267. Bradshaw, R.H., Parrott, R.F., Goode, J.A., Lloyd, D.M., Rodway, R.G. and Broom, D.M. 1996. Behavioural and hormonal responses of pigs during transport: effect of mixing and duration of journey. *Anim. Sci.*, 62, 547-554.

Behavioural and hormonal responses of pigs during transport: effect of mixing and duration of journey

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Abstract

Two experiments investigated the welfare of pigs during transport. In experiment 1, 12 groups of four 90-kg pigs were transported to slaughter in a commercial livestock lorry for 1·5 h. Half the animals were transported in their social groups (unmixed condition) and half were transported with groups of previously unfamiliar pigs mixed together (mixed condition). Behaviour was recorded, a general activity index scored and saliva samples taken at different stages of the journey for analysis of cortisol. Pigs spent most of their time standing in both conditions. The journey was very rough (as revealed by characterization with an accelerometer) and in the unmixed condition the pigs appeared to stand to reduce travel sickness. In contrast, in the mixed condition, this preference for standing seemed to be due to fighting which stressed and exhausted the animals (the general activity index was three times the unmixed condition). Levels of salivary cortisol were higher in the mixed condition at the beginning, middle and end of the journey. In experiment 2, six 35-kg pigs, prepared in advance with jugular vein catheters, were loaded onto a commercial livestock lorry (09.30 h) where they were individually penned. The vehicle remained stationary with the engine off and blood samples were taken at 30-min intervals during the next 8 h (control). Two days later this procedure was repeated while the vehicle was driven for 8 h (on main roads and motorways). Plasma concentrations of cortisol and beta-endorphin increased markedly in both conditions immediately after loading. Cortisol levels were greater (relative to control) at the beginning, in the middle and at the end of the journey. Concentrations of beta-endorphin did not differ between control and experimental conditions except during the final 180 min of the journey when the control levels were higher.

Keywords: *endorphins, hydrocortisone, mixing, pigs, transport.*

Introduction

Pigs are usually transported during the course of their life and various behavioural and physiological effects of the transportation process have been reported (e.g. Becker, Mayes, Hahn, Nienaber, Jesse, Anderson, Heymann and Hedrick, 1989; Nyberg, Lundström, Edfors-Lilja and Rundgren, 1988; Warriss, Bevis, Edwards, Brown and Knowles, 1991; Dalin, Magnusson, Häggendal and Nyberg, 1993; Geers, Bleus, van Schie, Villé, Gerard, Janssens, Nackaerts, Decuypere and Jourquin, 1994). Most research concerned with the welfare of pigs during transport has been directed at examining the effects of the physical environment (e.g. Lambooy and Engel, 1991; Randall, 1993), simple behavioural time budgets (e.g. Lambooy, 1988; Bradshaw, Hall and Broom, 1995) and levels of stress hormones in the blood (Spencer, Wilkins and Hallett, 1984; Dalin, Nyberg and Eliasson, 1988; Nyberg *et al.*, 1988; Dalin *et al.*, 1993; Geers *et al.*, 1994).

While these studies are important, the social environment should be considered no less important in relation to welfare. It is well known that unfamiliar pigs (when not being transported) fight and that this leads to elevated levels of cortisol (e.g. Parrott and Misson, 1989; Tan and Shackleton, 1990; Jensen, 1994). The practice of mixing unfamiliar pigs still commonly occurs during transportation (Geverink, Bradshaw, Lambooy and Broom, 1996) and there have been few studies which specifically examine whether the mixing of unfamiliar pigs during road transport is an important challenge to welfare. Guise and Penny (1989) showed that mixing at loading led to more carcass damage, which can be presumed to compromise welfare. Warriss and Brown (1985) showed that mixing led to increased skin damage and the greater the degree of damage the higher were the concentrations of cortisol, glucose and lactate in blood collected from pigs at slaughter.

Studies which have attempted to investigate changes in plasma hormone concentrations during transport have been frequently restricted to sampling before and after journeys (e.g. Dalin *et al.*, 1988; Geers *et al.*, 1994; Nyberg *et al.*, 1988; Rajanasthien, 1989) or sampling during journeys of short duration (e.g. Becker, Nienaber, DeShazer and Hahn, 1985). This is presumably due to the practical difficulties inherent with blood sampling by venipuncture when in transit. However, a recent study allowed pigs to be sampled during a short (1-h) journey by means of an indwelling venous catheter implanted in each animal (Dalin *et al.*, 1993). This procedure alleviates the stressful effects of venipuncture and allows samples to be taken during the journey without having to halt the transporter.

Two situations are described in this paper. The first investigated behavioural and salivary cortisol responses of pigs to road transport when loaded in small familiar social groups or when loaded and mixed with other unfamiliar pigs. The second used catheterized animals to examine stress hormone (cortisol and beta-endorphin) responses during a long distance (8-h) journey in which pigs were sampled during transit.

Material and methods

Experiment 1

Forty-eight 90-kg (Landrace × Large White) slaughter pigs, divided into 12 mixed sex groups of four, were used (13 male: 27 female). Each group consisted of three pigs which had been housed together for 20 weeks with a fourth individual introduced 1 week before transport. This procedure was a result of a separate study investigating the effects of mixing at the farm on welfare. Analysis of agonistic behaviour (from video tapes) revealed that fighting had ceased within 48 h of the introduction of the fourth pig. This procedure was followed for all groups of pigs. Pigs were given food from a trough twice a day at 08.00 h and 15.00 h (Dalgety Ultrabreed 16 nuts) and the quantity offered was 2 kg per pig per feed. All pigs were used to close contact with humans. A commercial livestock lorry (four-wheeler rigid chassis) was hired with internal penning such that pigs could either be transported in their individual groups of four (unmixed condition) or three groups could be mixed together (mixed condition). All pigs were penned at a stocking density of 0·49 m² per pig (regardless of whether they were mixed). This stocking density is lower than normal commercial practice and was due to constraints imposed by vehicle design (due to the small size of the groups). Food was withdrawn 2·5 h before transport (just after their morning feed). This decision was taken in order to exacerbate any potential signs of travel sickness and thereby render it detectable (since any more subtle signs of travel sickness may have been overlooked if the pigs failed to display overt signs of sickness).

In week 1, and again in week 2, three groups of four pigs were loaded onto the lorry (by driving up a 24° ramp onto the lower deck of the vehicle) at 10.30 h, mixed into a single pen (mixed condition) and tranported 1·5 h to slaughter. The procedure followed in weeks 3 and 4 was the same except that the animals were penned in their individual groups (unmixed condition). One pig in each condition (weeks 1 and 3 respectively) was deemed unfit to travel. Transporting pigs in such small groups does not reflect usual commercial practice and a small group size may be expected to maximize the effects of mixing and social disturbance (in the mixed condition).

The experimenter travelled with the pigs in the main body of the vehicle throughout the journey. Since the pigs were used to close human contact it is envisaged that the presence of the researcher had very little effect on the behaviour and physiology of the pigs. Pigs were scanned every 4 min throughout the journey and the number of animals standing and lying noted. A general activity index was also scored based on a qualitative assessment of the general levels of pig activity every 4 min (5 = high activity; 1 = low activity). Incidences of retching, vomiting and fighting were recorded as they occurred. Mean number of pigs standing or lying, mean activity index and total frequencies of retching, vomiting and fighting were calculated for each condition.

The use of saliva rather than blood for sampling for cortisol has been validated in the pig (Parrott, Misson and Baldwin, 1989; Parrott and Misson, 1989). Saliva samples were collected by allowing the pig to chew on two cotton buds (Johnson and Johnson, Slough, Berkshire) until they were thoroughly moistened (about 30 s). These were taken from each pig immediately before loading on the morning of transport at 09.45 h after loading (10.30 h), following 45 min of transport (11.15 h) and upon arrival at the slaughterhouse (12.00 h) before unloading. The cotton buds were stored in a test tube which was immediately placed on ice and subsequently centrifuged at 1800 r.p.m. for 5 min to remove the saliva which was then stored at −20°C. Saliva was always collected from the pigs in the same order. However, three pigs in each condition did not yield a sufficient quantity of saliva to allow analysis. Salivary cortisol was measured using an enzyme-linked immunosorbent assay (ELISA, Cooper, Trunkfield, Zanella and Booth, 1989). Two comparisons were made using a two-tailed *t* test. Firstly, cortisol concentrations were compared in mixed and unmixed conditions at each stage of the journey. Secondly, a comparison was made of cortisol concentrations at the beginning, middle and end of the journey relative to pre-loading levels. Following these analyses a one-tailed *t* test was also conducted (predicting higher levels in the mixed compared with the unmixed condition and higher levels at each stage of the journey relative to pre-loading levels).

The journey was characterized in week 4 by means of a triaxial accelerometer (EDR - 1s), temperature probe (Grant type CTU thermistor), sound meters (Cirrus CRL 221A integrating meter) and relative humidity probe (Vaisala HMP35A). Sensors recording air temparature (°C), sound (dBA) and relative humidity reported to Grant Squirrel data loggers. A reading was taken every 1 min and every 10 min these were averaged and logged. The accelerometer was set to log every acceleration event greater than 0·707 **g** and of duration greater than 0·012 s, in one or other of the planes *x* (fore and aft), *y* (side to side) or *z* (vertical). There was a period of 0·48 s after a reading before another reading could be logged.

Experiment 2

Six 35-kg male Large White pigs were surgically prepared under general anaesthesia, using sterile precautions, with jugular vein catheters. Surgery was carried out 2 weeks before the experiment and the pigs were kept in individual cages during this period where they became accustomed to close contact with people. Catheter patency was maintained by daily flushing with sterile heparinized saline and catheters were protected by elasticated bandages.

The pigs were then individually loaded onto a lorry at 09.30 h (food was withdrawn at 17.00 h the previous evening). The vehicle was a 3·5-t standard cattle lorry with ventilation louvres (open), metal floor and sides (straw was provided) with internal penning such that pigs were individually confined (pigs will ingest each others catheters if housed in a group). The vehicle remained stationary for the next 8 h; blood samples were collected in 10-ml heparinized sample tubes ('Monovette', Sarstedt Ltd, Beaumont Leys, Leicestershire) every 30 min. At the end of the sampling period (17.30 h), the pigs were individually unloaded and returned to their cages. After a 2-day interval this procedure was repeated with the difference that the vehicle was driven for 8 h on 'A' roads and a motorway, travelling a distance of 455 km. The experimenters travelled with the pigs in the body of the vehicle, power for centrifuges and lighting was provided by a portable generator, and physical data on acceleration events, temperature, humidity and sound were characterized as in the previous experiment. Once again, as the pigs were used to close human contact it is envisaged that the presence of the researchers had little effect on the behaviour and physiology of the pigs. The noise of the generator was not considered an important factor due to the generally high background noise when the lorry was in transit.

On both test days, blood was centrifuged and the resultant plasma divided into aliquots. These were frozen in dry ice and subsequently stored at −30°C pending radioimmunoassay for cortisol and beta-endorphin. Plasma cortisol was assayed as described in Parrott and Goode (1992). Beta-endorphin was assayed as described by Fordham, Lincoln, Ssewannyana and Rodway (1989) using porcine beta-endorphin (Bachem, USA) as iodinated tracer and standards. The antiserum was supplied by Dr G. Lincoln (Edinburgh). Plasma concentrations of cortisol and beta-endorphin averaged over each 3-h period (or 2·5 h in the case of the final period) under stationary and driven conditions were compared using paired *t* tests and the results expressed as two-tailed probability values.

Results

Experiment 1

Pigs, on average, spent all their time standing in the unmixed condition (number of pigs = 24) and most of their time standing in the mixed (number of pigs = 23). The mean activity index was more than three times greater in the mixed rather than in the unmixed condition (3·2 in mixed; 1 in unmixed). The frequency of fights was greater in the mixed, compared with the unmixed (mixed: 20; unmixed: 0) groups. Unmixed pigs became travel sick towards the end of the journey (and began to lie down). Thus frequency of retching and vomiting was much higher in the unmixed condition compared with the mixed (unmixed retching: 2; unmixed vomiting: 9 (a total of eight individuals); mixed retching and vomiting: 0) condition.

Figure 1 shows the mean concentration of salivary cortisol for mixed and unmixed pigs. Before loading (and before any pigs were mixed) there was no difference in levels of cortisol between conditions.

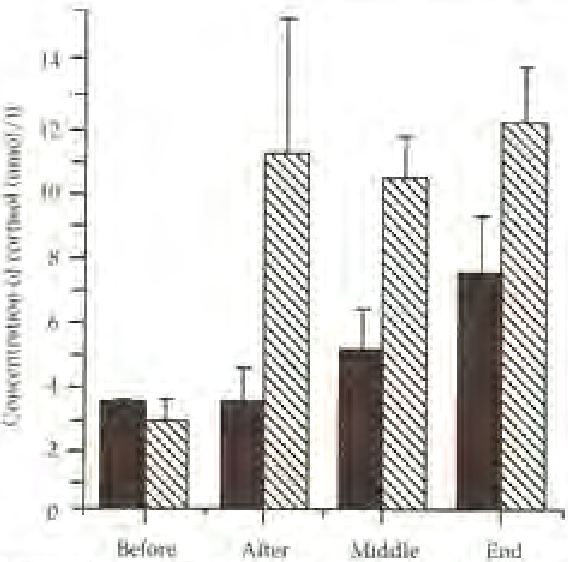

Figure 1 Concentration of salivary cortisol (nmol/L mean ± s.e. before loading (09.45 h), after loading (10.30 h), in the middle (11.15 h) and at the end (12.00 h) of a 1·5 h journey for unmixed (solid) and mixed (hatched) pigs. Mixed v unmixed comparison (i) two-tailed *t* test — levels significantly higher in the mixed condition in the middle ($P < 0.01$) and approached significance after loading ($P < 0.08$) and at the end ($P < 0.06$); (ii) one-tailed *t* test — levels significantly higher in mixed condition at all stages (beginning $P < 0.05$; middle $P < 0.01$, end $P < 0.05$). Before loading there was no difference between conditions ($P > 0.05$). Different stages of journey v. pre-loading levels (i) two-tailed *t* test — in the unmixed, no significant difference at all stages of the journey ($P > 0.05$) in the mixed levels approached significance immediately after loading ($P < 0.06$) and were significantly higher in the middle ($P < 0.01$) and end ($P < 0.01$); (ii) one-tailed *t* test — in unmixed, levels were significantly higher at the end only ($P < 0.05$), in the mixed levels were higher at all stages of the journey (beginning $P < 0.05$, middle $P < 0.01$, end $P < 0.01$).

Analysis using a two-tailed *t* test revealed that levels were significantly higher in the mixed condition (compared with the unmixed) in the middle of the journey ($P < 0.01$) and approached significance immediately after loading ($P < 0.08$) and at the end of the journey ($P < 0.06$). In the case of a one-tailed analysis, levels were significantly higher in the mixed condition at all stages of the journey (beginning $P < 0.05$; middle $P < 0.01$; end $P < 0.05$).

Analyses using *t* tests were also conducted to compare cortisol levels at different stages of journey relative to pre-loading levels. Using two-tailed *t* tests: in the unmixed condition concentrations of cortisol were not significantly higher relative to pre-loading levels at any stage of the journey ($P > 0.05$ for all stages); in the mixed condition, levels (relative to pre-loading levels) approached significance immediately after loading ($P < 0.06$) and were highly significant in the middle ($P < 0.01$) and at the end ($P < 0.01$) of the journey. In the case of a one-tailed analysis: in the unmixed condition levels were not significantly higher relative to pre-loading levels after loading or in the middle of the journey ($P > 0.05$) but were significantly higher at the end ($P < 0.05$); in the mixed condition levels were higher at all stages of the journey relative to pre-loading levels (beginning: $P < 0.05$, middle: $P < 0.01$, end: $P < 0.01$).

Duration of journey and the route taken were the same each week and there were no substantial changes in weather conditions between weeks. The physical characters recorded for week 4 were as follows: mean temperature 6·87°C; mean relative humidity 0·678; mean sound levels 83·65 dB. The number of acceleration events was 440. This compares with journeys lasting 1 h 20 min (2 × 40 min) characterized as 'rough' and 'smooth' (Bradshaw *et al* 1995) where a rough journey (averaged over four journeys) constituted 100 acceleration events (minor roads) and a smooth journey 25 acceleration events (main roads and motorway). Hence the journey in experiment 1 was particularly rough.

Experiment 2

Figure 2 shows concentrations of plasma cortisol under control (stationary) and experimental (moving) conditions. Thirty minutes after loading cortisol concentrations were substantially raised in both situations. In the control condition cortisol levels peaked at 90 min and rapidly decreased to pre-loading levels over the next 1·5 h period, whereas in the experimental condition, concentrations remained higher for longer and declined slowly. As a consequence, cortisol levels differed between conditions in the 0 to 180 min ($P < 0.05$), 180 to 360 min ($P < 0.01$) and 360 to 510 min ($P < 0.05$) experimental periods.

Figure 3 shows concentrations of plasma beta-endorphin for control and experimental conditions. Beta-endorphin concentrations were substantially raised 30 min after loading in both situations and then decreased over the remainder of the first 180 min period (and fell below the experimental condition after 90 min). The difference between treatments was not significant in the 0 to 180 or 180 to 360 min periods. However, hormone levels were higher ($P < 0.01$) on the control day in the 360 to 510 min period.

Physical characteristics were: mean temperature 6·48°C (stationary), 9·13°C (moving); mean relative

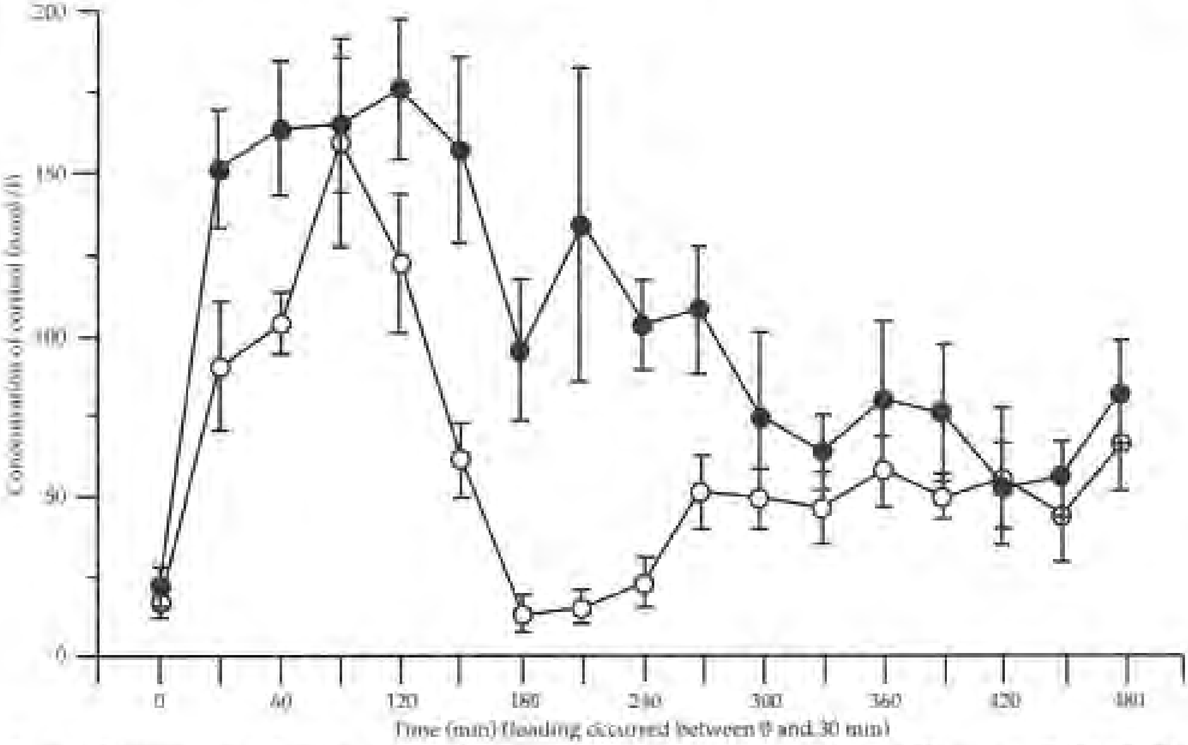

Figure 2 Concentration of plasma cortisol (nmol/l; mean ± s.e.) in pigs (no. = 6) sampled every 30 min from 09.30 to 17.30 h on experimental (moving) day (solid symbols) and control (stationary) day (open symbols). Using a two-tailed *t* test cortisol levels differed between conditions in the 0 to 180 min ($P < 0.05$), 180 to 360 min ($P < 0.01$) and 360 to 510 min ($P < 0.05$) experimental periods.

humidity 0·9040 (stationary), 0·5751 (moving); sound levels were not recorded due to a technical fault. The number of acceleration events during the journey was 52 (compared with 100 for the characterized rough journey and 25 for the characterized smooth journey in Bradshaw *et al.*, 1995).

Discussion

Pigs in experiment 1 spent the majority of their time standing which contrasts with the findings of Bradshaw *et al.* (1995) and Lambooy (1988) who found that pigs tended to lie down in transit. This may have reflected the relative difference in the roughness of particular types of journeys. In the present experiment, the number of acceleration events recorded was more than four times the number recorded during a journey characterized as rough (Bradshaw *et al.*, 1995). Even taking into consideration the fact that the previous study involved the use of a fixed-axle horse-trailer and that the journey was 10 min shorter, it is clear that the present journey was considerably rougher. Thus the finding that pigs preferred to stand does not necessarily contradict those of others (e.g. Lambooy, 1988) because whether the pigs stand or not may depend on the relative smoothness of the journey. The reason why pigs prefer to stand on rough journeys may be due to the type of flooring or in order to alleviate the effects of vehicle motion which can be manifested as travel sickness (Bradshaw *et al.*, 1995).

In the mixed condition pigs showed no obvious evidence of travel sickness whereas in the unmixed condition the pigs appeared to become travel sick. Since a total of eight individuals became travel sick in the unmixed condition and none in the mixed condition it appeared unlikely that some pigs were predisposed to vomiting and happened to be in the unmixed group. Thus in the mixed condition the direct effects of the journey appear to have been delayed possibly due to fighting while the unmixed pigs suffered the direct effects of the transport journey (and became travel sick). It is therefore envisaged that the mixed pigs might suffer the effects of the journey (and become travel sick) once aggressive behaviour ceased some time after the

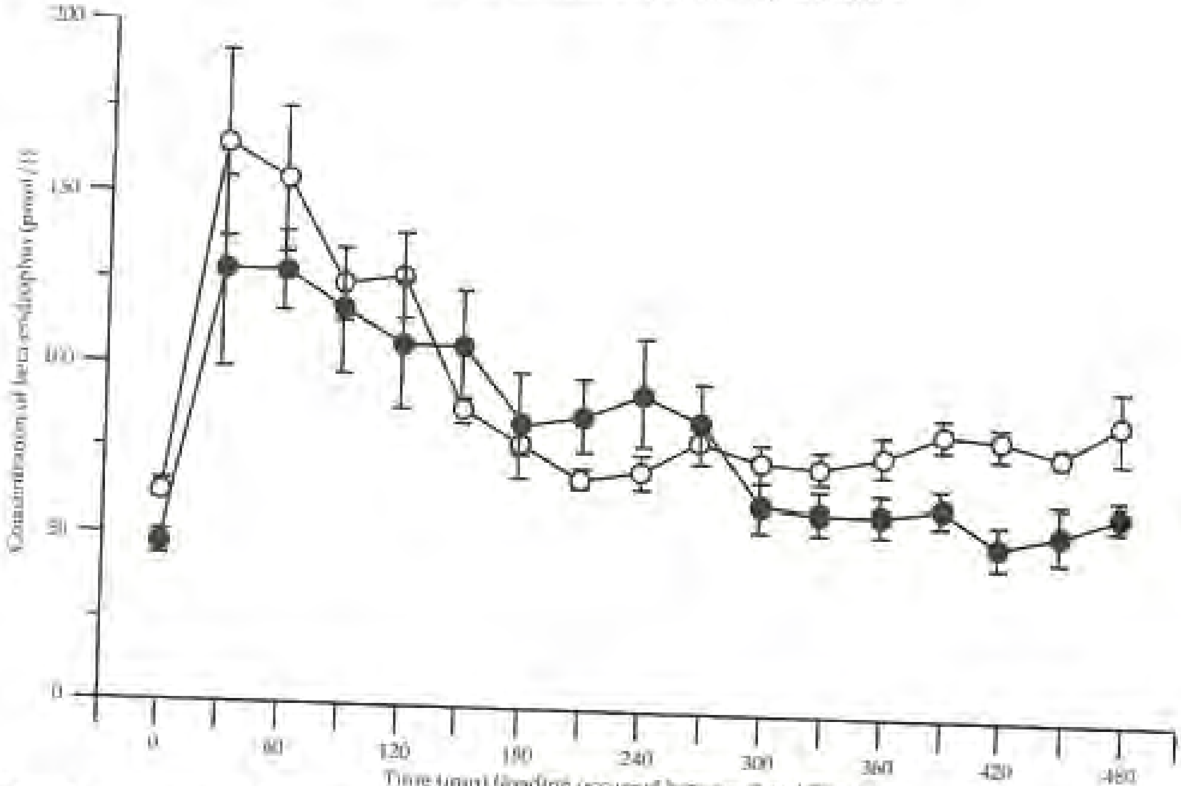

Figure 3 Concentration of plasma beta-endorphin (pmol/l, mean ± s.e.) in pigs (n = 6) sampled every 30 min from 09.30 to 17.30 h on experimental (moving) day (solid symbols) and control (stationary) day (open symbols). Using a two-tailed t test the difference between treatments was not significant in the 0 to 180 or 180 to 360 min periods. Hormone levels were higher ($P < 0.01$) on the control day in the 360 to 510 min period.

1 5-h period studied (by which time they would also be exhausted and stressed because of the fighting). Lambooy (1988) and Lambooy and Engel (1991) reported fighting at the beginning of journeys lasting up to 12 h and 25 h respectively. While the concentration of cortisol after loading in the unmixed condition was lower than the pre-loading levels there was substantial individual variation and concentrations of salivary cortisol remained significantly higher in the mixed compared with the unmixed condition at all stages of the journey. This may be attributed to fighting which is known to lead to increased levels of cortisol (e.g. Parrott and Misson, 1989), a higher level of activity (due to social disturbance) and to the stressful effects of the journey. The ramp angle (24°) was higher than the recommended 20° and loading procedures may therefore have been more stressful than those commonly in commercial use.

There have not been any studies comparable with experiment 2 in which pigs have been sampled at regular intervals during the course of a long journey. Large increases in plasma concentrations of cortisol and beta-endorphin during the first 150 min period of experiment 2 may be attributed to the effects of loading and the start of the journey. Loading was not conducted according to normal commercial practice by means of a ramp or lift (each pig was individually lifted from a cage, wheeled in a barrow 100 m to the lorry and placed in a separate pen) and may have been more stressful than normal commercial practice. However, the procedure may be seen as mimicking some of the effects of commercial loading which are known to be disturbing (Brown, Knowles, McKinstry, Edwards, Anil and Warriss, 1995). However, it is important to note that in experiment 1, there was no significant increase in levels of salivary cortisol in the unmixed condition after the pigs were loaded according to a conventional loading procedure using a ramp. In contrast, when pigs were mixed, levels of salivary cortisol increased substantially immediately after loading. This finding suggests that when pigs are not mixed, loaded with care and by stockmen familiar to them, the procedure may not be particularly stressful. The elevated levels of cortisol and beta-endorphin during loading in experiment 2 suggest that under these conditions, despite the fact the pigs were not mixed, they found the particular loading procedures adopted very stressful. Further explanations for these findings may be differences in breed (Landrace X Large White in experiment 1 and Large White in experiment 2) and size of pigs (90 kg in experiment 1 and 35 kg in experiment 2). Further research is needed to establish the least stressful situation for pigs during loading.

The stimulatory effect on cortisol release during the 8 h journey in experiment 2 is consistent with other studies relating to the effects of short journeys (e.g. Becker *et al.*, 1985; Dalin *et al.*, 1988 and 1993; Nyberg *et al.*, 1988). In the present study, long-term effects on cortisol secretion were also observed as levels remained higher than the control throughout the 8 h study. Concentrations of cortisol began to decline after an initial peak following loading (in both conditions) but the relative difference between control and experimental treatments only began to decline substantially after sample 10 (5 h). This suggests that the pigs remained stressed for a number of hours following loading and that this response may be directly attributed to the effects of the transportation process (and not to loading) since in the control condition, concentration of cortisol declined after loading.

The effects of the journey on plasma concentrations of beta-endorphin are less clear. Following an initial rise after loading concentrations decreased but there was no difference in concentration of beta-endorphin between conditions except during the last 3-h period when hormone concentrations were significantly lower in the experimental treatment. This may be attributed either to the fact that beta-endorphin is not a good measure of stress under transport conditions or that the pigs showed an increase in beta-endorphin to a novel situation on the control day and they were sufficiently habituated to the novelty on the treatment day that no further rise was recorded in response to transport. While a number of researchers have employed beta-endorphin as an indicator of stress in transport studies (e.g. Shaw and Tume, 1990; Geers *et al.*, 1994; Geers, 1995) the latter explanation seems unlikely because the cortisol response was very clear and persisted throughout the journey.

Finally, in experiment 2 food was withdrawn at 17.00 h on the night before transport mimicking usual commercial practice. One pig vomited during the experiment (after 4 h) and behaviour associated with travel sickness, previously observed in experiment 1, was observed during sampling in experiment 2. This included repetitive chewing, a slight foaming at the mouth and continual bouts of sniffing the air (often associated with standing) followed, after the 1st h, by the pigs lying down. These findings support those reported in Bradshaw *et al.* (1995) where travel sickness was more overt due to the withdrawal of food 4 h before transit and the same symptoms were noted before vomiting.

In conclusion, this study shows that pigs fight when mixed with other unfamiliar individuals during transport, and this fighting is very stressful. They show a marked increase in concentrations of cortisol and beta-endorphin in response to loading which is therefore indicated to be the most stressful phase of the journey. Levels of cortisol remain high for the first 5 h of an 8 h journey suggesting the animals find long distance travel stressful. This situation is further compounded by the effects of travel sickness.

Acknowledgements

We would like to thank Dr S. J. G. Hall for his considerable practical assistance in conducting experiment 2 and S. Kirkpatrick for carrying out the cortisol assay in experiment 1. We would also like to thank Mr P. Maltby, P. Carter and G. Maltby for their invaluable support throughout the study both with the husbandry of the pigs in experiment 1 and in the loading procedures. Funding was provided by the EC (AIR project 262).

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(*Received 25 July 1995—Accepted 22 November 1995*)