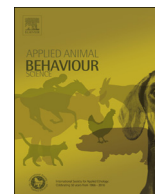




Contents lists available at ScienceDirect

## Applied Animal Behaviour Science

journal homepage: [www.elsevier.com/locate/applanim](http://www.elsevier.com/locate/applanim)

# The effects of regularity of simulated ship motions on the behaviour and physiology of sheep

Eduardo Santurtun\*, Clive J.C. Phillips

Centre for Animal Welfare and Ethics, School of Veterinary Sciences, The University of Queensland, Gatton, Queensland, 4343, Australia

## ARTICLE INFO

**Keywords:**  
Behaviour  
Interactions between sheep  
Regularity  
Motion  
Sheep  
Ship

## ABSTRACT

Floor movement influences sheep responses to transport, but the importance of movement regularity and interactions between sheep are unknown. To test this, sheep were restrained in pairs in a crate mounted on a moveable, programmable platform for 60 min periods, changing treatments over 12 consecutive days. In an initial experiment a repeated speed of movement and change in angle (regular movement) was compared to variable angles and speeds (irregular movement) of roll, pitch or combined movements, for sheep behaviour, heart rate and feed and water intake responses. Feed intake was increased by irregular roll + pitch motion ( $P = 0.04$ ). During irregular sequences sheep affiliated more, with their heads one above the other ( $P = 0.001$ ) and supported themselves against the crate ( $P < 0.001$ ) or kneeling ( $P = 0.03$ ). Irregular sequences and combined roll and pitch synergistically increased stepping behaviour, indicating loss of balance, and heart rate, possibly indicating stress ( $P < 0.001$ ). Heat rate data demonstrated that the RMSSD band was reduced during irregular movement ( $P = 0.04$ ), and LF/HF ratio increased during irregular sequences of roll + pitch ( $P = 0.007$ ), suggesting less parasympathetic nervous system activity. In a second experiment, we investigated the effects of these floor motion patterns with and without a barrier to separate the sheep. With no barrier or irregular motion, sheep stepped more to avoid loss of balance ( $P < 0.001$ ) and were again more affiliative. During irregular motion they supported themselves more against the crate ( $P < 0.001$ ). With no barrier there was more agonistic behaviour (body pushing ( $P = 0.02$ ), butting ( $P = 0.02$ ) and evading the other sheep ( $P = 0.001$ ) and less rumination ( $P = 0.02$ ), which together with a reduction in RMSSD and NN50 suggested that sheep welfare was reduced by the close proximity of the other sheep. The ratio of low to high frequency beats was highest ( $P = 0.005$ ) and the RMSSD and NN50 were lowest ( $P < 0.001$ ) during irregular motion and no barrier. Evidence is provided that sheep were both more stressed in this combination of treatments and also exercising more, through stepping behaviour. Thus irregular sequences and combined roll and pitch caused stress and increased activity to correct loss of balance, as well as increased affiliative behaviour. Separating sheep during irregular motion reduced body instability and stress, suggesting that close stocking is detrimental to their welfare.

## 1. Introduction

During transport, livestock continuously try to avoid contact with other individuals and the vehicle (Broom, 2003; Jones et al., 2010). Balance is maintained by stepping movements and support from vehicle structures (Broom and Fraser, 2007). Some research describes increased stress under loose stocking (Hall et al., 1998; Jones et al., 2010), however tightly stocked livestock may fall down when trying to avoid a fallen animal (Cockram et al., 1996; Das et al., 2001; Jones et al., 2010).

Unpredictable situations are likely to surpass animals' regulatory capacity, resulting in stress (Johannesson and Ladewig, 2000; Bassett

and Buchanan-Smith, 2007; Koolhaas et al., 2011). In particular, the regularity of floor motions may determine the stress impact if animals cannot predict or habituate to them, particularly if the time delay between exposures is significant (Johannesson and Ladewig, 2000; Abeyesinghe et al., 2001; Phillips and Rind, 2001).

We developed a moving platform to simulate ship motion and to monitor sheep responses to movement variables (Santurtun et al., 2014). With this, we demonstrated from heart rate evidence that a roll (side to side) motion stressed sheep, as well as requiring them to make regular posture changes (Santurtun et al., 2015). Pitch (end to end) motion did not evince such responses, but in combination with roll may

\* Corresponding author. Present address: Departamento de Etología, Fauna Silvestre y Animales de Laboratorio, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Ciudad Universitaria, 04510, Mexico City, Mexico.

E-mail address: [esanturtun@gmail.com](mailto:esanturtun@gmail.com) (E. Santurtun).

<https://doi.org/10.1016/j.applanim.2018.03.005>

Received 7 June 2017; Received in revised form 2 March 2018; Accepted 12 March 2018  
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increase the unpredictability of the movements.

The hypotheses of this study were that irregular sequences of movement, a combination of different movement types, and contact with other sheep would be stressful as sheep would be less able to cope with the changes of motion. The specific objectives of this study were twofold. First we examined the effects of regular and irregular sequences of roll and pitch on feed intake, behaviours, heart rate, and body posture. Second, to examine whether the effects were dependent on the ability of sheep to move around, we compared sheep that were either isolated or paired within a crate.

## 2. Materials and methods

Two experiments were conducted at the University of Queensland, Australia (27.3° S, 152.2° E) with approval from the University's Animal Ethics Committee (SVS/315/12).

### 2.1. Animals, housing and management

The design of novel methodology for exposing sheep to floor movement, including the programming of a movement platform, heart rate monitoring and video recording of behaviour, have been described in full elsewhere (Santurtun et al., 2014). Six merino cross wethers, approximately 34 months of age, weighing (mean  $\pm$  SEM) 44.2  $\pm$  0.1 kg and shorn over the front half of the body to facilitate heart rate monitor placement, were acquired from the University's flock. Before and after each trial, sheep were kept in a small paddock with ad libitum water and wheaten chaff and access to the experimental rooms. During the trials, sheep were restrained in pairs in a crate (Fig. 1) made with 3 tubular steel bars (0.87 m wide  $\times$  1.2 m long  $\times$  0.95 m high), divided in 2 by a removable barrier. The crate was covered with a sheet to reduce visual stimulation. Aluminium bowls and plastic bottles were attached to the outside of the crate for the first experiment only. A small external mesh barrier was placed to prevent sheep eating from their companion's bowl.

### 2.2. Regular and irregular roll and pitch motions

The motion platform was programmed to move in both regular and irregular sequences for roll and pitch independently or in combination, using two variables, amplitude and period. An irregular sequence

programme was constructed from thirty separate amplitude and period values that were randomly selected by the software Microsoft Visual Studio Solution C++ Express 2008. Regular roll and pitch sequences were programmed as the mean amplitude (4.3°) and period (235 ms) of the irregular roll and pitch sequence. A detailed explanation of the methods to obtain both regular and irregular sequences, including the programming commands, as well as the characteristics of the motion platform used to produce roll and pitch movements independently and in combination, is available in Santurtun et al. (2014).

### 2.3. Experimental protocols

Before the start of each experiment, over a period of 32 d sheep were habituated to the experimental conditions to minimise the confounding effects of other potential stressors preceding and during experimental trials. Potential stressors identified were handling of the sheep, use of a ramp to get them into the crate, drinking from a water bottle, adjustment to a new environment in the research facility, heart rate monitoring, the researchers' presence and a pelleted diet. The first step involved the reduction of fear of researchers by offering high quality pellets by hand as a positive reinforce for the sheep in triads every two hours a day for 10 d (Photo 4). The next stage involved different, simultaneous training procedures, including loading and unloading into the crate using a ramp (8 d), clipping the area of skin where the heart rate monitor electrodes would be placed (10 d), attaching the heart rate monitor (7 d), and 3–4 h inside the research facility for feeding, resting and use of crate (20 d). The training stopped when there were no obvious fear behaviours and the heart rate mean was close to resting rate.

During the initial Motion Regularity Experiment, sheep were exposed in pairs to six treatments with two factors: regular and irregular sequences of pitch, roll, and combined roll and pitch. During the second experiment, Interactions between Sheep in Irregular and Regular Motion, sheep were again exposed in pairs in a 2 factor design: factor 1, Regular or Irregular sequences of a combined pitch and roll motion, or a Control treatment with no motion, and factor 2, with or without a barrier between the two sheep.

Each treatment was applied to the sheep in the crate for a 60 min period in a 6  $\times$  6 Latin Square with one repetition, lasting 12 consecutive days (see Table 1 for Experiment 1 design). In total, each sheep was exposed to 12 treatment periods. Sheep experienced treatments in

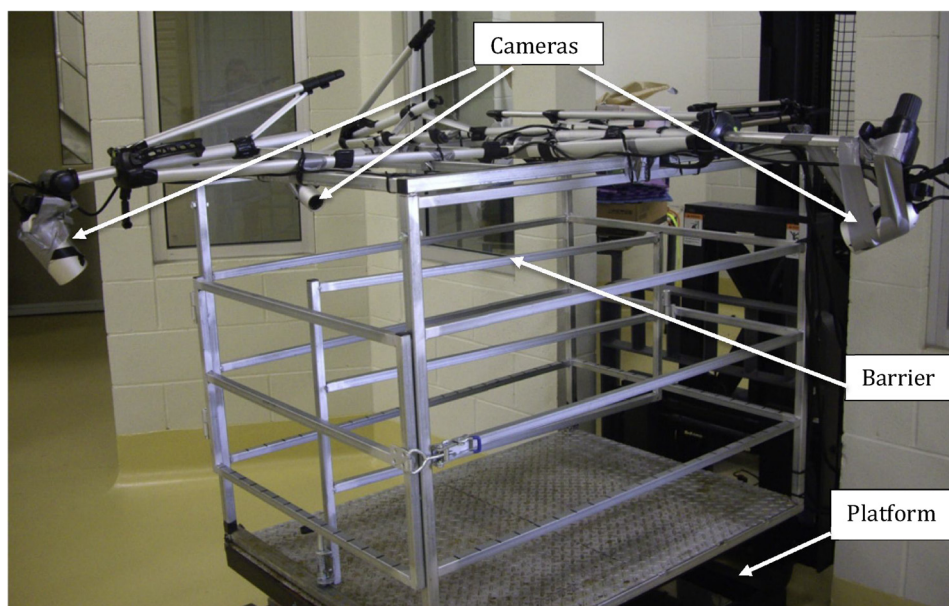


Fig. 1. Crate positioned on the platform showing the barrier between the two sides and camera positions depicted.

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