h period in which sheep were observed ruminating: 14°C (—△—); 21°C (- - ▲ - -).**

Post-treatment, there was a significant effect of time on eating, rumination, idling, lying and movement, but the only significant treatment effect on behaviour was a significant time x temperature x water interaction on the proportion of scans spent eating ($P < 0.01$). However, the general pattern of eating behaviour post-treatment was similar in all treatment groups (Figure 3). Immediately after the end of the treatment all groups ate hay and the time spent eating gradually decreased over the first 6h post-treatment.

Blood chemistry

The effects of temperature and water restriction on blood chemistry are shown in Table 1. There were no treatment effects on the plasma cortisol concentration. The (mean ± SEM) plasma β-hydroxybutyrate concentration during the treatment and post-treatment periods was slightly greater in sheep kept at 14°C (0.29 ± 0.05 mmol l^{-1}) than in those kept at 21°C (0.20 ± 0.05 mmol l^{-1}), $P < 0.001$. However, there was a significant temperature x water x time interaction on the plasma concentration of free fatty acids. After 14h without feed but with access to water, the plasma concentration of free fatty acids was greater in sheep at 21°C

Table 1 **Effects of temperature and water restriction on blood chemistry. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.**

	Significance of effects						
	Temperature	Water	Time	Temperature *x* Time	Water *x* Time	Temperature *x* Water	Temperature *x* Water *x* Time
Packed cell volume			***		*		
Plasma osmolality			***	***	*		***
Plasma concentration							
Vasopressin			***		*		*
Total protein			***				
Sodium			***				
Potassium			***				
Chloride			***	**			
Free fatty acids			***	***			*
ß-hydroxybutyrate	***						
Glucose			***		***		
Cortisol			***				

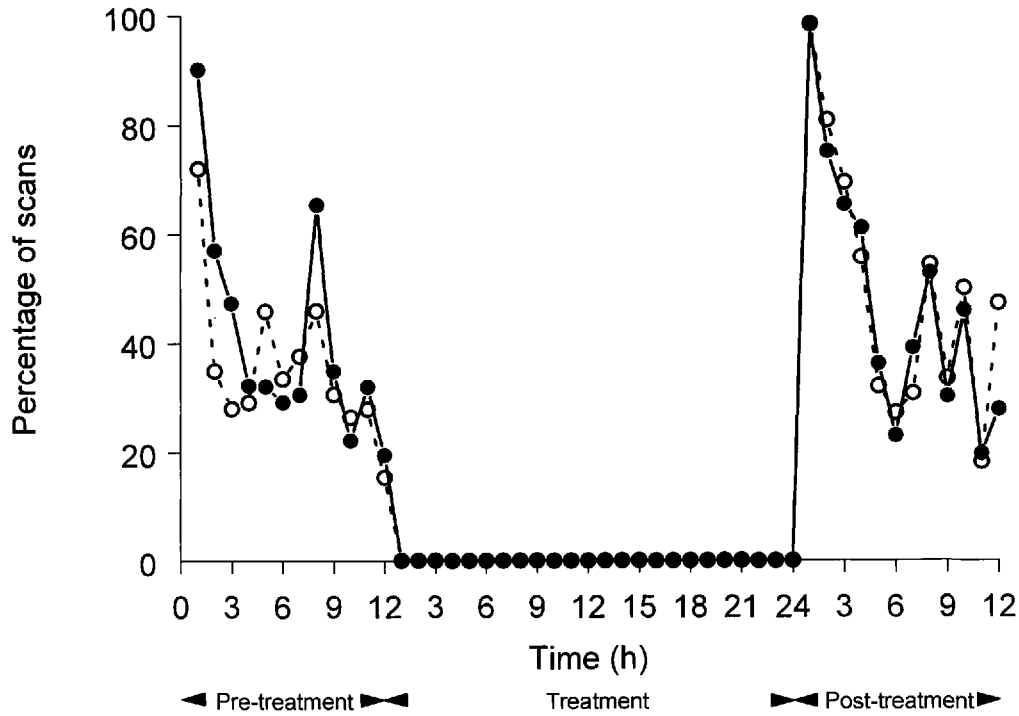

Figure 3 **Effect of water provision during 24h without feed (treatment) on the mean percentage of scans during each 1h period in which sheep were observed eating: with access to water (—●—); without access to water (- - ○ - -).**

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than in those at 14°C ($P < 0.05$; Figure 4). The plasma concentration of free fatty acids increased during the treatment period and then decreased during the 36h post-treatment period.

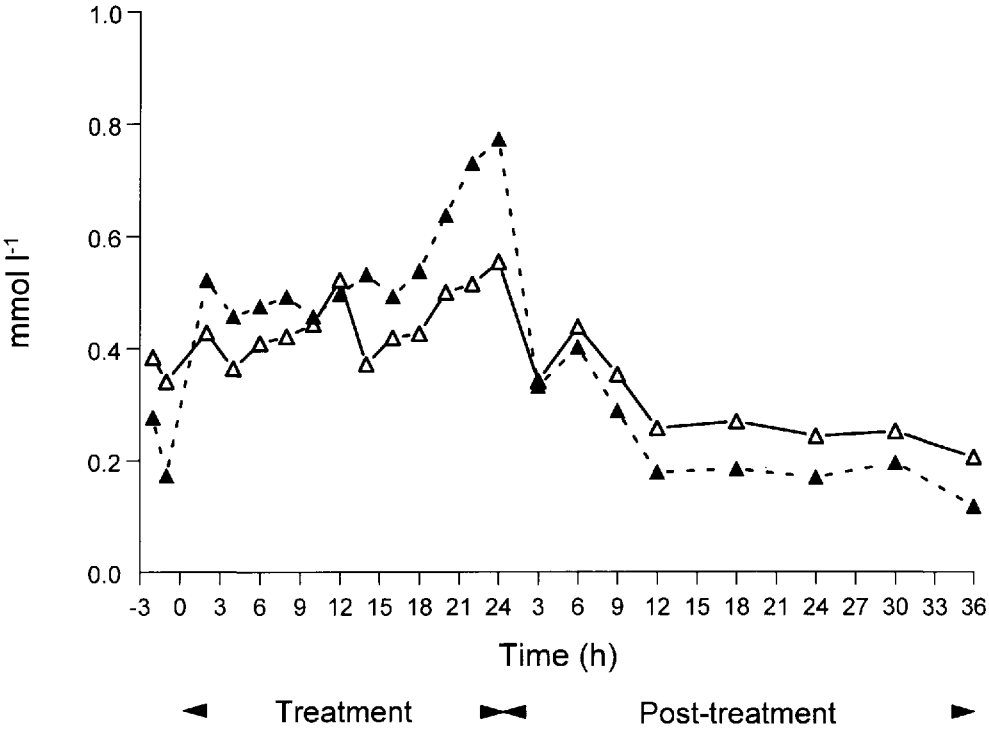

Figure 4 **Effect of temperature during 24h without feed (treatment) on the mean plasma concentration of free fatty acids: 14°C (—△—); 21°C (- - ▲ - -).**

There was no effect of temperature and no consistent effect of water restriction on the plasma concentration of glucose. However, the (mean ± SEM) plasma concentration of glucose was significantly lower during the second 12h of the treatment period (3·30 ± 0·05 mmol l^{-1}) than during the first 12h of the treatment period (3·49 ± 0·05 mmol l^{-1}), $P < 0·0001$.

There was a significant temperature x water x time interaction for both plasma osmolality and plasma vasopressin concentration. Apart from a slight rise in plasma osmolality after 20h of treatment in sheep at 21°C, as compared with those at 14°C, the plasma osmolality and the plasma vasopressin concentration were relatively stable throughout the period (Figures 5 and 6). However, 3h post-treatment, the plasma osmolality in sheep previously kept at 14°C, was significantly greater among those which had previously had access to water than in those kept without water; and it was also greater than in sheep previously kept at 21°C (either with or without water), $P < 0.001$. At 24h post-treatment, the plasma osmolality was significantly greater in sheep previously kept at 21°C than in those previously kept at 14°C ($P < 0.05$). For the first 18h post-treatment, the plasma vasopressin concentration in sheep previously kept without access to water was greater in those previously kept at 21°C than in those previously kept at 14°C ($P < 0.05$). Between 6h and 12h post-treatment, the plasma vasopressin concentration in sheep that had been previously kept at 21°C, was greater in those previously

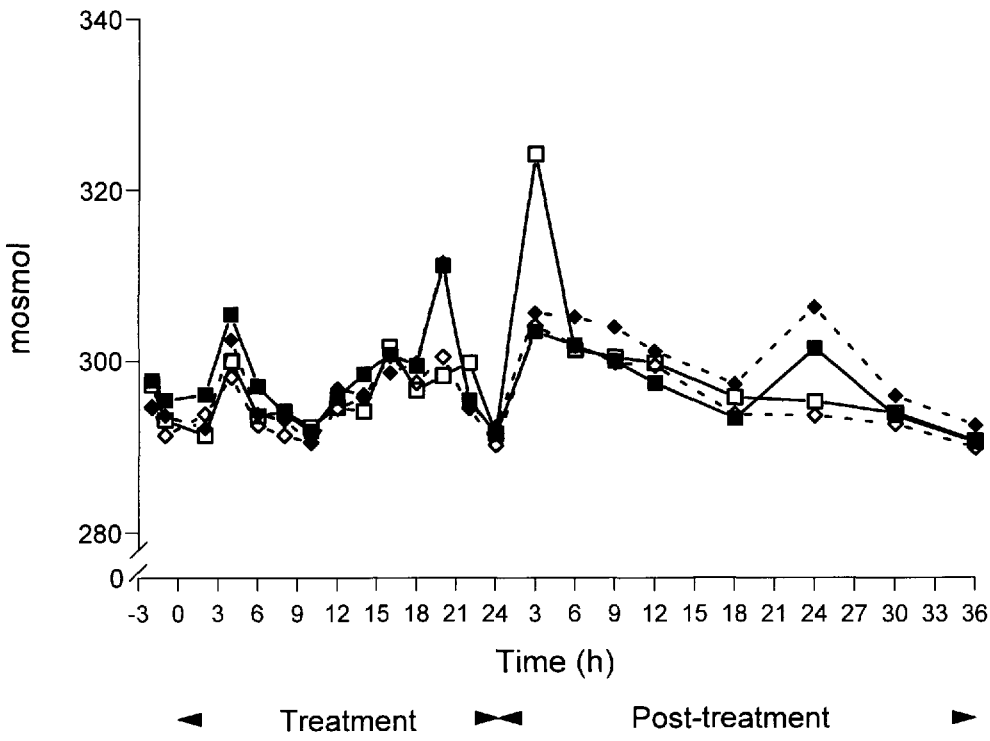

Figure 5 **Effects of temperature and water provision during 24h without feed (treatment) on the mean plasma osmolality: 14°C with access to water (— □ —); 14°C without access to water(- - ◇ - -); 21°C with access to water(— ■ —); 21°C without access to water (- - ◆ - -).**

kept without access to water than in those previously given access to water ($P < 0.05$). There were no treatment effects on the plasma concentration of total protein and no biologically or statistically important treatment effects on either the packed cell volume or the plasma concentrations of sodium, potassium and chloride.

Discussion

Due to technical problems with the environmental chambers the highest temperature (21°C) used in this study was not as high as originally intended, and the temperatures used probably fell within the thermoneutral zone of fully fleeced sheep (Alexander 1974). However, the temperature range in the experiments would be similar to that often experienced by sheep during transport (Knowles *et al* 1995). When the environmental temperature is above 25°C sheep increase evaporative heat loss via increased respiration and sweating (Degen & Shkolnik 1978). While the maximum temperature used in the current study was lower than 25°C, close confinement might have impaired the ability of sheep to lose heat. However, there was no evidence of panting or hyperthermia and no evidence of dehydration during the treatment period, which might have occurred if the sheep had lost large volumes of water through respiration and sweating. The lack of an effect of 24h of water deprivation on the range of biochemical measurements of dehydration used in this study supports the findings

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of Parrott *et al* (1996). Although based only on the measurement of plasma osmolality, they found that sheep, in the absence of feed, can compensate for insensible water loss without the need to drink for periods in excess of 24h. There was no evidence from the sheep which had access to water during the treatment period that thirst resulted in increased drinking during treatment. As in previous studies (Hecker *et al* 1964), water consumption during the period of food deprivation was low. However, the movement of the sheep from individual pens to a novel environment where they were kept in group pens may also have contributed to the low water intake during the treatment period.

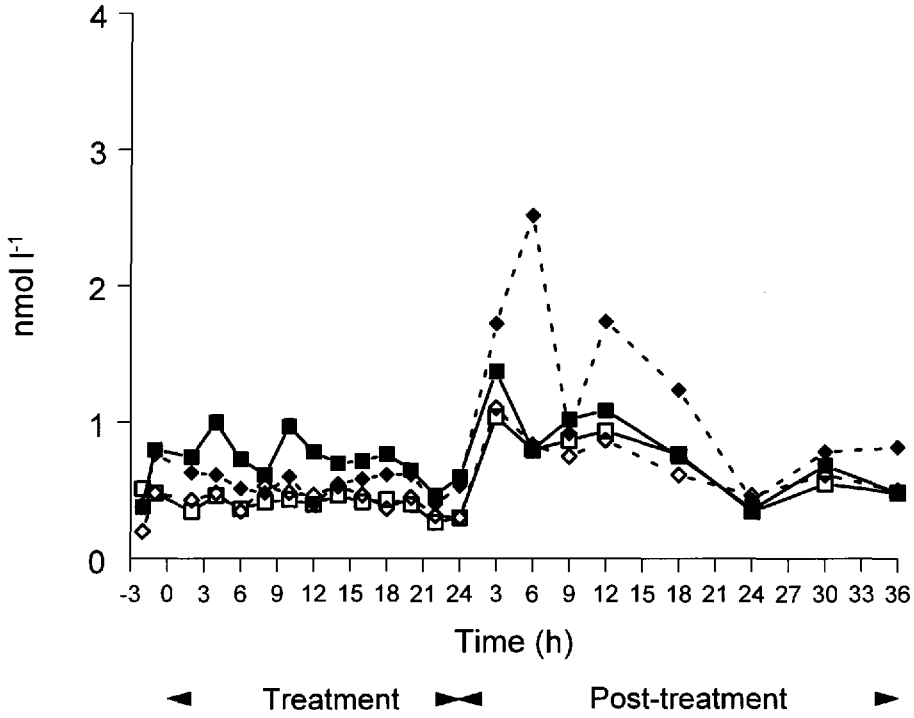

Figure 6 **Effects of temperature and water provision during 24h without feed (treatment) on the mean plasma concentration of vasopressin. For key legends see Figure 5.**

The lack of treatment effects on the plasma cortisol concentration confirms the findings of Parrott *et al* (1996). The plasma cortisol concentration during the treatment period was similar to that found during the pre-treatment period and suggests that confinement of sheep at temperatures of either 14°C or 21°C for 24h without food and with/without water is not associated with a physiological response that would indicate a possible welfare problem. However, the increased hay intake, and the increase in time spent feeding for 3–4 h post-treatment suggests that 24h without feed does stimulate sheep to eat readily. Although this rapid intake in feed could most readily be described as a response to hunger, the underlying mechanism for hunger in ruminants is not certain (Forbes 1995). In this study, the effects of 24h without food on the plasma concentrations of glucose and β-hydroxybutyrate in sheep were less than those reported by Warriss *et al* (1989). However, the increase in the plasma free fatty acid concentration during the treatment period was consistent with a mobilization

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of body energy reserves in response to fasting (Annison 1960) – and this suggests that sheep should receive feed at intervals of less than 24h.

The decrease in the proportion of scans spent ruminating during the treatment, as compared with the pre-treatment, period was similar to that found previously when sheep were confined in a stationary vehicle without feed (Cockram *et al* 1996). The increase in the proportion of scans spent moving at the beginning of the 24h treatment period was probably a response to mixing in a novel environment by the sheep, which had been previously penned individually (although they were kept as a group before housing).

The intake of hay after the treatment period produced biochemical signs of dehydration. After the intake of a large amount of dry food such as hay, a considerable volume of saliva is produced and there is an increase in rumen osmolality resulting in a movement of fluid into the rumen. This can increase plasma osmolality (Ternouth 1968) and result in the release of vasopressin (Stacy & Brook 1964). The plasma vasopressin concentration during the first 12h post-treatment was greatest in sheep which were likely to have lost the greatest amount of water during the treatment period, ie those kept at 21°C as compared with those kept at 14°C, and those without water as compared with those with access to water. The rapid intake of feed and the subsequent biochemical changes were probably responsible for the increased water intake during the 24h post-treatment compared with the pre-treatment period. This suggests that when feed is provided after a long journey without feed and water, sheep should be given sufficient time and access to drinking water to correct any imbalance of water within the body that could result from a large intake of feed post-transport.

As in previous studies (Cockram *et al* 1996; 1997), close confinement of sheep without feed affected their behaviour post-treatment: increasing the time spent standing and eating and decreasing the time spent lying and ruminating. The increase in feeding behaviour lasted for 3–4 h, which suggests that a 3–4 h lairage may be beneficial in enabling sheep to recover from a period of food deprivation lasting up to 24h. However, the sheep showed some biochemical signs of dehydration during this interval. This post-treatment dehydration highlights a potential problem with a short lairage period in the middle of a long journey, as sheep eat before they commence drinking (Cockram *et al* 1997). If sheep are kept in lairage for a short period and do not drink after feeding, this could increase the risk of dehydration during the continuation of any journey.

Animal welfare implications

The behavioural observations and the wide range of biochemical measures of dehydration used in this study confirm previous work suggesting that sheep do not appear to experience any obvious adverse effects during 24h of water deprivation. Provided that sheep are not subjected to conditions resulting in a greater than normal rate of water loss, the current European Council Directive (European Council 1995) that permits sheep to be transported for up to 14h before they must be given access to water appears to be reasonable. However, there are several potential problems with the requirements for the mid-journey 'rest' period in the Directive. The results of this study cast doubt on whether most sheep would drink water during a break (which may be as short as 1h) in a journey of up to 24h, when the temperature is below 21°C and they are placed in a novel environment without food. However, if feed such as hay is provided during the mid-journey 'rest' period, feed intake is likely to be high and the sheep will need to drink water to overcome the resultant period of temporary dehydration demonstrated in this study. As suggested by this and a number of previous studies (Parrott *et al* 1996; Hall *et al* 1997), and shown by Cockram *et al* (1997), if

feed is provided during a short break in a long journey and the sheep are not given sufficient time to drink after consuming large amounts of dry feed, this can result in dehydration during the remainder of the journey; consequently, their welfare may be worse than if they had not been offered feed.

Acknowledgements

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