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Behavioural and physiological responses of sheep of different breeds to supplementary feeding, social mixing and taming, in the context of transport

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Abstract

Three experiments investigated differences between sheep breeds in response to supplementary feeding and very close confinement with familiar and unfamiliar sheep; and differences between individuals in response to a taming procedure prior to transport in a trailer. The first two experiments were with a mixed flock of Clun Forest lambs and crossbred lambs from Orkney. An initial tendency to feed with others of the same breed diminished over 3 weeks. Responses of heart rate and salivary cortisol concentration when one sheep (the visitor) was penned in an enclosure of 1 m² with three other sheep of the same or the other breed (the trio group), did not depend on whether the visitor and the trio group were of the same or different breed. Clun Forest sheep showed little response while Orkney sheep showed increased heart rate and some elevation of salivary cortisol concentration. In the third experiment, 34 Beulah Speckled Face ewes were divided into two groups one of which was subjected to a taming procedure over a period of 3 weeks. Response of salivary cortisol concentration to subsequent journeys of 2 h in a livestock trailer did not differ between tamed and untamed sheep. There were individual differences in the response to taming and this was correlated with the cortisol response during transport, in that those sheep which responded most to taming showed the least elevation during transport.

Keywords: *sheep, stress, transport.*

Introduction

The physiological responses of sheep to transport and to events associated with transport such as marketing, assembly of animals into batches and lairage, have been of considerable recent interest (review: Hall and Bradshaw, 1998). In view of the great diversity of sheep breeds it is important to establish whether there are differences among breeds in these responses and whether prior experience of contact with humans can be influential. We report three experiments on these topics.

The first two experiments consider social mixing, under very different conditions both of relevance to transport. Previous studies have considered the social behaviour of sheep under extensive conditions (Hunter and Milner, 1963; McBride *et al.*, 1967; Jewell *et al.*, 1974; Arnold and Dudzinski, 1978; Hohenboken, 1986). We consider social behaviour under the artificial conditions of supplementary feeding and of very close confinement. Such conditions apply during, respectively, pre-transport maintenance of batches of sheep, for example in exporters' premises and during transport itself when sheep may be stocked at rates of around 0·25 m² per sheep (Hall and Bradshaw, 1998). In our third experiment, we investigated whether response to transport could be influenced by taming. This complements the other experiments in that, should certain genotypes of sheep show a strong response to transport, it may be possible to mitigate such effects by applying a taming regime.

For the first two experiments, two distinct groups of lambs were combined into a flock. An experiment involving supplementary feeding was conducted to elucidate whether sheep preferred to feed with others from their own group. An experiment involving close confinement was used to find out,

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first, whether there was a stress response if sheep were confined with sheep from the same or a different flock and secondly whether there were stress responses when a previously unacquainted sheep was introduced. For the third experiment a group of ewes was subjected to a taming procedure. Behavioural and salivary cortisol (Cooper *et al.*, 1989) responses to transport were compared with those of a group which had not been tamed.

In view of public interest in the transport of sheep and of its economic importance, there is an urgent need for an understanding of how welfare can be inferred from non-invasive, behavioural observation. Accordingly, our third experiment was also designed to provide new behavioural data. Sheep perform many behaviours during transport (e.g. Bradshaw *et al.*, 1996; Cockram *et al.*, 1996) but not enough is known about how to use such behavioural observations to assess welfare and some workers have been pessimistic as to the prospects of success (e.g. Ewbank and Kent, 1990). However it is very desirable to achieve this understanding because, as pointed out by Hargreaves and Hutson (1990a), 'individuals may differ as to whether physiology and behaviour are correlates or alternatives' in response and habituation to fearful stimuli, and non-invasive techniques have obvious advantages.

Lying in a relaxed position might be seen as an indicator of good welfare. Sheep during transport usually lie in sternal recumbency (S. J. G. Hall, personal observations) with or without rumination. Provision of enough space for all sheep to lie simultaneously during transport is sometimes taken as an absolute requirement (e.g. Cockram *et al.*, 1996) it being implied that unless sheep lie during transport their welfare will not be good. This issue has not been critically examined. Lying behaviour cannot be used to discriminate how poor welfare is when there is insufficient space to lie, as in normal commercial transport. Standing rumination might be of value for such studies but it can be difficult to detect in crowded conditions. Standing with the head below the level of the shoulders may have potential; preliminary analysis suggested that most, if not all, sheep perform this behaviour during transport, for variable lengths of time (bout lengths) and with no indication that they imitate one another. This behaviour is therefore considered in detail in the analysis of the third experiment.

Material and methods

Animals

Seventeen Clun Forest sheep (16 females, one castrated male) and 18 crossbred sheep purchased from a dealer in the Orkney Islands (10 females, eight castrated males) were grouped on 17 May 1995 in preparation for the experiment to start on 12 June. All sheep were born in 1994 and all were unshorn. The Clun Forest sheep had all been bred in the same flock in England. The Orkney sheep were of a wide range of genotypes. Some were of predominantly Shetland type (three of these were the only sheep in the group to possess horns) while others showed influence of Suffolk, Cheviot and other breeds. Body weights were 40 to 45 and 25 to 35 kg for Clun Forest and Orkney sheep respectively.

The second experiment consisted of the systematic introduction to the above sheep of six sheep with which they were unacquainted, from 18 June 1995. All were castrated males (wethers), of between 50 and 95 kg body weight and of various breeds. They are known here as the stranger sheep. For the third experiment, 34 Beulah Speckled Face post-breeding cull ewes were purchased from a farmer, to provide two control and two experimental groups each of eight sheep and two spares. All were from the same flock which had been bred and kept together under extensive conditions in eastern England but they had not been frequently handled. The body weights of the 17 that were assigned at random to the group to be tamed averaged 47·9 kg (range 31·7 to 67·9 (s.e. 1·95) kg).

Experiment 1: supplementary feeding

This experiment, on sheep which had been kept together for 4 weeks, took place between 12 June and 13 July 1995 (June 12-17, period 1; June 18-26, period 2; June 26-July 8, period 3; July 9-13, period 4). Drought conditions had led to a lack of grazing in the paddock (0·7 ha) occupied by the sheep. On 10 occasions during each period (i.e. twice daily on some days) the sheep were offered a pelleted diet (180 g protein per kg; H. and C. Beart Ltd, Kings Lynn) presented as 10 piles each of 800 g (basal diameter of pile approx. 24 cm). The sheep were admitted into the paddock where the food was on offer and on four scans during the next 5 min the breeds and number of the sheep at each pile of food were noted. With four scans being performed on 10 piles of food on 10 occasions in each period, 400 feeding groups were recorded in each period. Feeding groups were tabulated according to breed composition. Groups comprising two or three sheep were considered further and, following Hall (1986) the observed breed composition was compared with that expected had association been at random.

During the scans, some sheep were not sighted as they were not at a pile of food; if all 35 sheep had been sighted during every scan, a total of (35 × 4 × 10), i.e. 1400, sightings would have been made during each period. The sighting ratio was calculated for each period as the total number of sightings of

Clun Forest sheep divided by that of Orkney sheep. Mean group size for each period was calculated as the total number of all sightings divided by 400.

Experiment 2: confinement and social mixing

On 5 days during period 2, i.e. between 18 and 26 June, four trio groups were made up each of three sheep, all three being either Orkney or Clun Forest. On every day, the same single sheep in each group carried a Polar Sport Tester heart-rate monitor (Hopster and Blokhuis, 1994) set to log every 15 s. Sheep were confined in solid-sided, open-roofed pens each of floor area 1 m^2 for 8 h. Initially, one sheep of the same or a different breed (the visitor) was placed with each trio group and at hourly intervals thereafter this sheep was replaced by a different individual. Measurements were not made of the heart rates of visitors. Initial and subsequent manipulations were accompanied by sampling of saliva for the assay of cortisol (Cooper *et al.*, 1989). On day 1 and day 2, these additions and exchanges were not made, in order to determine a daily pattern of heart rate and salivary cortisol. On day 1, four groups each of four Clun Forest sheep were established, the remaining five being penned as one group of three and one group of two. On day 2 there were six groups each of four Orkney sheep. On both these days saliva samples were taken every hour.

On day 3, there were four trio groups each of three Clun Forest, and on day 4, three Orkney sheep. The sheep placed with the trio groups were of the other breed in most cases, according to the following pattern:

	Group 1	Group 2	Group 3	Group 4
Breed of initial visitor	other	other	other	same
Second visitor	same	other	other	other
Third visitor	other	same	other	other
Fourth visitor	other	other	same	other
Fifth visitor	other	other	other	same
Sixth visitor	other	other	other	same

Saliva samples were taken from each visitor sheep when it was introduced to a trio group and when it was removed.

On day 5, two trio groups were penned of each breed and each hour, a different member of the stranger group was added to each trio group.

Experiment 3: taming and transport

The 17 ewes to be tamed were placed in a paddock of 0·7 ha on 1 March 1996 and on 10 occasions, at the same time of day, over a period of 21 days (maximum interval between occasions, 5 days) were gathered into a pen (12 m^2). There, saliva was taken from each sheep on two large cotton wool buds, the same order of sampling being followed by the same experimenter every time. The sheep were left in the pen for 2 h without human company, saliva was sampled again and then sheep pellets were given in troughs. The mean times taken to collect all saliva samples were 19·3 min for the first sample and 18·1 min for the second. The other 17 ewes were placed in a paddock out of sight and were surveyed from a distance every day but were not handled.

On the first experimental day, which was the day after the 10th taming session, the sheep were penned, saliva was sampled from all 17 animals and eight were loaded into a four-wheeled trailer (Ifor Williams TA5G). The area in the trailer was 1·55 × 3·03 m (0·59 m^2 per sheep). After 2 h of driving over a variety of roads for approximately 90 km the sheep were returned to the pen and saliva taken from all 17 animals. This was repeated on the afternoon of the next day except that the eight sheep that had already been taken on the journey were left behind and eight of the others were taken instead. On the afternoon of the first experimental day, the 17 sheep which had not been tamed were gathered and saliva was sampled. Eight were then loaded and transported as described above. This was repeated, for the other eight untamed sheep, on the morning of the second experimental day. During transport, video recordings were made with a tripod-mounted Sony 3CCD camcorder.

Analysis of heart rate data

Some heart rate monitors yielded only incomplete records. Heart rates obtained during saliva sampling and manipulation of sheep were discarded. Heart rate data were available from seven separate periods, each of 40 min, on each of days 1 and 2. On days 3 to 5 they were available from six separate periods each of between 30 and 40 min. Median heart rates were calculated for each of these periods. The median heart rate for each sheep, measured during a given period on day 1 or day 2, was plotted against the heart rate of the same sheep during the corresponding period on days 3, 4 or 5. The χ^2 approximation of the Kruskal-Wallis test (Helwig and Council, 1979) was used to compare medians.

To investigate whether heart rates changed during a day, the median heart rate for a sheep during each 40 min period was found. These were standardized by expressing each hourly median as a percentage of the median for the 1st h. The Terpstra-Jonckheere ordered alternatives test (Neave and Worthington, 1988) was applied; significance would indicate a consistent change-over time in median heart rate.

Assay of salivary cortisol
Saliva samples were frozen and subsequently assayed by enzyme-linked immunosorbent assay (Cooper *et al.*, 1989). For experiment 2, salivary cortisol concentration after each 40-min period was investigated by analysis of covariance. The covariate was concentration before the period and there were two factors, namely breed (two levels: Orkney and Clun Forest) and visitor status (five levels, according to whether the sheep was (1) a member of a trio group being visited by the same or (2) the other breed, or by (3) a stranger sheep, or whether it was (4) visiting a trio group of the same or (5) the other breed).

For experiment 3, the progress of the taming procedure was followed by calculating, for each sheep, the regression against sample number (x) of salivary cortisol concentration (y). A significant and negative gradient for this regression would indicate attenuation of the cortisol response to handling. Multiple regression of salivary cortisol concentration after the journey on concentration before the journey and on the gradient of the response to taming, was calculated.

Analysis of behaviour
Videotapes were analysed twice, firstly to record for each sheep the time of onset (to the nearest 1 s) and termination of each bout of standing with the head below the level of the shoulders (standing head-down), and secondly to note, every min, the number of sheep that were lying. The Pearson correlation was calculated, for each sheep, between the length of bout of standing head-down and (a) the length of the preceding interval since the last bout of standing head-down, (b) length of subsequent interval, and (c) time elapsed since the start of transport. The equation given by Duncan *et al.* (1970) was used to test whether bout lengths of standing head-down were randomly distributed.

Results

Experiment 1
In each period, had every sheep been feeding while every scan was in progress, 1400 sightings would have been recorded. Total sightings during periods 1 to 4 were respectively 957, 1107, 1211, 1239. Although Orkney outnumbered Clun Forest sheep by 18 to 17, the latter were more frequently seen feeding. The sighting ratios for periods 1 to 4 (sightings of Clun Forest/sightings of Orkney sheep) were respectively 1·39, 1·22, 1·11, 1·09, and mean group sizes were 2·39, 2·77, 3·03, 3·10. On 96 occasions, a Clun Forest sheep fed alone, and on 99, an Orkney sheep. On 85 occasions, there were no sheep at a pile of food. Groups of four or more (maximum observed, nine on one occasion) numbered, in the four periods respectively, 74, 122, 148, 156.

Table 1 *Supplementary feeding experiment: χ^2 statistics comparing observed and expected incidences of the different pair and trio groups, according to period*†

	Pairs			Trios			
	CC	OO	CO	CCC	OOO	CCO	COO
Period 1	8·9	10·1	8·3	16·9	1·9	3·8	0·2
June 12-17	+	–	–	+		–	
Period 2	5·6	0·1	2·0	12·9	0·1	0·2	6·6
June 18-26	+			+			–
Period 3	1·4	1·6	3·0	1·2	0·01	0·2	0·9
June 26-July 8							
Period 4	0·9	0·1	0·2	1·1	1·4	4·8	3·9
July 9-13						+	+

† C indicates Clun Forest, O indicates Orkney; + indicates significant excess of observed over expectation, – indicates significant deficit.
Critical value of χ^2 for $P = 0·05$, d.f. 1, is 3·841.

Considering pairs and trios (Table 1), departures from expectation were most noticeable in periods 1 and 2. Then, groups comprising only Clun Forest sheep were in excess. There were initial deficits of mixed-breed groups and of pairs of Orkney sheep. In period 4 the only departures from expectation were that trios of mixed breeds were in excess.

Experiment 2: salivary cortisol concentrations
On day 1 and day 2 respectively, Clun Forest and Orkney sheep were kept in the pens for 8 h. Spearman coefficients indicated negative correlation between concentration of salivary cortisol and the serial number of the sample, for 15 of the 17 Clun Forest sheep ($P < 0·05$ for 10 cases) and for 12 of the 15 Orkney sheep ($P < 0·05$ for three cases). Concordance among these correlations was significant for the Clun Forest sheep ($\chi^2 = 40·562$, d.f. $= 7$, $P < 0·001$) but not for the Orkney sheep ($\chi^2 = 0·999$, d.f. $= 7$, $P > 0·05$). These results show that concentration of salivary cortisol declined during the day and that this effect was stronger and more uniform for the Clun Forest sheep.

The salivary cortisol concentration at the end of each 40 min period of confinement was most strongly influenced by that at the beginning (the covariate); the visitor status of the sheep did not itself have a significant effect but it did interact with breed (Table 2). Accordingly the breeds were analysed separately and this revealed that Orkney sheep showed a significantly greater response when visiting trio

Table 2 *Analysis of covariance tables of concentration of salivary cortisol (nmol/l) after period of introduction of sheep to penned trio group†*

(a) Both breeds. The factors (class variables) were breed and the visitor status of the sheep.		
Overall analysis of covariance: $F_{(10,238)} = 17{\cdot}32$, $P < 0{\cdot}001$		
Contribution of covariate:	$F_{(1,238)} = 155{\cdot}54$, $P < 0{\cdot}001$	
Contribution of breed:	$F_{(1,120)} = 0{\cdot}05$, $P > 0{\cdot}05$	
Contribution of visitor status:	$F_{(4,120)} = 1{\cdot}98$, $P > 0{\cdot}05$	
Contribution of interaction of factors:	$F_{(4,120)} = 2{\cdot}41$, $P < 0{\cdot}05$	
(b) Orkney sheep. The factor (class variable) was the visitor status of the sheep.		
Overall analysis of covariance: $F_{(5,120)} = 12{\cdot}17$, $P < 0{\cdot}001$		
Contribution of covariate:	$F_{(1,120)} = 46{\cdot}40$, $P < 0{\cdot}001$	
Contribution of factor:	$F_{(4,120)} = 3{\cdot}61$, $P < 0{\cdot}01$	
Least-squares means:	nmol/l	no.
Visiting trio group of Clun Forest	7·558[a]	17
Being visited by Stranger sheep	4·055[b]	35
Being visited by Clun Forest	3·782[b]	51
Being visited by Orkney	3·222[b]	18
Visited trio group of Orkney	2·641[b]	5
(c) Clun Forest sheep. The factor (class variable) was the visitor status of the sheep.		
Overall analysis of covariance: $F_{(5,117)} = 34{\cdot}09$, $P < 0{\cdot}001$		
Contribution of covariate:	$F_{(1,117)} = 167{\cdot}74$, $P < 0{\cdot}001$	
Contribution of factor:	$F_{(4,117)} = 0{\cdot}68$, $P > 0{\cdot}05$	
Least-squares means:	nmol/l	no.
Being visited by Orkney	5·047[a]	51
Visiting trio group of Orkney	4·627[a]	17
Being visited by Clun Forest	4·585[a]	17
Visiting trio group of Clun Forest	4·496[a]	6
Being visited by Stranger sheep	4·215[a]	32

† Least-squares means are given when appropriate (superscripts indicate the results of application of Duncan's test to the means; different superscripts indicate means are significantly different). In all cases the covariate was concentration before the period.

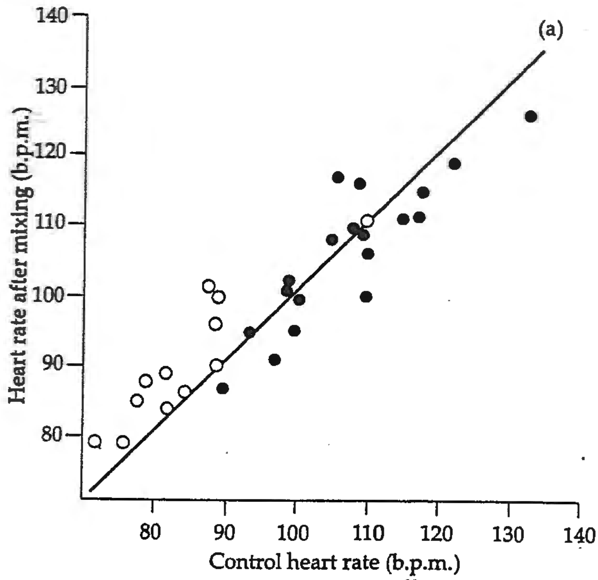
groups of Clun Forest sheep than during any other kind of visit.

Experiment 2: heart rates

The ordered alternatives test showed that for most penned groups of sheep, median heart rates declined significantly during all five days of observation (Terpstra-Jonckheere statistics for days 1 to 5 respectively: 27, $P < 0{\cdot}01$; 26, $P < 0{\cdot}01$; 17, $P < 0{\cdot}01$; 19.5, $P > 0{\cdot}05$; 7, $P < 0{\cdot}01$). When the median heart rates during mixing (days 3 to 5) were compared, period by period, with the median heart rates during corresponding periods on days 1 and 2 when there was no mixing (Figure 1a and b), a breed difference became evident. Clun Forest sheep sometimes responded to the introduction of a visitor with an elevation of heart rate and sometimes with a decline (Figure 1a); Orkney sheep in all except one comparison showed an increase in heart rate whether the visitor was Orkney or Clun Forest. For both breeds, response was not consistent when the visitor was a Stranger sheep (Figure 1b).

Experiment 3: salivary cortisol concentrations

During the 17 days of the taming process, most sheep showed a decline in salivary cortisol concentration; the gradient of the regression against sample number of salivary cortisol concentration was negative and significant for six sheep, negative and non-significant for five, positive and significant for none and positive and non-significant for five. Considering the experimental journeys after the period of taming, for all sheep combined, the multiple regression of salivary cortisol concentration after the journey on concentration before, and on the gradient of the response to taming, was very close to significance ($F_{2,13} = 3{\cdot}66$, $P = 0{\cdot}0549$, $R^2 = 0{\cdot}36$), with the latter factor being more important ($F_{1,15} = 5{\cdot}65$, $P < 0{\cdot}05$ compared with $F_{1,15} = 1{\cdot}67$, $P > 0{\cdot}05$). This implies that the cortisol response to transport was



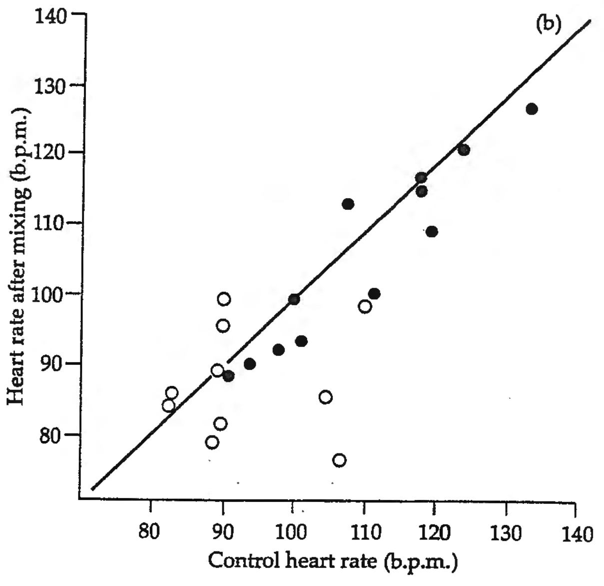


Figure 1 (a) comparison of median heart rate of each individual sheep, between a 40-min period on the control day on which no manipulation was made (day 1 for Clun Forest, day 2 for Orkney) and the corresponding period on the day when other sheep were introduced (day 3 for Clun Forest, day 4 for Orkney). ● : Clun Forest, O: Orkney. Points above the 45° line represent periods when the heart rate during mixing was greater than that on the corresponding period during the control day. (b) as for (a) but comparison is between the control day and day 5, when Stranger sheep were introduced.

partly predictable from the cortisol response to taming.

Experiment 3: behaviour
From the 8 h of driving, video tapes were obtained of 387 min. Very little lying was observed: at 35 of the 387 scans, one sheep was lying, i.e. of all the time recorded in the trailer, the proportion spent lying was 0·011. Proportions of time spent standing head-down ranged, for tamed sheep, from 0·018 to 0·869 (mean 0·316, s.e. 0·078, no. = 16) and for untamed sheep, between 0·002 and 0·732 (mean 0·209, s.e. 0·051, no. = 16). These means do not differ ($t = 1{\cdot}159$, $P > 0{\cdot}05$). Mean bout length of standing head-down also varied widely among sheep (tamed sheep, range of means of 16 sheep 4·0 to 324·5 s; untamed sheep, 2·0 to 96·8 s). No differences in bout length of standing head-down were apparent between tamed and untamed sheep so for further consideration of this behaviour, all sheep were grouped. For 29 sheep, calculations were made of correlation between length of bout of standing head-down (standing head-down) and of the preceding interval. For the other three sheep, there were too few sample points. Only one such correlation was significant ($r = 0{\cdot}278$, d.f. = 127, $P < 0{\cdot}01$). Only one correlation between standing head-down and following interval was significant ($r = 0{\cdot}369$, d.f. = 10, $P < 0{\cdot}01$). Five correlations between standing head-down and time elapsed since the start of observations were significant (respectively r = 0·486, 0·489, 0·491, 0·682, 0·532, d.f. = 132, 127, 100, 18, 44).

Tests of fit to the exponential distribution showed that for the total of observations of all sheep, and for each sheep considered individually, the exponential model did not fit. There was a total of 1767 bouts of mean length 19·26 s, with a sizeable deficit of very long bout lengths (>200 s; 356 expected, 34 observed) and an excess of very short (<5 s). Considering bouts less than and greater than 5 s, of the former, 651 were observed and 315 expected, and of the latter, 1116 were observed and 1452 expected ($\chi^2 = 436{\cdot}15$, d.f. = 1, $P < 0{\cdot}001$).

Discussion

In this study, the tendency of sheep in a mixed flock to remain with others of their own kind was investigated in the context of supplementary feeding and the responses to forced confinement with sheep of different origins were investigated by studying heart rate and salivary cortisol concentration. Finally, the response to transport of sheep that had undergone a taming programme was compared with that of sheep which had not been tamed.

The supplementary feeding experiment revealed a strong breed difference, as might be expected from the distinctiveness of the sheep studied. The Clun Forest sheep were all from the same, purebreeding flock. This breed is long-established and has been selected in the context of lowland, high-input, high-output husbandry (Hall and Clutton-Brock, 1988). In contrast the Orkney sheep were from several flocks and most were crossbreds of hardy, extensively farmed northern breeds. The Clun Forest sheep were normally observed to be feeding together and appeared to be pre-adapted to the regime of supplementary feeding. It was only in period 3, i.e. 2 weeks after the experiment started, that random association at the piles of food became evident. Before then there were more pairs and trios consisting entirely of Clun Forest sheep than would be expected by chance, and in period 4 trios of mixed breeds were in excess. This implies that at first Orkney sheep were either unable to exclude Clun Forest sheep from their feeding groups, or were unable to insinuate themselves into a group of Clun Forest sheep. However, in period 4, trios of mixed breeds were in excess, which implies that the Orkney sheep had acquired one or both of these abilities. Group size and the number of sightings of Orkney sheep at the food relative to Clun Forest both increased, indicating an increased competitive ability on the part of the former. A practical consequence is that sheep should, especially when new groupings have been established, be provided with adequate space for all to eat at the same time.

Studies of physiological responses during the confinement experiment showed a tendency (more uniform in Clun Forest) for both heart rate and salivary cortisol concentration to decline during the day. An increase in heart rate was exhibited by Orkney sheep on introduction of another sheep but this increase was not shown by the physically larger Clun Forest sheep. Orkney sheep only showed a significant rise in salivary cortisol concentration when they were introduced to Clun Forest trio groups, while the latter were relatively unresponsive in this respect.

There is therefore evidence of breed differences in these responses, the Clun Forest being less likely to show elevation of salivary cortisol concentration and heart rate. It seems likely that the increase in heart rate shown by the Orkney sheep was not associated with greater physical activity as movement in the confined space of the pen was difficult. Behavioural studies of analogous breeds have produced similar results; Romeyer and Bouissou (1992) described the Romanov (a northern, relatively unimproved and small bodied breed) as more 'fearful' than the Ile-de-France (a lowland, large-bodied breed); perhaps greater responsiveness of behaviour and physiology to social challenges could be characteristic of relatively primitive, northern breeds of sheep.

It seems reasonable to suggest that these differences have a genetic basis, as although evidence is lacking in livestock of heritability of heart rate and of salivary cortisol concentration, there is evidence in humans (Degaute *et al.*, 1994; Kirschbaum *et al.*, 1992). Additionally, several previous studies have concluded that behavioural differences in sheep could have a genetic basis (Hohenboken, 1986; Lynch *et al.*, 1992).

Compared with those of other species, stress responses of sheep to social mixing seem mild. The mixing of animals from different herds is well known to be stressful in the case of pigs (e.g. Bradshaw *et al.*, 1996) and farmed red deer (Pollard *et al.*, 1993; Hanlon *et al.*, 1995). When sheep are mixed in farms, the incomers usually remain with their previous acquaintances, and interact relatively little with strangers (X. Manteca, unpublished observations).

The finding that there is not a marked stress response to confinement with strangers is of relevance to discussion on how best to organize breaks during long journeys (Anonymous, 1995). While a short break may or may not have value for realimentation and rehydration (Hall *et al.*, 1997; Knowles *et al.*, 1996), it seems clear that the social mixing consequent on unloading and reloading is unlikely to cause any welfare problem, provided there is sufficient space for all animals to eat and drink.

The taming regime that was applied to the Beulah Speckled Face ewes reduced their salivary cortisol response to handling but did not lead to a general reduction in cortisol response during transport. However, there was evidence that the individual sheep which responded more to taming were less stressed during transport. That sheep do respond to taming was also shown by Hargreaves and Hutson (1990a and b). It is a matter of speculation which aspect of taming was most effective in the present study but for these extensively husbanded ewes the confinement in a pen may have been important. Indeed many sheep farmers maintain that sheep are easier to load, and travel better, if they are kept in close confinement overnight, with food and water, before being loaded.

In this final experiment, the most interesting behavioural result was that for approximately one quarter of the time during transport the sheep were standing with the head below the level of the shoulders. This behaviour was mentioned by

Cockram *et al.* (1996) but its significance was not discussed. It was not among the behaviours classified as 'fearful' by Romeyer and Bouissou (1992). Its occurrence was very slight in other studies of mature sheep in a stationary trailer (S. J. G. Hall, unpublished results). Cockram *et al.* (1996), however, observed it more with lambs in a pen than during transport. Standing with the head down was found to be very variable in occurrence among sheep, in terms of length and number of bouts. Its bout length shows no correlation with time elapsed since the journey began, with interval since the last such bout, or with interval to the next. These findings imply that it is not an expression of fatigue, at least on short journeys. On a model of randomness, more long bouts are expected than are observed, implying that sheep tend to curtail these bouts. This implies that while at least some sheep have a need to perform this behaviour during transport, they may be inhibited from doing so by the existence of motivation to remain in the normal head-up posture. While its functional significance is not yet clear, it seems possible that this behaviour might indicate stress and it is therefore likely to repay further investigation.

Transport of sheep is associated with many procedures that could cause stress and further study is needed of breed differences in this respect. A very large proportion of slaughter lambs in Britain is, genotypically, half Suffolk (Ministry of Agriculture, Fisheries and Food, 1982) so some uniformity in genetically controlled behavioural responses might be expected in meat lambs, but particularly among cull ewes and meat lambs from hill flocks substantial effects of breed are to be predicted.

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