BEHAVIOURAL EFFECTS OF SOCIAL MIXING AT DIFFERENT STOCKING DENSITIES IN PREPUBERTAL LAMBS

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Abstract *Animal Welfare 1999, **8:*** 117-126

The behavioural effects of mixing individuals from two different flocks were studied in prepubertal lambs of about 20kg body weight kept at either low (1 animal m^{-2}) or high (3.3 animals m^{-2}) stocking densities. At both densities, flock mates associated preferentially with one another over the three experimental days. The social mixing conditions decreased the total number of aggressive interactions (including head-to-head clashes, head-to-body buttings and mountings). Since animals associated preferentially with flock mates, aggressive behaviours were also preferentially directed towards individuals from the same flock. Males initiated significantly more aggressive interactions than females. The total number of aggressive interactions received was similar for males and females, but females received more mountings than males. Stocking density, therefore, had no effect on aggressive behaviour. These results are discussed as they relate to transport and it is suggested that social mixing may not be a welfare problem in prepubertal lambs.

Keywords: *aggression, animal welfare, mixing, sheep, stocking density, transport*

Introduction

Mixing of unacquainted animals leads to an increase in agonistic activity in several species, including pigs (McBride *et al* 1964; Shenton & Shackleton 1990) and cattle (Grandin 1978; Mench *et al* 1990). It is widely accepted that this increase in aggression is related to the establishment of dominance relationships between individuals previously unknown to one other (Fraser & Rushen 1987).

Fighting may cause injuries and is also known to be a potent activator of the pituitary-adrenal axis (Arnone & Dantzer 1980; Blecha *et al* 1985). Particularly when its effects are compounded by other stressors, mixing animals may have deleterious effects on both productivity and welfare (Shenton & Shackleton 1990). Furthermore, mixing shortly before slaughter can lead to reduced carcase and meat quality in pigs (Guise & Penny 1989) and cattle (Price & Tennessen 1981).

Although mixing of unfamiliar sheep may occur during transport and lairage, the effects of this practice upon sheep behaviour and welfare are not known. Sheep have been reported to show individual recognition (Baldwin & Meese 1977; Kendrick 1990 pp 144-156) and, therefore, the possibility exists that they can recognize unfamiliar animals and respond to them at mixing. However, the effects of any dominance relationships in sheep seem to be relatively weak, particularly between animals of similar ages (Lynch *et al* 1992 Chapter 2), and young sheep show a low level of agonistic interactions (Schaller & Miraz 1974 pp 306-323). It has been shown that when sheep from two different flocks are penned together, each individual tends to associate only with its flock mates for some considerable time (McBride *et al* 1967). This would decrease the possibility of social interactions taking place between unknown animals. It has to be stressed, however, that the previous study involved adult sheep kept at very low densities; therefore its results may not be fully applicable to younger animals under the conditions commonly encountered during transport.

The aim of this study was to assess the behavioural effects of mixing lambs from two different flocks. Since we were particularly interested in this problem as it relates to transport and lairage, the age and weight of the animals used was selected to match those of lambs commonly slaughtered in Spain. In addition, since reducing stocking density can also lead to an increase in aggression (Fraser & Rushen 1987) the effects of mixing at two different stocking densities were compared.

Animals, materials and methods

The subjects for the trials were 80 lambs (43 males and 37 females) of the Ripollesa breed (an autochthonous breed from Northern Spain), 20–22 kg in body weight and 10–12 weeks old. Sixty animals came from one flock (A) and 20 from another (B). Animals from flock A were randomly divided into six groups of 10 animals and animals from flock B were randomly divided into two groups, each containing 10 animals. During non-experimental periods, the animals were kept in a straw-bedded pen (allowing $2m^2$ per animal) and taken to graze outside during the day. They were given good quality hay, commercial feed for growing lambs and water *ad libitum*. Animals were marked with letters on their backs and sides to allow individual identification.

Four experimental trials were undertaken: i) low stocking density without social mixing (LD, 1 animal m^{-2} and two groups of 10 lambs, both coming from the same flock); ii) low stocking density plus social mixing (LDM, 1 animal m^{-2} and two groups of 10 lambs, each coming from a different flock); iii) high stocking density without social mixing (HD, 3.3 animals m^{-2} and two groups of 10 lambs, both coming from the same flock); and iv) high stocking density plus social mixing (HDM, 3.3 animals m^{-2} and two groups of 10 lambs, each coming from a different flock). In all experimental trials the shape and size (5x4 m) of the pens were the same.

Each experimental trial consisted of two adaptation days followed by three experimental days. On adaptation days, the experimental pen was divided into two areas of the same size by means of a solid barrier which prevented visual contact. Each group of animals spent one adaptation day on each side of the barrier. This method allowed the animals to explore the whole pen, thus reducing exploratory behaviour during the experimental days. At the beginning of the third day, the barrier was removed and behavioural observations commenced.

On each experimental day behaviour was recorded during two, 1.5h sessions (from 0830h to 1000h and from 1600h to 1730h). This gave a total of 9h of behavioural recording, for each experimental trial. Behavioural observations were recorded by means of a video camera

(Sony CCD-V600-E, Sony Supplier, Barcelona, Spain) and the analyses of behaviour were always performed by the same person. The following variables were measured:

i) Degree of association. This was measured by recording whether an animal's nearest neighbour was a group mate. This was recorded 50 times per animal on each experimental day (ie every 10min), giving a total of 3000 observations per experimental trial (50 observations per animal and experimental day x 20 animals per trial x 3 experimental days per trial). The degree of association was calculated as the percentage of observations in which the observed animal's nearest neighbour was a group mate.

ii) Aggressive behaviour. This included head-to-head clashes (HHC), head-to-body buttings (HBB) and mountings (MOU). Although mounting is not always an aggressive behaviour, it was included here following other studies on sheep social behaviour (Lynch *et al* 1992 Chapter 2). Bouts of the same type of aggressive behaviour between two individuals were treated as being one interaction. Aggressive behaviour was recorded as the number of aggressive interactions initiated by each animal and as the number of aggressive interactions received by each animal. This method avoided statistical dependence among data. Since animals were individually marked, the direction of aggressive interactions was also recorded, enabling determination of whether the two animals involved were group mates, as well as the sex of the animals involved.

Statistical analysis

The degree of association was analysed using a chi-square test. The expected degree of association was 0.474 (9 group mates from a total of 19 animals). The number of aggressive interactions was analysed using a two-way ANOVA test. Sex differences were analysed using a Mann-Whitney *U* test, pooling data from the four experimental trials. The direction of aggressive interactions was analysed using a chi-square test and correlations between the different types of initiated aggressive interactions were analysed by Spearman rank-order correlation coefficients. All statistical analyses were conducted on SPSS©, version 7.5.2S, software (SPSS Inc, Chicago, USA).

Results

Degree of association

The observed degree of association was higher than expected in LDM and HDM trials ($P < 0.001$), while no difference was found between the observed and the expected values in LD and HD trials (Table 1). Therefore, it appears that when mixed with unfamiliar groups, animals associated preferentially with group mates.

Table 1 **Observed and expected degrees of association over three experimental days. (See text for description of abbreviations.) ns – not significant; ******P* **< 0.001.**

	Trial			
	LD	**LDM**	**HD**	**HDM**
Observed	0.473	0.762	0.469	0.781
Expected	0.474	0.474	0.474	0.474
P	ns	***	ns	***

Total number of aggressive interactions

The total number of aggressive interactions (TNA) initiated per animal over the three experimental days was higher in trials without social mixing than in trials with social mixing ($P < 0.05$). Stocking density had no effect on the total number of aggressive interactions (Table 2).

Table 2 Mean (± SEM) number of aggressive interactions initiated per animal over three experimental days. (See text for description of abbreviations.) ns – not significant; *$P < 0.05$; **$P < 0.01$.

	Trial				ANOVA		
Behaviour	LD	LDM	HD	HDM	Stocking density	Social mixing	Stocking density-social mixing interaction
HBB	5.95 (1.76)	4.60 (1.74)	8.25 (2.92)	4.25 (1.59)	ns	ns	ns
HHC	4.45) (0.85	2.10 (0.49)	2.50 (0.67)	1.15 (0.29)	*	**	ns
MOU	2.30 (0.72)	2.40 (0.81)	3.80 (1.17)	0.80 (0.24)	ns	ns	ns
TNA	12.70 (2.72)	9.10 (2.44)	14.55 (3.81)	6.20 (1.89)	ns	*	ns

The number of HHC initiated per animal over the three experimental days was affected by stocking density ($P < 0.05$) and social mixing ($P < 0.01$): it was higher in trials at low stocking density as compared with trials at high stocking density, and in trials without social mixing as compared with trials involving social mixing (Table 2). The levels of initiated HBB and MOU were not affected by stocking density or by social mixing (Table 2).

The TNA received per animal over the three experimental days was higher in trials without social mixing as compared with those involving social mixing ($P < 0.001$). Stocking density had no effect on the total number of aggressive interactions (Table 3).

Table 3 Mean (± SEM) number of aggressive interactions received per animal over three experimental days. (See text for description of abbreviations.) ns – not significant; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

	Trial				ANOVA		
Behaviour	LD	LDM	HD	HDM	Stocking density	Social mixing	Stocking density-social mixing interaction
HBB	5.95 (1.01)	4.60 (0.91)	8.25 (0.81)	4.25 (0.45)	ns	**	ns
HHC	4.45 (0.94)	2.10 (0.50)	2.50 (0.58)	1.15 (0.36)	*	**	ns
MOU	2.30 (0.56)	2.40 (0.63)	3.80 (0.75)	0.80 (0.28)	ns	*	**
TNA	12.70 (1.79)	9.10 (1.48)	14.55 (1.15)	6.48 (0.69)	ns	***	ns

The number of HBB received per animal over the three experimental days was higher in trials without social mixing than in trials involving social mixing ($P < 0.01$). Stocking density had no effect on the total number of interactions (Table 3).

The number of HHC received per animal over the three experimental days was affected by stocking density ($P < 0.05$) and social mixing ($P < 0.01$): it was higher in trials at low stocking density as compared with trials at high stocking density, and in trials without social mixing as compared with trials involving social mixing (Table 3).

The number of MOU received per animal over the three experimental days was affected by social mixing ($P < 0.05$) and by an interaction between social mixing and stocking density ($P < 0.01$): levels were lower at low stocking density than at high stocking density in trials without social mixing, whereas in trials with social mixing, they were higher at low stocking density than at high stocking density (Table 3).

As we were particularly interested in the welfare problems related to transport and lairage, we also analysed aggressive behaviour on the first experimental day. Since the total numbers of each type of aggressive interaction recorded during the first experimental day were low, it was not possible to analyse each type of aggressive interaction independently, so the total of all aggressive interactions was analysed.

The total number of aggressive interactions initiated per animal on the first experimental day was affected by an interaction between stocking density and social mixing ($P < 0.05$): in trials without social mixing, it was lower at low stocking density than at high stocking density, whereas in trials with social mixing, it was higher at low stocking density than at high stocking density (Table 4).

Table 4 Mean (± SEM) of the total number of aggressive interactions initiated and received during the first experimental day. (See text for description of abbreviations.) ns – not significant; *$P < 0.05$; **$P < 0.01$.

	Trial				ANOVA		
	LD	**LDM**	**HD**	**HDM**	**Stocking density**	**Social mixing**	**Stocking density-social mixing interaction**
Aggressive interactions initiated	3.75 (0.84)	5.00 (1.72)	7.35 (1.98)	1.40(0.45)	ns	ns	*
Aggressive interactions received	3.75 (0.68)	5.00 (1.09)	7.35 (0.85)	1.40 (0.27)	ns	**	**

The total number of aggressive interactions received per animal on the first experimental day was affected by social mixing ($P < 0.01$) and also by an interaction between stocking density and social mixing ($P < 0.01$): in trials without social mixing, it was lower at low stocking density than at high stocking density, whereas in trials with social mixing, it was higher at low stocking density than at high stocking density (Table 4).

Sex differences

Males initiated significantly more aggressive interactions than females (TNA, $P < 0.001$; HBB, $P < 0.001$; HHC, $P < 0.001$; MOU, $P < 0.05$). Females received more MOU than males ($P < 0.05$). No difference was found between males and females for TNA received, number of HBB received, and number of HHC received (Figure 1).

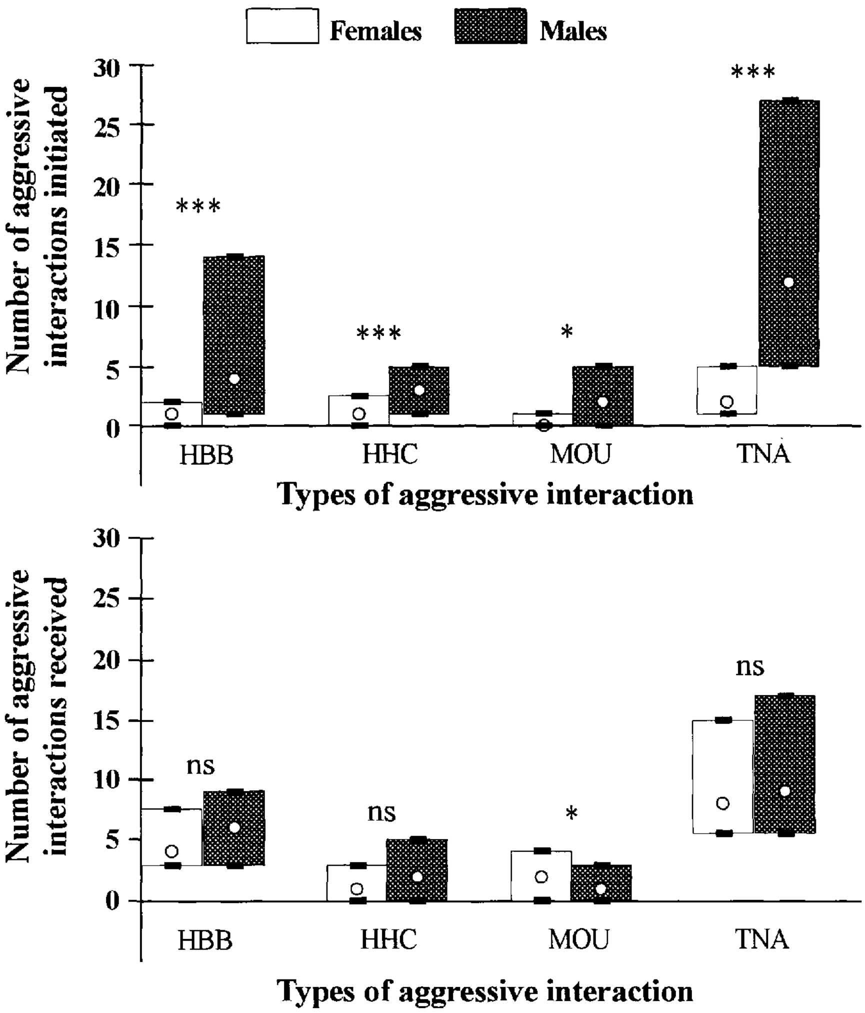

Figure 1 Median (circles) and upper and lower quartiles (tops and bottoms of bars) for three types of aggressive interactions initiated and received by males and females over three experimental days. (See text for description of abbreviations.) ns – not significant; *$P < 0.05$; ***$P < 0.001$.

Direction of aggressive interactions

In trials without social mixing, the proportion of aggressive interactions between group mates was similar or lower than expected by chance. In trials with social mixing, the proportion of aggressive interactions between group mates was similar or higher than expected (Table 5).

Table 5 **Observed percentages[1] of different types of aggressive interactions between group mates over three experimental days. (See text for description of abbreviations.) ns – not significant; *P < 0.05; ** P <0.01; ***P < 0.001.**

	Trial			
Type of aggressive interaction	**LD**	**LDM**	**HD**	**HDM**
HHC	41.2% ns	60.9% **	49.1% ns	57.6% ns
HBB	36.0% *	81.0% ***	40.0% ns	43.5% ns
MOU	50.0% ns	75.0% ***	43.4% ns	100.0% [2]
TNA	40.9% *	69.2% ***	46.0% ns	60.5% **

[1] Expected percentage in each class = 47.4 per cent.
[2] Mountings only observed between group mates, so no statistical comparisons were possible.

Correlations between types of initiated aggressive interactions

Correlations between each type of aggression initiated by a given animal were calculated by pooling the data from the four experimental trials. When males and females were considered together, significant correlations were found between HBB and HHC (P < 0.01), HBB and MOU (P < 0.01), and HHC and MOU (P < 0.001). Among males, significant correlations were found between HBB and HHC (P < 0.05) and between HHC and MOU (P < 0.001). No significant correlations were found among the females (Table 6).

Table 6 **Spearman rank-order correlation coefficients (r_s) between the number of different types of aggressive interactions initiated in four experimental trials (each lasting three days) and their statistical significance. (See text for description of abbreviations.) ns – not significant; * P < 0.05; ** P <0.01; *** P < 0.001.**

Sex	**HBB-HHC**	**HBB-MOU**	**HHC-MOU**
Females + males (n = 70)	0.377 **	0.315 **	0.541 ***
Females (n = 29)	0.210 ns	0.325 ns	0.308 ns
Males (n = 41)	0.315 *	0.031 ns	0.581 ***

Discussion

In general, our results suggest that mixing unacquainted groups of lambs does not increase aggressiveness, but rather decreases it. This is a rather different situation from that encountered in pigs (McBride *et al* 1967; Shenton & Shackleton 1990) and cattle (Grandin 1978; Mench *et al* 1990). Differences between sheep and other ungulates in this respect may be a consequence of the fact that dominance relationships tend to be rather weak in sheep, particularly when all animals are the same age (Lynch *et al* 1992). The association between familiar animals may also help to explain the results. Our results show that lambs preferentially associate and interact with flock mates. A similar result was obtained by McBride *et al* (1967) for adult animals kept on pasture. In trials with social mixing, the number of familiar animals a given animal can interact with is smaller than in trials without social mixing (ie in our trials, after social mixing, each lamb had only 9 flock mates to interact with, compared with 19 flock mates in trials without social mixing).

As we were particularly interested in transport and lairage, results from the first experimental day were analysed independently. Due to the low number of each type of aggressive interaction initiated and received per animal on any one day, aggressiveness on the first experimental day was analysed using the total number of aggressive interactions (Table 4). Our results show that at low stocking density, social mixing increased the number of aggressive interactions, whereas at high stocking density, it reduced their number. Since the stocking density commonly found during transport and lairage is likely to be rather high (Broom 1984), our results suggest that mixing is not likely to be a major welfare problem during the transport of prepubertal lambs.

The only type of aggressive interaction affected by stocking density was HHC, which were less frequent at high stocking density than at low stocking density. This may be due to the fact that they require a considerable amount of space. A similar effect has been described for the so-called parallel-inverse pressing behaviour of pigs (Baxter 1985).

Social mixing decreased the number of HBB, HHC and MOU received, whereas it only decreased the number of initiated HHC. This difference between initiated and received aggressive interactions is mainly due to variability between individuals (the SEMs were up to 3.6 times greater for initiated than for received aggressive interactions). Furthermore, over 80 per cent of aggressive interactions were initiated by 10 per cent of the animals, while the same percentage of animals received up to 60 per cent of the aggressive interactions. This variability was partially explained by the sex of the animals, since males initiated more aggressive interactions than females, but both sexes received the same number of aggressive interactions overall (with the exception of MOU which were predominantly received by females).

The Spearman rank-order correlation coefficients between initiated aggressive interactions suggest that the three types of aggressive interaction share a common motivation. Furthermore, the highest correlation appeared between HHC and MOU in males, and these behaviours are known to be sex-related (ie controlled by male sex hormones [Lynch *et al* 1992 Chapter 2]).

From the above discussion, it is tempting to infer that mixing unacquainted individuals should not pose a serious welfare concern during sheep transport and lairage. However, at least three points should be considered before reaching such a conclusion:

i) Behavioural measures may not always be a reliable indicator of an animal's response to social challenges (Hinde 1985); therefore work should be undertaken to assess the effect of social mixing upon physiological measures such as heart rate.

ii) It has been shown in other species (Lyons *et al* 1988), that the effects of a given stressor, such as human approach or handling, may be partly dependent on the presence of familiar conspecifics. This would be particularly relevant to transport issues and, therefore, we suggest that further work aimed at studying whether the effects of handling are socially modulated in sheep would be of interest.

iii) Puberty in sheep occurs at 25–30 weeks of age (or about 40kg liveweight [Cunningham 1992]), but this work was carried out with prepubertal animals (10–12 weeks old and weighing 18–20 kg). Therefore, our results cannot be extrapolated to mature animals, in which sexual hormones may cause an increase in aggression (Lincoln & Davidson 1977). In this sense, it is remarkable that even in our experimental conditions, males were more aggressive than females. These differences in young animals have also been found in play behaviour (Schubert & Scheibe 1993) and could be due to the organizing effects of male sexual hormones during the prenatal period (Breedlove 1992).

Animal welfare implications

It is widely accepted that mixing unacquainted individuals is one of the main stressors for animals during transport and lairage. Experimental data has shown this to be true in pigs and cattle. This study suggests that the situation may be different in prepubertal lambs. Our results also emphasize the point, that recommendations to improve animal welfare should be tailored not only to each particular species but also to each type of animal within a species.

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