

# EFFECTS OF A 15H JOURNEY FOLLOWED BY EITHER 12H STARVATION OR *AD LIBITUM* HAY ON THE BEHAVIOUR AND BLOOD CHEMISTRY OF SHEEP

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## Abstract

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*In a 2x2 factorial design, (n=6) sheep were either transported by road for 15h or kept in their home pens, and then either starved for 12h with access to water or offered hay ad libitum, with access to water. All groups were offered hay and water 12h after transport. Behavioural observations and measurements of dehydration and feed restriction were made before, during, and for 24h post-transport, to evaluate the implications of these procedures for the welfare of sheep.*

*After the journey, the immediate priority for the sheep was to eat. Consumption of hay increased water intake and reduced the time spent lying down. The plasma cortisol concentration was greater in sheep which had been starved during the 12h post-transport period, than in those offered hay during this time; and the plasma free fatty acid concentration was greater in sheep which had been transported than in those which had not. Although transported sheep kept without hay during the first 12h post-transport drank more water than those which had not been transported, the mean time before they drank was greater than 7h. During the transport period, there was less lying behaviour in transported sheep than in non-transported sheep but transported sheep did not lie down more post-transport than non-transported ones. This work suggests that sheep should be offered both feed and water after a 15h journey. However, when feed was not available after a 15h journey, drinking and resting did not appear to be immediate priorities.*

**Keywords:** *animal welfare, behaviour, blood chemistry, sheep, transport*

## Introduction

The effects of transport on the welfare of sheep 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 Farm Animal Welfare Council's 'five freedoms' (Farm Animal Welfare Council 1994) provide a basis for the evaluation of the welfare of sheep during and after transport (Cockram *et al* 1996; 1997). One of the many potential factors that could affect the welfare of sheep during and after transport is feed and water restriction. If sheep are to be slaughtered within 12h of arrival at a slaughterhouse, they might not be offered feed in the slaughterhouse lairage, and the period of feed restriction associated with the journey to the slaughterhouse could also be extended by up to 12h under the *Welfare of Animals (Slaughter or Killing) Regulations* (GB Parliament 1995). Although sheep can be starved for at least 48h without showing a plasma cortisol response (Parrott *et al* 1996), it is possible that a greater plasma cortisol response may be seen during starvation post-transport. The effect on the welfare of sheep of this extra period of feed restriction after a journey has not been previously reported.

After a 12h road journey, the immediate priority for sheep is often to eat rather than to drink or lie down (Cockram *et al* 1996; 1997). Parrott *et al* (1996) found no evidence of dehydration (measured by plasma osmolality) in sheep during 48h without feed and water. Although there was no change in plasma osmolality, it was possible that the sheep experienced thirst during this time. The increased water intake and drinking behaviour previously observed after a 12h transport (Cockram *et al* 1996; 1997) may have been the result of thirst, either due to water restriction during the journey or secondary to an increased feed intake post-transport. The behaviour of sheep with no feed after transport would indicate: i) whether sheep drink more post-transport in response to a water deficit accumulated during transport rather than after eating; and ii) whether they rest sooner after transport if they are not occupied by standing and eating. This experiment investigated the effects on the behaviour and blood chemistry of sheep of 15h of transport without feed and water followed by 12h starvation with access to water.

## Materials and methods

### *Animals and design*

In a 2x2 factorial design, (n = 6) weaned lambs were either transported by road for 15h or kept in their home pens and then either starved for 12h with access to water or offered *ad libitum* hay with access to water (Table 1). All procedures were conducted under Home Office licences, and the 15h journey time was chosen as, at the time of study, this was the maximum time sheep could be transported without feed under the *Welfare of Animals During Transport Order* (GB Parliament 1994).

Forty-eight, 16-week-old, full-fleeced, Suffolk x Greyface lambs with a liveweight of between 30kg and 40kg were randomly divided into eight groups of six, balanced by sex (equal numbers of females and castrated males) and liveweight. For each treatment combination, six sheep were used for blood sampling and were penned individually on wood shavings, and six sheep were used for behavioural observations and were penned in a group on wood shavings. They were housed for 1 week before the start of the treatment and provided with hay *ad libitum* (924 g kg<sup>-1</sup> dry matter [DM], 334 g kg<sup>-1</sup> DM acid detergent fibre, 70 g kg<sup>-1</sup> DM crude protein, 62 g kg<sup>-1</sup> DM ash and 938 g kg<sup>-1</sup> DM organic matter) and water. Sheep without access to hay during the first 12h post-transport were visually isolated from those with access to hay. The hay intake of individually penned sheep was recorded

over two, consecutive 12h periods during the behavioural observations before the start of the transport treatment and over the two, consecutive 12h periods immediately following the transport treatment. The liveweight of the sheep was recorded immediately before transport, immediately after the 15h transport treatment and, 12h and 24h after the end of the transport treatment.

**Table 1** Transport and feeding treatments during the transport period and the two, 12h post-transport treatment periods and the types of measurements made.

Transport treatment	Availability of feed and water			Sheep in group pens used for behavioural observations	Sheep in single pens used for blood sampling and feed and water intake measurements
	During transport period	First 12h post-transport	Second 12h post-transport		
<i>15h transport</i>	No Feed No Water	Water No Feed	Feed and Water	+	+
<i>15h transport</i>	No Feed No Water	Feed and Water	Feed and Water	+	+
<i>No transport</i>	Feed and Water	Water No Feed	Feed and Water	+	+
<i>No transport</i>	Feed and Water	Feed and Water	Feed and Water	+	+

### Procedures

On the day before the start of treatment, jugular cannulae were inserted into the sheep whose blood was to be sampled and 7ml of blood was manually collected into Monovette tubes (Sarstedt Ltd, Leicester, UK) containing lithium heparin (as described by Cockram *et al* 1996) at 6h, 5h, 4h, 3h and 0.5 h before the start of the transport period; at 3h, 6h, 9h, 12h and 15h after the start of transport; and at 3h intervals for 24h post-transport. Direct observations of the behaviour of the sheep were made from an elevated platform before and after the transport treatment, and from an observation compartment within the vehicle during transport. Individual sheep were identified by using colour marker sprays (Net-Tex Agricultural Ltd, Meopham, UK). The sheep were scanned at 6min intervals, for 24h before and after the transport period, and during the transport period. The following behaviours were recorded: standing, moving, lying, eating, rumination, investigation (Cockram *et al* 1996) and foraging (nosing the bedding). Drinking behaviour was recorded by continuous observation for 24h before and after the treatment period. The following potentially traumatic (behavioural) events were recorded for 12 sheep during transport: loss of balance, falling, being ridden, being trampled (Cockram *et al* 1996), or being butted (struck by another sheep with the head); any other potentially traumatic events were also recorded. The observations were recorded on a Psion Organiser LZ64 (Psion Instruments, London, UK) using behavioural observational software (Noldus Information Technology 1993; 1994).

At 1500h on the day of treatment, 12 sheep (two groups of sheep used for blood sampling) were loaded into the front pen (1.08x2.22 m) and 18 sheep (two groups of six sheep used for behavioural observations plus six additional sheep) were loaded into the back pen (1.6x2.22 m) of a livestock vehicle (Cockram *et al* 1996). They were transported for 15h at a space allowance of 0.2m<sup>2</sup> sheep<sup>-1</sup> and at a speed of 60–80 km h<sup>-1</sup>. Stops were made after a 3h circuit to allow blood sampling. The vehicle was stationary for a total of 1.56h during the 15h journey. A similar route was followed on each circuit and consisted of A (ie major)

roads for 87 per cent of the time: there were a median of 27 roundabouts, seven sharp corners and two rapid brakes per circuit. Two groups of group-penned sheep (used for behavioural observations) and two groups of individually penned sheep (used for blood sampling) were not transported and remained in their pens with *ad libitum* access to hay and water. At the end of the transport period (0600h), the transported sheep were returned to their original pens: one group of sheep (used for behavioural observations) and one group of sheep (used for blood sampling) had *ad libitum* access to hay and water; and one group of sheep (used for behavioural observations) and one group of sheep (used for blood sampling) had access to water, but no feed for the first 12h post-transport. At 0600h the hay was also removed for 12h from one group of non-transported sheep (used for behavioural observations) and one group of non-transported sheep (used for blood sampling). Twelve hours post-transport (at 1800h), all sheep were offered fresh hay and water.

#### **Laboratory analyses**

Using the methods described by Cockram *et al* (1996), the packed cell volume and plasma osmolality of samples were measured, as were the plasma concentrations of sodium, potassium, chloride, cortisol and  $\beta$ -hydroxybutyrate; and the plasma activity of creatine kinase was analysed. The plasma samples were also analysed for free fatty acids (Randox Laboratories Kit FA/115S; Randox Laboratories, Co Antrim, UK), urea (Randox Laboratories Kit UR456,) and albumin (Bayer Diagnostics Kit 01137702; Bayer Diagnostics, Basingstoke, UK) on a Bayer Diagnostics RA-2000 random access chemistry analyser at 37°C. Total plasma protein was estimated by the biuret method (Gornall *et al* 1949).

#### **Environmental recordings**

Air temperature and humidity were recorded at 5min intervals using Tinytalk Data Loggers (Orion Components Ltd, Chichester, UK). The mean ( $\pm$  SEM) air temperature in the experimental accommodation was  $18 \pm 0.1$  °C (range 12–25 °C) and the mean relative humidity was  $81 \pm 0.3$  per cent. The mean air temperature in the transporter was  $22 \pm 0.2$  °C (range 19–27 °C) and the mean relative humidity was  $74 \pm 0.8$  per cent.

#### **Statistical analysis**

A repeated measures analysis of covariance (Laird & Ware 1982) using the mixed procedure within SAS, version 6 (Statistical Analysis Systems (SAS) Institute Inc, Cary, USA) was used to examine the effects of transport, feed and time on blood chemistry. The main factors were 'Transport' (15h transport or no transport), 'Feed' (post-transport feeding management, ie no feed during the first 12h post-transport vs feed during the first 12h post-transport) and 'Time' (each blood sampling time). The value obtained before the start of the treatment was used as the covariate. Where there were interactions between time, transport and post-transport feeding management, the differences between least-square means were examined within the following periods: transport, the first 12h post-transport, and the second 12h post-transport. The treatment effects on the post-transport behaviour of the sheep (the percentage of scans per hour in which the sheep were performing a particular behaviour) were analysed as described above for blood chemistry, but with 'Time' represented by each 1h period post-transport. Where there were interactions between time, transport and post-transport feeding management, the differences between least-square means were examined within the first 12h post-transport and the second 12h post-transport. However, this analysis was based on the assumption that the individual behaviour of the sheep within each group was independent of that of the others in the group. This may not have always been the case.

The effects of transport and post-transport feeding management on hay intake, water intake and liveweight change were examined using two-way analyses of variance (ANOVA).

## Results

The results of the covariance analyses determining the significance of the effects of transport and feeding post-transport on blood chemistry and post-transport behaviour are shown in Table 2 and Table 3, respectively. These results determined which comparisons could be made within each treatment period.

**Table 2** Significance of effects of transport and post-transport feeding on blood chemistry. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

	Significance of effects						
	Transport <sup>1</sup>	Feed <sup>2</sup>	Time <sup>3</sup>	Transport x Time	Feed x Time	Transport x Feed	Transport x Feed x Time
Packed cell volume			***	***	***		**
Plasma osmolality			***	***	***		
Plasma concentration							
Total protein			***	**			
Albumin			***	*			
Sodium			***	***	***		
Potassium	*		***	**	**		
Chloride			***	***	***		**
Free fatty acids	***	***	***	***	***	**	
$\beta$ -hydroxybutyrate			***	*	***	*	
Urea			***	***	***		***
Cortisol		*	***	***	**		**
Plasma activity							
Creatine kinase			***				

<sup>1</sup> Either a 15h journey or no journey.

<sup>2</sup> Post-transport feeding management in the first 12h post-transport either hay *ad libitum*, or no feed.

<sup>3</sup> Blood sampling time.

**Table 3** Effect of transport and post-transport feeding on behaviour (% of scans per 1h period) during the post-transport period. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

Behaviour	Significance of effects						
	Transport <sup>1</sup>	Feed <sup>2</sup>	Time <sup>3</sup>	Transport x Time	Feed x Time	Transport x Feed	Transport x Feed x Time
Lying		*	***	**	***		***
Moving			***		**		
Eating		***	***	*	***		**
Rumination	***	***	***		***	**	*
Foraging		***	***	***	***		***
Investigating the environment		***	***	***	***	**	***
Investigating other sheep			**	*			

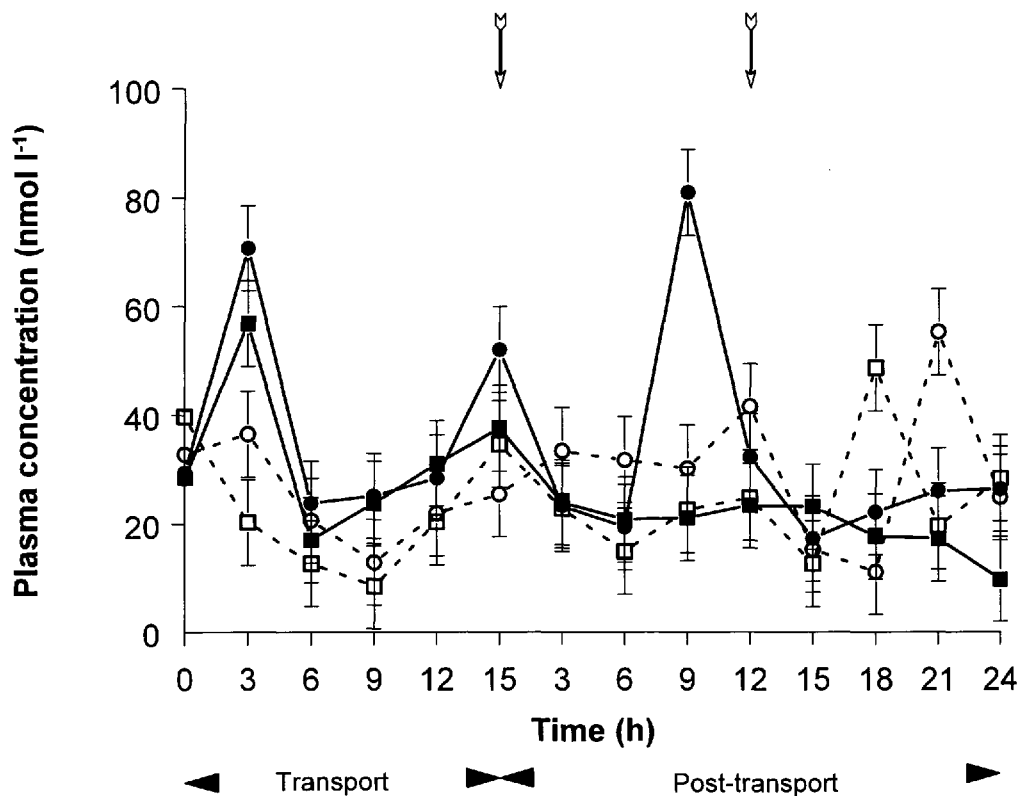
<sup>1</sup> Either 15h journey or no journey.

<sup>2</sup> Post-transport feeding management in the first 12h post-transport either hay *ad libitum*, or no feed.

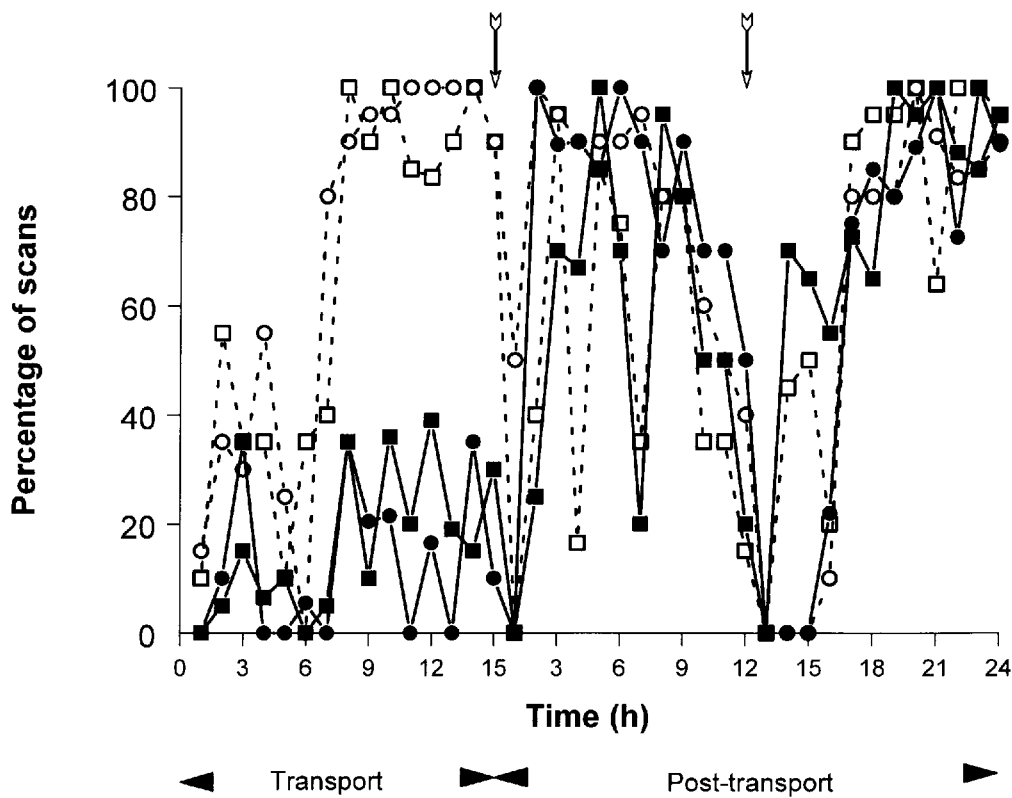
<sup>3</sup> Hourly periods post-transport.

**Transport period**

The plasma cortisol concentration (mean  $\pm$  SEM) was greater in transported sheep ( $37 \pm 5.6$  nmol l<sup>-1</sup>) than in non-transported sheep ( $21 \pm 5.6$  nmol l<sup>-1</sup>),  $P < 0.001$ , with the greatest response after 3h of transport (Figure 1). Transported sheep lay down less than non-transported sheep (Figure 2). The median (or 2nd quartile, Q2) numbers of potentially traumatic events experienced during the journey by sheep in the front pen of the transporter were: 31 losses of balance (Q1 = 18, Q3 = 33), 0 falls (Q1 = 0, Q3 = 1), 0 hits against the side of the vehicle (Q1 = 0, Q3 = 0), 2 riding events (Q1 = 0, Q3 = 4), 7 trampling events (Q1 = 3, Q3 = 12) and 1 butting event (Q1 = 0, Q2 = 3). However, there was no significant effect of transport on plasma creatine kinase activity.



**Figure 1** Effects of transport and feeding post-transport on the mean plasma cortisol concentration (nmol l<sup>-1</sup>). No transport and feed available during first 12h post-transport (- - □ - -); no transport and no feed available during first 12h post-transport (- - ○ - -); 15h transport and feed available during first 12h post-transport (—■—); 15h transport and no feed available during first 12h post-transport (—●—). Bars denote SEMs; the vertical arrows indicate the start and end of the 12h post-transport period.



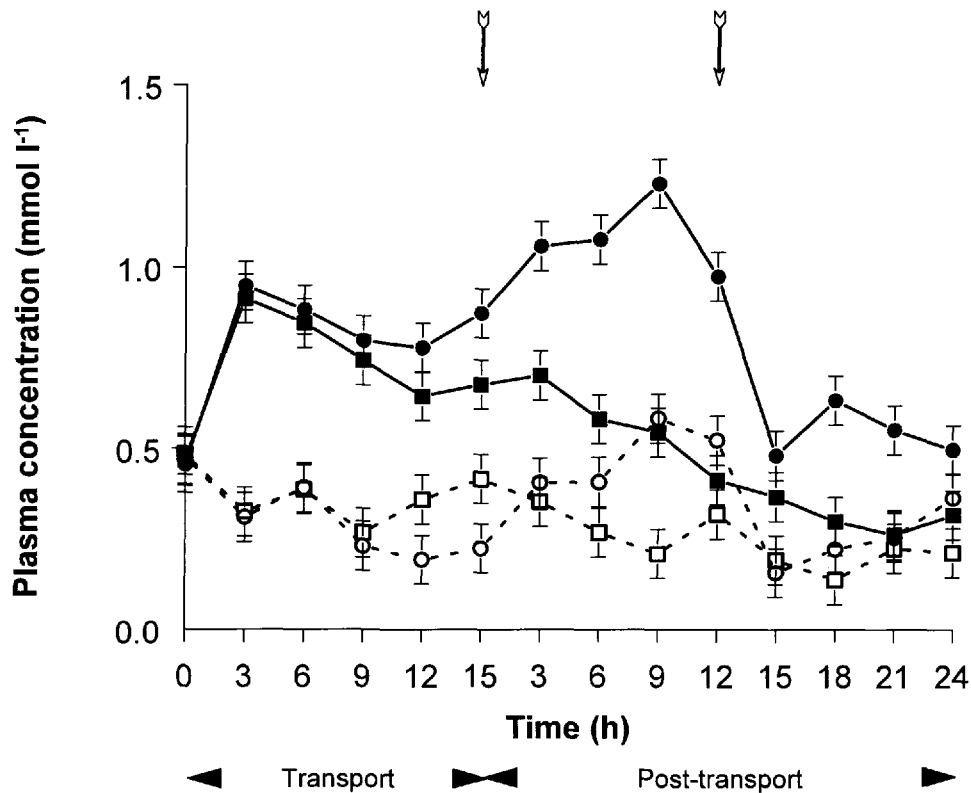
**Figure 2** Effects of transport and feeding post-transport on the mean percentage of scans per 1h period during which the sheep were observed lying down. For key legends see Figure 1. Vertical arrows indicate the start and end of the 12h post-transport period.

The plasma free fatty acid concentration (mean  $\pm$  SEM) was greater in transported sheep ( $0.81 \pm 0.048$  mmol l<sup>-1</sup>) than in non-transported sheep ( $0.31 \pm 0.048$  mmol l<sup>-1</sup>),  $P < 0.01$ , (Figure 3). The plasma concentration (mean  $\pm$  SEM) of  $\beta$ -hydroxybutyrate after 9h of transport was slightly greater in transported ( $0.31 \pm 0.024$  mmol l<sup>-1</sup>) than in non-transported sheep ( $0.24 \pm 0.024$  mmol l<sup>-1</sup>),  $P < 0.05$ . There was no effect of transport on liveweight change (mean  $\pm$  SEM) during the transport period ( $-2.5 \pm 0.82$  % liveweight).

There was no physiological evidence of dehydration (as assessed by changes in the packed cell volume, plasma osmolality, and the plasma concentrations of albumin, total protein and sodium) during the 15h journey.

#### *First 12h post-transport*

Sheep without access to feed had a greater (mean  $\pm$  SEM) plasma cortisol concentration ( $39 \pm 5.6$  nmol l<sup>-1</sup>) than those with access to feed ( $22 \pm 5.6$  nmol l<sup>-1</sup>),  $P < 0.01$ . However, in sheep which were starved for 12h, there was no significant difference in plasma cortisol



**Figure 3** Effects of transport and feeding post-transport on the mean plasma free fatty acid concentration ( $\text{mmol l}^{-1}$ ). For key legends see Figure 1. Bars denote SEMs; the vertical arrows indicate the start and end of the 12h post-transport period.

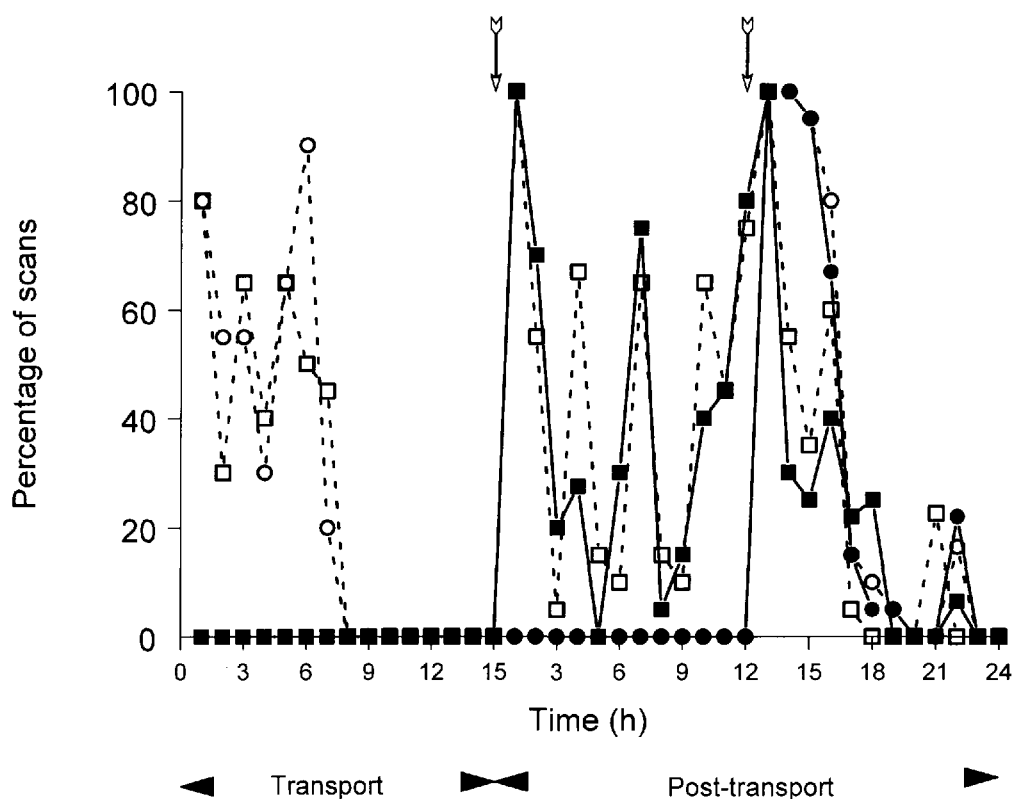
concentrations between sheep which had been transported ( $39 \pm 7.9 \text{ nmol l}^{-1}$ ) and those which had not been transported ( $34 \pm 7.9 \text{ nmol l}^{-1}$ ), see Figure 1.

Sheep lay down more (mean  $\pm$  SEM) if kept without hay ( $75 \pm 4.7\%$  of scans) than if given access to hay ( $51 \pm 4.7\%$  of scans),  $P < 0.001$ . However, there was no significant difference in the lying behaviour of sheep without access to hay, between those which had been transported ( $76 \pm 6.7\%$  of scans) and those which had not been transported ( $75 \pm 6.7\%$  of scans),  $P > 0.05$ , (Figure 2). Sheep without hay lay down sooner ( $0.5 \pm 0.19 \text{ h}$ ) than those with hay ( $1.6 \pm 0.08 \text{ h}$ ),  $P < 0.001$ ; but there was no effect of transport on the time taken to lie down ( $P > 0.05$ ).

The sheep offered hay at the start of the post-transport period immediately started to eat it, but there was no significant effect of transport on either eating behaviour (Figure 4) or hay intake (Table 4). There was a greater liveweight increase (mean  $\pm$  SEM) in sheep that had access to hay ( $5.9 \pm 1.45\%$ ) than in those that did not have access to hay ( $0.5 \pm 0.82\%$ ),  $P < 0.01$ . In sheep offered hay, there was more (mean  $\pm$  SEM) rumination in sheep which had been transported ( $45 \pm 8.8\%$  of scans) than in those which had not been transported ( $33 \pm$



8.8 % of scans),  $P < 0.05$ . Sheep with access to hay ruminated more ( $39 \pm 6.3$  % of scans) than those with no access to hay ( $27 \pm 6.3$  % of scans),  $P < 0.05$ . There was more (mean  $\pm$  SEM) foraging ( $6 \pm 1.3$  % of scans) and investigation of the environment ( $7 \pm 1.6$  % of scans) in sheep without access to hay than in those with access to hay (foraging,  $0.0 \pm 1.3$  % of scans; investigating the environment,  $2 \pm 1.6$  % of scans); both  $P < 0.01$ . Transported sheep without access to hay investigated their environment ( $9 \pm 2.3$  % of scans) and foraged ( $8 \pm 1.8$  % of scans) more than non-transported sheep without access to hay (investigating the environment,  $4 \pm 2.3$  % of scans; foraging,  $4 \pm 1.8$  % of scans); both  $P < 0.001$ .



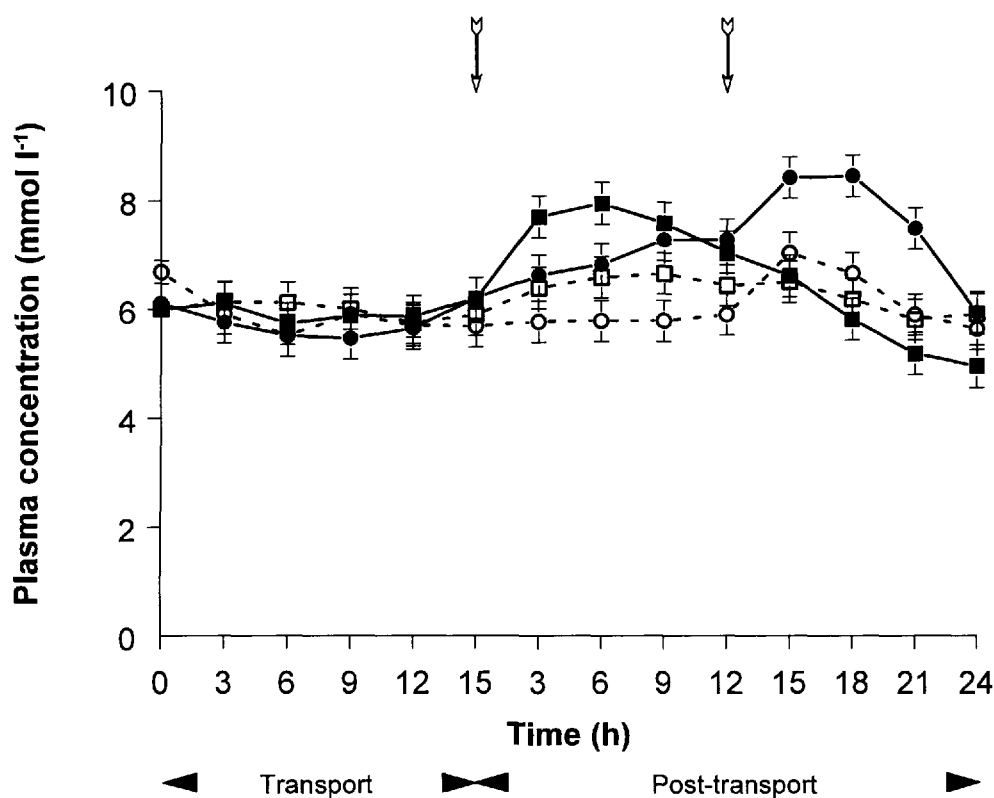
**Figure 4** Effects of transport and feeding post-transport on the mean percentage of scans per 1h period during which the sheep were observed eating. For key legends see Figure 1. Vertical arrows indicate the start and end of the 12h post-transport period.

In sheep which had not been transported, the plasma free fatty acid concentration (mean  $\pm$  SEM) was greater in sheep without access to hay ( $0.48 \pm 0.068$  mmol l<sup>-1</sup>) than in those with access to hay ( $0.29 \pm 0.068$  mmol l<sup>-1</sup>),  $P < 0.01$ . In sheep which had been transported, the plasma free fatty acid concentration was greater in sheep without access to hay ( $1.08 \pm 0.068$  mmol l<sup>-1</sup>) than in those with access to hay ( $0.56 \pm 0.068$  mmol l<sup>-1</sup>),  $P < 0.0001$ . In sheep with access to feed, the plasma free fatty acid concentration in sheep which had been transported was greater than in non-transported sheep ( $P < 0.0001$ ), see Figure 3. Figure 5 shows the

**Table 4** Feed and water intakes (mean  $\pm$  SEM) pre- and post-transport for individually penned sheep.

	No transport				Transport			
	Feed		No feed		Feed		No feed	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
<b>Hay intake (kg sheep<sup>-1</sup>)</b>								
First 12h pre-transport	0.4	0.05	0.5	0.04	0.4	0.07	0.4	0.05
Second 12h pre-transport	0.4	0.05	0.4	0.04	0.3	0.04	0.4	0.07
First 12h post-transport	0.4 <sup>a</sup>	0.04	0		0.6 <sup>a</sup>	0.09	0	
Second 12h post-transport	0.4 <sup>a</sup>	0.02	0.7 <sup>b</sup>	0.03	0.3 <sup>a</sup>	0.05	0.6 <sup>b</sup>	0.06
<b>Water intake (l sheep<sup>-1</sup>)</b>								
First 12h pre-transport	1.4	0.13	2.0	0.21	1.4	0.29	1.7	0.4
Second 12h pre-transport	0.4	0.16	0.6	0.18	0.5	0.19	0.7	0.23
First 12h post-transport	2.3 <sup>a</sup>	0.19	0.8 <sup>b</sup>	0.07	2.1 <sup>a</sup>	0.37	1.8 <sup>a</sup>	0.25
Second 12h post-transport	0.3 <sup>a</sup>	0.17	1.5 <sup>b</sup>	0.23	0.7 <sup>a</sup>	0.10	1.3 <sup>b</sup>	0.25

<sup>a,b</sup> Means with different superscripts within the same row are significantly different ( $P < 0.05$ ).



**Figure 5** Effects of transport and feeding post-transport on the mean plasma urea concentration (mmol l<sup>-1</sup>). For key legends see Figure 1. Bars denote SEMs; the vertical arrows indicate the start and end of the 12h post-transport period.

plasma concentration (mean  $\pm$  SEM) of urea was greater in sheep which had been transported ( $7.29 \pm 0.27$  mmol l<sup>-1</sup>) than in those which had not been transported ( $6.17 \pm 0.27$  mmol l<sup>-1</sup>),  $P < 0.001$ .

Sheep with feed took less time (mean  $\pm$  SEM) before starting to drink ( $3.5 \pm 1.02$  h) than those without feed ( $8.4 \pm 1.99$  h),  $P < 0.01$ . Although there was a tendency for transported sheep to drink sooner ( $4.4 \pm 1.06$  h) than non-transported sheep ( $7.5 \pm 1.95$  h), there was no significant effect of transport on the time taken before the sheep drank after transport ( $P > 0.05$ ). Among non-transported sheep, those with access to hay (feed), drank more than those without access to hay ( $P < 0.05$ ). In sheep with access to hay, there was no significant effect of transport on water intake. Among sheep without hay, those which had been transported drank more than those which had not ( $P < 0.05$ ), see Table 4. The (mean  $\pm$  SEM) plasma osmolality ( $306 \pm 1.9$  mosmol) and the sodium concentration ( $145 \pm 0.7$  mmol l<sup>-1</sup>), were greater in sheep which had been transported and had access to feed than in the other treatment groups (osmolality,  $297\text{--}301 \pm 1.9$  mosmol; sodium,  $142 \pm 0.7$  mmol l<sup>-1</sup>); both  $P < 0.01$ .

### **Second 12h post-transport**

The hay intake was greater among sheep kept without hay during the previous 12h than in those given access to hay previously ( $P < 0.001$ ), but there was no significant effect of transport on hay intake (Table 4). Sheep kept without access to hay during the previous 12h spent more (mean  $\pm$  SEM) time eating ( $37 \pm 4.2$  % of scans) than those which had been given access to hay during the previous 12h ( $24 \pm 4.2$  % of scans),  $P < 0.001$ , (Figure 4). Among sheep which had been transported and had been kept without feed during the previous 27h, the (mean  $\pm$  SEM) plasma free fatty acid concentration ( $0.54 \pm 0.068$  mmol l<sup>-1</sup>) and the plasma urea concentration ( $7.58 \pm 0.38$  mmol l<sup>-1</sup>) were greater than in the other treatment groups ( $0.19\text{--}0.31 \pm 0.068$  mmol l<sup>-1</sup> and  $5.65\text{--}6.31 \pm 0.381$  mmol l<sup>-1</sup>, respectively); both  $P < 0.05$ , see Figures 3 and 5.

### **Discussion**

Although there was no replication of the transport treatment in this study, the behavioural and physiological responses of the sheep were similar to those found in eight similar journeys that formed part of previous studies (Cockram *et al* 1996; 1997). After the 15h journey, the immediate priority for the sheep was to eat. The consumption of hay post-transport affected their biochemistry, increased drinking and reduced the time spent lying down. The absence of feed during the 15h journey was associated with increases in the plasma concentrations of free fatty acids and  $\beta$ -hydroxybutyrate, indicating a mobilization of the body's energy reserves (Annison 1960; Warriss *et al* 1989). The most probable reason for the transported sheep starting to eat immediately post-transport was the effect of the previous 15h without feed. However, non-transported sheep were also stimulated to eat by the provision of fresh hay after a period of reduced feeding between 0000h and 0600h. The intake of hay in previously transported sheep was associated with a rise in plasma urea concentration (Cole *et al* 1988). The decline in the plasma free fatty acid concentration in sheep which had been transported and then offered hay during the first 12h post-transport, the rise in those which had been transported and then starved, and the smaller rise in non-transported sheep starved during this period, suggest that starvation post-transport resulted in an additional mobilization of body energy reserves. Post-transport starvation also resulted in

greater foraging and investigation of the environment, greater hay intake and more time spent eating when hay was offered.

There does not appear to have been a cortisol response to hunger associated with the duration of starvation, as there was no difference in the plasma cortisol concentration between sheep which had been transported for 15h and starved for a further 12h and those starved for only 12h. The greater plasma cortisol concentration of sheep starved during the 12h post-transport period than of those offered hay during this time was influenced by the sample taken after 9h. This rise in plasma cortisol concentration may have been a psychological response to either the expectation of feeding or non-visual stimuli associated with other groups feeding/receiving feed during this time. This suggestion is supported by the results of Parrott *et al* (1996), where the 48h starvation of sheep previously offered hay and concentrates twice a day was not associated with a rise in the plasma cortisol concentration; however, in sheep previously offered feed *ad libitum* and then starved for 18–20h, a rise in the plasma cortisol concentration has been reported, although this response diminished after repeated periods of fasting (Purchas 1973).

Consumption of hay post-transport was followed by drinking. The hay intake had a greater effect on water intake than did water restriction during the 15h journey (at a mean temperature of 22°C). There was no evidence that water loss during the journey had a significant effect on biochemical measurements of dehydration during the journey, but there was some evidence of temporary dehydration during post-transport feeding. The increased intake of hay during the first 12h post-transport in both transported and non-transported groups, was associated with increased plasma osmolality (Ternouth 1968) and an increased water intake. Although most of the increased post-transport water intake was secondary to the feed intake, there was evidence that the sheep required drinking water to replace a water deficit accumulated during the 15h journey. During the first 12h post-transport, transported sheep without access to hay drank more water than those which had not been transported. However, drinking was not an immediate priority after transport (the mean time taken by sheep without access to hay to obtain their first drink being more than 7h).

An increase in the plasma cortisol concentration during the initial stage of a journey has been reported previously (Cockram *et al* 1996) and was probably associated with vehicular motion. However, the journey did not appear to have caused significant injury to the sheep. The number of potentially traumatic events observed during transport was low and this was reflected in the lack of change in the plasma activity of creatine kinase, which can rise after muscular damage (Boyd 1988). Although there was less lying behaviour in transported sheep than in non-transported ones, this did not result in greater lying behaviour post-transport in transported sheep than in non-transported sheep. In previous work (Cockram *et al* 1996), eating was shown to be a greater priority post-transport than resting. In the current experiment there was no evidence from the sheep without feed to suggest that lying was an important priority immediately post-transport. When feed was not available post-transport, the mean time taken by transported sheep to lie down was about 45min, whereas non-transported sheep lay down within 14min of the disturbance caused by the removal of their hay. Although it seems that the sheep were not physically tired after 15h of transport, it was possible that sheep without access to hay may have delayed resting until the possibility of obtaining feed had been explored. However, both transported and non-transported sheep without hay lay down for similar amounts of time during the 12h post-transport.

**Animal welfare implications**

Eating is an immediate priority for sheep after a 15h journey. Starvation of sheep for an additional 12h after a 15h journey without feed further increased the mobilization of body energy reserves. If sheep are not immediately slaughtered, their welfare during lairage would be improved if both feed and water were offered on arrival at a slaughterhouse. When feed was not available after a 15h journey, drinking and resting were not immediate priorities and the absence of feed did not encourage resting behaviour. However, transported sheep did drink and rest post-transport and appropriate facilities for eating, drinking and resting should be provided after a journey.

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