BEHAVIOURAL AND PHYSIOLOGICAL RESPONSES OF SUCKLING LAMBS TO TRANSPORT AND LAIRAGE

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Abstract

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The effects on suckling lambs of two stocking densities during transport (high stocking density: eight lambs per m^2; low stocking density: four lambs per m^2) and two periods of lairage before slaughter (1.5 h and 3 h) were studied. The behaviour of lambs transported for short-duration journeys at the two stocking densities was recorded. At slaughter, blood samples were obtained after a lairage period and some physiological parameters related to stress were analysed: lactate dehydrogenase (LDH), creatine kinase (CK), calcium (Ca^{2+}), cortisol, and packed cell volume (PCV). There was a significant effect of stocking density on lambs' behaviour during transit. The number of lambs walking was greater at low than at high density (84 and 20 observations, respectively; $P < 0.001$) whilst the number standing was lower at low density (852 and 366 observations, respectively). There was a significant effect ($P < 0.001$) of stocking density on the plasma concentration of LDH. The length of the period of lairage significantly affected plasma concentrations of LDH ($P < 0.001$), CK ($P < 0.05$), Ca^{2+} ($P < 0.001$) and cortisol ($P < 0.05$), as well as PCV ($P < 0.01$). The results show that stocking density affects the lambs' behaviour during transport. After the longer lairage period, plasma cortisol concentration is lower; meanwhile, the activity of LDH and CK is higher.

Keywords: *animal welfare, lairage, stocking density, suckling lambs,*

Introduction

There have been several studies concerning the behavioural and physiological responses of sheep to transport, but none concerning young lambs. In some European Mediterranean countries, the slaughter of young lambs at about one month of age is very common because of the large number of dairy sheep and the high demand for this type of lamb. In Spain, 24 per cent of the sheep slaughtered in 1997 were young lambs.

Even though the most stressful aspects of the journey are loading and the initial period in the lorry (Knowles *et al* 1995), the length of the journey and the conditions under which the animals are carried have a significant bearing on their welfare (Knowles *et al* 1998). The effect of transport on sheep may vary with the relative roughness of the journey (Bradshaw *et al* 1996).

Stocking density of lambs during commercial transport is determined by European Communities Directive 95/29/EC. In Spain, it is defined by RD 1041/1997, which allows less than 0.2 m^2 per lamb for young lambs weighing under 55 kg but does not specify the minimum space allowance during transport. Indeed, transport involving distances of less than 50 km is exempt from EU legislation. The floor space allowed to each animal during transport is a very important welfare consideration. Dimensions of individual animals can provide a guide to the minimum acceptable dimensions of a penned area, but these dimensions do not necessarily provide adequate room for the animals to change posture or position (Randall 1993). Transport at stocking densities at which the animals are unable to lie down comfortably can lead to severely reduced animal welfare (Knowles *et al* 1995). If sheep are transported at high stocking densities, they may not all be able to lie down at the same time (Cockram *et al* 1996). The ideal is probably a density at which all can just lie down together. The effects of stress may be assessed by changes in the plasma levels of cortisol (Fordham *et al* 1989). A variety of physiological parameters also change in response to factors associated with stress and physical fatigue, such as creatine kinase (CK), lactate dehydrogenase (LDH) and Ca^{2+} concentration (Broom *et al* 1996, Warriss *et al* 1992). Decreases in packed cell volume (PCV) may be related to dehydration after transport or any stress situation. Sheep can also lose weight during transport (Knowles *et al* 1993, 1995).

There has been no study to date about this issue in young lambs. The effect of transport on young lambs could be different from that described in older lambs and sheep, even for short journeys. Further information on the behavioural and physiological responses of young lambs to transport is required in order to assess the potential of transport to lead to welfare problems. In the following study, an attempt is made to establish how the welfare of suckling lambs is affected by the stocking density during transport on short-duration journeys and by the subsequent period of time spent in lairage.

Materials and methods

Experimental procedure

Forty-eight suckling lambs (mean live weight 13.2 ± 0.3 kg), aged 35–45 days of age, of both sexes of Rubia de El Molar breed (a Spanish dairy breed), were used in a 2 x 2 factorial design. The lambs were from single and double births. They had remained with their dams until they were loaded into the experimental lorry. The lambs were randomly allocated to four groups of transport, two groups at high (8 lambs m^{-2}) and two groups at low (4 lambs m^{-2}) stocking density. Each group was transported on consecutive days in November.

The lambs were loaded at 0800h into an experimental cage measuring 2 x 1 x 1 m (length x width x height). The four walls were covered with an aluminium sheet, one of which was mobile and was used to load the animals. Straw was provided as bedding over a non-slip floor. The ceiling was barred so that the lambs could not jump out. The vehicle was a small experimental lorry (two-wheeler rigid chassis), with ventilation holes located at a height of 1.6 m which were opened. The cage was situated in the back of the lorry.

The temperature during loading ranged from –1°C to 0°C and the average temperature during transport was 8 ± 0.5°C, which did not represent a thermal challenge to the lambs. The vehicle was driven for 30 min, during which a distance of 41 km was travelled.

After transport, the lambs were unloaded and held in lairage for one of two time periods: either 1.5 h or 3 h. Lambs transported at each stocking density were submitted to each lairage condition according to a 2 x 2 design. During lairage, they received neither food nor water. Lambs' live weights prior to loading and immediately upon arrived at the abattoir were recorded on a commercial scale. The loss of live weight was expressed as a proportion of live weight before loading.

After the lairage period, the lambs walked to the slaughter area. They were stunned with low voltage (110 V) while being held individually in a restrainer. Blood samples were taken at exsanguination from eight lambs in each of the four groups (32 blood samples). The blood samples were drawn into heparinised and non-heparinised tubes and refrigerated for analysis over the following 24 h.

Laboratory analyses

The blood samples that were collected into heparinised tubes were used within 24 h to analyse PCV. The non-heparinised blood samples were centrifuged and the serum was separated and stored at 2°C for subsequent analysis. Levels of isozymes of LDH and CK were measured on a SBA autoanalyser, using Boehringer Mannhein reagents. Ca^{2+} was measured using a CBR kit (Boehringer Mannhein) and cortisol by enzyme immunoassay (Radim, Roma).

Behaviour measures

Lambs were scanned every minute throughout each journey by direct observation, and the number of lambs performing the following mutually exclusive behaviours was noted: walking, standing, and lying. In addition, it was noted if the animals performed rumination. The observations were made from the roof of the cage, reducing any effect on the behaviour of the lambs. These behaviours were also recorded by Bradshaw *et al* (1995).

Statistical analysis

All analyses were performed using Statgraphics Plus (1994) software for parametric and non-parametric data. A chi-square test was used to compare the numbers of lambs performing particular behaviours between both stocking densities (high and low) for the total journey time (30 min). An unpaired *t*-test was performed to examine the effect of the stocking density on the percentage of weight loss. The blood parameters were analysed statistically by analysis of variance using the model:

$$y_{ijk} = \mu + SD_i + L_j + SDxL_{ij} + e_{ijk}$$

where y_{ijk} is the blood measurement, μ is the population mean, SD_i is the stocking density, L_j is the lairage condition, $SDxL_{ij}$ is the interaction between factors, and e_{ijk} is the error variance. The Newman-Keuls method was used for multiple means comparison. LDH, CK, Ca^{2+} and PCV were normally distributed, whereas the cortisol concentrations were normally distributed when $\log_{10}$ transformed. Simple correlations and linear regression equations were calculated on LDH, CK and Ca^{2+} to describe any relationships among these parameters.

Results

The total numbers of lambs walking and standing during transport were significantly different ($P < 0.001$) depending on the stocking density, but there was no difference between the stocking densities in the numbers of lambs lying ($P > 0.05$; see Table 1). The number of lambs walking during transport was greater at low stocking density. In contrast, the number of lambs standing up during transport was greater at high stocking density.

Stocking density did not significantly affect the loss of live weight during transport ($P > 0.05$) although there was an insignificant trend towards higher live weight loss in lambs transported at low than at high stocking density (Table 2).

Table 1 Number of observations for the total journey in each stocking density.

Behaviours	8 lambs m^{-2} (32 lambs x 30 min = 960)*	4 lambs m^{-2} (16 lambs x 30 min = 480)*	χ^2	*P* value
Walking	20	84	113.51	0.0000
Standing	852	366	38.34	0.0000
Lying	88	30	3.62	0.0571

*Calculation of the total number of observations

Table 2 Differences in live weight losses (mean ± SE) of lambs during transport.

	8 lambs m^{-2}	4 lambs m^{-2}	*t*-value	*P* value
Live weight losses (%)	0.59 ± 0.14	0.81 ± 0.10	–1.26	0.2162

The plasma concentration of LDH isozymes was significantly ($P < 0.001$) higher in lambs transported at low than at high stocking density, whereas there were no significant effects ($P > 0.05$) of stocking density on the rest of the analysed blood variables (Table 3). The plasma activity of LDH was also significantly greater for the 1.5 h lairage group than the 3 h lairage group ($P < 0.001$) for both stocking densities. There was no significant interaction between stocking density and lairage ($P > 0.05$) on the plasma LDH concentration.

Table 3 Differences in blood parameters of lambs transported at two stocking densities and two lairage periods.

Stocking density	8 lambs m^{-2}		4 lambs m^{-2}			*P* values		
Lairage (h)	1.5	3	1.5	3	MSE	SD	L	Interaction SD x L
LDH (U litre^{-1})	1427.75	1221.38	2056.25	1594.88	75.32	0.0000	0.0001	0.1016
CK (U litre^{-1})	571.50	948.62	567.75	630.12	84.64	0.0673	0.0148	0.0735
Ca^{2+} (mg dl^{-1})	9.14^{a}	13.00^{c}	9.46^{a}	11.51^{b}	0.30	0.0630	0.0000	0.0054
PCV (%)	25.60^{a}	38.67^{b}	31.91^{ab}	32.25^{ab}	2.17	0.9795	0.0046	0.0067
Cortisol (log_{10} µg dl^{-1})	0.48	0.16	0.28	0.03	0.10	0.1293	0.0117	0.7500

L, lairage; MSE, mean square error; SD, stocking density

Means in the same row with different superscript letters are different ($P < 0.05$) with respect to interaction

The plasma concentrations of CK isozymes and Ca^{2+} were significantly affected by the lairage period ($P < 0.05$ and $P < 0.001$, respectively). Plasma levels of CK and Ca^{2+} were greater in the 3 h lairage group than in the 1.5 h group. There was a significant interaction between stocking density and lairage ($P < 0.01$) in plasma Ca^{2+} concentration, and lambs transported at high stocking density and with 3 h lairage showed the greatest plasma concentration of Ca^{2+}.

The PCV was also significantly affected by lairage ($P < 0.01$), but there were significant differences among PCV values in lambs transported at high stocking density only, being higher after 3 h lairage than after 1.5 h. There was a significant interaction between stocking density and lairage ($P < 0.01$).

The plasma concentration of cortisol was significantly ($P < 0.05$) higher in the 1.5 h lairage group at both stocking densities. There was no significant interaction between lairage and stocking density ($P > 0.05$).

Data for correlation analyses were found to be homogeneous (and of similar magnitude and direction) for both stocking density and lairage; therefore, data were pooled. The relationship between CK and Ca^{2+} concentration was positive ($r = 0.63$, $P < 0.001$). Lambs with higher serum CK had higher serum Ca^{2+} concentrations. LDH and serum Ca^{2+} values were negatively correlated ($r = -0.45$, $P < 0.01$). Lambs with higher LDH had lower Ca^{2+} concentration than did lambs with lower LDH.

Discussion

The behaviour of lambs is shown to be significantly affected by stocking density during transit. The majority of lambs stood up for the duration of the transportation period, but there were more lambs standing at high stocking density than at low. Other authors have also found a trend for more animals to remain standing at higher stocking densities (Knowles *et al* 1998). The greater number of lambs observed to be walking at low stocking density might be a result of the higher proportion of space per animal, hence increasing their capacity for movement, and factors such as lorry movement which might have caused them to move to retain their balance. Adult sheep have been shown to spend the first four hours of the journey (Knowles *et al* 1995) or most of their transport time standing (Bradshaw *et al* 1996).

The number of lambs lying down was not significantly different between stocking densities. However, some authors have found that after 3 h of transport, the proportion of sheep lying down was significantly lower at a space allowance of 0.22 m^2 per sheep than at other higher space allowances (Cockram *et al* 1996). This disagreement with respect to lying behaviour could be because lying behaviour may not be a good welfare indicator when there is insufficient space to lie down. In addition, in sheep this behaviour shows both a high imitative component towards others lying down and a strong individual component (Hall *et al* 1998).

Rumination behaviour is usually shown when animals are at leisure, and many factors — such as transport — may cause its cessation (Fraser & Broom 1990). The lack of rumination behaviour during transport was likely to be because the lambs were too young; they might resemble young calves in this respect, which have been shown not to ruminate in the first hour after loading (Kent & Ewbank 1986).

The loss of live weight was minimal, without significant differences between stocking densities. This is what would be expected for short journeys, where weight changes are produced only by defecation and urination (Knowles *et al* 1995).

LDH was the only blood parameter significantly affected by stocking density. LDH is associated with muscle fatigue (weariness) and also increases with physical stress (Knowles *et al* 1993). The higher LDH activity observed at the lower stocking density may be attributable to the higher number of lambs walking, suggesting that an increased space allowance per lamb results in higher levels of plasma LDH because of the greater amount of exercise of these lambs. The lower LDH concentration after 3 h lairage suggests that the lambs had begun to recover from the direct effects of the physical exertion caused by the journey, perhaps because of a decrease in anaerobic metabolism after the muscular exertion of transport and unloading.

There was no significant effect of stocking density during transport on the mean plasma activity of CK and Ca^{2+} concentration, in agreement with Cockram *et al* (1996). Others found a marked increase in CK levels only in lambs transported at the highest stocking density during the winter months (Knowles *et al* 1998). This enzyme leaks from muscle cells because of the increased permeability resulting from physical exertion (Knowles *et al* 1993), particularly when damaged (Broom *et al* 1996). This result, therefore, indicates that there is no difference in the muscular damage produced to lambs transported at the different stocking densities. The higher plasma concentration of CK after 3 h lairage suggests that CK has had time to reach a greater level of activity than after the shorter lairage period, as the effects of previous transport and handling have been shown to continue to manifest themselves because of long-term changes in the permeability of muscle cell membranes (Warriss *et al* 1992). On the other hand, Knowles *et al* (1995) did not find detectable differences in plasma CK levels among groups of lambs after journeys of different durations. There is a positive correlation between CK and Ca^{2+}, because they are both released into the blood when muscle cell walls are damaged. Furthermore, the negative correlation between LDH and Ca^{2+} might reflect the quick recovery of aerobic metabolism although muscle cell wall damage persists.

The plasma concentration of cortisol was not affected by stocking density, supporting earlier observations (Cockram *et al* 1996), although some workers have shown differences between stocking densities but offered no clear interpretation (Knowles *et al* 1998). High levels of plasma cortisol are usually associated with short-term stress, being marked at the beginning of a journey and becoming less obvious as the journey proceeds (Cockram *et al* 1996), implying adaptation as the journey progresses (Hall *et al* 1998; Knowles *et al* 1995). The higher plasma concentration of cortisol after the shorter lairage period might reflect the effects of prior transport or handling. Meanwhile, lambs held in lairage for 3 h may have begun to recover their basal cortisol levels and also begun to adapt to their novel pen and strange environment. Because of this adaptation, the stress resulting from different stocking densities cannot be accurately assessed through measurement of cortisol after 3 h or more of lairage time has elapsed.

There was no significant effect of stocking density on PCV, in agreement with previous studies (Cockram *et al* 1996; Knowles *et al* 1998). Some authors found a significant systematic increase in PCV during transport in lambs (Knowles *et al* 1995, 1998). In the present study, the higher PCV observed after the longer lairage and higher stocking density might be only mildly related to dehydration of the animals; it may be more strongly related to excitement, caused by transport and handling, which releases erythrocytes into the circulation as a result of sympatho-adrenal stimulation causing a contraction of the spleen (Crookshank *et al* 1979; Knowles *et al* 1995).

The effects of space allowance and group size may be confounded in the present experimental design. This may lead one to suppose that the greater group size of the high-density groups could affect the behaviour of the lambs; however, sheep of similar age show relatively weak dominance relationships (Lynch *et al* 1992). Moreover, no effects of group size on activity levels of pigs, which have a strong social hierarchy, have been found (Spoolder *et al* 1999). Considering the small group size used here, and the fact that the same social groups were maintained throughout the study, it is therefore tempting to infer that group size has a minimal effect.

The lambs used in this study were separated from their dams at the moment of loading and their live weights were only about 13 kg, so it is possible that they endured the transport and lairage worse than more mature animals. Some authors have suggested that calves are relatively unable to adapt physically to transport in the way that is observed in more mature animals (Knowles *et al* 1997); in addition, strong negative correlations between age at transport and mortality rate have been found (Knowles *et al* 1995). Weaning and handling increase CK and LDH values in young calves (Crookshank *et al* 1979) so they could also have influenced the high results for LDH and CK obtained in the present study.

Although blood collected at exsanguination is not ideal for estimating the effects of transport, this method can be valuable when animals are submitted to commercial conditions (Moss & Rob 1978; Warriss *et al* 1998, Brown *et al* 1999). Moreover, it has to be considered that electrical stunning might cause muscular damage (Gregory 1998) and might increase plasma cortisol concentration in sheep (Pearson *et al* 1977).

In summary, the behaviour of suckling lambs during transport has been shown to differ as a result of stocking density. From the results obtained, it seems that neither of the analysed stocking densities significantly affects the welfare of suckling lambs, affecting only the plasma concentration of LDH isozymes, probably as a result of the greater physical exertion of lambs transported at lower stocking density. It seems that a longer lairage period allows lambs to recover from psychological stress, because their cortisol levels decrease, but the plasma activity of CK continues to increase during the lairage period. The observed differences from previously published results could be attributable to the age of the lambs used in this work, although many other factors may be involved. Further work is required under commercial conditions to study the effects of transport in young lambs, and to determinate the ideal stocking density and conditions of transport, as well as to evaluate the effects of lairage prior to slaughter.

Animal welfare implications

Transport is a stressful experience for all farm livestock, including young lambs, and it has behavioural and physiological effects. In this study, the welfare of suckling lambs transported for short-duration journeys was studied. The results show that the behaviour of young lambs is affected by stocking density in that lambs are submitted to more physical exercise at lower stocking densities. As a consequence, it will be necessary to define the minimum space requirements for young lambs transported short distances. A lairage period of three hours may allow lambs to recover from psychological stress, but not from physical stress.

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