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Effects of sea transport motion on sheep welfare

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Abstract

The effects on sheep welfare of motion produced by a ship during sea transport have not been reviewed, but there is comprehensive knowledge of adverse effects in humans. The overall aim of this project was to describe the impact that roll, pitch and heave motions have on sheep welfare through behavior and heart rate measurements. A novel method was developed to create these motions using a programmable simulator platform that generated roll and pitch motions replicating ship movements, and comparing regular and irregular movement sequences. An electric forklift was used to produce heave motion.

The first study measured individually Roll, Heave and Pitch. Heave reduced the time that sheep spent ruminating, compared with the other three treatments ($P < 0.001$). The two sheep spent more time during Heave with their heads one above the head of the other ($P < 0.001$) compared with sheep in roll, pitch or control treatments, and looking towards their companion ($P = 0.02$) compared with sheep in roll and control treatments. Sheep spent more time during Heave standing with their back supported on the crate ($P = 0.006$) and less time lying down ($P = 0.01$) compared with sheep in roll, pitch or control treatments. Roll caused more stepping motions than pitch and control ($P < 0.001$). Heave and Roll had increased heart rates and reduced inter-beat intervals, compared to Control ($P < 0.001$). The inter-beat intervals of sheep in the heave treatment had an increased ratio of low to high frequency duration ($P = 0.01$), indicating reduced parasympathetic control of stress responses. Therefore Heave and Roll caused stress, with sheep experiencing Roll requiring apparently coping better by regular posture changes and Heave causing the sheep to seek close presence to their companion.

The second study measured whether Roll and Heave affects sheep feed intake, behaviors, and heart rate measurements, and whether providing antiemetic drugs attenuates motion effects in the sheep. There was no evidence of effects of the motion on feed intake, but the Heave treatment made the sheep eat faster ($P = 0.006$), with fewer mastication bites ($P = 0.004$), which could reduce the efficiency of digestion. Antiemetic provided limited evidence of improved balance, since the sheep spent less time with their head against a dividing mesh in the crate ($P= 0.01$), and they increased prehension biting rate in the Heave treatment ($P = 0.002$). Sheep in the Heave treatment spent longer with their head against the mesh than those in the Control treatment ($P = 0.009$). It is concluded that simulated ship motion did not cause reduced feed intake over short periods, but that there was limited evidence that the motion had adverse effects on balance which could be attenuated by an antiemetic.

The third study measured the effects of regular and irregular sequences of roll and pitch, or a combination of the two on sheep feed and water intake, heart rate measurements and body posture.

Feed intake was increased by irregular sequences in the combined Roll and Pitch motion (P=0.04). The two sheep spent more time during Irregular sequences with their heads one above the other (P= 0.001), and facing down (P = 0.001) than Regular sequences. The combined Roll and Pitch also increased the time sheep spent with their heads down (P = 0.007) compared to Roll and Pitch motions. Sheep spent more time during Irregular sequences standing with their back supported on the crate (P < 0.001) or kneeling (P = 0.03) compared to Regular sequences. Irregular sequences, the combined roll and pitch and the interaction between the two produced more stepping behaviour. Sheep exposed to Irregular sequences of combined Roll and Pitch had increased heart rates (P < 0.001). In the combined Roll and Pitch motion the ratio of Low to High Frequencies was increased (P = 0.007) by Irregular compared to Regular sequences. Therefore Irregular sequences and the combination of Roll and Pitch caused stress, loss of balance and more affiliative behaviour between sheep.

The final study measured the effects of regular and irregular combined roll and pitch motions and a barrier to separate sheep on heart rate measurements and behavior. Stepping to avoid loss of balance was more frequent when sheep had no barrier (P < 0.001) and during irregular motion (P < 0.001). Without the barrier (P = 0.03) and during irregular motion (P < 0.001), sheep spent more time with their head under or above the other sheep compared to barrier in place, and control motion. During irregular motion they supported themselves more against the crate (P <0.001) compared to Regular and Control. When the barrier was removed there was increased agonistic behavior, including pushing with the body (P = 0.02), butting (P = 0.02) and evading the other sheep (P = 0.001). Sheep ruminated more when the barrier was in place (P = 0.02). There was evidence of stress caused by removal of the barrier since the square root of the mean of the sum of the squares of differences between successive inter-beat intervals (RMSSD) and the number of pairs of successive inter-beat intervals (NN50) and rumination were all reduced. Irregular sequences increased the ratio of high to low frequency beats (P = 0.03). 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, indicating that sheep were most stressed in this combination of treatments. Therefore when providing an opportunity for sheep to interact during irregular motion caused stress and body instability.

Our findings provide sufficient evidence to conclude that sea transport motions represent a potential stressor to sheep.

Declaration by author

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

I have clearly stated the contribution of others to my thesis as a whole, including statistical assistance, survey design, data analysis, significant technical procedures, professional editorial advice, and any other original research work used or reported in my thesis. The content of my thesis is the result of work I have carried out since the commencement of my research higher degree candidature and does not include a substantial part of work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution. I have clearly stated which parts of my thesis, if any, have been submitted to qualify for another award.

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Publications during candidature

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Phillips, CJC	Designed experiments (40%) Edited paper (60%) Statistical analysis (20%)

Contributions by others to the thesis

The concept for, and design of, this research project, as well as analysis and interpretation of data were achieved through discussions and consultations with my principal advisor Professor Clive Phillips. Miss Valerie Moreau contributed with the platform programming design and first experiment preparation and data collection.

Statement of parts of the thesis submitted to qualify for the award of another degree

Behavioral and food consumption analysis from Chapter 5 was submitted by Hala Taha Khidr Taha Al-Zubiedi as part of her Masters of Science degree at the School of Agriculture & Food Sciences, The University of Queensland in 2012. Analysis, literature review and discussion were carried out independently by each student. The experimental design was prepared by the PhD student. The Master's student participated during the preparation and in the experimental trials.

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behavior, heart rate variability, heave, motion sickness, pitch, roll, sheep, ship motions, stress, transport

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List of Abbreviations used in this thesis

B	Beam
C	Empirical constant
FFT	Fast Fourier Transformation
GM_T	Transverse metacentric height
H^{amp}	Heave amplitude
HF	FFT high frequency band
IBIs	Inter-beats intervals
LF	FFT low frequency band
L_{PP}	Length between perpendiculars
MS	Motion sickness
NN50	Number of pairs of successive IBIs differing by more than 50 ms
OIE	World Organisation for Animal Health
RMSSD	Square root of the mean of the sum of the squares of different successive IBIs
T_{roll}	Roll period
T_P	Pitch period
T_H	Heave period
Ψ	Pitch amplitude
Φ	Roll amplitude

CHAPTER 1. SHEEP LIVE EXPORT

1.1 Introduction

Sheep are raised around the world for different production uses like meat or wool (Cockram, 2007), and China has the biggest population with 176.9 million, India the second biggest with 73.9 million and Australia has the third place with 68.1 million (Fig. 1) (FAOSTAT, 2010).

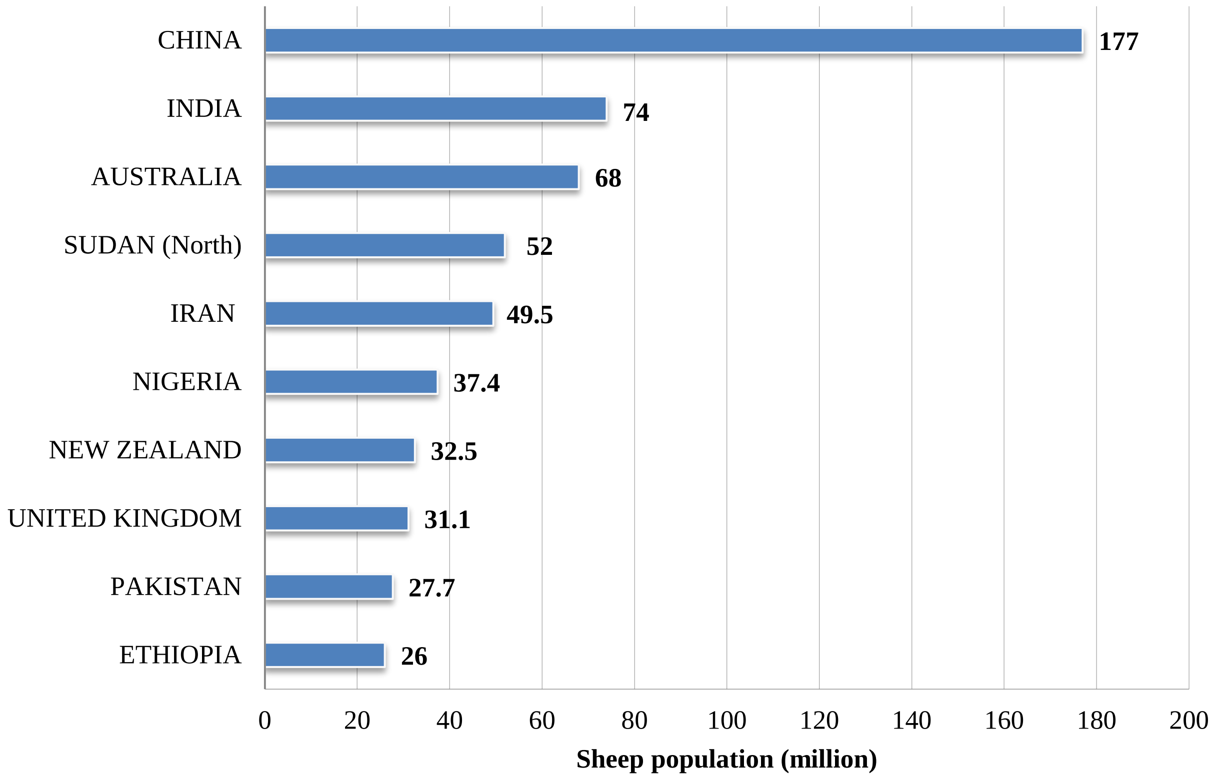


Fig. 1. World leaders in national sheep population statistics in 2010 (FAOSTAT, 2010).

In each country, a high proportion of all farmed animals are transported at some period in their lives for different reasons (Broom, 2005; Cockram, 2007). Consequently, transportation is a critical component of the infrastructure of the livestock industry (Speer et al., 2001). During long distance transportation, sheep are exposed simultaneously to a variety of stressors in a relatively short period of time (Grandin, 2000; Cockram, 2007). Transportation represents a combination of several stressors that can have additive and negative effects on the animal welfare (Broom, 2005; Maria, 2008). Besides the novelty of many aspects of transportation, sheep can be exposed to a variety of changes in their physical and social environment during each stage of transportation (Cockram, 2007), which could cause them psychological and physical stress (Grandin, 1997; Knowles and Warriss, 2000). Such stressors include water deprivation, mixing of unacquainted individuals, human handling, exposure to novel environment, noise, motion, excessive physical exercise, extremes temperatures, humidity, among others. Live export sea transport process involves several steps that start with the collection of the stock using dogs, horses, helicopters, or all-terrain vehicles, curfewing or restriction of food and water one night before transport of livestock[1], continues with the road or rail transport to the port where loading and the journey represent two important potential stress events[2], days at a pre-export assembly depot, road transport to port, loading into a ship, a sea journey, discharge phase or unloading of livestock into the different ports and ends with animal slaughter in the country of destination (Hall et al., 1998; Norris and Norman, 2003; Norris, 2005; Petherick, 2005; Phillips, 2008). The whole process can last between one and two months (Phillips, 2008). The risk factors for this type of transport, besides those mentioned above are: consumption of pelleted food, farm group, animal age, time of the year, fatness, total duration of the journey, pen high humidity, ship motions, among others (Norris, 2005; Cockram, 2007; Phillips, 2008). All these stressors potentially have synergistic and additive effects which may reduce the welfare of livestock (Phillips, 2008). It is therefore essential to understand the impact that each of these stressors has on the welfare of livestock transported during sea transport. It is important to emphasize that some stressors have been well studied in land transport but little is known about sea transport stressors, such as the motions produced by ship and its impact on sheep welfare (Norris, 2005; Phillips, 2008). Nonetheless, Phillips and Santurtun (2013) in a review of sea transport's impact on livestock welfare concluded that long distance transport exposes livestock to several potential stressors that could affect their welfare.

1.2 Australian sheep live export industry

In recent decades a major trade in exporting livestock from economically wealthy countries to the main consumption areas has become established, which means in some circumstances, long distance transport. Many animals are exported alive for different reasons, and the economic perspective is one of the most important (Phillips, 2008), but also other factors such as increase of urban population are important (Li et al., 2008). In relation to sheep export, over fifteen million sheep were exported around the world in 2011, representing a value of USD $1.5 billion, with Sudan being the main live sheep exporter in the world, followed by Australia, Somalia, Romania and France (Table 1).

[1] In Australia this period must be no less than 12 hours of green feed and up to 12 hours for water.

[2] Australian standards establish a journey time limit for sheep according to maximum water deprivation time (30 hrs. for mature stock, 20 hrs. for young stock less than 6 months old).

Table 1. Top ten sheep world exporters in 2011 (FAOSTAT, 2011).

	Country	Export quantity (Head)	Export value (USD$1000)
1.	Sudan (North)	2,566,266 [R]	239,688 [R]
2.	Australia	2,308,449 [O]	317,954 [O]
3.	Somalia	2,007,934 [R]	89,907 [R]
4.	Romania	1,791,595 [O]	166,269 [O]
5.	France	837,988 [O]	71,766 [O]
6.	Bulgaria	777,293 [O]	71,219 [O]
7.	Hungary	643,689 [O]	61,155 [O]
8.	Saudi Arabia	528,832 [O]	80,365 [O]
9.	Jordan	371,393 [O]	109,579 [O]
10.	Spain	356,994 [O]	39,393 [O]

[O] Official data, [R] Estimated data using trading partners database

The live sheep trade from Australia started in 1845 (ACIL Tasman, 2009), but it was not until the 1970s that, as a result of an expansion of the oil industry in the Middle East, the trade from this region increased significantly (Kelly, 1995). Today, Australia is the second world's largest exporter of live sheep with an average of 3.2 million per year (2008-2012 period, Fig. 2), although export volumes have reduced during recent years as a result of the limited stock availability (Phillips, 2005; LiveCorp, 2010), price of animals (Fig. 3) (Hassal & Associates Australia, 2006; Drum and Gunning-Trant, 2008), shipping costs and currency exchange variation (Phillips, 2005; Hassal & Associates Australia, 2006).

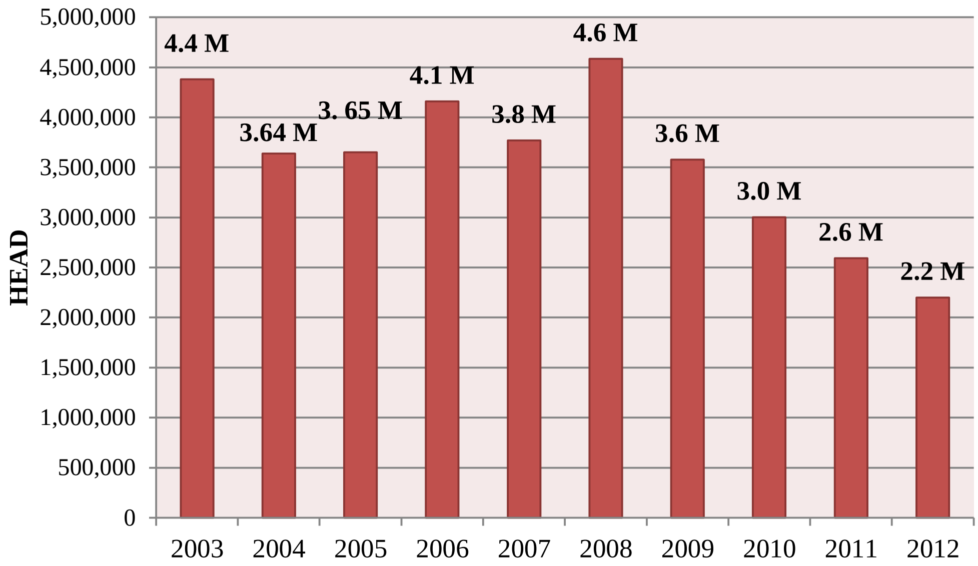


Fig. 2. Export of sheep from Australia, 2003-2012 (head) (DAFF, 2013).

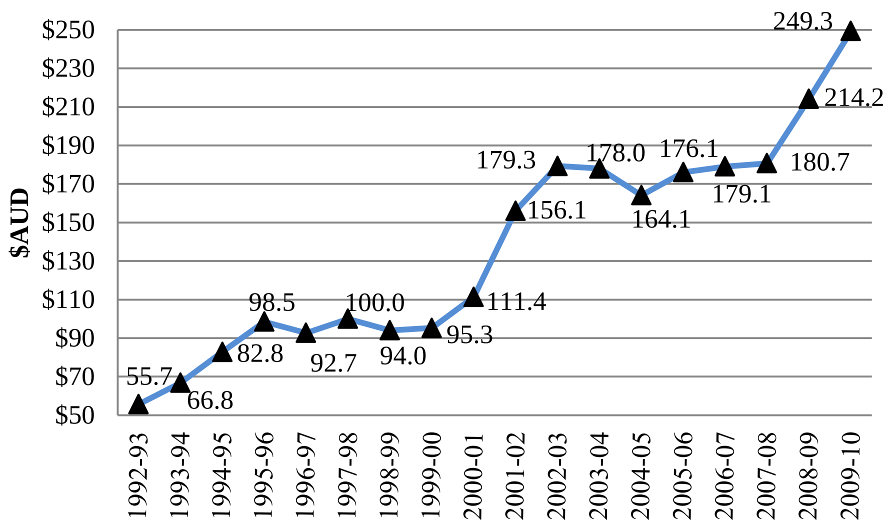


Fig. 3. Live sheep export prices in Australia 1992-2010 period (ABARES, 2010).

1.2.1 Export destination and value

Sheep are transported from Australia to different destinations, primarily to the Middle East (Table 2), which represents 99% of Australian sheep exports (Norris, 2005; AGRA, 2008; Appleby, 2008; Phillips, 2008), with 99.86% of sheep are imported to be slaughtered and 0.14% for breeding purposes (ABARES, 2010). In relation to the economic value, three million sheep exported in 2010 represented a value of AUD$323 million, but the cattle live export industry is the greater with a value of AUD$678 million in 2010 (Gilbertson, 2011). The main markets, Kuwait, Bahrain and

Qatar, represented 64% of the total value of Australian live exported in 2010 with a value of AUD $206.5 million (Gilbertson, 2011). Saudi Arabia, on the other hand, was the main live sheep market for Australia during the 1980´s, but the trade was disrupted in 1990 and 2003 as a result of rejected shipments. The trade started again in 2005 and has been described to have the potential to turn into the leading market for live sheep from Australia (Lightfoot, 2008). Currently Saudi Arabia represents 9.3% of the market with a AUD$FOB value of $30.2 million (Gilbertson, 2011).

Table 2. Australia live sheep exports to Middle East and South East Asia in 2010 (Gilbertson, 2011).

Country	Head Exported	Total Australian exports (%)
I. Middle East		
Kuwait	1,076,455	36.15
Bahrain	535,731	17.99
Qatar	321,415	10.79
Jordan	265,986	8.93
Saudi Arabia	262,500	8.81
Turkey	224,285	7.53
United Arab Emirates	78,747	2.64
Libya	75,026	2.52
Oman	69,073	2.32
Israel	42,000	1.41
Total Middle East	***2,951,218***	***99.10***
II. South East Asia		
Malaysia	19,000	0.6
Singapore	7,401	0.2
Philippines	18	0.0006
Total South East Asia	***26,419***	***0.88***

1.2.2 Religious and cultural beliefs.

Live sheep demand from Middle East continues and has increased over the recent years (Drum and Gunning-Trant, 2008; LiveCorp, 2010). This region prefers live animals as a result of their religious beliefs (i.e. halal slaughter method) and traditions (i.e. sheep slaughtered at traditional markets or ‘souk’ in presence of customer) (Shiell, 2003; Drum and Gunning-Trant, 2008), and since their domestic production is not enough to cover the regional demand caused by the arid regional environment, they need to import primarily from Australia, Iran and North Africa (Drum and Gunning-Trant, 2008). In contrast, South East Asia demand live animals mainly as a result of infrastructure deficiencies to maintain meat, but also due to religious values (Drum and Gunning-Trant, 2008; ACIL Tasman, 2009).

1.2.3 Sheep breed.

The Middle East market prefers lean carcasses of 8-12 kilograms of weight which is typical of breeds such as Awassi (named “fat tail type”). In this sense some producers have introduced fat tail breeds in Australia because of their higher price in relation to merinos; nonetheless, merinos are the main breed exported to this region because of stock availability in Australia (Drum and Gunning-Trant, 2008; ACIL Tasman, 2009).

1.2.4 Transport methods.

The main method used to export sheep is sea transport (99.2% of head exported), although air transport has increased significantly in recent years, from 3,074 sheep exported by air in 2007 to 22,286 in 2010 (Gilbertson, 2011). In relation to sea transport, the main ports in Australia that export sheep are in declining importance Fremantle located in Western Australia (80.8% Market Share), Portland located in Victoria (14.61% Market Share), and Port Adelaide located in South Australia (3.85% Market Share) (Gilbertson, 2011). Sea length voyages to the South East Asia generally have a duration of 7 to 10 days, and 14 to 23 days to the Middle East (Drum and Gunning-Trant, 2008; Phillips, 2008).

1.2.5 Livestock carriers.

In relation to the capacity and characteristics of the livestock vessels, the Australian livestock carriers have undergone significant changes. In the 1980´s, mainly general cargo ships around the world were converted to livestock carriers, but were required to fulfil national animal welfare regulations (Skraastad, 1983). In recent years, new vessels have been constructed specifically for livestock transportation. These ships have been acquired by national live export companies who looked for smaller, faster and better equipped vessels to transport livestock (Phillips, 2008). The livestock vessels capacity increased from the 1970´s when they had a maximum capacity to carry 50,000 sheep, changing in 1980´s to a capacity of 92,000 and 125,000 (Australian Bureau of Animal Health, 1981). Currently, the new vessels used by Australian exporters have different livestock cargo capacity. Table 3 shows some of the livestock vessels constructed by the Italian company *Siba Ships* and owned by Wellard Rural Exports which is one of Australia´s largest livestock exporter.

Table 3. Livestock carriers employed in Australia for livestock export (SIBA Ships, 2005; Wellard Rural Exports, 2010 a,b).

Ship name	Sheep capacity	Cattle Capacity	Pens	Decks	Length (m)	Speed (knots)	Launched (year)
MV Ocean Swagman	26,000	6,500	Sheep: UK Cattle: UK	7	130	17	2010
MV Ocean Drover (former MV Becrux)	75,000	17,000	Sheep: 779 Cattle: 1416	9	176.7	20	2002
MV Ocean Shearer (former Stella Deneb)	125,000	23,000	Sheep: 2,072 Cattle: 2,072	14	213.2	24	2002 (restored)

UK: Unknown

In relation to international sea livestock transport legislation and standards, no international convention has been adopted worldwide that includes transport of live animals on ships (Shultz-Altmann, 2008). However, some legal instruments established in Australia like the Marine Orders Part 43, Issue 6, includes engineering and equipment requirements regarding the carriage of livestock for ship safety (AMSA, 2006; Shultz-Altmann, 2008). In addition, the Australian standards for the long distance transport of animals (DAFF, 2011) cover different animal welfare aspects, and the World Organisation for Animal Health (OIE) developed the OIE Terrestrial Animal Health Code guidelines (Norris, 2005) which includes animal welfare standards during the different stages of sea transport[3], but not compulsory for the industry.

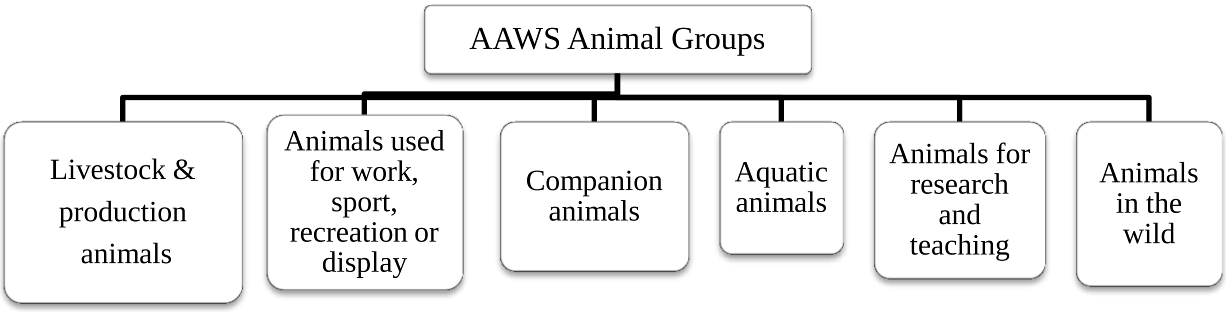
1.3 Animal welfare and the Australian Live Export Industry

Animal welfare legislation enforcement responsibility in Australia resides with states and territories. The federal government on the other hand is responsible for international trade issues such as live export (de Witte, 2009). In relation to animal welfare regulations, Australia has different mechanism and legal instruments that covers the welfare of livestock during transport such as the *Australian Animal Welfare Strategy* and the *Australian Standards for Long Distance Transport*, which are described below.

[3] See: OIE (2012). World Organisation for Animal Health Terrestrial Animal Health Code: Chapter 7.2. Transport of Animals by Sea. http://www.oie.int/en/internationalstandard-setting/terrestrial-code/access-online/ (accessed 21 August 2012).

1.3.1 Australian Animal Welfare Strategy

The Australian Animal Welfare Strategy (AAWS), launched in 2004, was established as one of the recommendations of the National Consultative Committee on Animal Welfare (NCCAW) and has as its main objective the establishment of a national framework to improve the animal welfare in Australia (DAFF, 2008; Animal Health Australia, 2009). This strategy covers the following animal groups:



In relation to transport, this strategy also covers the welfare of animals transported based on international standards and guidelines from OIE, International Air Transport Association (IATA), among others (DAFF, 2008).

1.3.2 Australian standards for long distance transport

The Australian standards were created after some major incidents where many cattle and sheep died such as the MV Cormo Express 2003 (voyage No.93) incident where a shipment of 57,973 sheep that departed from Fremantle, Western Australia on August 5th, 2003 was rejected from the port of Jeddah, Saudi Arabia, due to concern from local authorities that 6% of sheep apparently had scabby mouth disease. After several negotiations between the Australian government and numerous countries of the region, finally the Eritrean government accepted the sheep, and after 80 days of travel the unloading started in Massawa on October 24th 2003. As a result, 5691 sheep died (9.82%) during the voyage of almost three months and the trade with Saudi Arabia was suspended (Keniry et al., 2003; Phillips, 2005; Stinson, 2008; Thornber, 2008). Another mayor incident happened in 2002 on the maiden voyage of the MV Ocean Drover (former MV Becrux), called “world´s most technologically advanced livestock carrier” (Wellard Rural Exports, 2010b). Almost 70,000 sheep and 1,995 cattle were exported to the Middle East and after a 27 day voyage a 28.5% and 2% mortality rate of cattle and sheep was reported, respectively (More et al., 2003). The main cause of death reported was heat stress, with inadequate ventilation playing a major role (More et al., 2003; Stinson, 2008). Simultaneously, OIE in 2002 identified sea transport of animals as one of the key animal welfare areas and started the development of a terrestrial code specific in 2003 for this area (Norris, 2005). The incidents mentioned previously as well as other situations generated a response from the government and a review of the live export industry ("*The Keniry Review*"), concluding that animal welfare must be considered and accomplished in each of the stages of the livestock export chain, reducing as much as possible each of the stressor events that occur during this process (Keniry et al., 2003). After this review, the Australian Government elaborated *The Australian Position Statement on the Export of Livestock* which provided a framework for the development of the *Australian Standards for the Export of Livestock (ASEL) (DAFF, 2006; Stinson, 2008)*. These standards introduced in 2004, replaced *The Australian Livestock Export Standards* (Keniry et al., 2003; Stinson, 2008), and have been modified in the last years. The latest version (2.3) covers different livestock species: cattle, sheep, goats, buffalo, deer and camelids, and include six areas of the export chain (DAFF, 2011):

- I — **Sourcing and on-farm preparation of livestock**
- II — **Land transport of livestock for export**
- III — **Management of livestock in registered premises**
- IV — **Vessel preparation and loading**
- V — **On-board management of livestock**
- VI — **Air transport of livestock**

In relation to sheep welfare, Appendix 1 summarizes those standards that include animal welfare issues relevant to sheep.

1.3.3 Australian sheep live export mortalities.

Livestock mortality is one of the main animal welfare indicators used by the live export industry, although mortality is not the best animal welfare indicator as the animals could be experiencing low welfare levels without dying (Petherick, 2005). Livestock mortalities for exports by sea are compiled by the Australian Maritime Safety Authority (AMSA) from the ship masters' reports and sent to the Australian Parliament every six months (Drum and Gunning-Trant, 2008; DAFF, 2013). This obligation was established from the modifications made to the Australian Meat and Livestock Industry Act 1997 (DAFF, 2013). In relation to the mortality rate of sheep exported from Australia, it has declined over recent years from 1.34% reported in 2000, to a level of 0.88 % in 2012, the reduction occurring during the first three years of the last decade (2000-2002) with the highest percentage of mortality rate and the lowest in 2011 with a mortality rate of 0.74% (Fig. 4). This could be explained by several reasons such as the transport of younger sheep, welfare recognition, enhanced situation, seasonal variation, among others (Norris and Norman, 2005; Phillips, 2008). Mortality varies according to season as occurred during 2010 where 67% of sheep losses took place during the period of September-November (Fig. 5). Similar season variation was reported by Norris and Norman (2003) where highest mortality occurred during the second semester of the year in sheep exported from Fremantle and Portland to the Middle East.

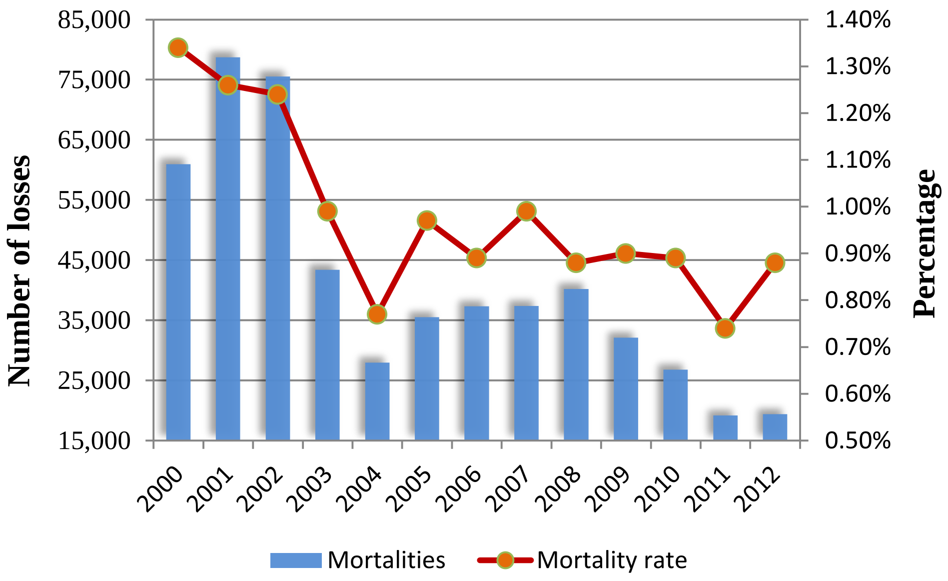


Fig. 4. Australian live sheep export by number and percentage mortality during the period 2000-2012 (DAFF, 2013).

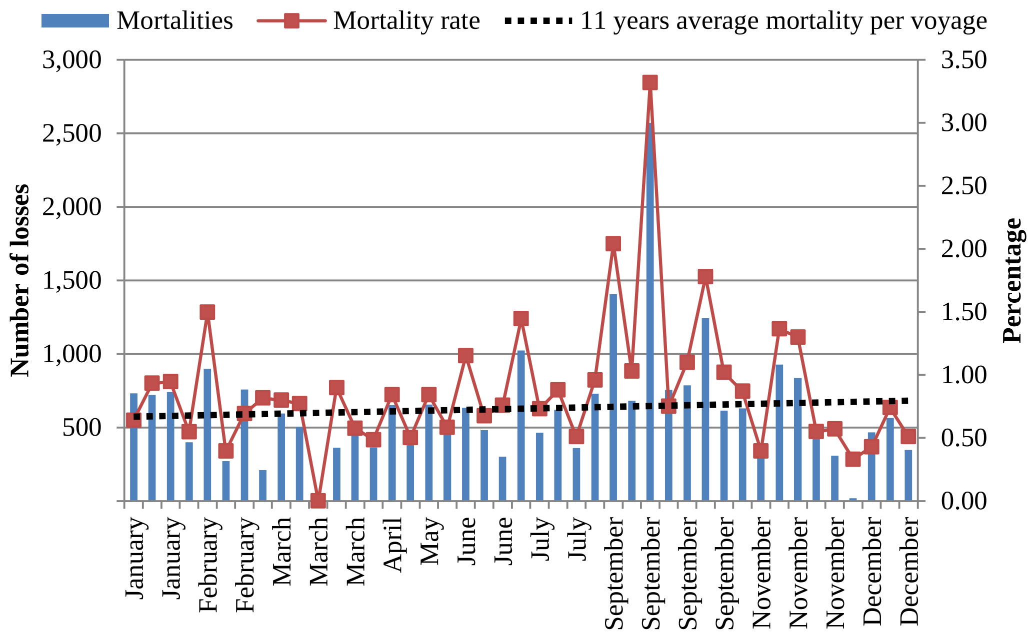


Fig. 5. Australian live sheep export by number and percentage mortality during each of 43 sea voyages in 2010 (DAFF, 2013).

1.3.4 Australian sheep live export mortality etiology.

Mortality of sheep occurs mainly on ship, approximately 75%, and the rest take place at the discharge port. A few deaths occur at the pre-export assembly depot as a result of salmonellosis (Norris, 2005). There are two main causes of sheep mortality during the long distance transport to the Middle East, representing 75% of total deaths aboard ship: a) failure to eat. This is the main reason of mortality during live export representing almost 50% of total deaths (Phillips, 2008), b) salmonellosis. This disease contributes to 25% of total deaths (Phillips, 2008). Heat stress and high humidity conditions have also been reported to cause mortality on sheep transported by sea (Gardiner and Craig, 1970).

1.3.5 Australian sheep live export criticism.

The long distance transport of farm animals has been criticized and reviewed by different groups inside and outside of Australia where ethical, economic and welfare arguments have been expressed (Kelly, 1995; Keniry et al., 2003; Phillips, 2005; Animals´Angels, 2011). In particular, the *Cormo Express* incident in 2003 raised several concerns from the local and international community about the welfare of livestock during long distance transport (Keniry et al., 2003; Phillips, 2005). This concern continues until today, where different organizations have criticized the live export process and the partial enforcement of standards (Animals´Angels, 2011). Phillips (2005) on the other hand discussed some ethical perspective of the Australian live export (summarized on Table 4) where it was concluded that the discussion about live export should consider the interest and welfare of the entire actors involved during live export where the information must be available for everyone and research should be done to understand, identify and reduce the welfare problems that sheep have to face during long distance transport.

Table 4. Stakeholders ethical consequences of the Australian sheep live export trade (Phillips, 2005).

Actor interest	**Trade positive ethical consequences**	**Trade negative ethical consequences**
Animal	1. Adequate food and water quality & quantity 2. Decreased mortality 3. Sheep life prolonged	1. Mortality rate greater than farm situation 2. Have to face several stressors 3. Poor slaughter conditions and disembarkation at Middle East 4. Risk of disease transmission
Trade stakeholders*	1. Higher prices for live export animals 2. Without trade, profit margin would be seriously affected 3. Found a market for mature wool-producing wethers	1. Cognitive dissonance may arise from farmers to distance themselves from the export process 2. Repeated presentation of cruelty may cause farmers to habituate to cruelty
Consumers	1. Meat affordable for Middle East 2. Ritual slaughter in Middle East involves social ethic issues 3. If not from Australia, could be from countries with poor welfare standards	1. Jobs are potentially lost in countries due to overseas suppliers 2. Muslims may not support the acquisition of goods from a Christian country
Australian Public	1. Farmers' jobs are maintained 2. Rural environment is preserved 3. Purchase from Muslims could help to increase contact with Christians	1. Trade infringes Australian´s moral responsibilities 2. Australians do not control animal management at foreign countries such as slaughter practices without stunning 3. Extensive systems contribute to land degradation 4. Australian abattoir jobs could be reduced

*Farmers, stockmen, veterinarians on ships, stevedores, ship owners and exporters

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CHAPTER 2. THE IMPACT OF VEHICLE MOTION DURING TRANSPORT ON ANIMAL WELFARE

Abstract. Motion sickness is a common response in humans and farm livestock during transport, but research on the impact of motion has been primarily focused on the use of animal models for humans and human research. Most of the research on livestock has occurred mainly during road transport. Two theories have been proposed to explain motion sickness. The first emphasizes the capacity of an individual to maintain balance and stability, and the second the relevance of motion signals transmitted by sensory receptors such as the vestibular system and the eyes. During livestock transportation, animals seek to reduce uncontrolled movements to reduce energy consumption and keep their posture, avoiding falls which might cause injuries and be fatal in some cases. Road and sea transport of livestock are two situations than can stimulate the vestibular system and produce motion sickness responses. Roll, pitch and heave motions are primarily responsible for motion sickness in humans during sea transport, and probably in animals too. The symptoms in humans include nausea, vomiting and loss of appetite, mostly as a result of autonomous nervous system activation. Road transport research in domestic animals such as dogs or livestock have described travel sickness behaviours such as vomiting and, specifically in the case of ruminant livestock, a stress-related reduction in rumination, but there have been no investigations of the impact of sea transport motion. It is concluded that, despite the paucity of data on livestock, there is sufficient evidence to believe that motion could be playing a key role in determining animal welfare when animals are transported by road or sea, and therefore it is relevant to investigate this topic in those animals that are regularly transported by humans, such as farm animals.

Keywords: Animal welfare; Livestock; Motion; Transport; Travel sickness; Stress

2.1 Introduction

As described in Chapter 1, failure to eat is the main cause of mortality during sea transport of sheep to the Middle East. This has been explained as a result of lack of adaptation to pellets offered at the assembly depot (Phillips and Santurtun, 2013), or high stocking densities (Black, 1996), and/or ammonia concentrations (Phillips et al., 2012) on the ship. Sea transport motion effects on sheep welfare are also a possibility, but these have not been investigated despite the evidence about their impact on different systems (e.g. digestive) in humans and other animals (Lang et al., 1999; Bos et al., 2008; Cai et al., 2010). In this sense, there has been limited research into the impact that motion of the vehicle or vessel during transport has on the welfare of livestock. This potential stressor is in most cases relatively new for livestock, which are infrequently transported (Weeks, 2007). One of the most common and important consequences that both non-human animals (hereafter animals) and humans experience during transport is motion sickness (MS). This term has been used mainly in humans to refer to discomfort associated with atypical patterns of passive motion (not initiated by the individual) during sea transport (sea sickness) (Aranda et al., 2005; Shupak and Gordon, 2006) road transport (train or truck sickness) (Lackner, 2009), space transport (space sickness) (Muth, 2006), as well as more recent phenomena in which there is no vehicle involved, such as cybersickness and simulator sickness (Bonnet et al., 2006). MS is a physiological reaction to motion patterns (Caillet et al., 2006), which integrates multiple responses from different physiological systems (Doweck et al., 1997) and affects most humans at least once in their lives (Fukutake and Hattori, 2000), in particular females (Lawther and Griffin, 1986). MS has been investigated in animal models mainly for human benefit (Chen et al., 2010), such as the use of fish as the experimental model to study space motion sickness, a research area of particular human interest to evaluate astronauts' performance (Anken and Hilbig, 2004).

MS has been studied and demonstrated in many different animal species, including squirrel monkeys (Brizzee et al., 1980), rats (Cai et al., 2010), dogs (Doring-Schatzl and Erhard, 2004; Cannas et al., 2010), cats (Crampton and Lucot, 1991; Lang et al., 1999) and the house musk shrew (*Suncus murinus*), an insectivore species that has been used as the animal model for motion-induced emesis (Uchino et al., 2001; du Sert et al., 2010). Other species for which MS has been described are fish (Anken and Hilbig, 2004), guinea pigs (Ossenkopp and Ossenkopp, 1990), pigs (Randall and Bradshaw, 1998), horses (Lee et al., 2001), sheep (Hall et al., 1998), seals and birds (Money, 1970). Some species of lower vertebrates, such as amphibians, are believed to not be capable of experiencing MS because of the absence of the relevant brain structures, which are possessed by mammals (Lychakov, 2012).

This review includes human research literature on motion sickness because of its relevance for other species and knowledge obtained in this field, as well as the research on animals for the purposes of investigating animal transport. Throughout, implications for livestock welfare are the primary focus of the review. Both ship and road transports are considered where relevant because some limited information is available. No information is available for air travel. This topic is increasing in importance as the value of livestock exports have increased from 5 to 20 billion US$ in the last 20 years (FAOSTAT, 2014).

2.2 Motion sickness theories

There are two main MS theories. The first, broadly known and accepted, is the sensory conflict theory (Reason and Brand, 1975; Oman, 1982; Warwick-Evans et al., 1998), alternatively known as sensory rearrangement theory (SRT) developed by Reason and Brand (1975). This states that 'all situations which provoke motion sickness are characterized by a condition of sensory rearrangement in which the motion signals transmitted by the eyes, the vestibular system and the non-vestibular proprioceptors are at variance either with one another or with what is expected based upon previous experience'. Some authors partially agree with SRT, but still emphasize that subjective vertical experience by individuals is the major component (Bles et al., 1998; de Graaf et al., 1998). Some authors have rejected it because of its low predictive validity (Riccio and Stoffregen, 1991; Draper et al., 2001).

The second MS theory emphasizes control of body orientation (Bles et al., 1998), and is known as the postural instability theory. Environments that generate a prolonged postural instability will produce motion sickness, and individual behaviour responses are a key aspect of motion sickness aetiology (Riccio and Stoffregen, 1991; Owen et al., 1998; Stoffregen et al., 2010). Although this theory does not predict the type of environments that will produce long periods of postural instability, it is a useful alternative instrument to study MS (Draper et al., 2001); however, some authors have observed that postural instability is not a condition sine qua non for motion sickness (Warwick-Evans et al., 1991; Faugloire et al., 2007; Bos, 2010). In all likelihood, it is probably just a contributing not causative factor, but the scale of the contribution in animal MS is unknown. The lack of resolution of these two theories emphasizes that despite its universal occurrence in humans and several animal species (Griffin, 1990), the numerous causes and mechanisms that produce MS are poorly understood. Table 5 illustrates some of the many elements implicated in the causation of motion sickness (Griffin, 1990). The processing of the signals begins with activation of the visual and vestibular systems, causing awareness and then interpretation of the motion, followed by emergence of clinical signs, sweating, nausea, pallor, hypersalivation and gastrointestinal disturbances (Griffin, 1990).

Table 5. Theoretical factors involved in causation of motion sickness, adapted to livestock transport (Griffin, 1990).

Motion characteristics	Animal factors
Acceleration	Experience
Frequency	Emotional state
Amplitude	Posture
	Age
	Sex
	Species/genotype

The theoretical basis for MS does not address the aetiology of the condition. In this sense, Bowins (2010) considers that MS cannot be explained by a disease model and proposed instead an evolutionary anomaly explanation, a theory that MS evolved, like pain, as a negative reinforcement mechanism to terminate an unusual motion. If individuals cannot eliminate or escape from a situation that produces MS, they exhibit behaviours to reduce MS effects such as lying down in humans when travelling by boat (Bowins, 2010). However, the fundamental process that produces MS has not yet been confirmed (Buyuklu et al., 2009).

2.3 Motion sickness symptoms and clinical signs

Susceptible humans show different symptoms when experiencing MS that include evidence of autonomic nervous system activity, mainly from the sympathetic branch, such as pallor, headaches, loss of appetite, cold sweating, apathy, nausea, depression and reduction in cognitive function (Buyuklu et al., 2009; Lackner, 2009; Macefield, 2009; Burton et al., 2010; Chen et al., 2010). MS incidence fluctuates according to individual susceptibility and stimulus intensity (Buyuklu et al., 2009). Susceptibility to MS in humans has been studied through questionnaires and experimental tests (Lackner, 2009), whereas in animals only experimental tests are possible (Kaji et al., 1990). However, humans and animals show similar gastrointestinal symptoms and clinical signs associated with MS, including hypersalivation, pica (craving for and consumption of non-nutritive substances), nausea, intestinal peristalsis, defecation and vomiting (Lang et al., 1999; Bos et al., 2008; Cai et al., 2010); nonetheless, not all motion sickness results in vomiting (Bowins, 2010). Further research on the relationship between MS and digestive disorders is warranted (Lang et al., 1999).

2.3.1 Nausea

Nausea is a negative sensation associated with the urge to vomit, which is less understood compared with the act of vomiting in mammals (Andrews, 2009). This is primarily because it has not been widely accepted which sensory requirements an animal needs to have to experience MS, the criteria for experiencing nausea (Holmes et al., 2009), and because it is difficult to quantify it as a feeling (Lang et al., 1999). However, there are 'behavioural equivalents' (Andrews, 2009) of nausea in animals, for example pica (McCaffrey, 1985), which are useful research tools to study nausea and MS in animals. As with MS, the sensory experiences cannot not be studied in the same way in animals as in humans, for whom the use of questionnaires is commonplace (Golding, 2006a). In this sense, vomiting is an important and useful research indicator of MS (Kaji et al., 1990) in those animal species that can perform this behaviour; however, in humans at least, there is an important percentage of individuals that experience MS but do not vomit (Shupak and Gordon, 2006).

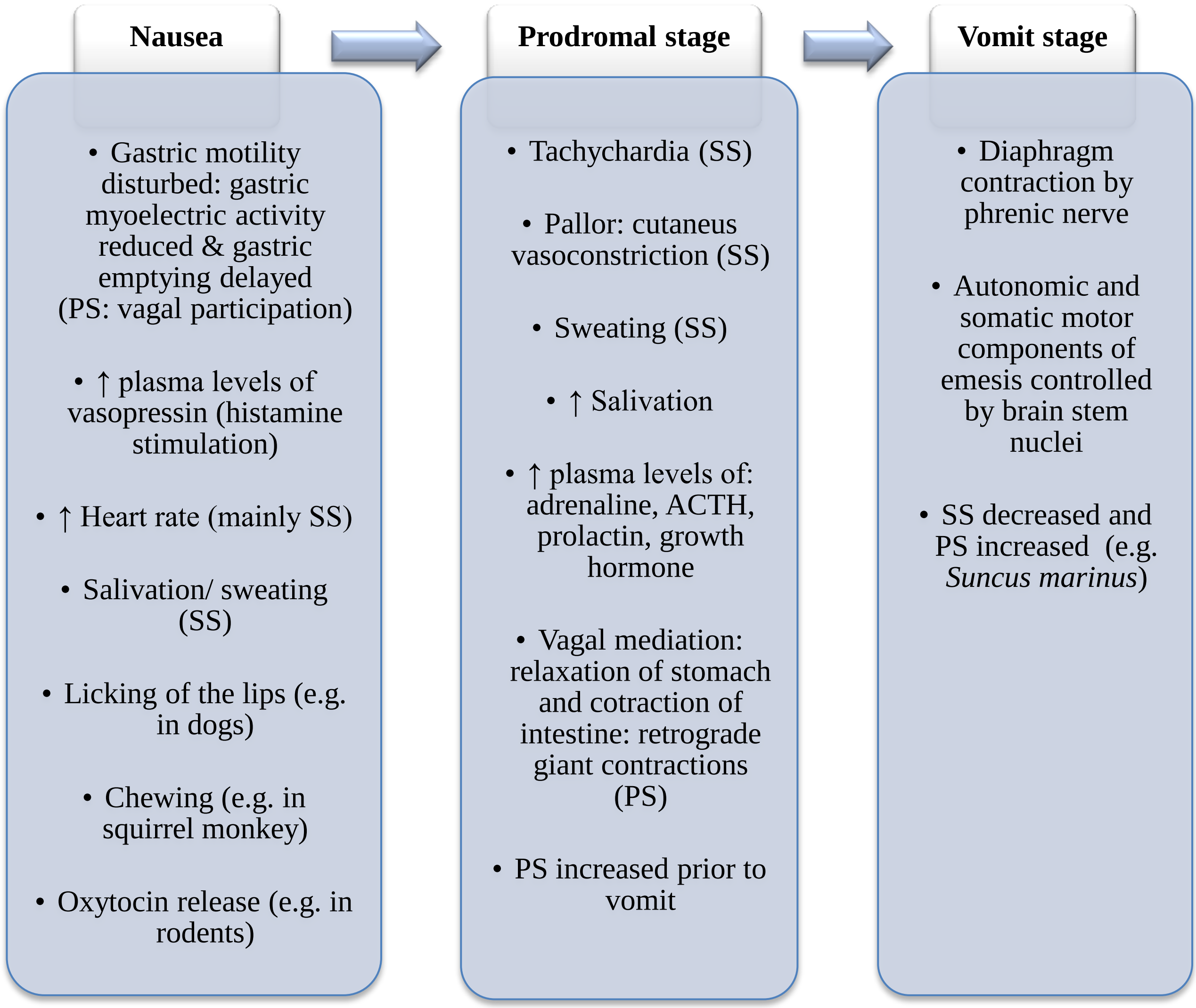
2.3.2 Vomiting

Vomiting, or emesis, is a protective response and coordinated reflex where upper gastrointestinal tract contents are forcefully ejected from the mouth (Frandson et al., 2009; Holmes et al., 2009). In relation to the animal species that experience MS, there are few mammalian species (house musk shrew, cat, dog, pig, marmoset, sperm whale, ferret) and even fewer bird (pigeon and petrel), amphibian (salamander and frog), reptile (snake and crocodile) or fish (shark and tuna) species that can vomit (Ebenezer et al., 1989; Wassersug et al., 1993; Andrews, 2009; Holmes et al., 2009; du Sert et al., 2010). Some animal species, for example rats (Ebenezer et al., 1989; Lee et al., 2010), mice and rabbits (Holmes et al., 2009), cannot vomit because they do not have the necessary reflex action (Andrews, 2009) as a result of their physiological and anatomical characteristics (Lee et al., 2010). Farm animals also rarely vomit because of anatomical characteristics (e.g. the horses' cardiac sphincter tone). Sheep, cows and goats hardly ever eject gastrointestinal contents from the mouth, except in some cases where plant, soil and mineral toxins have been consumed (Smith and Magdesian, 2002; Andrews, 2009). Nonetheless, in ruminants an 'internal vomit' may occur in which the contents of the abomasum are ejected into the rumen (Smith and Magdesian, 2002), with discharge from the mouth being very rare (Reece, 2009). Some differences that have been mentioned between monogastric and ruminants' gastrointestinal system and their capacity to vomit are the lack of complex neural connection in ruminants brain that could coordinate muscles during vomiting, and a reduced esophageal and diaphragm muscle strength. Further research in ruminants should describe better these responses.

2.4 Autonomic responses associated to MS

Many of the physiological responses associated with MS are mediated by the autonomic nervous system (ANS), including sympathetic nervous system activation and a general reduction in parasympathetic nervous system activity (apart from vagal participation). The exception to this rule is in the gastrointestinal system where the ANS changes differ prior to and during vomiting as a result of parasympathetic nervous system activity and a reduction of stomach activity by the sympathetic system (Uchino et al., 2001; Muth, 2006; Hasler, 2013). Much of the change is associated with stress (Lackner, 2009). In this sense, there is no general rule about which process starts first, motion sickness or stress, or whether they act in concert as a result of activation of the autonomous nervous system during motion sickness, which contributes to a normal stress response (Yates et al., 1998). Some visual (Wilkins and Evans, 2010) or gastrointestinal stressors (Chouker et al., 2010) have been described to generate motion sickness responses. Other authors have reported motion sickness and stress physiological responses occurring at the same time (Bradshaw et al., 1996a). Nonetheless, physiological responses from nausea to the emesis stage described in Fig. 6 (Money, 1970; Yates et al., 1998; Uchino et al., 2001; Li et al., 2005; Muth, 2006; Ohyama et al., 2007; Andrews, 2009; Macefield, 2009; Hasler, 2013) could be used as indicators to evaluate the impact on animal welfare as a result of a stress or motion sickness situation.

Monitoring of heart rate to evaluate MS has been used in humans as an additional indicator, as it increases during nausea as well as during the MS progression as a result of an increase in sympathetic stimulation (Holmes and Griffin, 2001). Similar conclusions were made by LaCount et al. (2009) using heart rate variability, in which they found a gradual sympathetic activation with increasing nausea, and an increase in vagal tone just before a strong nausea was registered.



SS=Sympathetic system activity; PS=Parasympathetic system activity

Fig. 6. Autonomic nervous system responses during motion sickness in mammals.

2.5 Postural balance and locomotion

Postural balance is maintained by multiple interactions of sections of the nervous system with the biomechanical design of animals (Biewener, 2003; Cuthbert, 2006). The vestibular system, vision and locomotion somatoreceptors are part of the equilibrium system, and its interactions (Bles, 1998; Cuthbert, 2006) are summarized in Fig. 7.

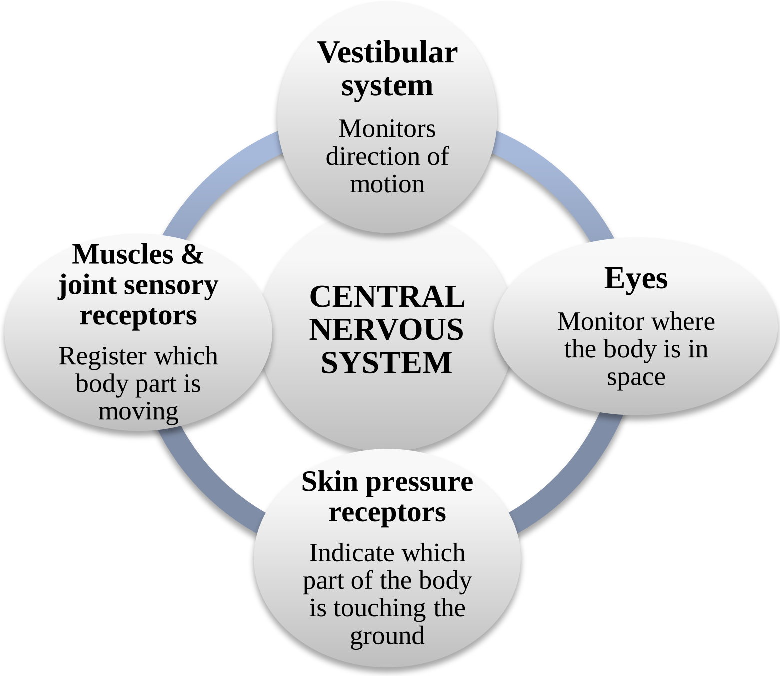


Fig. 7. Equilibrium structures and their interactions with the central nervous system to maintain postural balance.

As a basic principle, animals seek to avoid uncontrolled movements and use minimum energy in order to maintain their posture (Riccio and Stoffregen, 1991; Biewener, 2003). Stabilizing mechanisms exist to assist them when their support and balance have been adversely affected to provide dynamic stabilization of the body (Biewener, 2003). There are situations in animal transport where, although there is not complete postural control failure, animals are not able to stop the interactions with the environment and the persistent instability may lead to stress and potentially MS (Riccio and Stoffregen, 1991; Biewener, 2003). For example, Ruiz de la Torre et al. (2001) found that lambs transported on rough roads were more stressed than those transported on highways as their cortisol and heart rate was higher. Driving events such as braking and cornering are associated with losses of balance in several different livestock species, including cattle (Kenny and Tarrant, 1987; Knowles, 1999), goats (Das et al., 2001) and deer (Grigor et al., 1998). Space allowance has an impact on the motion that animals experience which affects their body postural balance and welfare. Maintenance of balance, and therefore minimisation of motion experienced, is achieved by regular stepping movements and support from other animals and vehicle fixtures. However, research to identify the optimum space allowance to minimise loss of balance has produced equivocal results. Some research suggests increased stress under loose stocking (Hall et al. 1998; Jones et al., 2010), but it has also been observed that goats transported by road with a reduced space allowance mostly fell down when trying to avoid a fallen animal (Das et al., 2001).

Orientation is important for animals to maintain balance during transport. If an animal is able to modify this to control postural instability and thereby escape from a destabilizing environment, they do not become sick (Riccio and Stoffregen, 1991). In this sense, Clark et al. (1993) observed that horses facing to the direction of travel in parallel formation lost their balance more and had more impacts with the trailer compartment compared to individuals facing backwards, probably because they had more space to move their heads and hence maintain balance. However, in other horse studies orientation during transport had no effect on locomotion (Toscano and Friend, 2001) or heart rate (Smith et al., 1994). In relation to other domestic species, Das et al. (2001) observed that goats transported by road spent most of the time parallel to the direction of travel but with frequent changes to diagonal and perpendicular orientation to maintain balance. Similar results in steers transported by road have been reported (Kenny and Tarrant, 1987). This situation is likely to be different during sea transport as the type and amount of movements differ from road transport, being primarily heave, pitch and roll (Santurtun et al., 2014). In contrast to this, in road transport surge, derived from rapid acceleration and deceleration, is a dominant motion type, and heave much less likely.

2.6 Vestibular function during motion sickness

The vestibular system located in the inner ear is composed of two sensory organs, the semicircular canals to detect angular acceleration and the otolith organs, utriculus and sacculus, to detect linear accelerations and head tilts (Stevens and Parsons, 2002; Glover, 2004). The vestibular system's main functions are spatial orientation and changes in body posture to maintain balance and vision stabilization (Javid and Naylor, 1999; Golding, 2006b). The system is very sensitive to angular motion and linear acceleration (Riccio and Stoffregen, 1991; Glover, 2004) and hence is fundamental in the development of motion sickness (Bos et al., 2007). Individuals with bilateral vestibular dysfunction do not usually develop MS (Golding, 2006b; Buyuklu et al., 2009), except during exposure to optokinetic stimulation (Lackner, 2009).

Situations that stimulate the vestibular system (i.e. the otolith organs) during transport are capable of inducing MS (Shupak and Gordon, 2006; Buyuklu et al., 2009). This is the case in sea sickness, described mainly in humans, which is characterized by low frequency, complex linear and angular accelerations (Buyuklu et al., 2009). MS in humans travelling by ship is mainly caused by heave (up and down) motion, but is also associated with the pitch (angular fore-aft) and roll (angular sideways) motions (Wertheim et al., 1998; Shupak and Gordon, 2006; Joseph and Griffin, 2008). This is an important consideration when sea transport vessels are designed (Fang and Chan, 2007), but is rarely considered in relation to animal cargo. Although roll and pitch motions by themselves can cause MS in humans (Wertheim et al., 1998; Howarth and Griffin, 2003), the worst situation for development of MS has been measured when humans experienced combined roll and pitch magnitudes of between 3.6° and 7.3°, compared with a lower magnitude (1.8°) (Joseph and Griffin, 2008), and when roll and pitch are combined with heave (Wertheim et al., 1998; Joseph and Griffin, 2008).

2.7 Animal behaviour related to motion sickness in vehicles

Ewes have been observed during transport to show active, coping behaviours such as teeth grinding and pawing at the ground because they were stressed by vehicle movements and lack of food and water (Schmiddunser, 1994, 1995). Following an intravenous injection of cholecystokinin, which induces nausea in sheep (Ebenezer et al., 1989; Greenough et al., 1998), a reduction in locomotion and defecation has been observed (Ebenezer et al., 1989), suggesting that these behaviours may be elevated during motion sickness.

Other species like pigs exhibit travel sickness related behaviours such as foaming, chomping, retching and vomiting (Bradshaw et al., 1996a; Bradshaw et al., 1996b; Randall and Bradshaw, 1998; Bradshaw et al., 1999). Bradshaw et al. (1996a) found that some pigs transported by road exhibited some behaviours and an increase in plasma lysine vasopressin that are commonly associated with travel sickness; however other scientists have suggested that these could be the result of food withdrawal, and emotions like anxiety and fear (Bradshaw et al., 1996a; Phillips, 2008). When travelling by car, dogs exhibit behaviours related to MS, such as swallowing, vomit and panting (Cannas et al., 2010). The potential for confounding factors indicates a need for scientists to take a reductionist approach to travel stress in animals, exposing animals to individual components and evaluating responses. There is only limited evidence of this: in some studies animals in moving transporters are compared with a group in an identical stationary vehicle, or with a group on the farm, and in others animals have been exposed in the laboratory to single components of the travel, e.g. ship motion (Santurtun et al., 2014) or ammonia (Phillips et al., 2010).

Rumination has been reported to reduce when livestock or wildlife are transported by road (Kenny and Tarrant, 1987; Grigor et al., 1998; Das et al., 2001), indicating a stress response but also linked to travel sickness (Kenny and Tarrant, 1987). As mentioned before, food ejection from the mouth has only been described in ruminants when an intoxication event occurs (Smith and Magdesian, 2002; Baert et al., 2005). Nonetheless with regard to the impact of sea sickness on ruminants, the Australian Bureau of Animal Health has reported (ABAH, 1981) that two percent of

294 dead sheep that were examined presented external signs of vomit, and two percent of 190 dead sheep examined presented with inhaled ingesta. One of the main reasons for sheep mortality during long distance transport by sea (e.g. in the Australia live export trade) is inappetence (Phillips, 2008). MS could be contributing to this situation, at least in susceptible individuals, as an additional stressor in sea transport. If some sheep experience MS during sea transport, this could activate the emetic system and, as concluded by Provenza et al. (1994), this system in sheep produces malaise and a reduction of food consumption, as part of an aversive feedback system. Further research should review the impact of sea transport motion on the digestive system as this situation has not been properly investigated yet.

2.8 Conclusions

The research reviewed confirms that motion may play an important welfare role during animal road and sea transport, producing motion sickness and stress responses. Our understanding of MS mainly comes from research on humans or animal models for human activities, and there is a need to investigate MS in livestock and other animal species used by humans. Transport motion research has been conducted in livestock mainly during road transport and there is an absence of research on sea transport motion, even though it has potential to stimulate the vestibular system and produce body instability, both precursors of motion sickness. Further research should use autonomic nervous system and behavioural responses to determine responses of this condition, leading to a better understanding of how this additional stressor could affect livestock during long distance transport.

2.9 Research project hypotheses and overall objectives

In relation to the knowledge reviewed in this chapter and the possible impact that sea transport motion may have on sheep welfare, the overall objectives and hypotheses of the research project were:

Hypotheses:

I) Sheep exposed to roll, heave and pitch with similar amplitude and period conditions of a commercial livestock transport vessel would demonstrate effects on their physiology and behavior.

II) Antiemetic drugs would alleviate adverse effects on feeding and position behaviours in sheep exposed to roll and heave in simulated ship transport.

III) Irregular sequences and the combination of Roll and Pitch motions may be the most stressful to sheep as they will not be able to habituate.

IV) Interactions between sheep would be stressful, as they will not be able to cope with changes of motion.

Objectives:

I) To examine the effects of roll, pitch and heave on the behavior, heart rate and its variability, rumination and body posture of sheep.

II) To examine the effects of antiemetic drugs in feed intake, feeding behaviours, heart rate, rumination and body posture.

III) To examine the effects of sequence regularity and roll and pitch motions in combination or individually on sheep feed and water intake, heart rate and its variability and body posture.

IV) To examine the effects of a combination of irregular and regular pitch and roll motions on sheep behaviours and heart rate variability.

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CHAPTER 3. ENGINEERING CONSIDERATIONS IN DEVELOPING A NOVEL METHOD TO MEASURE THE IMPACT OF SEA TRANSPORT MOTION ON SHEEP BEHAVIOUR AND PHYSIOLOGY

Abstract. Sheep are subjected to multiple stressors during commercial sea transport, including ship motion, ammonia, novel social dynamics and feed, high stocking density and multiple handling, all of which make it difficult to measure sheep responses to ship motion in isolation during a voyage. A practical method for measuring the impact of ship motions on the welfare of sheep on land was therefore developed, which exposed them to the three most important motions, roll (sideways), heave (vertical) and pitch (fore-aft). Roll and pitch motions were created using a programmable flight simulator platform, and heave motion was simulated elevating the entire apparatus with an electric forklift. Two main methods were developed to investigate the effect of these motions on sheep behaviour, physiology, balance, body posture, heart rate variability, rumination and feed intake. The first method evaluated each of the motions independently, replicating the frequency and magnitude of typical ship movements, taking into consideration the dimensions of a commercial vessel. The second method compared regular and irregular (random) movement sequences to investigate the importance of movement predictability on stress responses from sheep. The behaviour of sheep on the platform was similar to that which has been observed on ship. It is concluded that a detailed understanding of the responses of sheep to ship motion can be obtained by subjecting them to the different components of simulated transport using land-based equipment.

Keywords: Heave, Pitch, Roll, Sheep, Ship motions, motion simulator

3.1 Introduction

Live sheep export from Australia to the Middle East involves multiple stages and significant stressors over the three to four weeks of the journey, from mustering in the paddock to arrival in a feedlot or abattoir at the destination country (Phillips, 2008). Most concern surrounds the sea journey, when mortality increases as the animals are exposed to the potential stressors of ship motion, ammonia, novel social dynamics and feed, high stocking density, heat stress and multiple handling. Although some factors, such as ammonia (Pines & Phillips, 2013), have been examined in detail, the effects of ship motion have not been studied (Phillips & Santurtun, 2013). However, the complexity of interacting factors makes study on ships difficult.

Generally, motion impact on animals can be measured by evaluating their body posture and balance behaviours (Biewener, 2003; Riccio & Stoffregen, 1991), physiological parameters such as cortisol, vasopressin, packed-cell volume, heart rate and its variability, the latter indicating sympathetic and parasympathetic system responses (Holmes & Griffin, 2001; Knowles & Warriss, 2007; LaCount et al., 2009), and feed intake and rumination assessments (Das, Srivastava, & Das, 2001; Kenny & Tarrant, 1987). Feed intake is one of the main reasons for sheep mortality during long distance transport (Phillips, 2008), but ship motion could be contributing to this through motion sickness and stress responses to motion.

The World for Animal Health (OIE)[4] uses the following definition of animal welfare: "*State of an animal as regards its attempts to cope with its environment and includes both the extent of failure to cope and the ease or difficulty in coping*". In this sense the Ethograms used throughout the project (Appendix 7) include behaviours related to the body posture and balance strategies that sheep used to cope with the motion during experimental trials such as: a) head positions, b) standing and lying positions, c) stepping and other events (pawing, butting, pushing). Head position and standing were used to measure changes on body posture and stepping for balance. As described on Chapter 2, if animals do not cope with transport motion this could produce physiological stress and motion sickness responses. Physiological responses to stress were measured through the analysis of heart rate and its variability, which are useful tools to evaluate autonomic nervous system activity. In addition feeding behaviours (eating, drinking, rumination, prehension and mastication) were measured to evaluate the potential impact of motions on feed intake and digestive system. In summary, body posture and balance behaviours were used to describe how easy or difficult it was for sheep to cope with the sea transport motions. Heart rate and HR variability measurements were used to mainly examine the activity of the parasympathetic system in stress responses.

[4] http://www.oie.int/fileadmin/Home/eng/Internationa_Standard_Setting/docs/pdf/ENG_WG_September_2007_final_report.pdf

3.1.1 Sea transport motions

Ships typically experience six major motion types (Baniela, 2008; Ibrahim & Grace, 2010; McDonald, 1993): three displacement motions: heave (up and down motion along the vertical 'z' axis), sway (linear lateral motion along the transverse 'y' axis), and surge (linear longitudinal motion along the 'x' axis), plus three angular motions: yaw (rotational motion about the vertical 'z' axis), pitch (oscillatory motion about the transverse 'y' axis), and roll (oscillatory motion about the longitudinal 'x' axis) (Fig. 8).

Heave, pitch, and roll have most relevance to ship security and human health situations, such as motion sickness (MS) (Stevens & Parsons, 2002; Wertheim, Bos, & Bles, 1998). Heave motion in particular has been demonstrated to produce MS in various animal species, including humans (Lang, Sarna, & Shaker, 1999; Shupak & Gordon, 2006). Roll and pitch motions alone can also cause MS (Howarth & Griffin, 2003; Wertheim et al., 1998), but they have their greatest effect when combined with heave (Joseph & Griffin, 2008; Shupak & Gordon, 2006; Wertheim et al., 1998). The consequences of these motions have been mainly described in humans, but have also been studied in cats (Lang et al., 1999) and livestock (European Commission, 2002). Since the inner ear of sheep is similar to humans (Schnabl et al., 2012; Seibel, Lavinsky, & Irion, 2006) and is central to the development of MS (Bos, Damala, Lewis, Ganguly, & Turan, 2007), it is possible that sheep are affected by sea transport motions.

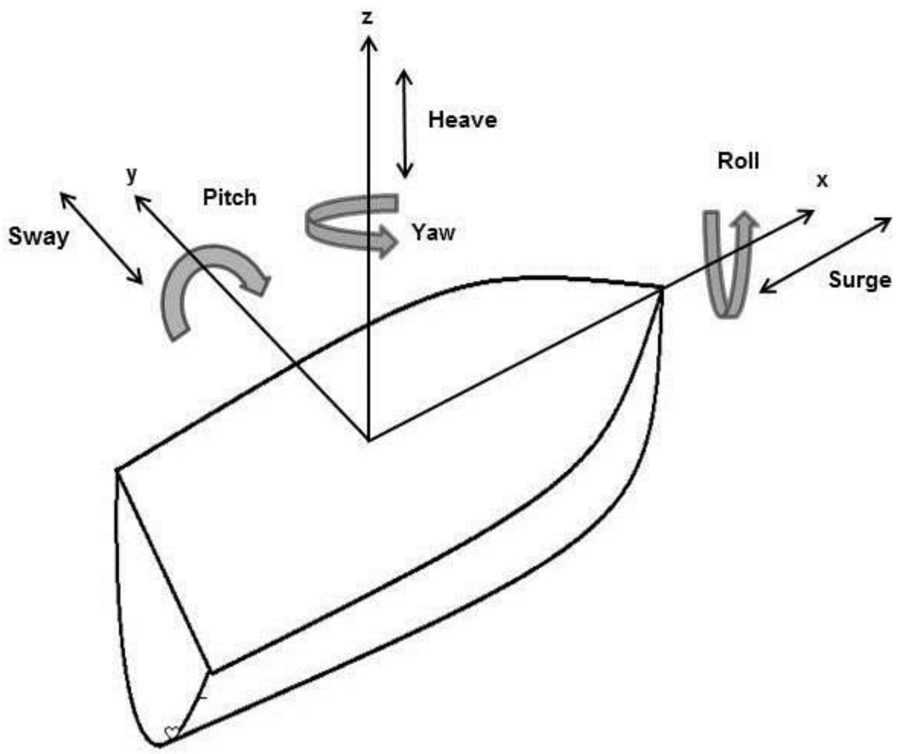

Fig. 8. Schematic diagram of ship showing the six motions, yaw, pitch, roll, heave, surge, sway.

Due to the complexity of ship stressors and motions, a laboratory-based ship motion simulator was developed that could replicate the frequency and amplitude of roll, heave and pitch motions independently and in combination, as well methods to register balance, body posture, feed intake behaviours and heart rate variability of sheep. The objectives in this paper were:

- describe the methodology used to determine the impact that roll, pitch and heave independently have on sheep welfare, taking into consideration the dimensional characteristics of a typical live export vessel.

- describe the methodology used to determine the impact that roll and pitch in regular and irregular sequences have, both in combination and independently.

- describe the methods to measure body posture, balance, feed intake and rumination behaviours, as well as heart rate variability.

3.2 Material and methods

The Animal Ethics Committee of the University of Queensland, Australia, approved all sheep-related procedures used during experiments.

3.2.1 Equipment used to simulate roll, pitch and heave motions

Roll and pitch platform

A motion platform (Model T2sMP, CKAS Mechatronics Pty Ltd, Melbourne, Australia, Photo 1) capable of producing roll and pitch movements either in combination or independently was obtained. The platform measured 800 x 1180 mm and sat 300 mm from the floor, on top of a 60 Hz, 10 A, 200-240 V electric motor which required single phase power. The motor drove two crank arms to generate pitch and roll operated by low noise pistons capable of moving the platform at 18^{o} s^{-1} to maximum 8^{o}, with an acceleration of 160^{o} $s^{-2.}$ The motor was connected to a desktop computer (Dell Optiplex 960, Texas, USA) through a Hi-Speed USB 2.0 cable (Belkin Ltd, Tuggerah, Australia) in order to send programmed motion instructions.

Photo 1. Two motion programmable platform.

3.2.2 *Platform programming*

Comparison of different motions

Initially sheep were exposed in pairs to independent roll, pitch and heave motions, taking into consideration the characteristics of a commercial live export ship. Dimensions of a typical and frequently used vessel, MV Ocean Drover, were used to determine the amplitude and frequency of these motions. This livestock carrier was one of the first large carriers constructed for this trade, with capacity for 75,000 sheep and 18,000 cattle, and an overall length of 176.7 m, length between perpendiculars of 164.9 m, and moulded (maximum) breadth of 31.0 m (Siba Ships, 2011; Wellard Rural Exports, 2010). Roll, pitch and heave amplitude and period were calculated using formulae for the construction of livestock carriers (AMSA, 2006; Baniela, 2008; McDonald, 1993; Skraastad, 1983) (Table 6 and 7).

Table 6. Amplitude and period formulae used for determining ship motions.

Motion	Amplitude	Period	References[c]
Roll	$\phi = [14.8+3.7\ L_{PP}/B]e^{-0.0023Lpp}$	$T_{roll} = C*B/\sqrt{GM_T}$	(1, 2)
Pitch	$\psi = 12e^{-0.0033LPP}$	$T_P = 0.5\ \sqrt{L_{PP}}$	(1)
Heave	$H^{amp} = L_{PP}/80$	$T_H = 0.5\ \sqrt{L_{PP}}$	(1)
	Symbols:	***Units:***	
H^{amp}	Heave amplitude	M	(1,3)
ψ	Pitch amplitude	°	(1,3)
ϕ	Roll amplitude	°	(1,3)
T_{roll}	Roll period	s	(1,3)
T_P	Pitch period	s	(1,3)
T_H	Heave	s	(1,3)
L_{PP}	Length between perpendiculars	Distance on a ship from the 'forward' to its 'after' perpendicular	(3)
C	Empirical Constant [a]	0.7	(1)
B	Maximum ship's beam	m	(1,2)
GM_T	Transverse metacentric Height [b]	m	(2,4)

[a] 0.7 is recommended for livestock carriers. Data were not available for MV Ocean Drover.
[b] Distance between centre of gravity and ship's metacentre (2m), derived from a vessel with similar dimensional characteristics to the MV Ocean Drover (McDonald, 1993).
[c] (1) Skraastad, 1983; (2) Baniela, 2008; (3) McDonald, 1993; (4) AMSA, 2006.

Table 7. Amplitude and period used for roll, pitch and heave motions.

	Amplitude	Period
Roll	8.0° each side	15 seconds (7.5s left, 7.5s right)
Pitch	2.3° each side	6 seconds (3s left, 3s right)
Heave	67 cm up + 67cm down	6 seconds (3s up, 3s down)

The amplitude for roll, pitch and heave motions represented 33 % of the maximum tolerance recommended for conversion of a cargo vessel to a livestock carrier (Table 7) (Skraastad, 1983). This maximum was determined in part by the capacity of the motion platform for roll, but was also similar to that found in a study of a ship with similar dimensions to the MV Ocean Drover (McDonald, 1993).

Microsoft Visual Studio Solution C++ Express 2008 software was used to programme the platform, which was achieved by calibrating the computer-recorded positions to angles using an inclinometer (DNM 60L Professional Digital Level, accuracy $\pm$ 0.05^{o}, ®Bosch, Stuttgart, Germany). The platform was programmed by instructing it to move to positions that were at increasing angles to the level position (Appendix 2). For Roll motion to achieve 8^{o} amplitude and a period of 15 s (right and left side movements, each of 7.5 s) experimentation with angular movements of 0.095, 0.14 and 0.19^{o} demonstrated that 0.143^{o} movements gave the smoothest transition to maximum roll, with 56 movements of 67 ms each, and a total time of 3752 ms (which with a return time of 3752 ms gave a total time for movement on one side of 7.5 s). For pitch experimentation with the same angular movements indicated that 0.114^{o} for each movement gave the smoothest transition, with 20 movements of 75 ms each, giving a total time of 1500 ms.

Comparison of regular and irregular motion sequences

The regularity of the motion sequences may determine the stress impact on the animal; therefore the platform was programed to move in both regular and irregular sequences for roll and pitch, independently or in combination, using two variables, amplitude and period. Irregular sequences for roll and pitch were constructed from thirty amplitude and period values that were randomly selected by the software (Table 8). The duration of these irregular roll and pitch sequences used an increment of three positions.

Regular roll and pitch sequences were determined from the mean amplitude and period for the irregular roll and pitch sequences (Table 9). The delay was determined from the following formula:

$$\text{Delay} = (\text{positional increment} \times 1\ \text{s})/(\text{speed in}\ ^{o}\,\text{s}^{-1} \times \text{positional increment}\ ^{o\,a})$$

[a]21.0 and 17.4 platform units for roll and pitch, respectively

Equation 1**.**

The platform positions were linked to angles as previously described. Appendix 3 and 4 give the programme commands for regular combinations of roll/pitch and irregular combinations, respectively.

Table 8. Amplitude and period values used during irregular pitch and roll sequences (Speed and delay before each movement are specified for the various period values).

IRREGULAR ROLL			IRREGULAR PITCH		
Amplitude (Degree)	Period		Amplitude (Degree)	Period	
	Delay (ms)	Speed (°sec^{-1})		Delay (ms)	Speed (°sec^{-1})
8.0	8	18	8.0	10	18
7.75	9	16	7.75	11	16
7.5	10	14	7.5	12	14
7.25	11	13	7.25	13	13
7.0	12	12	7.0	14	12
6.75	13	11	6.75	16	11
6.5	14	10	6.5	17	10
6.25	16	9.0	6.25	19	9.0
6.0	18	8.0	6.0	22	8.0
5.75	20	7.0	5.75	25	7.0
5.5	24	6.0	5.5	29	6.0
5.25	29	5.0	5.25	34	5.0
5.0	36	4.0	5.0	43	4.0
4.75	41	3.5	4.75	49	3.5
4.5	44	3.25	4.5	53	3.25
4.25	48	3.0	4.25	57	3.0
4.0	57	2.5	4.0	69	2.5
3.75	63	2.25	3.75	77	2.25
3.5	71	2.0	3.5	86	2.0
3.25	95	1.5	3.25	115	1.5
3.0	114	1.25	3.0	138	1.25
2.75	143	1.0	2.75	172	1.0
2.5	159	0.9	2.5	192	0.9
2.25	179	0.8	2.25	216	0.8
2.0	204	0.7	2.0	246	0.7
1.75	238	0.6	1.75	287	0.6
1.5	286	0.5	1.5	345	0.5
1.25	357	0.4	1.25	431	0.4
1.0	476	0.3	1.0	575	0.3
0.75	714	0.2	0.75	862	0.2

Table 9. Mean amplitude and period values used during regular roll and pitch sequences.

Type of Sequence	Amplitude (Degree)	Period	
		Delay (ms)	Speed (°sec^{-1})
Regular roll	4.4	117	1.22
Regular pitch	4.3	141	1.22
Combined roll & pitch	4.3	235	1.21

3.2.3 Heave equipment

An electric forklift (Walkie Reach Stacker model SHR 5500 series, Crown Equipment Corporation, New Bremen, OH, USA) was used to elevate the platform and produce the heave motion. The forklift had a maximum elevation of 3200 mm, and a lifting speed of 0.18 and 0.28 ms^{-1}, with and without load, respectively. The forklift was covered with egg boxes and foam mats to reduce the noise output to 65 dB (Digital Sound Level Meter, Q1362, Dick Smith Electronics Pty, NSW, Australia, ± 0.1 dB accuracy) and set to reach the height and speed needed. The platform was loaded onto a pallet to facilitate insertion of the forks (Photo 2). To avoid differences in noise levels between motion treatments, the forklift was withdrawn from under the platform but still operated when sheep were exposed to other treatments: i.e. roll, pitch and control (immobile platform).

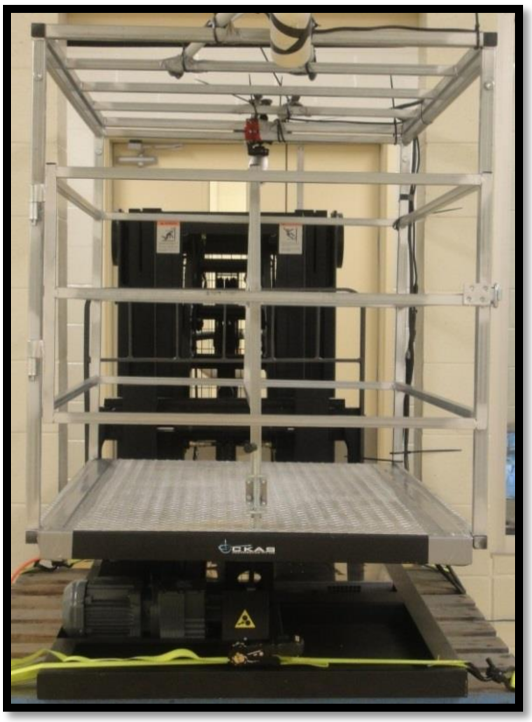

Photo 2. Crated attached to the programmable platform.

3.2.4 Animals and housing

Four to six Merino cross wethers from the School of Veterinary Science, University of Queensland, flock were used in four experiments. At all times, except when they were introduced to the platform, the sheep were kept in a paddock with ad libitum access to water, lucerne chaff and 1 kg of lucerne pellets daily.

For safe exposure of the sheep to the motions, a steel crate 0.85 m wide x 1.2 m long and 0.95 m height was attached to the platform (Photo 2), providing 0.51 m^2 $sheep^{-1}$. This compares with 0.22 m^2 required in Australian standards for a 40 kg sheep (DAFF, 2011), allowing the sheep to move and facilitating behaviour observation. In tests requiring provision of feed (Chapter 5 and 6) and water, aluminium and plastic bowls, inset into a wooden frame, were provided for this purpose (Photo 3). When providing feed during tests, 1.5 kg of lucerne pellets (® Lockyer Lucerne Products PTY. LTD, Queensland, Australia) were offered which contained 90.5% DM, 18.6% crude protein, 13% digestible protein, 33% ADF, digestible DM 64.9%, TDN 64.7%, and 9.3 MJ/kg DM. During tests of the platform, sheep were exposed to treatments arranged in a Latin Square design (Appendix 6), with all sheep experiencing each treatment four times (factorial designs) or twice (i.e. not single factor designs), with 30-60 min of exposure to treatment each day for 8-12 d to determine either the impact of roll, pitch and heave independently (Appendix 6, Experiment 1 and 2), or the impact of roll and pitch in regular and irregular sequences, in combination and independently (Appendix 6, Experiment 3 and 4). The experimental design allowed for two exposure periods in the morning and two in the afternoon to enable all of the sheep to be tested in one day.

Photo 3. Feed and water devices used during experimental trials.

3.2.5 Habituation protocol

This process was essential to reduce the stress from other potential stressors preceding and during experimental trials and to obtain a valid representation of the impact of ship motions alone on the sheep. The potential stressors included handling of the sheep, use of a ramp to get them into the crate, forklift noise, drinking from a water bottle, adjustment to a new environment in the research facility, heart rate monitoring, the researchers' presence and a low quality diet. The first step involved the reduction of fear of researchers by offering high quality pellets by hand 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), three to four hours inside the research facility for feeding, resting and use of crate (20 d), one hour every for 7 d experiencing the forklift noise. The training stopped when there were no obvious fear behaviours and the heart rate mean was close to the resting rate. The entire process lasted 32 d.

Photo 4. Feeding of sheep by hand using pellets.

3.2.6 Validation of the motion platform

Behaviour

Behaviour was recorded by four video cameras (Kobi CCD Video Camera, Model K-32HCVF, Ashmore, QLD, Australia) attached to tripods and to each side of the crate to record the position of the head, legs, body, and the back of each sheep, as well as feeding and drinking intake behaviours (Photo 5). A digital video recorder (Kobi H.266, Model XQ-L 900H, Ashmore, QLD, Australia) was used to record the images. Video footage was analysed using behaviour coding software (CowLog versions 1.1 and 2.0, University of Helsinki, Finland), which added time codes to behaviours during replay and registered this in a data file (Hänninen & Pastell, 2009). For behaviours like prehension, mastication and rumination it was imperative to use the front camera and the video footage was analysed at one third of the normal speed.

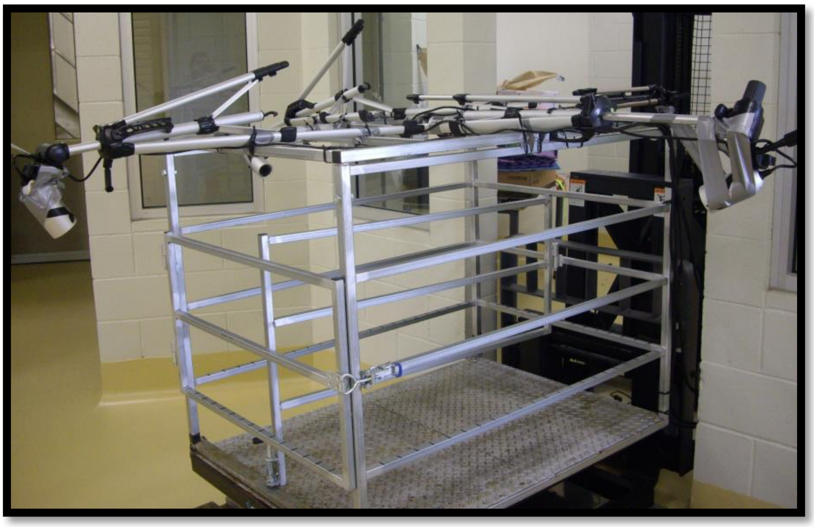

Photo 5. Video cameras attached to the crate.

Heart rate and its variability (HRV)

Hear rate monitors (Polar Heart Rate 810i Meter, Kempele, Finland) were attached to each sheep one week prior to the start of experiments so they could be habituated to the device. This type of monitor has been validated for assessment of heart rate, interbeat (R-R) interval and standard deviation of R-R intervals (Gamelin, Baquet, Berthoin, & Bosquet, 2008) and used on cattle, horses and sheep (von Borell et al., 2007).

The monitors consisted of a watch and a transmitter, attached with a plastic cinch, where the positive electrode was placed caudal to the posterior border of the scapula and the negative electrode caudal to the olecranon process (Photo 6). The electrode sites were shaved every afternoon after tests and water was used to optimise electrode-skin contact. Each monitor was activated at the beginning of each experimental trial, placing each sheep 5 m from the nearest recorded sheep in order to avoid interference. After activation, interference was not experienced and sheep were taken to the crate without any technical problems. Detection of heart rate and inter-beats intervals (IBI) was continuous for 30-60 min periods in the crate, and the resulting data on intervals between beats were transmitted wirelessly and stored in a data logger. These data were then downloaded onto a computer using the Polar Equine Software 3.0. Sections of 512 beats were extracted as non-overlapping segments of approximately 6 min, as recommended for time domain and spectral analysis (von Borell et al., 2007). Kubios HRV version 2.1 (Tarvainen & Niskanen, 2012) was used for the analysis of HRV variables. Data anomalies were detected by the software using a predictive algorithm and only data with less than 5 % of anomalies was selected, as recommended for the analysis of heart rate data (von Borell et al., 2007). Correction of the anomalies in selected sections of data was by the cubic spline interpolation method at default rate of 4 Hz, with smoothing within a window of 256 s (Tarvainen & Niskanen, 2012). Four time domain variables: IBIs mean, SDNN (standard deviation of all IBIs), RMSSD (square root of the mean of the sum of the squares of differences between successive IBIs) and NN50 count (number of pairs of successive IBIs differing by more than 50 ms) and a Fast Fourier transformation (FFT) analyses were carried out to measure changes of the sympathovagal balance. Frequency bands for FFT were modified according to sheep heart rate frequency range, as recommended by von Borell et al. (2007).

Photo 6. Position of the heart rate electrodes and transmitter.

3.2.7 Statistical analysis

During analyses, all data were checked for normal distribution of residuals using the Anderson-Darling test. For data not satisfying the Anderson-Darling test, log_{10} transformations were made and back-transformed means are reported in addition to transformed data. Preliminary analyses determined that sheep within pair was not a significant predictor of behavior or heart rate results, and individual sheep were therefore considered valid as replicates. For this purpose a general linear model produced residuals that were not normally distributed; therefore Mood median tests were conducted for all variables to check the independence of the companion animal variable. This indicated no statistical significance (P > 0.05) in any variable, indicating that identity of the companion sheep did not have any effect on any treatment effects. Proportion of time spent and frequency of each behavior, HRV time domain and Fast Fourier transformed data were analysed using a general linear model with the following factors: treatment, day, sheep (nested with treatment), and treatment-day interactions using the statistical package Minitab (version 16). For all tests, probability levels are two-tailed and are considered significant when P< 0.05. Post hoc Tukey's tests were used to identify which means were significantly different from each other.

3.3 Results and validation

Results of the general linear model of data from the first experiment, fitted with day, sheep, treatment, and treatment-day interactions as factors, from the first experiment are presented in Table 10. In this, four merino cross wethers, approximately 24 months of age, weighing (mean ± SEM) 37.4 ± 0.1 kg) were exposed in pairs to four treatments, pitch, roll, heave and control (immobile platform) in the crate for 30 min periods. Sheep spent a mean of 66 and 33 % of their time standing and lying, respectively, and 38 % of their time ruminating. There were no significant day effects (P > 0.05) but there tended to be differences between sheep for standing (P = 0.050) and lying (P = 0.047), but not ruminating (P = 0.11). In a second experiment, six merino cross wethers, approximately 34 months of age, weighing (mean ± SEM) 44.2 ± 0.1 kg were exposed in pairs to six treatments, regular and irregular sequences of pitch, roll, and combined roll and pitch in the crate for 60 min periods and were offered feed. They spent a mean of 60 % of time feeding, again with a significant sheep effect (P = 0.002).

Table 10. Standing, lying and ruminating behaviours during 30 min treatment periods.

	Standing	Lying	Ruminating
Proportion of time (%)	65.7	33.3	38.3
P value of Day effect	0.24	0.24	0.10
P value of Sheep effect	0.05	0.047	0.11

The two experiments together show that sheep engaged in normal behaviours for sheep in ships: feeding, lying, standing and ruminating. In a report of sheep behaviour on board a live export shipment from Australia to the Middle East, sheep spent a mean of 4, 29, 64, and 24 % of a 10 day voyage, feeding, lying, standing and ruminating, respectively (Pines & Phillips, 2013). A further report of sheep behaviour on ship from New Zealand to the Middle East indicated that sheep spent a mean of 17, 24, 45, and 12.1 % of their time spent feeding, lying, standing and ruminating (Black, Matthews, & Bremner, 1994), suggesting some variation in the time spent in these three major activities. A comparison between the first two experiments and what has been described in these studies is shown on Table 11.

Table 11. Mean of time spent feeding, standing, lying and ruminating during three types of studies.

Studies	Feeding	Standing	Lying	Ruminating
Motion platform experiments	60	65.7	33.3	38.3
Pines and Phillips, 2013	4	64	29	24
Black et al., 1994	17	45	24	12.1[a]

[a] Mean of two enclosures.

The short term exposure of sheep in our crate and the high quality of the pellets, which were deliberate to evaluate treatment differences in feeding behaviour, probably explain the long-time sheep spent feeding in our second experiment, compared with observations on ship, which were either continuous over 24 h (Pines & Phillips, 2013) or concentrated into the major feeding and resting periods (Black et al., 1994).

Heart rate and its variability provide potent measures of stress responses in sheep (von Borell et al., 2007) and they were used in this experimental program to identify how the sheep's physiology responded to simulated ship motion. Heart rate declined over the first four days of the first experiment ($P<0.001$) (Figure 9). A similar result was observed ($P < 0.001$) in the second experiment seven months later. Heart rate variability, as measured by the $\log_{10}$ RMSSD, increased over days (Fig. 9), suggesting increased parasympathetic activity of the autonomous nervous system (von Borell et al., 2007). With the resting heart rate of a sheep being 70-80 beats min^{-1} (West, 1995) and stressful events commonly elevating sheep heart rate to over 100 beats min^{-1} (Parrott, Hall, & Lloyd, 1998), we may conclude that heart rate in this apparatus was close to resting rate. However, the results also suggest some adaptation to the stress of enclosure in the cage over time, including an increased activity of the parasympathetic nervous system (Friedman & Thayer, 1998; Porges, 2003; von Borell et al., 2007), and a long period of adaptation is therefore recommended.

After dividing the 30 min period into quartiles, it was found in the first experiment that the mean heart rate was greater in the first quartile (83.8) than the last three quartiles (80.5, 81.5 and 81.5, respectively) (P = 0.05). Lengthening the habituation period may overcome this problem.

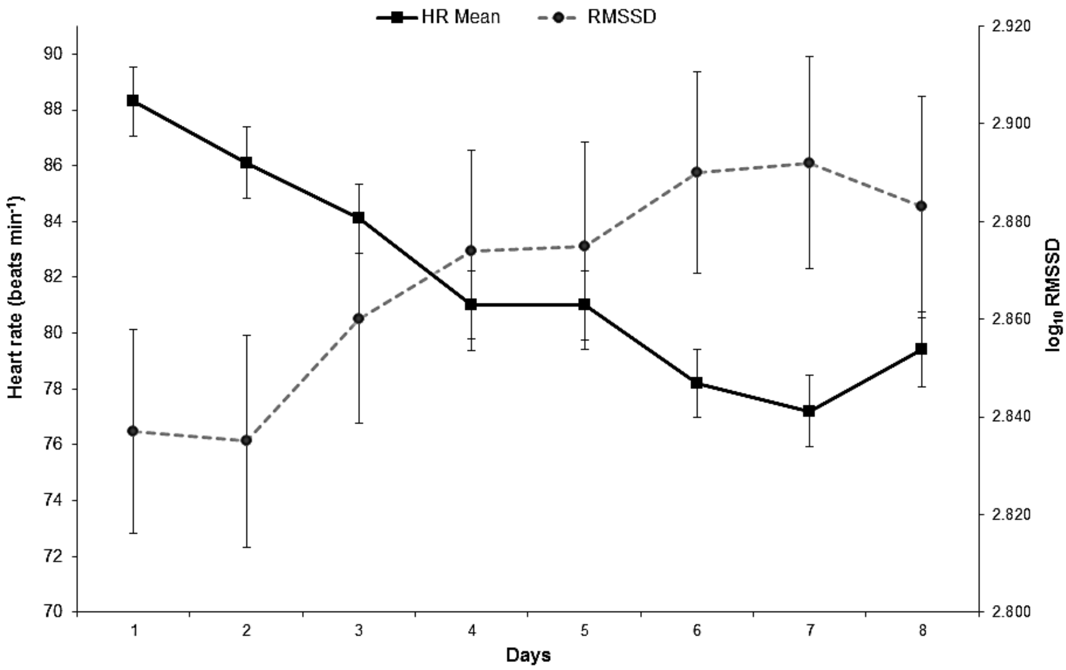

Fig. 9. Least square means and ± SE of heart rate and $\log_{10}$ RMSSD between inter-beat intervals over an eight day experiment (difference between days P<0.001).

3.4 Conclusion

A motion platform that exposed sheep separately or in combination to roll, pitch and heave motions, which represent the major motions experienced on ships was developed. The device was successfully programmed to simulate regular and irregular motion sequences. Sheep performed similar behaviours in the crate to those performed in a ship, and it was possible to provide feed and measure feeding behaviour. The heart rate responses suggest that there were initially minor elevations due to novelty during exposure to treatments, but that these could be overcome with a period of adaptation of the sheep to the apparatus.

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CHAPTER 4. PHYSIOLOGICAL AND BEHAVIORAL RESPONSES OF SHEEP TO SIMULATED SEA TRANSPORT MOTIONS

ABSTRACT: The motion of ships can cause discomfort and stress in humans, but little is known about the impact on sheep welfare, despite many sheep travelling long distances by ship during live export. We tested whether exposing sheep to roll (side to side movement), heave (up and down movement) and pitch (front to back movement) with similar amplitude and period conditions to a commercial livestock transport vessel would affect their behavior and physiology. Specifically, we tested the effects of these motions, and a control treatment, on behavior, heart rate variability, rumination, body posture and balance of sheep. Four sheep (37 ± 0.1 kg) were restrained in pairs in a crate that was placed on a moveable and programmable platform that generated roll and pitch motions. An electric forklift was used to produce heave motion. The treatments were applied for 30 min each time in a changeover design with one repetition over eight consecutive days. Sheep behavior was recorded continuously from video records and heart rate monitors were attached to determine heart rate and its variability. Heave reduced the time that sheep spent ruminating, compared with the other three treatments ($P < 0.001$). The two sheep spent more time during heave with their heads one above the head of the other ($P < 0.001$) and looking towards their companion ($P = 0.02$), indicating greater affiliative behavior. Sheep spent more time during heave standing with their back supported on the crate ($P = 0.006$) and less time lying down ($P = 0.01$). Roll caused more stepping motions than pitch and control, indicating loss of balance ($P < 0.001$). Heave and roll had increased heart rates and reduced inter-beat intervals, compared to Control ($P < 0.001$). The inter-beat intervals of sheep in the heave treatment had an increased ratio of low to high frequency duration ($P = 0.01$), indicating reduced parasympathetic control of stress responses. Therefore there was both behavioral and physiological evidence that heave and roll caused stress, with sheep experiencing roll requiring apparently coping better by regular posture changes and heave causing the sheep to seek close presence to their companion.

Key words: balance, behavior, heart rate variability, sea motions, sheep, transport

4.1 INTRODUCTION

Several stressors have been studied during the road transport of livestock but little is known about sea transport stressors, including the motions produced by ships (Norris, 2005; Phillips, 2008). One common consequence that animals and humans experience during sea, land and air transport is motion sickness (**MS**) (Javid and Naylor, 2002; Stevens and Parsons, 2002; Lackner, 2009). Humans and animals show various symptoms such as loss of appetite, vomiting and tachycardia (Bos et al., 2008; Buyuklu et al., 2009; Lackner, 2009; Macefield, 2009; Burton et al., 2010; Cai et al., 2010; Chen et al., 2010). Autonomic responses that take place during MS are associated with stress responses (Lackner, 2009) but it is unclear which comes first. Transport has also been reported to affect body posture (Cockram et al., 2004) and rumination (Cockram, 2007).

In relation to sea transport motion, heave (up and down motion along the vertical axis), roll (oscillatory motion about the longitudinal axis) and pitch (oscillatory motion about the transverse axis) (Ibrahim and Grace, 2010), are most studied because of their relevance to ship security and human health (Wertheim et al., 1998; Stevens and Parsons, 2002). Heave is the most relevant motion to MS in humans (Lawther and Griffin, 1988; Wertheim et al., 1998; Shupak and Gordon, 2006), and its impact has been described in cats (Lang et al., 1999), squirrel monkeys (Holmes, 2009) and rats (Cai et al., 2010). Nonetheless, roll and pitch motions can by themselves cause MS responses (Wertheim et al., 1998; Howarth and Griffin, 2003).

The hypothesis of this study was that exposing sheep to roll, heave and pitch with similar amplitude and period conditions of a commercial livestock transport vessel would affect their physiology and behavior. Specifically, the objective was to examine the effects of roll, pitch and heave on the behavior, heart rate and its variability, rumination and body posture of sheep.

4.2 MATERIALS AND METHODS

The study was conducted at the University of Queensland, Australia (27.3° S, 152.2° E). Approval for this research was obtained from the University's Animal Ethics Committee (SVS/443/10).

4.2.1 Animals, Housing and Management

The design of novel methodology for exposing sheep to floor movement, programming the movement platform, heart rate monitoring and video-recording of behavior have been described in full elsewhere (Santurtun et al., 2014). In brief, 4 Merino cross wethers, approximately 24 months of age, weighing (mean ± SEM) 37.4 ± 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 of 3 tubular steel bars (0.87 m wide × 1.2 m long × 0.95 m high), divided in 2 by a 3-barred division, which prevented them from turning round. This provided 0.56 m^2 /sheep, almost twice that of the national standard (0.285 m^2 for half wool sheep, DAFF, 2011), to allow expression of behavior. The crate was covered with a white cotton sheet to reduce near distance visual stimulation, which might encourage motion sickness (Bos et al., 2005). Chaff and water were not offered when the sheep were in the crate but were available ad libitum in racks at all other times. In addition 1 kg of lucerne pellets was offered at 1600 h daily.

4.2.2 Simulating Sea Transport Motions

Amplitude and period.

The dimensions of the MV Ocean Drover, the world's largest purpose-built livestock carrier, were used to determine typical amplitude and duration of roll, heave and pitch to which the sheep would be exposed. The amplitude used for this experiment represented 33% of the maximum tolerance required when a ship is converted from a cargo to a livestock carrier (Skraastad, 1983). The resultant amplitudes and durations (**Table 7**) were equivalent to the expected dynamic environment of a ship with dimensional characteristics similar to the MV Ocean Drover in moderate seas (McDonald, 1993).

Pitch, roll and heave equipment.

The crate was positioned on a 0.8 m wide × 1.2 m long motion platform (Model T2sMP, CKAS Mechatronics Pty Ltd, Melbourne, Australia) capable of producing roll and pitch movements independently or in combination with the aid of two crank arms. The platform moved in two directions to simulate roll and pitch, with movement duration determined from computer commands (Santurtun et al., 2014). It responded to a computer sending motions programming commands through a BELKIN® Hi-Speed USB 2.0. An electric forklift (Model SHR5550 series, Crown Equipment Corporation, New Bremen, OH, USA) was used to elevate the platform to produce heave motion. The characteristics of the apparatus have been described in more detail in Santurtun et al. (2014).

4.2.3 Experimental protocol

Sheep were habituated through positive reinforcement with feed pellets to the different potential stressors they would face during the experiment, including handling, heart rate monitor fitting and wearing, forklift noise, ramp for loading and unloading, experimental rooms and the crate over a period of 32 d. During the experiment, sheep were exposed in pairs to four treatments, pitch, roll, heave and control (immobile platform) in the crate for 30 min periods. The treatments were applied in a 4 × 4 Latin Square with one repetition/d for 8 d (Appendix 6, **Experiment 1.**). In total each sheep was exposed to 16 treatment periods, 8 in the morning and 8 in the afternoon. Sheep experienced treatments in 4 possible pairs (1+2, 3+4, 2+3 and 1+4) so that pair effects could be evaluated.

Behavior recording

Sheep behavior was recorded continuously in real time by four video cameras (Kobi CCD Video Camera, Model K-32HCVF, Ashmore, QLD, Australia) during exposure to treatment. A digital video recorder (Kobi H.266, Model XQ-L 900H, Ashmore, QLD, Australia) was used to record the images, and the video data were then analysed using a continuous recording of each animal and Cowlog 2.0 behavior software for coding of behaviors (Hänninen and Pastell, 2009), according to the ethogram in Appendix 7 (**Experiment 1.**). The duration of time spent in the following mutually exclusive states was continuously recorded: standing (not ruminating): no support, or supporting their body against the crate or the barrier; lying (not ruminating); standing or lying ruminating. In addition the duration of various head positions, which has been shown to relate to emotional state (Hall et al., 1998; Hemsworth et al., 2011) was recorded as up, middle, down (relative to withers), above/ under the companion sheep, looking towards companion sheep, turned around, looking towards side bars. Finally stepping was recorded an event. After each exposure to treatment, sheep were taken to an adjacent paddock and eating pellets, drinking, standing and walking behavior were recorded by an instantaneous scan every ten s by a single observer outside the paddock for 30 min to determine post-treatment behavior.

Heart rate variability.

Heart rate monitors (Polar S810i, Kempele, Finland) were attached to each sheep for detection of heart rate and inter-beats intervals (IBI) during the 30 min exposure to treatment in the crate. Four sections of 512 beats (approximately 6 min) were extracted from each treatment for time and frequency domain analysis. Kubios HRV 2.1 software (Tarvainen et al., 2014) was used to detect anomalies and obtain heart rate variability (HRV) variables. The following 3 time domain variables were examined to measure changes to the sympathetic and parasympathetic branches of the autonomous nervous system: IBIs mean (RR mean); square root of the mean of the sum of the squares of different successive IBIs (RMSSD), which reflects the integrity of vagus nerve-mediated autonomic control of the heart, and the number of pairs of successive IBIs differing by more than 50 ms (NN50), which is correlated to RMSSD and hence also reflects vagal activity. IBI mean is less descriptive and provides general variability information (von Borell et al., 2007). In addition a frequency domain analysis was done using a Fast Fourier Transformation (FFT) obtaining high (HF) and low (LF) frequency bands, expressed in normalized units (n.u.), and as a ratio (LF/ HF). HF has been associated with vagal activity (Malliani et al., 1994) and LF with both sympathetic and vagal activity (Cerutti et al., 1995; von Borell et al., 2007). Frequency bands widths (LF: 0.04-0.2 Hz, HF: 0.2-0.4 Hz) were assigned according to sheep recommended ranges (von Borell et al., 2007).

4.2.4 Statistical analysis

During analyses, all data were checked for normal distribution of residuals using the Anderson-Darling test. For data not satisfying the Anderson-Darling test, $\log_{10}$ transformations were made and back-transformed means are reported in addition to transformed data. Preliminary analyses determined that sheep within pair was not a significant predictor of behavior or heart rate results, and individual sheep were therefore considered valid as replicates. For this purpose a general linear model produced residuals that were not normally distributed; therefore Mood median tests were conducted for all variables to check the independence of the companion animal variable. This indicated no statistical significance ($P > 0.05$) in any variable, indicating that identity of the companion sheep did not have any effect on any treatment effects. Proportion of time spent and frequency of each behavior, HRV time domain and Fast Fourier transformed data were analysed using a general linear model with the following factors: treatment, day, sheep (nested with treatment), and treatment-day interactions using the statistical package Minitab (version 16). For all tests, probability levels are two-tailed and are considered significant when $P< 0.05$. Post hoc Tukey's tests were used to identify which means were significantly different from each other.

4.3 RESULTS

4.3.1 Behavior

Sheep spent more time during heave with their heads under or above the head of their companion ($P < 0.001$) and towards the companion sheep ($P = 0.02$) than in the roll, pitch or control treatments (Table 12). No significant treatment differences were observed for head up, middle, down, turned around or towards side bars. Sheep spent more time standing during heave with their body supported on the crate (P = 0.006), compared with sheep in roll, pitch or control treatments. During roll and pitch they spent almost no time unsupported, whereas those in heave and control treatments spent more time without than with support (P < 0.001). Little time was spent standing against the division for support, and there were no treatment differences (P = 0.59). Sheep spent less time lying when experiencing heave in relation to the other treatments (P = 0.01) (Table 12). Roll produced more stepping in total than pitch and control, with heave intermediate (P < 0.001). Heave reduced the time sheep spent ruminating in relation to the other treatments (P < 0.001) (Table 12).

Table 12. Mean time spent by sheep in different head positions, standing, lying and ruminating, and mean of frequency of stepping events during roll, heave, pitch and control treatments.

	Treatments					
Behavior	**Roll**	**Heave**	**Pitch**	**Control**	**SED**	**P-value**
Head position						
Under/above, s/30 min[1]	1.22[b] (16.6)	2.34[a] (218.8)	1.21[b] (16.2)	1.03[b] (10.7)	0.110	<0.001
Towards companion, s/30 min	151.8 [b]	309.1 [a]	193.4[ab]	140.4[b]	27.10	0.02
Turned around, s/30 min[1]	0.82 (6.6)	0.71 (5.1)	0.70 (5.0)	0.89 (7.8)	0.138	0.88
Up, s/30 min	63.9	38.9	43.6	43.3	16.80	0.11
Middle, s/30 min	326.7	215.2	250.5	214.9	45.01	0.57
Down, s/30 min	408.6	427.3	381.6	334.2	43.41	0.73
Towards side bars, s/30 min	49.0	65.9	61.4	36.8	13.41	0.70
Standing[2]						
Against crate, s/30 min	169[b]	408[a]	190[b]	131[b]	37.1	0.006
No support, s/30 min[1]	0.33[b] (2.1)	2.97[a] (933)	0.45[b] (2.8)	2.70[a] (501)	0.071	<0.001
Against barrier, s/30 min	0.25	0.26	0.00	0.82	0.305	0.59
Lying[2], s/30 min	574[a]	212[b]	743[a]	910[a]	97.1	0.01
Stepping rate, number/30 min[1]	2.32[a] (209)	1.98[ab] (96)	1.65[bc] (45)	1.50[c] (32)	0.064	<0.001
Ruminating, s/30 min	809[a]	166[b]	839[a]	941[a]	70.0	<0.001

[a-c] Least square means within rows with different superscripts are significantly (P < 0.05) different by Tukey's test.

[1] Statistical analyses were performed on $\log_{10}$ transformed data. Least squares mean were calculated and then back transformed for presentation in this table in parentheses. [2] Not ruminating. Degrees of freedom: 3.

4.3.2 Heart rate measurements

The inter-beat interval was shorter, and thus heart rate was higher, for sheep experiencing roll and heave treatments compared with control, with pitch intermediate (Table 13) (P < 0.001).

RMSSD was lower for the Heave treatment (P = 0.04) compared to Pitch. The inter-beat intervals in the Heave treatment had a reduced high frequency band (P = 0.003) and increased low frequency band (P = 0.004) and ratio (P = 0.010) compared to Control. A trend for a decreased NN50 was observed during Heave relative to Control (P = 0.09) (Table 13).

Table 13. Heart rate measurements during roll, heave, pitch and control treatments.

	Treatments					
Measure	**Roll**	**Heave**	**Pitch**	**Control**	**SED**	**P-value**
HR mean, beats/min	83.5^{a}	84.0^{a}	81.0^{ab}	79.2^{b}	0.70	<0.001
IBIs mean, ms[1]	2.860^{b} (724.4)	2.858^{b}(721.1)	2.870^{ab}(741.3)	2.885^{a} (767.4)	0.003	<0.001
RMSSD, ms[1]	1.57^{ab} (37.1)	1.55^{b} (35.5)	1.60^{a} (39.8)	1.59^{ab} (38.9)	0.011	0.04
NN50, count	1.77	1.73	1.83	1.85	0.188	0.09
HF, n.u.	28.3^{ab}	26.8^{b}	31.3^{ab}	33.1^{a}	0.97	0.003
LF, n.u.	71.4^{ab}	72.8^{a}	68.5^{ab}	66.6^{b}	0.98	0.004
LF/HF[1], ms[2]	0.473^{ab} (2.9)	0.496^{a} (3.1)	0.404^{ab} (2.5)	0.359^{b} (2.3)	0.0241	0.010

[a-c] Least square means within rows with different superscripts are significantly (P < 0.05) different by Tukey's test.

[1] Statistical analyses were performed on $\log_{10}$ transformed data. Least squares means are provided (with back transformed means). Degrees of freedom: 3.

4.3.3 Post treatment behavior

In the residual period no sheep were observed to lie down, but sheep that had been exposed to the control treatment ate for less time (P = 0.01) and stood for longer (P = 0.01) than sheep exposed to the other three treatments (Table 14). No treatment differences were observed for walking (P = 0.24) and drinking (P = 0.63).

Table 14. Mean time spent (s/30 minutes) by sheep in post-treatment behaviors following motion and control treatments.

	Treatments					
Behavior, s/30 min	**Roll**	**Heave**	**Pitch**	**Control**	**SED**	**P-value**
Eating	$176^{\mathbf{ab}}$	$183^{\mathbf{a}}$	$186^{\mathbf{a}}$	$158^{\mathbf{b}}$	6.42	0.01
Drinking	3.9	6.1	3.8	4.4	1.93	0.63
Standing	$18.5^{\mathbf{b}}$	$12.8^{\mathbf{b}}$	$9.8^{\mathbf{b}}$	$34.0^{\mathbf{a}}$	5.54	0.01
Walking	11.4	7.9	10.8	13.2	2.41	0.24

[a,b] Least square means within rows with different superscripts are significantly (P < 0.05) different by Tukey's test.

4.4 DISCUSSION

The hypothesis that roll, heave and pitch at similar levels to those experienced during commercial transport would affect sheep physiology, balance and behavior was confirmed. The results suggest that heave first, and secondly roll could have a negative impact on sheep welfare. This agrees with research on the capacity of these motions to affect humans (Lawther and Griffin, 1988; Wertheim et al., 1998; Shupak and Gordon, 2006; Joseph and Griffin, 2008a).

Heave greatly reduced the time spent ruminating in relation to roll, pitch and control treatments. This could derive from activation of the vestibular system (i.e. otolith organs), which is linked to the vagus nuclei, area postrema and other areas of the brain (Balaban, 1996; Lackner, 2009; Hasler, 2013). The RMSSD and HF band, indicating vagus nerve mediated autonomic control of the heart was significantly reduced in this treatment. Further research is required to determine if these sheep are experiencing malaise and inappetence (Kenny and Tarrant, 1987; Provenza et al., 1994; Muth, 2006). In humans and other monogastric animal species, the central involvement of the autonomic nervous system during motion sickness has been suggested to change the gastrointestinal physiology (Muth, 2006; Hasler, 2013). Within the autonomic nervous system, the sympathetic adrenal medullary system reduces stomach activity and produces an emetic response, with the parasympathetic system reversing these responses (Muth, 2006; Trentini et al., 2008; Hasler, 2013). Heart rate variability analysis suggests a decrease of the parasympathetic activity in the heave treatment, supporting the hypothesis that heave reduced rumination as a result of motion sickness. However, rumination could have been reduced as a result of stress, as reported in road transport studies (Das et al., 2001; Grigor et al., 1998; Cockram, 2004) or in combination with travel sickness responses (Kenny and Tarrant, 1987).

Heave and roll were more important than pitch for their capacity to affect body posture and balance, as they are for humans (Shupak and Gordon, 2006). During heave, sheep positioned their head above or below their companion's head as well as having their head turned towards their companion twice as long as in the other three treatments. This affiliative behavior has not been reported previously but it could be a result of stress, with sheep being a highly gregarious species, and is similar to sheep putting their head under the withers of other sheep during stress associated with transport or slaughter (Hall et al., 1998; Hemsworth et al., 2011). This behavior could also have the purpose of reducing the impact of the motion as described in humans (Bittner and Guignard, 1985), improving balance, as observed previously in sheep (Jones et al., 2010). Alternatively, it could be residual infant behavior such as sucking (Dwyer and Lawrence, 1998) or a submissive position under stressful conditions (Done-Currie et al., 1984; Nowak et al., 2008). The interpretation of these behaviors requires further motivational and neurophysiological investigation

(Rushen, 2000). Nonetheless, considering that the identity of the companion sheep did not affect the treatment effects and that sheep do not like to be in contact during transport (Broom and Fraser, 2007; Jones et al., 2010), the lowering of the head beneath the body of the conspecific may have a balance function as well as a possible stress response. The sheep did not brace themselves against the division during heave, only against the crate, suggesting that body contact was deliberately avoided perhaps because of potential loss of balance if the conspecific moved suddenly. It would be interesting to learn if the dominant animal has their head above or below the subordinate conspecific.

Sheep spent more time supporting their body on the crate during heave and reduced time lying down. Body movement control is limited during lying down, as the sheep is effectively like a cylinder on a moving floor, whereas when standing the four feet are used to maintain position. Support of the body against the crate during heave, but not roll, may have been due to less predictability in the heave movements caused by variation in motor speed. In comparison roll movements were smooth and sheep were observed to sway in response to these. Decreased resting behavior in sheep that are being transported has been considered a source of stress (Cockram, 2004). The stress in this case could be exacerbated by the postural instability and loss of body control associated with motion sickness (Riccio and Stoffregen, 1991; Stoffregen et al., 2010).

Roll impacted on sheep stepping more than control and pitch and during this motion the sheep spent less time with their back supported on the crate than heave and control treatments. This was probably to overcome the potential loss of balance during roll. Roll is an angular motion, less natural to the locomotion mechanism of a sheep and their hooves' design copes well with back and forward motions like heave or pitch rather than side angular motions (Alexander, 2003).

Responses to pitch were not different statistically to the control treatment for behavior and heart rate variability, except that sheep experiencing pitch spent less time standing unsupported than those in the control treatment. This suggests mild risk to loss of balance. The absence of major negative effects of this motion may have been due to its low amplitude compared to roll, as experienced on ships, but may also reflect the fact that the worst motion sickness and stress situations occur when pitch is combined with roll and heave motions (Wertheim et al., 1998; Joseph and Griffin, 2008b). Further experiments should measure the combination of these motions.

Heave and roll heart rate and heart rate variability analyses suggested physiological stress responses as a result of these motions. The mean heart rate during both of these motions was not elevated to levels experienced during previous livestock road transport studies (Hall et al., 1998; Andronine et al., 2008). This could be because the sheep were accustomed to the apparatus, whereas the neophobic response of sheep to road vehicles probably exacerbated the effects of the movement. HR mean is of limited value to assess sympathovagal input as it summarizes the contribution of all components of the cardiac activity (Tulppo et al., 1998). The interval between beats and most importantly the HRV measurements that represent vagal regulatory activity, in particular RMSSD and the High Frequency band indicated a reduction in the parasympathetic nervous system activity (which counteracts stress effects) with heave (von Borell et al., 2007). Only heart rate elevation gave an indication of a stress response to roll, suggesting the stress response was greatest in the heave treatment.

Post treatment behavior analysis showed sheep spending more time eating and less time standing doing nothing after exposed to motion in relation to control treatment. This may be comfort eating as a result of the stress in heave and roll. It has been reported that stress increases food intake in other animal species (Ortolani et al., 2011; McMillan, 2013). However, if some sheep experienced motion sickness, the amplitude, frequency and duration experienced might have been insufficient to reduce appetite after thirty minutes of trials exposure. Further experiments should evaluate feeding behaviours during motion over long periods.

4.5 CONCLUSIONS

These results indicate that heave and roll require sheep to make regular behavioral and physiological adjustments to body instability. Sheep exposed to heave showed affiliative behavior towards their partner in the crate and rumination was greatly reduced, both suggesting stress. Heart rate measurements also suggested that sheep in this treatment experienced the greatest stress level, and probably reduced parasympathetic activity. Roll primarily affected balance, requiring the sheep to make corrective stepping movements, but there was some evidence of stress from an elevated heart rate. Sheep were not greatly affected by the pitch movement, probably because the amplitude in our experiment, which simulated the amplitude of the movement observed in ships, was lower than roll.

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CHAPTER 5. THE EFFECTS OF ANTIEMETIC ON SHEEP BEHAVIOUR AND PHYSIOLOGY DURING SIMULATED SHIP TRANSPORT

Abstract: Heave and roll have been described to be the most important sea transport motions because of their capacity to produce motion sickness and stress responses in humans and animals affecting the digestive system and producing diverse symptoms from nausea, vomit, and inappetence. We tested whether exposing sheep to roll (side to side movement) and heave (up and down movement), with similar amplitude and period conditions to a commercial livestock transport vessel, affects their feed intake, feeding behaviour, heart rate and body posture, and whether providing antiemetic drugs attenuates motion effects in the sheep. Six sheep were restrained in pairs in a crate that was placed on a moveable and programmable platform that generated roll motion. An electric forklift was used to produce heave motion. The treatments were applied for 60 min each time in a changeover design over 12 consecutive days. There was no evidence of effects of the motion on feed intake, but the Heave treatment made the sheep eat faster ($P = 0.006$), with fewer mastication bites ($P = 0.004$), which could reduce the efficiency of digestion. Antiemetic provided limited evidence of improved balance, since the sheep spent less time with their head against a dividing mesh in the crate, and they increased prehension biting rate. Sheep in the Heave treatment spent longer with their head against the mesh than those in the Control treatment, confirming previous observations of increased affiliative behaviour caused by this treatment. It is concluded that simulated ship motion did not cause reduced feed intake over short periods, but that there was limited evidence that the motion had adverse effects on balance which could be attenuated by an antiemetic.

Key words: antiemetic drugs, feed intake, motion sickness, sea transport motions, sheep

5.1 INTRODUCTION

Motion sickness (MS) is a commonly consequence experienced during transport by animals and humans (Javid and Naylor, 2002; Lackner, 2009), including various digestive disorders, such as loss of appetite, nausea, vomiting, intestinal peristalsis and defecation (Bos et al., 2008; Buyuklu et al., 2009; Lackner, 2009; Macefield, 2009; Burton et al., 2010; Cai et al., 2010; Chen et al., 2010). In ruminants the effects are difficult to quantify both because the sensation of nausea is little understood (Holmes et al., 2009) and because the impact of the ruminant anatomical and physiological characteristics on vomiting are unclear (Andrews, 2009). Loss of appetite and reduction of rumination are two symptoms that are easier to quantify and have been reported in livestock transport studies. Rumination declines when livestock are transported as a result of a physiological stress response (Das et al., 2001) and/or travel sickness responses (Kenny and Tarrant, 1987). Loss of appetite is one of the main causes of sheep mortality when they are exported alive from Australia to the Middle East (Richards et al., 1989), which has been suggested to result from lack of adaptation to pellets (Phillips and Santurtun, 2013), high ammonia concentrations (Phillips et al., 2012), and/or metabolic diseases (Norris, 2005).

One of the sea transport stressors that could contribute to inappetence is ship motion (Phillips and Santurtun, 2013). Sea sickness in humans is primarily caused by heave (up and down) motion, which is exacerbated in combination with roll (side to side) and pitch (fore to aft) motions (Lawther and Griffin, 1987; Shupak and Gordon, 2006; Joseph and Griffin, 2008). These motions are capable of stimulating the vestibular system (Wertheim et al., 1998), which in turn is linked to the area postrema in the brain to produce malaise or appetite reduction (Muth, 2006; Lackner, 2009; Hasler, 2013). Antiemetic drugs such as dexamethasone, metoclopramide and diphenhydramine have been used on sheep to investigate 1) if feed intake could be increased (Adams and Sanders, 1992), 2) cardiovascular effects on pregnant ewes (Eisenach and Dewan, 1996), 3) to reduce perinatal mortality (Miller et al., 2009) and 4) metabolic and organ pathways (Kumar et al., 1999). To my knowledge only one has investigated the combination of these drugs and provided some evidence that they might have an antiemetic function in sheep to food aversion but did not prove that they functioned as in humans (Provenza et al., 1994).

The hypothesis of this study was that antiemetic drugs would alleviate adverse effects on feeding and position behaviours in sheep exposed to roll and heave in simulated ship transport. To test this we monitored antiemetic drugs impact on feed intake, feeding behaviors, heart rate, rumination and body posture.

5.2 MATERIALS AND METHODS

The study was conducted at the University of Queensland, Australia (27.3° S, 152.2° E) with approval from the University's Animal Ethics Committee (SVS/443/10).

5.2.1 Animals, Housing and Management

The design of novel methodology for exposing sheep to floor movement, programming the 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 30 months of age and weighing (mean ± SEM) 39.1 ± 0.1 kg, 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 of 3 tubular steel bars (0.87 m wide × 1.2 m long × 0.95 m high), divided in 2 by a 3-barred division, which prevented them from turning round. The crate was covered to reduce visual stimulation (Appendix 5, **Photo 7**) and aluminium bowls and plastic bottles were attached to provide feed and water respectively during experimental trials. A mesh was attached to the division to avoid sheep eating from their companion's bowl (Appendix 5, **Photo 8**).

5.2.2 Sea Transport Motions

Amplitude and period.

The dimensions of the MV Ocean Drover were used to determine typical amplitude and duration of roll and heave to which the sheep would be exposed. Exposing the sheep to pitch was discounted because previous research had shown limited effects of this motion (Santurtun et al., 2014). The platform moved in two directions to simulate roll, with movement duration determined from computer commands (Santurtun et al., 2014). The amplitude used for this experiment represented 33% of the maximum tolerance required when a ship is converted from a cargo to livestock carrier (Skraastad, 1983). The resultant amplitudes and durations (Table 15) were equivalent to the expected dynamic environment of a ship with dimensional characteristic similar to the MV Ocean Drover in moderate seas (McDonald, 1993).

Table 15. Period and amplitude used for roll and heave motions.

	Amplitude	Duration
Roll	8.0º each side	15 seconds (7.5s left, 7.5s right)
Heave	67 cm up + 67cm down	6 seconds (3s up, 3s down)

Roll and heave equipment

The crate was positioned on a 0.8 m wide × 1.2 m long motion platform (Model T2sMP, CKAS Mechatronics Pty Ltd, Melbourne, Australia) capable of producing roll movements independently or in combination with the aid of two crank arms. The platform responded to a computer sending motions programming commands through a BELKIN® Hi-Speed USB 2.0. An electric forklift (Model SHR5550 series, Crown Equipment Corporation, New Bremen, OH, USA) was used to elevate the platform to produce heave motion. The characteristics of the apparatus have been described in more detail in Santurtun et al. (2014).

5.2.3 Experimental protocol

Sheep were habituated through positive reinforcement to different potential stressors they would face during the experiment such as handling, heart rate monitor, forklift noise, ramp for loading and unloading, experimental rooms and crate over a period of three weeks. In addition, the entire group was habituated to new situations such as the use of plastic bottles and aluminium bowls to drink water and feeding respectively, and eat pellets with molasses which was used later to provide the antiemetic (Appendix 5, **Photo 9**). During the experiment, sheep were exposed to six treatments, roll, heave and control in pairs in the crate for 60 min periods, providing antiemetic on half of the treatments. The treatments were Heave, Roll and Control, with and without antiemetic, applied for 60 minute periods in two consecutive 6 x 6 Latin Squares over 12 consecutive days (Appendix 6, **Experiment 2.**). In total each sheep was exposed to 12 treatment periods in 6 possible pairs (1+6, 2+4, 3+5, 1+4, 2+5, and 3+6), in order that pair effects could be evaluated.

Feeding

In the crate sheep had ad libitum access to water and 1.5 kg of lucerne pellets for each 60 min period (® Lockyer Lucerne Products PTY. LTD, Queensland, Australia[5]). Pellets contained

[5] http://www.lockyerlucerne.com.au/pellet.htm

90.5% DM, 18.6% crude protein, 13% digestible protein, 33% ADF, digestible DM 64.9%, TDN 64.7%, and 9.3 MJ/kg DM.

Antiemetic drugs

Sheep received two doses of the following combination of drugs: diphenhydramine hydrochloride at 1.19 mg/kg BW, metoclopramide monohydrochloride at 0.95 mg/kg BW, and crystalline dexamethasone at 0.19 mg/kg BW. These were given at both 90 and 1 minute before starting the treatment. This combination of drugs and doses was used for sheep by Provenza et al. (1994) without any detrimental consequences for the animals. The tablets of each of the drugs were crushed and given with 20 g of lucerne pellets and 30 ml of sugar cane molasses to avoid being rejected from sheep. For those treatments without antiemetic, only the pellets with molasses were given.

Behavior recording

Sheep behavior was recorded continuously by 3 video cameras/sheep cameras (Kobi CCD Video Camera, Model K-32HCVF, Ashmore, QLD, Australia) during each treatment period. Continuous video record of each animal was processed using Cowlog 2.0 software for coding of behaviors (Hänninen and Pastell, 2009) according to the ethogram in Appendix 7 (**Experiment 2.**). The duration of the following behaviours was continuously recorded: a) eating, b) drinking, c) feed prehension (taking feed into the mouth with the lips), d) feed mastication, e) head position: up, middle, down (relative to withers), above the companion sheep, looking towards companion sheep and side bars of the crate, on mesh separating the 2 sheep, f) standing, g) lying, h) kneeling. For prehension and mastication the number of bites and time spent in these behaviours was recorded during the first min from each 5 min, with the video footage played at 0.33 x real time. From the measurements, feed intake rate (g DM/min), and prehension and mastication biting rates (/min) were calculated. After each exposure to treatment, sheep were taken to an adjacent room (Appendix 5, **Photo 10**) and standing, walking and lying behaviours were continuously video recorded for 30 min.

Heart rate

Heart rate monitors (Polar Heart Rate 810i Meter, Kempele, Finland) were attached to each sheep for detection of beats every five seconds during the 60 minutes period in the crate to obtain mean values. Anomaly corrections were carried out using Polar heart rate monitor software.

5.2.4 Statistical analysis

During analyses, all data were checked for normal distribution of residuals using the Anderson-Darling test. For data not satisfying the Anderson-Darling test, $\log_{10}$ and square root transformations were made as appropriate and back-transformed means are reported in addition to transformed data.

Preliminary analyses determined that sheep within pair was not a significant predictor of behaviour or heart rate results, and individual sheep were therefore considered valid as replicates. For this purpose a general linear model produced residuals that were not normally distributed; therefore Mood median tests were conducted for all variables to check the independence of the companion animal variable. This indicated no statistical significance ($P > 0.05$) in any variable, indicating that identity of the companion sheep did not influence treatment effects.

Proportion of time spent and frequency of each behavior, together with HR mean data, were analysed using a general linear model with the following factors: motion type, use of antiemetic, movement-antiemetic interactions and sheep, using the statistical package Minitab version 16. A period term was introduced in the analyses of mastication and prehension biting rate and time spent in these activities. For all tests, probability levels are two-tailed and are considered significant when $P < 0.05$. Post hoc Tukey's tests were used to identify significant differences between individual means.

5.3 RESULTS

There were no significant ($P < 0.05$) or close to significant ($P > 0.05<0.10$) effects of provision of antiemetic on behaviour or heart rate (Table 16). There was no effect of motion on feed ($P = 0.72$) or water intake ($P = 0.45$) or the time that the sheep spent eating ($P = 0.11$) or drinking ($P = 0.48$). Sheep subjected to Heave had a faster eating rate ($P = 0.006$), compared to Control sheep, and fewer mastication bites ($P = 0.004$) (Table 17). The sheep in treatment Heave also spent longer with their head on the mesh ($P = 0.009$) and tended to spend longer looking towards their companion ($P = 0.09$) (Table 17). Body position was not affected by motion type and heart rate was affected by neither antiemetic nor motion type.

Neither feed ($P = 0.29$) nor water intake ($P = 0.24$) were affected by administration of antiemetic (Table 18), however, there was a tendency ($P = 0.06$) for the antiemetic to increase the time that sheep spent drinking. Antiemetic reduced the time that sheep spend prehending their feed ($P = 0.001$) and the total number of prehension bites ($P = 0.003$) (Table 18), but the prehension biting rate was increased ($P = 0.002$) by the antiemetic in the Heave treatment (Table 16). Antiemetic did not affect the rate or number of mastication bites ($P = 0.14$) (Table 18), but in the Control treatment without antiemetic the mastication biting rate was increased ($P = 0.002$) compared to heave with antiemetic and roll with antiemetic drugs (Table 16).

Head and body position were not affected by the antiemetic except that it caused the sheep to spend less time looking towards the side bars ($P = 0.01$) and with their head on the mesh ($P = 0.01$) (Table 18).

Post-treatment analysis showed that during Control treatment sheep that had received the antiemetic tended to walk ($P = 0.05$) and stood less ($P = 0.09$) and lie down for longer ($P = 0.07$) than those that had not received the antiemetic (Table 16).

Table 16. Significant or close to significant effects on feeding and residual behaviours as a result of interactions between administration of antiemetic drugs and motion type position.

	Movement*Antiemetic							
	✓			X				
	Control	Heave	Roll	Control	Heave	Roll	SED	P-value
Feeding								
Prehending bite rate, √(n/min prehension)[1]	1.65[ab] (2.7)	1.74[a] (3.0)	1.62[b] (2.6)	1.67[ab] (2.8)	1.62[b] (2.6)	1.63[b] (2.6)	0.013	0.002
Mastication bite rate, √(n/min chewing)[1]	2.27[ab] (5.1)	2.32[ab] (5.4)	2.20[b] (4.8)	2.45[a] (6.0)	2.18[b] (4.7)	2.35[ab] (5.5)	0.031	0.002
Post-treatment behaviors								
Walking, s/30 min	77	93	105	117	86	84	7.4	0.05
Standing, s/30 min	812	1043	1049	1083	966	968	51.5	0.09
Lying, s/30 min	911	663	647	600	748	748	56.3	0.07

[a,b] Least square means within rows with different superscripts are significantly ($P < 0.05$) different by Tukey's test.
[1] Statistical analyses performed on square root transformed data. Least squares mean were calculated and then back transformed. Degrees of freedom: 2.

Table 17. Effects of motion treatment on feeding, head position, position, residual behavior, and heart rate in sheep (n = 6) exposed for 60 min position.

	Motion				
	Control	**Heave**	**Roll**	**SED**	**P-value**
		Feeding			
Feed intake, g/h	741	770	769	23.9	0.72
Water intake, ml/h	1326	1168	1268	72.1	0.45
Eating time, s/h	1098	1032	1138	29.0	0.11
Drinking time, s/h	411	299	349	52.8	0.48
Eating rate, g DM/min eating	26.4[b]	30.1[a]	27.5[ab]	0.65	0.006
Prehending time, √(s/min)[1]	3.39	3.08	3.29	0.100	0.11
Prehending bite rate, √(n/min prehension)[1†]	1.66[ab] (2.7)	1.68[a] (2.8)	1.62[b] (2.6)	0.014	0.03
Total prehension bites, n/min	40.7	34.6	37.7	2.35	0.24
Mastication time, s/min	19.2[ab]	17.2 [b]	21.8[a]	0.85	0.002
Mastication bite rate, n/min chewing[†]	2.36	2.25	2.27	0.033	0.07
Total mastication bites √(n/min)[1]	6.4[ab] (41)	5.9[b] (35)	6.7[a] (45)	0.15	0.004
		Head position			
Up, log_{10} (s/h)[1]	1.03 (11)	0.76 (5.7)	0.75 (5.6)	0.081	0.07
Middle, s/h	1273	1293	1248	64.0	0.92
Down, log_{10}(s/h)[1]	1.8 (63)	2.0 (100)	1.9 (79)	0.12	0.76
Above companion, log_{10} (s/h)[1]	0.42 (2.6)	0.55 (3.5)	0.39 2.4)	0.09	0.57
Looking towards companion, s/h	310	426	318	33.4	0.09
Looking towards side bars, log_{10} (s/h)[1]	1.7 (50)	1.5 (32)	1.6 (40)	0.07	0.14
On mesh, s/h	55.9[b]	162.3[a]	107.3[ab]	19.3	0.009
		Body position			
Standing, s/h	3500	3512	3572	35.6	0.46
Lying, s/h	38	4	14	18.8	0.55
Kneeling, s/h	62	84	13	30.0	0.39
		Post-treatment behavior			
Walking, s/30 min[†]	97	90	95	7.4	0.85
Standing, s/30 min[†]	948	1005	1008	51.5	0.75
Lying, s/30 min[†]	756	706	697	56.3	0.81
		Heart rate			
Heart rate mean, beats/min	104	106	105	1.2	0.55

[a,b] Least square means within rows with different superscripts are significantly ($P < 0.05$) different by Tukey's test.
[1] Statistical analysis performed on log_{10} and square root transformed data. Least squares mean were calculated and then back transformed. Degrees of freedom: 2
[†] Significant interactions between motion and antiemetic are presented in Table 15.

Table 18. Effects of antiemetic drugs on feeding, head position, position, residual behavior, and heart rate in sheep (n = 6) exposed to different motion treatments for 60 min.

	Antiemetic			
	✓	X	SED	P-value
Feeding				
Feed intake, g/h	742	778	23.9	0.29
Water intake, ml/h	1315	1193	72.1	0.24
Eating time, s/h	1055	1124	29.0	0.10
Drinking time, s/h	424	282	53.0	0.06
Eating rate, g DM/min eating	28.4	27.6	0.65	0.38
Prehending time, √(s/min)[1]	3.04 (9.2)	3.46 (11.9)	0.098	0.001
Prehending bite rate, √(n/min prehension)[1†]	1.67 (2.8)	1.64 (2.7)	0.015	0.10
Total prehension bites, n/min	33.3	42.0	2.41	0.003
Mastication time, s/min	18.9	19.9	0.85	0.36
Mastication bite rate, n/min chewing[†]	2.27	2.32	0.036	0.14
Total mastication bites √(n/min)[1]	6.16 (38)	6.46 (42)	0.171	0.14
Head position				
Up, $\log_{10}$ (s/h)[1]	0.85 (7.1)	0.85 (7.1)	0.080	0.97
Middle, s/h	1274	1269	63.9	0.96
Down, $\log_{10}$ (s/h)[1]	1.99 (98)	1.88 (76)	0.125	0.54
Above companion, $\log_{10}$ (s/h)[1]	0.42 (2.6)	0.48 (3.0)	0.092	0.68
Looking towards companion, s/h	362	341	33.4	0.66
Looking towards side bars, $\log_{10}$ (s/h)[1]	1.43 (26.9)	1.71 (51.6)	0.074	0.01
On mesh, s/h	73.4	143.6	19.29	0.01
Body position				
Standing, s/h	3534	3522	35.5	0.81
Lying, s/h	10	27	18.8	0.52
Kneeling, s/h	56	51	30.1	0.91
Post-treatment behavior				
Walking, s/30 min[†]	92	96	7.3	0.69
Standing, s/30 min[†]	968	1006	51.5	0.61
Lying, s/30 min[†]	740	699	56.3	0.60
Heart rate				
Heart rate mean, beats/min	106	105	1.2	0.59

[1] Statistical analyses performed on $\log_{10}$ and square root transformed data. Least squares mean were calculated and then back transformed

[†] Significant interactions between motion and antiemetic are presented in Table 15. Degrees of freedom: 1

5.4 DISCUSSION

There was no evidence that feeding motivation was adversely affected by simulated ship motion, with no effects on feed intake. A large reduction in rumination in sheep exposed to Heave but not offered feed was previously observed (Chapter 4), but the presence of feed in this experiment occupied the sheep sufficiently to prevent rumination in all sheep, despite the lengthening of exposure period to 60 min. Sheep in Heave ate faster, prehending it from the trough more rapidly and masticating it less than sheep in the Control and Roll treatments, but this appears likely to have derived from the stress caused by this motion. In humans, Heave is considered a greater stressor than Roll during ship transport (Stevens and Parsons, 2002; Shupak and Gordon, 2006). The effects of Heave on feeding behaviour could reduce the effectiveness of the initial stages of digestion in the buccal cavity, including less comminution of feed particles and addition of saliva that is essential to buffer acid produced during the ruminal digestion process. Such effects would not have had time to influence feed intake during the 60 min periods of this experiment, but could be significant over a longer period. Further research could investigate the impact of motion on rumen function over longer periods, to determine whether it is implicated in the observed inappetence during sea transport of sheep (Richards et al., 1989).

Despite the limited effects of the motion treatments, the antiemetic did affect feeding behaviour by increasing rate of feed prehension in the Heave treatment. This may have been due to better balance, allowing the sheep to get their lips around the feed and withdraw it into the buccal cavity faster. The overall tendency for prehension time to be decreased and prehension biting rate increased may also reflect more efficient feed prehension with the antiemetic, which may be due to better balance or increased appetite as a result of reduced nausea, rather than increased stress as in the case of Heave effects on prehension rate. Possible improved balance effects of the antiemetic may be suspected, as in the Control treatment without antiemetic drugs speed of masticating the feed was increased, which is more likely to result from a better coping of motion. Nevertheless, the antiemetic was not sufficient to increase feed intake, which has been previously reported (Adams and Sanders, 1992; Provenza et al., 1994) or to diminish the stress responses which may also reduce feed consumption (Kronberg et al., 1993).

Further evidence for improved balance in sheep treated with the antiemetic was provided by reduced time spent with their head against the mesh. The head has a major influence on balance in quadrupeds (Smit, 2002), and the antiemetic could have helped to inhibit the stimulation of the vestibular system by motion. Heave is one of the main sea transport motions that produces sickness (Bles et al., 2000). It was previously observed that sheep in the Heave treatment spend longer with their heads one above the other (Chapter 4), and concluded that this was due to the greater stress experienced in Heave than other treatments. In this experiment, we added a mesh to stop the sheep putting their heads into the neighbouring enclosure and steeling food. Observing that sheep in Heave spent longer with their head against the mesh leads to the same conclusion as in the first experiment, that Heave increases the tendency towards affiliative behaviour between sheep, which almost certainly occurs as a result of increased stress. Evidence for increased stress in the Heave treatment in the first experiment was increased heart rate and its variability, compared to the Control treatment. This increase in heart rate in the same Heave (and Roll) treatments, compared to Control, in Experiment 1 was in sheep exposed for 30 min. The use of the same sheep as in the previous experiment and the longer exposure period in this experiment suggest that habituation may have occurred. However, this is not supported by the higher heart rate in this experiment (105 bpm) than in the previous one (81 bpm). Although antiemetic did not affect heart rate in this experiment, research has indicated that in rabbits dexamethasone decreases heart rate (Mokra et al., 2008), in sheep metoclopramide increases heart rate (Eisenach and Dewan, 1996) and in humans diphenhydramine has no effect under normal conditions (Zareba et al., 1997). Nonetheless, heart rate alone is inadequate to describe changes in the autonomous nervous system (von Borell et al., 2007) and variability will be examined in future.

Post treatment behavior analysis showed that antiemetic tended to increase lying and reduce activity in the Control sheep, which may suggest a better ability to rest, whereas those without antiemetic may reasonably be expected to have remained agitated. Those sheep in the motion treatments receiving antiemetic drugs tended to rest less and have more activity than those in the control treatment, probably as a result of a positive effect of antiemetic drugs on balance and body posture and hence less exhaustion as a result of corrective motions.

The limited impact of the antiemetic may partly derive from the doses given, the motions being experienced independently and the short duration of the treatments. The antiemetic combination and the doses were determined from a previous study with lambs (Provenza et al., 1994), using a lower dose suggested for humans. This is an important aspect to further be reviewed as it is possible that during this experiment the drugs did not produce a full antiemetic effect and only helped to improve balance. Further experiments could first conduct basic pharmacokinetic studies to determine the most efficient doses (Fan and de Lannoy, 2014). The other factor that may have affected the response to the antiemetic was that roll and heave experienced independently may have been not sufficient to produce nausea or other physiological responses. These two motions experienced in combination produce greater stress and motion sickness responses in humans (Joseph and Griffin, 2008). Further experiments should combine these motions and extend the treatment duration or magnitude. The tendency for the antiemetic to increase drinking is likely to be due to the drugs themselves, rather than any specific effects on nausea, since metoclopramide (Ljungberg, 1989) and dexamethasone (Liu et al., 2010) have both been reported to reduce water intake, although diphenhydramine increases it (Pyle, 2011).

5.5 CONCLUSIONS

There was only limited evidence that the motions to which the sheep were exposed, in simulated ship travel, resulted in adverse effects on feeding behaviour and no effect on feed intake was observed. As a result the antiemetic did not affect these parameters, but evidence that balance may have been improved was obtained from the fact that the sheep spent less time with their head against a mesh on the side of the crate. There was also an increase in prehension rate in sheep experiencing Heave that may derive from better balance. This experiment confirmed previous observations that Heave causes an increase in affiliative behaviour between sheep.

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CHAPTER 6. THE EFFECTS OF REGULARITY OF ROLL AND PITCH MOTIONS ON SHEEP BEHAVIOR AND PHYSIOLOGY DURING SIMULATED SHIP TRANSPORT

Abstract: Roll and pitch sea transport motions have been described to produce motion sickness and stress responses in animals. This study aims to identify the effects of regular (a standard angle and speed) and irregular (random selections of thirty angles and speeds) sequences of two types of floor movement: roll (side to side) and pitch (end to end), or a combination of the two on sheep feed and water intake, heart rate and its variability and body posture. Six sheep were restrained in pairs in a crate that was placed on a moveable and programmable platform that generated roll and pitch motions for 60 min in a changeover design over 12 consecutive days. Feed intake was increased by irregular sequences in the combined Roll and Pitch motion (P=0.04). The two sheep spent more time during Irregular sequences with their heads one above the other (P= 0.001), indicating greater affiliative behaviour, and facing down (P = 0.001). The combined Roll and Pitch also increased the time sheep spent with their heads down (P = 0.007). Sheep spent more time during Irregular sequences standing with their back supported on the crate (P < 0.001) or kneeling (P = 0.03). Irregular sequences, the combined roll and pitch and the interaction between the two produced more stepping behaviour, indicating loss of balance. Sheep exposed to Irregular sequences of combined Roll and Pitch had increased heart rates (P < 0.001). Therefore there was both behavioral and physiological evidence that Irregular sequences and the combination of Roll and Pitch caused stress, loss of balance and more affiliative behaviour between sheep.

Key words: behavior, predictability, regularity, motion, sheep, ship transport

6.1 INTRODUCTION

Roll and pitch motions during sea transport can cause motion sickness in humans (Wertheim et al., 1998; Joseph and Griffin, 2008), and have been studied in detail because of ship security (Ibrahim and Grace, 2010) and human health concerns (Stevens and Parsons, 2002). Motion sickness responses have been focused on effects on humans (Santurtun et al., 2014) and habituation by humans has been investigated regularly over the last 40 years (e.g. McCauley et al., 1976; Li et al., 2012). Accurate predictions of the motions and their effects is also fundamental for ship performance and design (Scamardella and Piscopo, 2014). In relation to animals, unpredictable and uncontrollable situations are believed to surpass their regulatory capacity and as a result they experience stress (Johannesson and Ladewig, 2000; Bassett and Buchanan-Smith 2007; Koolhaas et al., 2011). Regularity of the motion sequences may determine the stress impact on the animal if they are not capable of predicting or habituating to them. Previous studies on the capacity of animals to habituate to potential stressors have suggested that this may not be achievable if the time delay between exposure is too great, for example in feeding anticipation in birds (Abeyesinghe et al., 2001) and cattle (Phillips and Rind, 2001), or if regular feeding is not established in calves (Johannesson and Ladewig, 2000).

The objective of this study was to examine the effects of Roll, Pitch and their combination during Regular and Irregular motion sequences on feed intake, behaviors, heart rate and its variability, and body posture. The hypothesis was that Irregular sequences and the combination of Roll and Pitch motions may be the most stressful to sheep as they will not be able to habituate. Habituation was assessed through repeated exposure to the movements and monitoring the responses over time.

6.2 MATERIALS AND METHODS

The study was 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).

6.2.1 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 ± SEM) 44.2 ± 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 of 3 tubular steel bars (0.87 m wide × 1.2 m long × 0.95 m high), divided in 2 by a 3-barred division, which prevented them from turning round. The crate was covered with a white sheet to reduce visual stimulation that might affect predisposition to motion sickness. Aluminium bowls (Appendix 5, **Photo 11**) and plastic bottles were attached to the outside of the crate for feed and water, respectively, during the experimental trials. An external mesh was placed (Appendix 5, **Photo 12**) to prevent sheep eating from their companion's bowl.

6.2.2 Simulating regular and irregular roll and pitch motions

Amplitude and period.

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 sequences programme was constructed from thirty amplitude and thirty period values that were randomly selected by the software Microsoft Visual Studio Solution C++ Express 2008. Regular roll and pitch sequences were determined from the mean amplitude and period of the irregular roll and pitch sequence (**Table 7**). A detailed explanation of the methods to obtain both regular and irregular sequences and the programming commands is available in Santurtun et al. (2014).

Pitch and roll equipment.

The crate was positioned on a 0.8 m wide × 1.2 m long motion platform (Model T2sMP, CKAS Mechatronics Pty Ltd, Melbourne, Australia) capable of producing roll and pitch movements independently or in combination with the aid of two crank arms, with movement duration determined from computer commands (Santurtun et al., 2014). It responded to a motion programming commands sent from a computer through a BELKIN® Hi-Speed USB 2.0.

6.2.3 Experimental protocol

Before the study, sheep were habituated through positive reinforcement to different potential stressors they would face during the experiment over a period of 20 days. During the experiment, sheep were exposed in pairs to six treatments, regular and irregular sequences of pitch, roll, and combined roll and pitch in the crate for 60 min periods. The treatments were applied in a 6 × 6 Latin Square with one repetition on 12 consecutive days (Appendix 6, **Experiment 3.**). In total each sheep was exposed to 12 treatment periods. Sheep experienced treatments in 6 possible pairs (1+2, 3+4, 5+6, 1+4, 3+6 and 2+5) so that pair effects could be evaluated. During the experimental trials, sheep had ad libitum access to water and a container with 1.5 kg of lucerne pellets (® Lockyer Lucerne Products PTY. LTD, Queensland, Australia).

Behavior recording.

Sheep behavior was recorded continuously in real time by 3 video cameras/sheep (Kobi CCD Video Camera, Model K-32HCVF, Ashmore, QLD, Australia) during exposure to treatment. A digital video recorder (Kobi H.266, Model XQ-L 900H, Ashmore, QLD, Australia) was used to record the images, and the video data were then analysed using a continuous recording of each animal and Cowlog 2.0 behavior software for coding of behaviors (Hänninen and Pastell, 2009), according to the ethogram in Appendix 7 (**Experiment 3.**). The duration of time spent in the following mutually exclusive states was continuously recorded: standing, no support or supporting their body against the crate; kneeling; the duration of various head positions was recorded as up, middle, or down (relative to withers), above/ under the companion sheep, looking towards companion sheep or side bars; time spent drinking, eating and licking the bowl feeder. Stepping, pawing, and butting were recorded as events.

At the end of each experimental trial, food and water intake were obtained. After each exposure to treatment, sheep were taken to an adjacent room, and standing, walking and lying behaviours were continuously video- recorded for 30 min to determine residual effects on behavior.

Heart rate and its variability.

Heart rate monitors (Polar S810i, Kempele, Finland) were attached to each sheep for detection of heart rate and inter-beats intervals (IBI) throughout the 60 min exposure to treatment in the crate. Eight sections of 512 beats (approximately 6 min each) were extracted from each treatment for time and frequency domain analysis. Kubios HRV 2.1 software (Tarvainen et al., 2014) was used to detect anomalies and obtain heart rate variability (HRV). The following 3 time domain variables were examined to estimate responses in the sympathetic and parasympathetic branches of the autonomous nervous system: IBIs mean (RR mean); square root of the mean of the sum of the squares of different successive IBIs (RMSSD), which reflects the integrity of vagus nerve-mediated autonomic control of the heart, and the number of pairs of successive IBIs differing by more than 50 ms (NN50), which is correlated to RMSSD and hence also reflects vagal activity (von Borell et al., 2007). In addition a frequency domain analysis was done using a Fast Fourier Transformation (FFT) to obtain high (HF) and low (LF) frequency bands, expressed in normalized units (n.u.), and as a ratio (LF/ HF). HF has been associated with vagal activity (Malliani et al., 1994) and LF with both sympathetic and vagal activity (Cerutti et al., 1995; von Borell et al., 2007). Frequency bands widths (LF: 0.04-0.2 Hz, HF: 0.2-0.4 Hz) were assigned according to sheep recommended ranges (von Borell et al., 2007).

6.2.4 Statistical analysis

During analyses, all data were checked for normal distribution of residuals using the Anderson-Darling test. For data not satisfying the Anderson-Darling test, $\log_{10}$ transformations were made and back-transformed means are reported in addition to transformed data. Preliminary analyses determined that sheep within pair was not a significant predictor of behavior or heart rate results, and individual sheep were therefore considered valid as replicates. For this purpose a general linear model produced residuals that were not normally distributed; therefore Mood median tests were conducted for all variables to check the independence of the companion animal variable. This indicated that there was no statistical significance ($P > 0.05$) in any variable, hence identity of the companion sheep did not have any effect on any treatment effects. Proportion of time spent and frequency of each behavior, HRV time domain and Fast Fourier transformed data were analysed using a general linear model with the following factors: treatment, day, sheep, treatment, section (only for HRV analysis), sequence, treatment-sequence interaction using the statistical package Minitab (version 16). For all tests, probability levels are two-tailed and are considered significant when $P< 0.05$. Post hoc Tukey’s tests were used to identify which means were significantly different from each other.

6.3 RESULTS

6.3.1 Behavior

Irregular sequences of Roll and Pitch increased feed intake compared to the same combined motion with regular sequences (P = 0.04) (Table 19), which tended to be reflected in time spent eating (P=0.06). Sheep spent more time drinking (P <0.001) and consumed more water (P = 0.001) when experiencing regular sequences (Table 20).

During Irregular sequences sheep spent more time with their heads under /above the other sheep (P= 0.001) and orientated down (P = 0.001), and spent less time with their head up (P = 0.04) and in the middle (P <0.001) compared with during Regular sequences (Table 20). Sheep spent more time with their head down when experiencing Roll or the combination of Roll and Pitch, compared with just pitch (P=0.007), and tended to place the head in the middle mainly during pitch motion (P = 0.08) (Table 21). Sheep spent more time standing without support during Regular sequences (P=0.001), and during Irregular sequences they spent more time supported on the crate (P <0.001) and kneeling (P = 0.03) (Table 20). Motion type did not affect body position (Table 21).

Stepping behaviour was increased during Irregular sequences of Roll and Pitch, compared with Regular sequences of the same motion (P= 0.001) (Table 19). Sheep pawed more during the combined Roll and Pitch motions, compared with just pitch (P=0.02) and tended to spend less time butting (P = 0.06) (Table 21). Butting also tended to be more common during Irregular than Regular sequences (P = 0.08) (Table 20). Licking their bowl was more common during Regular than Irregular sequences (P = 0.01) (Table 20), and no significant treatment differences (P = 0.59) were observed as a result of motions (Table 21).

Post-treatment analysis showed no significant treatment differences in walking or standing as a result of sequences (Table 20) and motions (Table 21). However, sheep tended to lie less (P = 0.08) following Irregular sequences (Table 20).

6.3.2 Heart rate measurements

Heart rate was highest for Irregular sequences of combined Roll and Pitch and least for Regular sequences of the same motion (P < 0.001) (Table 21). Regardless of sequence, heart rate was lower for Roll than Pitch. The inter-beat interval showed the reverse pattern. RMSSD was less for the Irregular than Regular sequences (P = 0.04) (Table 19), and for Pitch than Roll (P = 0.04) (Tables 20). No significant treatment differences were observed during motions and sequences interaction. In the combined Roll and Pitch motion NN50 was reduced (P = 0.04) and the ratio of Low to High Frequencies was increased (P = 0.007) by Irregular compared to Regular sequences (Table 21).

Table 19. Significant or close to significant effects on stepping, feed intake and eating time as a result of interactions between type of sequence and motion type.

	Motions * Sequence							
	Regular sequences			Irregular sequences				
	Roll	Pitch	Roll & Pitch	Roll	Pitch	Roll & Pitch	SED	P-value
Feed intake, g/h	632[ab]	548[ab]	463[b]	599[ab]	569[ab]	714[a]	107.6	0.04
Eating time, s/h	643	657	531	690	589	820	138.5	0.06
Stepping, n/h	118[c]	145[bc]	142[bc]	208[b]	190[b]	315[a]	28.5	0.001
Heart rate measurements								
HR mean, log_{10}	1.919[cd]	1.937[ab]	1.889[e]	1.906[d]	1.928[bc]	1.946[a]	0.0022	<0.001
(beats/min)[1]	(83.0)	(86.5)	(77.4)	(80.5)	(84.7)	(88.3)		
IBIs mean, ms	726[bc]	706[cd]	777[a]	751[ab]	714[cd]	692[d]	4.2	<0.001
NN50, count	162[ab]	154[abc]	171[a]	157[abc]	138[bc]	136[c]	4.0	0.04
HF,	1.39[a]	1.35[a]	1.41[a]	1.33[ab]	1.32[ab]	1.26[b]	0.013	0.005
log_{10} (n.u.)[1]	(24)	(22)	(26)	(21)	(21)	(18)		
LF, n.u.	69.5[cd]	72.3[bcd]	68.0[d]	75.5[ab]	73.6[abc]	77.1[a]	0.74	0.01
LF/HF,	0.44[bc]	0.49[bc]	0.41[c]	0.54[ab]	0.53[abc]	0.62[a]	0.018	0.007
($log_{10} ms^2$)[1]	(2.8)	(3.1)	(2.6)	(3.5)	(3.4)	(4.2)		

[a,b,c,d,e] Least square means within rows with different superscripts are significantly (P < 0.05) different by Tukey's test.
[1] Statistical analysis performed on log_{10} transformed data. Least squares mean were calculated and then back transformed. Degrees of freedom: 2

Table 20. Effects of Regular and Irregular sequences on feeding, head position, position, residual behavior, and heart rate variability in sheep (n = 6) exposed for 60 min position.

	Sequences			
	Regular	**Irregular**	**SED**	**P-value**
Feeding				
Feed intake, g/h	548	628	32.5	0.10
Water intake, ml/h	203	101	19.4	0.001
Eating time, s/h	610	699	41.8	0.15
Drinking time, s/h	611	249	52.0	<0.001
Head position				
Under/above, s/h	173	467	55.6	0.001
Up, $\log_{10}$ (s/h)[1]	0.49 (3.1)	0.27 (1.9)	0.0718	0.04
Middle, s/h	965	683	50.4	<0.001
Down, s/h	713	1028	63.1	0.001
Looking				
Towards companion, $\log_{10}$ (s/h)[1]	2.29 (195)	2.21 (162)	0.060	0.40
Towards side bars, s/h	143	150	15.7	0.76
Body position				
Standing				
No support, $\log_{10}$ (s/h)[1]	3.52 (3311)	3.45 (2818)	0.014	0.001
Against crate, s/h	162	624	62.6	<0.001
Kneeling, $\log_{10}$ (s/h)[1]	0.075 (1.2)	0.340 (2.2)	0.0829	0.03
Others				
Stepping, number/h	135	238	8.6	<0.001
Pawing, $\log_{10}$ (number/h)[1]	0.68 (4.8)	0.87 (7.4)	0.08	0.14
Butting, $\log_{10}$ (number/h)[1]	0.12 (1.3)	0.26 (1.8)	0.0522	0.08
Licking bowl, s/h	79.2	22.5	14.26	0.01
Post-treatment behavior				
Walking, s/30 min	38	48	4.3	0.11
Standing, s/30 min	1013	1188	71.5	0.10
Lying, s/30 min	749	564	72.1	0.08
Heart rate measurements				
HR mean, $\log_{10}$ (beats/min)[1]	1.91 (81)	1.92 (83)	0.002	<0.001
IBIs mean, ms	734	719	3.7	0.001
RMSSD, $\log_{10}$ (ms)[1]	1.70 (50)	1.68 (48)	0.007	0.04
NN50, count	162	143	3.4	<0.001
HF, $\log_{10}$ (n.u.)[1]	1.38 (24)	1.31 (20)	0.011	<0.001
LF, n.u.	70	75	0.64	<0.001
LF/HF, $\log_{10}$ (ms^2)[1]	0.45 (2.8)	0.56 (3.6)	0.014	0.001

[1] Statistical analysis performed on $\log_{10}$ transformed data. Least squares mean were calculated and then back transformed. Degrees of freedom: 1

Table 21. Effects of Roll, Pitch and their combination on feeding, head position, position, residual behavior, and heart rate variability in sheep (n = 6) exposed for 60 min position.

	Motions				
	Roll	**Pitch**	**Roll & Pitch**	**SED**	**P-value**
Feeding					
Feed intake, g/h	615	558	588	36.7	0.61
Water intake, ml/h	136	146	174	21.9	0.57
Eating time, s/h	666	623	676	47.3	0.82
Drinking time, s/h	338	444	508	58.8	0.17
Head position					
Under/above, s/h	365	401	194	62.8	0.18
Up, $\log_{10}$ (s/h)[1]	0.369 (2.3)	0.416 (2.6)	0.365 (2.3)	0.0811	0.93
Middle, s/h	753	955	763	57.0	0.08
Down, s/h	951[a]	618[b]	1044[a]	71.3	0.007
Looking					
Towards companion, $\log_{10}$ (s/h)[1]	2.28 (190)	2.25 (178)	2.23 (169)	0.068	0.91
Towards side bars, s/h	150	179	110	17.8	0.15
Body position					
Standing					
No support, $\log_{10}$ (s/h)[1]	3.47(2951)	3.48 (3020)	3.50 (3162)	0.016	0.56
Against crate, s/h	445	456	277	70.7	0.31
Kneeling, $\log_{10}$ (s/h)[1]	0.265 (1.84)	0.143 (1.39)	0.216 (1.64)	0.0937	0.71
Others					
Stepping, number/h	163[b]	167[b]	228[a]	9.7	<0.001
Pawing, $\log_{10}$ (number/h)[1]	0.78 (6)[ab]	0.49 (3)[b]	1.04[a] (11)	0.095	0.02
Licking bowl, s/h	62.9	53.2	36.5	16.12	0.59
Butting, $\log_{10}$ (number/h)[1]	0.15 (1.4)	0.34 (2.2)	0.08 (1.2)	0.0589	0.06
Post-treatment behavior					
Walking, s/30 min	37	46	46	4.9	0.35
Standing, s/30 min	1064	1133	1105	80.9	0.85
Lying, s/30 min	699	621	649	81.5	0.81
Heart rate measurements					
HR mean, $\log_{10}$ (beats/min)[1]	1.913[b] (81.8)	1.932[a] (85.5)	1.918[b] (82.8)	0.0024	<0.001
IBIs mean, ms	739[a]	710[b]	734[a]	4.1	<0.001
RMSSD, $\log_{10}$ (ms)[1]	1.706[a] (51)	1.675[b] (47)	1.699[ab] (50)	0.008	0.04
NN50, count	159	146	153	3.8	0.08
HF, $\log_{10}$ (n.u.)[1]	1.361 (23)	1.338 (22)	1.334 (21)	0.0129	0.30
LF, n.u.	72.5	72.9	72.5	0.71	0.92
LF/HF, $\log_{10}$ (ms^2)[1]	0.49 (3.1)	0.51 (3.2)	0.52 (3.3)	0.017	0.50

[a,b] Least square means within rows with different superscripts are significantly ($P < 0.05$) different by Tukey's test.

[1] Statistical analyses performed on $\log_{10}$ transformed data. Least squares mean were calculated and then back transformed. Degrees of freedom: 2

6.4 DISCUSSION

The hypothesis that Irregular sequences and the combination of Roll and Pitch motions would be the most stressful to sheep as they could not be able to habituate was confirmed. The results suggest that Irregular sequences first, and secondly the combination of Roll and Pitch could have a negative impact on sheep welfare. The latter is consistent with the capacity of this combination to adversely affect humans during sea transport (Wertheim et al., 1998). In addition irregular waves are normally present during severe sea conditions (Clauss and Kühhnlein, 1997) which aggravates motion sickness in humans (Li et al., 2012). Although this study did not replicate sea and waves conditions, the irregularity and combination of ship motions used could also have stimulated the vestibular system and produce motion sickness and stress responses.

Although there was no evidence that feeding behaviors were adversely affected by the type of sequences and motions independently, when Roll and Pitch combination interacted with Irregular sequences sheep ate more and tended to spent more time eating pellets. The RMSSD band, indicating vagus nerve activity, was significantly reduced during Irregular sequences, and LF/HF ratio increased when Roll and Pitch combination interacted with Irregular sequences. These results suggest that the parasympathetic nervous system could not counteract stress effects, and as a result sheep may have eaten more to reduce negative emotions (Ortolani et al., 2011; McMillan, 2013). We previously observed that sheep that had been exposed to Heave motion, considered the most stressful, ate more in the observation period after exposure to treatment (Chapter 4). Conversely water intake and time spent drinking were reduced during Irregular sequences, probably because it was more difficult for sheep to walk towards the dispenser and maintain a fixed body position and pressure with their tongue on the dispenser to obtain water. Further evidence of reduced balance was provided by time spent by sheep supporting their body on the crate and increased frequency of stepping during Irregular compared to Regular sequences. This is confirmed in later observations (Chapter 7), and in Chapter 4 where sheep also supported themselves more against the crate during heave, the most stressful motion to which they were exposed.

Irregular sequences and the combination of Roll and Pitch had a synergistic effect on the sheep's capacity to adversely affect body posture. During Irregular sequences, sheep positioned their head above or below the companion sheep's body, a behaviour that we have observed previously during the most severe of motions, heave, and during more space allowance (Chapter 4, 5, 6), and down (Chapter 7) whereas during Regular sequences sheep were able to maintain better balance by positioning the head in the middle. In this experiment this affiliative behavior, and head down during the Irregular sequences coincided with an increased heart rate and heart rate variability, and a tendency for an increased time butting, both situations related to stress conditions (von Borell et al., 2007; Gougoulis et al., 2010). Similar results were later observed (Chapter 7) where sheep had more agonistic behaviors when more space was provided. Sheep positioning their head down has been associated to stress during transport (Hall et al., 1998) but this apparently stress-related affiliative behaviour potentially demonstrates increased gregariousness during stress, with consequent implications for preferred stocking density. Additional evidence of an unstable body posture and stress is provided by the fact that sheep stepped and pawed more during the combination of Roll and Pitch. These behaviors have been related to stress-related environments (Cockram et al., 1994) and nervous and anxious individuals (e.g. cattle) (Wenzel et al., 2003). These results coincide with the ones previously observed (Chapter 4) in which sheep stepped more during Roll motion. Despite this, the observed swaying response of the sheep to Roll and reduced heart rate and increased RMSSD, compared with Pitch, both indicate that Roll may have had a calming effect.

Post treatment behavior analysis showed a tendency on sheep to spend more time lying following Irregular sequences. This may suggest that this type of sequence had more impact on sheep fitness and therefore sheep had to rest more due to physical exertion, in particular increased stepping. This confirms later residual observations (Chapter 7) with Irregular sequences where sheep lay more.

6.5 CONCLUSIONS

Irregular sequences, particularly during a Roll and Pitch combination, required sheep to make behavioral and physiological adjustments to maintain body stability. Sheep exposed to Irregular sequences showed affiliative behavior towards their partner in the crate. Heart rate variability measurements suggested that sheep during Irregular sequences of a combination of Roll and Pitch experienced the greatest stress level, probably as a result of reduced parasympathetic activity, and an increase of food eaten, probably in an attempt to mitigate this effect. Roll and Pitch combinations adversely affected balance, requiring sheep to make corrective stepping movements, positioning the head down and pawing, but there was no evidence of stress from heart rate mean, except when interacting with Irregular Sequences. Regular sequences and motions experienced individually did not have a major impact on behavior or physiology, but a synergistic effect of both parameters demonstrated that the ability to predict motion is important in an ability to invoke suitable coping measures.

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CHAPTER 7. DO INTERACTIONS WITH CONSPECIFICS STRESS SHEEP DURING TRANSPORT MOTION?

Abstract. Interactions between animals play an important role in the responses of sheep to transport motion, and these may make it harder for sheep to cope with irregular movement patterns. We investigated the effects of floor motion patterns and a barrier to separate sheep on behavior and heart rate parameters. Six sheep were restrained in pairs in a crate that was placed on a programmable platform generating combined roll and pitch motions at identical angle and frequency (regular) or a random pattern of angles and frequencies (irregular). The treatments were applied for 60 min in a changeover design over 12 consecutive days. Stepping to avoid loss of balance was more frequent when sheep had no barrier ($P < 0.001$) and during irregular motion ($P < 0.001$). Without the barrier and during irregular motion, sheep spent more time with their head under or above the other sheep, demonstrating affiliative behaviour, and during irregular motion they supported themselves more against the crate ($P < 0.001$). When the barrier was removed there was increased agonistic behavior, including pushing with the body ($P = 0.02$), butting ($P = 0.02$) and evading the other sheep ($P = 0.001$). Sheep ruminated more when the barrier was in place ($P = 0.02$).There was evidence of stress caused by removal of the barrier since RMSSD, NN50 and rumination were all reduced. 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, indicating that sheep were most stressed in this combination of treatments. Therefore there was both behavioral and physiological evidence that providing an opportunity for sheep to interact during irregular motion caused stress and body instability.

Key words: balance, behavior, heart rate, sea motion, sheep, transport

7.1 INTRODUCTION

During transport, livestock continuously try to avoid contact with other individuals and the vehicle (Broom, 2003; Jones et al., 2010). Maintenance of balance is achieved by stepping movements (Broom and Fraser, 2007) and support from vehicle structures. However, research on balance during road transport to identify the optimum space allowance has produced ambiguous results. Some research described increased stress under loose stocking (Hall et al. 1998; Jones et al., 2010), however at low space allowances animals have been observed to fall down when trying to avoid a fallen animal (Cockram et al., 1996; Das et al., 2001; Jones et al., 2010).

Pitch and roll sea transport motions produce motion sickness in humans (Wertheim et al., 1998) and in combination the physiological responses are increased (Joseph and Griffin, 2008). A lack of regularity of these motions could be an additional stressor if the animals are unable to habituate, as it has been reported that many animals species have a limited capacity to learn and anticipate events or procedures (Johannesson and Ladewig, 2000; Abeyesinghe et al., 2001; Phillips and Rind, 2001). The hypothesis of this study was that interactions between sheep would be stressful to sheep as they will not be able to cope with changes of motion. We tested this by evaluating the effects of a combination of irregular and regular pitch and roll motions on sheep behaviour and heart rate parameters.

7.2 MATERIALS AND METHODS

The study was 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).

7.2.1 Animals, Housing and Management

The design of equipment for monitoring sheep responses to floor movement, including programming a movement platform, heart rate measurements and video recording of behaviour have been described in full elsewhere (Santurtun et al., 2014). Six merino cross wethers, approximately 35 months of age, weighing (mean ± SEM) 44.2 ± 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 (0.87 m wide × 1.2 m long × 0.95 m high) constructed of 4 steel bars set at 33, 58, 79 and 100 cm above ground level, and divided equally in 2 by a removable barrier with bars at the first 3 heights (Appendix 5, **Photo 13**). This provided 0.56 m^2 /sheep, almost twice the Australian mandated allowance (0.285 m^2 for half wool sheep, DAFF, 2011). The crate was covered with a sheet to reduce visual stimulation.

7.2.2 Regular and irregular roll and pitch motions

Amplitude and period.

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 sequences programme was constructed from thirty amplitude and thirty 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^o) and period (235 ms) of the irregular roll and pitch sequence. A detailed explanation of the methods to obtain both regular and irregular sequences and the programming commands are available in Santurtun et al. (2014).

Pitch and roll equipment.

The crate was positioned on a 0.8 m wide × 1.2 m long motion platform (Model T2sMP, CKAS Mechatronics Pty Ltd, Melbourne, Australia) capable of producing roll and pitch movements independently or in combination with the aid of two crank arms, with movement duration determined from computer commands (Santurtun et al., 2014). It responded to motion programming commands sent from a computer through a BELKIN® Hi-Speed USB 2.0.

7.2.3 Experimental protocol

Sheep were habituated to laboratory, crate and heart monitoring equipment before the start of this experiment. During the experiment, sheep were exposed in pairs for 60 min periods to 6 treatments 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 barrier between the two sheep. The treatments were applied daily in a 6 × 6 Latin Square (Appendix 6, **Experiment 4.**) with one repetition, over 12 consecutive days. Sheep experienced treatments in 6 possible pairs (1+2, 3+4, 5+6, 1+4, 3+6 and 2+5) so that pair effects could be evaluated.

Behavior recording

Sheep behavior was recorded continuously in real time by 3 video cameras/sheep (Kobi CCD Video Camera, Model K-32HCVF, Ashmore, QLD, Australia) during exposure to treatment. A digital video recorder (Kobi H.266, Model XQ-L 900H, Ashmore, QLD, Australia) was used to record the images, and the video data were then analysed using a continuous recording of each animal and Cowlog 2.0 behavior software for coding of behaviors (Hänninen and Pastell, 2009), according to the ethogram in Appendix 7 (**Experiment 4.**). The duration of time spent in the following mutually exclusive states was continuously recorded: standing (not ruminating), no support, or supporting their body against the crate; lying and kneeling, not ruminating; and standing or lying ruminating. In addition the duration of various head positions was recorded as level (level with withers) or down (below withers), above or under the companion sheep's head or body, and looking towards or opposite the companion sheep. Stepping, pawing, butting, or pushing the companion sheep with the body, or moving to evade touching the other sheep were recorded as events.

After each exposure to treatment, sheep were taken to an adjacent room, and standing, walking and lying behaviours were continuously video- recorded for 30 min to determine residual effects on behavior. Lucerne pellets and water were offered during this period, and food and water intake were recorded.

Heart rate and its variability

Heart rate monitors (Polar S810i, Kempele, Finland) were attached to each sheep for detection of heart rate and its reciprocal, inter-beats intervals (IBI), throughout the 60 min exposure to treatment in the crate. Eight sections of 512 beats (approximately 6 min each) were extracted from each treatment for time and frequency domain analysis. Kubios HRV 2.1 software (Tarvainen et al., 2014) was used to detect anomalies and obtain heart rate variability (HRV). The following 3 time domain variables were examined to estimate responses in the sympathetic and parasympathetic branches of the autonomous nervous system: IBIs mean (RR mean); square root of the mean of the sum of the squares of different successive IBIs (RMSSD[6]), which reflects the integrity of vagus nerve-mediated autonomic control of the heart, and the number of pairs of successive IBIs differing by more than 50 ms (NN50), which is correlated to RMSSD and hence also reflects vagal activity (von Borell et al., 2007). In addition a frequency domain analysis was done using a Fast Fourier Transformation (FFT) to obtain high (HF) and low (LF) frequency bands, expressed in normalized units (n.u.), and as a ratio (LF/ HF). HF has been associated with vagal activity (Malliani et al., 1994) and LF with both sympathetic and vagal activity (Cerutti et al., 1995; von Borell et al., 2007). Frequency bands widths (LF: 0.04-0.2 Hz, HF: 0.2-0.4 Hz) were assigned according to recommended ranges for sheep (von Borell et al., 2007).

[6] Root mean square of successive differences

7.2.4 Statistical analysis

During analyses, all data were checked for normal distribution of residuals using the Anderson-Darling test. For data not satisfying the Anderson-Darling test, square root transformations were made and back-transformed means are reported in addition to transformed data.

Preliminary analyses by the Mood median test determined that sheep within pair was a significant predictor of behavior results, and therefore the mean of paired sheep behaviors were used for analysis. Heart rate data were analysed using individual sheep. In both cases 4 sections of 6 minutes with a 12 min interval were analysed as follows: min 0-6, 18-24, 36-42, 54-60.

Proportion of time spent in each behavior, frequency of behaviour, heart rate data and Fast Fourier transformed heart rate variability data were analysed using a general linear model with the following factors: day, barrier, motion, section, and barrier interaction with motion, using the statistical package Minitab (version 16). For all tests, probability levels are two-tailed and are considered significant when $P < 0.05$. Post hoc Tukey's tests were used to identify which means were significantly different from each other.

7.3 RESULTS

7.3.1 Behavior

Sheep without the barrier spent more time with their head under or above the other compared with those with the barrier (P = 0.03). Those without the barrier also spent less time looking towards their companion (Table 22). Sheep in either regular or irregular motion (P <0.001) spent more time with their heads under or above the other sheep, compared to control sheep (Table 23). Sheep in irregular motion spent more time with their head looking down, compared with control sheep (P = 0.02). Control sheep spent more time with their head level, if there was no barrier, compared with sheep with the barrier that were experiencing Irregular motion (P = 0.04) (Table 24).

Sheep experiencing irregular motion spent longer supporting themselves against the crate (P< 0.001) and did more stepping (P< 0.001) compared with regular motion and control sheep (Table 23). Those with the barrier stood without support most if the motion was regular (P = 0.004) (Table 24), and they did less stepping (P < 0.001) and kneeling (P = 0.06) than those without the barrier (Table 22).

The barrier decreased the prevalence of one sheep pushing the other with their body (P = 0.02), butting (P = 0.02), evading the other sheep (P = 0.001)(Table 22), whereas motion type did not affect agnostic behaviors (Table 23). Sheep spent more time ruminating (P = 0.02) when the barrier was in place (Table 22).

Post treatment sheep that had been in irregular or regular motion on the platform lay down for longer than those that had been in the Control treatment (P = 0.03)(Table 23). There was a tendency for Control sheep without the barrier to spend longest standing (P = 0.07) (Table 24). There were no residual effects of treatment on feed or water intake.

7.3.2 Heart rate measurements

When the barrier was in place sheep in irregular motion had increased heart rate (P = 0.03), RMSSD (P = 0.02) and NN50 (P = 0.007), compared with regular motion (Table 24). Without the barrier these differences in heart rate and RMSSD were not evident, and the NN50 was less in the irregular treatment. LF/HF values were lower and NN50 values higher (P < 0.001) for Control sheep with the barrier compared to sheep experiencing regular motion (Table 24).

Table 22. Effects of a barrier on head and body position, agonistic and residual behavior, and heart rate measurements in sheep (n = 6) exposed for 60 min.

	Barrier			
	✓	X	SED	P-value
Head position				
Under/above, √(s/min)[1]	3.4 (1.93)	4.6 (3.52)	0.06	0.03
Level, s/min	19.0	21.5	1.38	0.24
Down, s/min	16.2	18.7	1.35	0.23
Looking				
towards companion, s/min	7.3	4.0	0.50	<0.001
away from companion, √(s/min)[1]	5.0 (4.2)	4.7 (3.7)	0.05	0.49
Body position				
Standing				
No support, s/min	45.3	48.8	1.43	0.09
Against crate, √(s/min)[1]	4.6 (3.5)	4.7 (3.7)	0.07	0.79
Lying, s/min	7.7	4.8	1.65	0.26
Kneeling, √(s/min)[1]	0.36 (0.022)	1.26 (0.267)	0.0505	0.06
Stepping, √(number/min)[1]	2.07 (0.72)	2.90 (1.40)	0.018	<0.001
Agonistic/others				
Push body, √(number/min)[1]	0.34 (0.018)	0.81 (0.110)	0.021	0.02
Butting, number/min	0.04	0.21	0.043	0.02
Evading, number/min	0.065	0.383	0.0583	0.001
Pawing, number/min	0.63	0.78	0.283	0.71
Ruminating, s/min	31	24	2.0	0.02
Post-treatment behavior				
Walking, s/30 min	29	32	2.7	0.52
Standing, s/30 min	1697	1687	26.0	0.79
Lying, √(s/30 min)[1]	5.6 (31)	4.8 (23)	1.51	0.69
Feed intake, g/ 30 min	802	840	33.0	0.44
Water intake, ml/30 min	645	574	93.0	0.61
Heart rate measurements				
HR mean, $\log_{10}$(beats/min)[1]	1.89 (77.6)	1.90 (79.4)	0.003	0.31
IBIs mean, ms	763	760	4.5	0.59
RMSSD, $\log_{10}$ (ms)[1]	1.66 (46)	1.62 (42)	0.015	0.02
NN50, count	132	117	3.9	0.007
HF, n.u.	26.5	25.2	0.78	0.26
LF, n.u.	73	75	0.8	0.25
LF/HF, $\log_{10}$ (ms^2)[1]	0.508 (3.2)	0.500 (3.1)	0.0209	0.77

[1] Statistical analyses performed on $\log_{10}$ and square root transformed data. Least squares mean were calculated and then back transformed to s/min for presentation in parentheses. Degrees of freedom: 1

Table 23. Effects of Irregular, Regular and control motion treatments on head and body position, agonistic and residual behavior, and heart rate measurements in sheep (n = 6) exposed for 60 min.

	Treatment				
	Irregular	**Regular**	**Control**	**SED**	**P-value**
	Head position				
Under/above, √(s/ min)[1]	5.8[a] (5.7)	4.6[a] (3.5)	1.7[b] (0.5)	0.07	<0.001
Level s/min	17.0	21.5	22.3	1.57	0.09
Down, s/min	21.0[a]	17.8[ab]	13.5[b]	1.52	0.02
Looking					
towards companion, s/min	5.8	6.2	5.0	0.57	0.48
away from companion, √(s/min)[1]	4.8 (3.8)	5.2 (4.5)	4.6 (3.5)	0.05	0.49
	Body position				
Standing					
No support, s/min	44.2[b]	51.3[a]	46.0[ab]	1.63	0.02
Support, √(s/min)[1]	7.3[a] (8.8)	4.2[b] (3.0)	2.4[b] (1.0)	0.08	<0.001
Lying, s/min	4.8	3.8	10.3	1.9	0.15
Kneeling , √(s/min)[1]	1.05 (0.18)	0.81 (0.11)	0.57 (0.05)	0.056	0.73
Stepping, √(number/min)[1]	3.1[a] (1.6)	2.4[b] (0.9)	2.0[b] (0.7)	0.02	<0.001
	Agonistic/others				
Push body, √(number/min)[1]	0.38 (0.02)	0.66 (0.07)	0.67 (0.07)	0.024	0.39
Butting, number/min	0.06	0.17	0.13	0.049	0.35
Evading, number/min	0.15	0.29	0.23	0.065	0.36
Pawing, number/ min	0.92	0.53	0.70	0.315	0.74
Ruminating, s/min	26.2	24.3	31.8	2.32	0.18
	Post-treatment behavior				
Walking, s/30 min	31.9	27.7	32.3	3.11	0.61
Standing, s/30 min	1628[b]	1687[ab]	1761[a]	28.2	0.04
Lying, √(s/30 min)[1]	8.5[a] (72)	6.6[ab] (43)	0.49[b] (0.24)	1.70	0.03
Feed intake, g/30 min	802	790	872	37.3	0.45
Water intake, ml/30 min	639	727	462	103	0.35
	Heart rate measurements				
HR mean, $\log_{10}$ (beats/min)[1]	1.907 (80.7)	1.896 (78.7)	1.901 (79.6)	0.0032	0.07
IBIs mean, ms	751	768	766	5.2	0.08
RMSSD, $\log_{10}$ (ms)[1]	1.628 (43)	1.625 (42)	1.671 (47)	0.0129	0.09
NN50, count	122	119	134	4.45	0.14
HF, n.u.	24[b]	25[ab]	28[a]	0.90	0.02
LF, n.u.	75.9[a]	74.5[ab]	71.4[b]	0.91	0.02
LF/HF, $\log_{10}$ (ms^2)[1]	0.55[a] (3.5)	0.51[ab] (3.2)	0.44[b] (2.7)	0.023	0.03

[1] Statistical analyses performed on $\log_{10}$ and square root transformed data. Least squares mean were calculated and then back transformed to s/min for presentation in parentheses. Degrees of freedom: 2

Table 24. Significant or close to significant effects on behavior and heart rate measurements due to interactions between motion type and barrier presence.

	Barrier*motion							
	✓			X				
	Irregular	Regular	Control	Irregular	Regular	Control	SED	P-value
				Head position				
Level, s/min	13.5[b]	24.0[ab]	19.6[ab]	20.4[ab]	18.9[ab]	25.2[a]	1.51	0.04
				Post-treatment behaviors				
Standing								
No support, s/min	39.8[b]	54.8[a]	41.5[b]	48.5[ab]	47.8[ab]	50.5[ab]	1.75	0.004
				Heart rate measurements				
HR mean,	1.913[a]	1.890[b]	1.895[ab]	1.902[ab]	1.903[ab]	1.907[ab]	0.0031	0.03
$\log_{10}$ (beats/min)[1]	(82)	(78)	(78)	(80)	(80)	(81)		
IBIs mean, ms	740[b]	777[a]	774[ab]	762[ab]	760[ab]	758[ab]	5.1	0.03
RMSSD, $\log_{10}$ (ms)[1]	1.68[a]	1.59[b]	1.71[a]	1.58[b]	1.66[ab]	1.63[ab]	0.012	<0.001
	(48)	(39)	(51)	(38)	(46)	(43)		
NN50, count	142[ab]	107[cd]	149[a]	102[d]	131[abc]	119[bcd]	4.51	<0.001
HF, n.u.	25.2[ab]	23.2[b]	30.7[a]	22.3[b]	27.3[ab]	26.0[ab]	0.90	0.007
LF, n.u.	74.3[ab]	76.6[a]	69.0[b]	77.5[a]	72.5[ab]	73.7[ab]	0.91	0.008
LF/HF, $\log_{10}$ (ms^2)[1]	0.52[ab]	0.59[a]	0.41[b]	0.59[a]	0.44[ab]	0.48[ab]	0.023	0.005
	(3.3)	(3.9)	(2.6)	(3.9)	(2.7)	(3.0)		

[1] Statistical analyses performed on $\log_{10}$ transformed data. Least squares mean were calculated and then back transformed. Degrees of freedom: 2.

7.4 DISCUSSION

The hypothesis that interactions between sheep during irregular motion would be the most stressful to sheep was confirmed. The results suggest that irregular motion and interactions between sheep allowed by removal of the barrier both caused behavioural evidence of loss of balance, and body position adjustment (head down, stepping, supporting themselves against the crate). Lowering their head also lowers their centre of balance, helping to protect against loss of balance. Agonistic behaviors, which are useful welfare measures during transport (Broom, 2003), were particularly observed when sheep had no barrier and hence greater opportunities to be in contact with their partner. This suggests that agonistic intent may have been greater than observed in our earlier experiments with this apparatus. However, extrapolation of these results to commercial sea transport conditions should be done carefully, as the space allowance used was significantly higher than the one recommended by the Australian government (DAFF, 2011). In support of the behavioural responses to the removal of the barrier, the decreased RMSSD and NN50 provided physiological evidence of stress and a possible reduction of the parasympathetic system which may suggest a withdrawal of the vagal tone rather than a dominance of the sympathetic branch (Langbein et al., 2004; von Borell et al., 2007). In addition to reducing the stress of motion, the Control sheep had low LF/HF values and high NN50 with the barrier, both suggesting low stress levels. Despite this apparent increase in stress with the barrier removed, there was greater affiliative behaviour, as well as agonistic behaviour. Sheep spent almost twice as long with their head under or above their partner when the barrier was removed, whereas when the barrier was in place they spent almost twice as long looking towards their companion. As expected, when there was no barrier there was evidence from the NN50 measure that irregular motion was more stressful than regular motion.

Rumination was reduced when sheep had the opportunity to interact with their companion. This could be because the sheep had to face additional potential stressors, such as having more contact with the companion sheep, which resulted in more agonistic behaviors and a prolonged effort to avoid the other sheep. Stress during road transport conditions also reduces time spent ruminating (Das et al., 2001; Cockram, 2004).

Irregular motion and absence of the barrier had major effects on body posture and balance, especially that sheep positioned their head above or below their companion's head or body. It was previously observed (Chapter 4 to 6) that this behavior may be as a result of a stress response to motion, which is supported by the increased LF/HF ratio during irregular motion, and a reduction of RMSDD and NN50 (von Borell et al., 2007) without the barrier. Sheep also spent more time with their head down during irregular motion, which has been associated with stress situations (Hall et al., 1998) and a submissive posture (Nowak et al., 2008). Additional evidence of an unstable body posture was that the sheep stepped more when they had no barrier and were in irregular motion, the latter having been observed previously (Chapter 6). Conversely when there was no barrier, sheep tended to spend more time kneeling, which may have increased stability or protected them from aggressive interactions with their companion. Crucially, when there was no barrier and the sheep had the opportunity to be in contact with their companion sheep, there was no record of them ever supporting each other. This coincides with a previous study of road transport in which sheep did not support each other even if they had space to do so (Jones et al., 2010). It is commonly believed that 'animals should be loaded tightly enough to give each other mutual support' (Wythes, 1994), but this study shows that the uncertainty surrounding other animals' reactions during motion clearly mitigates against any benefit being derived from mutual support.

The increased lying time of sheep that had experienced motion, as opposed to the Control treatment, demonstrates that physical exertion during the 60 min treatment periods was sufficient to increase the motivation to rest. It has been observed (Phillips, 2008) that sheep at high stocking densities are reluctant to lie down during the early stages of the voyage, probably because of the fear of other animals closing over them, and this work suggests that the motion experienced will increase their need for rest.

7.5 CONCLUSIONS

Irregular motion required sheep to make physiological and behavioral adjustments to maintain body stability, whereas a barrier between pairs of sheep reduced the need for this. Sheep able to contact their partner showed agonistic behaviors towards them and a reduction in rumination, probably as a result of stress. Further evidence of this was provided by a reduction of RMSSD and NN50 from heart rate measurements and an increase in time spent kneeling on the floor. Irregular motion and opportunities to interact with their companion also affected balance, requiring sheep to make corrective stepping movements, and encouraged them to position their head under/above their companion sheep, which may indicate affiliative behavior. In addition, sheep positioned their head down and supported themselves more on the crate during irregular motion. They never supported themselves against the companion sheep, demonstrating that the common justification of high stocking densities as providing greater opportunities for mutual support is erroneous. Heart rate variability measures demonstrated that sheep were stressed during irregular motion and when there was no barrier, and especially during the interaction of these two variables.

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CHAPTER 8. GENERAL DISCUSSION

The study of livestock welfare transported long distances involves investigation of several stressors that have been studied mainly during road transport conditions. Few studies have investigated the stressors that livestock have to face during sea transport conditions (e.g. ammonia, Phillips et al., 2012), and other potential stressors such as sea transport motion (Phillips and Santurtun, 2013) has not been studied for its impact on livestock even though there is comprehensive knowledge of adverse effects in humans. One challenge of studying these potential stressors during sea transport conditions is to determine their additive effect, which could be done under laboratory conditions investigating each of them separately and in combination. This research project makes an important contribution to current knowledge of sea transport motion impact on sheep welfare because it provides novel behavioral and physiological motion impact evidence. The research project aimed to describe the impact that Roll, Heave and Pitch sea transport motions have on the behavior and physiology of sheep. The project achieved its aim by developing a novel method that simulated sea transport motions (Chapter 3), measured individually Roll, Heave and Pitch impact on behavior and physiology (Chapter 4), and feeding behaviors with the provision of antiemetic drugs (Chapter 5) in conditions replicating aspects of a commercial livestock transport, and finally measuring the effects of Roll and Pitch regularity (Chapter 6), and when providing opportunity for interaction with conspecifics (Chapter 7).

The first stage of the project comprised the development of a practical and affordable method that could replicate three of the most important motions in terms of their capacity to produce stress and motions sickness responses in humans and other animals (Stevens and Parsons, 2002; Shupak and Gordon, 2006). One of the main challenges was to acquire a platform that could replicate roll, pitch and heave motions together, to provide an amplitude and duration required by each experiment, using software to programme the platform. In the end, it was decided to purchase a platform that replicated roll and pitch motions because the ones that replicate the three motions together were expensive and with inadequate heave features[7]. Heave equipment selection was critical because the different options available were either very expensive (e.g. car lift), which is not suitable to reach the height and speed needed, or potentially affected the animals (e.g. fuel trucks producing fumes). In the end, an electric forklift was chosen to replicate heave motion because it could be reprogrammed to reach the height and speed needed and did not produce fumes. The only problem with the heave equipment was that it produced some vibrations that could potentially have had an impact on key physiological systems such as the vestibular one. Future research that includes heave motion should try to use equipment that can replicate this motion without vibrations.

[7] Examples from CKAS mechatronics PTY LTH: http://www.ckas.com.au/

Nonetheless, as described in Santurtun et al. (2014), the proposed method was of some value as sheep behaved similarly to what has been described on ships, and it could be successfully programmed to replicate motion independently and in combination. Modern sea transport motion simulators (computation programs or complex and expensive ship sections simulators) incorporate several other aspects such as wave composition and behavior, ship design, sea and wind conditions, patterns inside a harbor (Bos et al., 2005; Ueng et al., 2008), among other characteristics that the model used during the research project was not capable of when simulating motion. However, as mentioned before, the aim was to create a practical method, not expensive, and one that only considered two aspects (amplitude and duration of motions) and that replicates the most important motions. It is important to emphasize that roll, heave and pitch motions are continuously monitored by ships using sensors as they are important for security[8]. In this sense the results obtained from the research project (Chapter 4 & 5) could be compared with future research data obtained from a ship voyage. Future research could also determine the range of amplitude and duration of each motion that has a major impact on sheep welfare, which may be used by the authorities to determine protocols or modify current standards that could reduce motion's impact. Until today, for example in Australia, the standards for the export of livestock (DAFF, 2011) does not include any reference related to this issue. The World Organisation for Animal Health (OIE) Terrestrial Animal Health Code[9] only mentions that the sea journey planning should take into account expected sea conditions and weather. I believe the results from this research project are enough to suggest the surveillance of heave, roll and pitch (and the combination) during sea voyages should be monitored in terms of their capacity to affect livestock as they are currently considered for humans (Stevens and Parsons, 2002) and not just for security reasons.

The proposed method included two measurements to evaluate sheep welfare: behavior and heart rate. Both resulted in practical and useful methods to evaluate motion's impact on sheep welfare, however they represented some challenges to implement them. In both cases a key issue was the habituation of the animals to several other potential stressors (facilities, heart rate monitor, etc.), which took more than expected but worthwhile to ensure that we measured only motion effects. Overall the cost of both measurements were not high because DVR, security cameras and some heart rate monitors already were in place, however, if the duration of experimental trials increased, other physiological indicators of stress could be included such as cortisol, plasma creatine kinase, metabolic hormones, among others (Knowles and Warris, 2007).

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[8] Example of roll, pitch and heave sensor on: http://www.kongsberg.com/

[9] See: OIE (2012). World Organisation for Animal Health Terrestrial Animal Health Code: Chapter 7.2. Transport of Animals by Sea. http://www.oie.int/en/internationalstandard-setting/terrestrial-code/access-online/ (accessed 21 August 2012).

As described on Chapter 3, sheep experienced in pairs ship transport motions. In this sense, it is important to stress that the potential dependence of any particular sheep can be divided into two types. First a dependence on particular sheep and second a flock size effect. I tested the effects of individual animals on focal animals' responses and there was none. Even if there was a general flock size effect (i.e. two animals responded differently to one animal), this means that individual animals can be considered as independent of any particular companion, which is the requirement for model testing by ANOVA. A flock size effect would be expected but would not invalidate ANOVA and it would be unrealistic to hold individual sheep in the pen as stress levels would be too high (Apple et al., 1995). As has been described previously (Phillips, 1998, 2000, 2002), some behaviours would not be expected to be influenced by individuals, e.g. the stepping behaviour recorded in this study, but other behaviour, e.g. the aggressive behaviour recorded in this study, might be affected by identity of the companion sheep.

The first experiment showed three main outcomes related to the impact of roll, heave and pitch on sheep behavior and physiology: 1) Heave motion reduced significantly the time spent ruminating, 2) Heave and secondly roll affected sheep body position and balance, and 3) Heave apparently produced a reduction of parasympathetic system.

The reduction of time spent in rumination represents a significant result from this study. As described previously, one of the main reasons of mortality of sheep exported to the Middle East from Australia is inappetence (Phillips, 2008). Failure to eat pellets during sea transport voyages has been associated to several risk factors such as age, time of the year and fatness (Higgs et al., 1991; Norris, 2005). Preparation of sheep that have experienced pellets before sea voyage (e.g. pre-export facilities) is an important factor for food adaptation (Phillips, 2008); nonetheless, it has been reported that sheep start eating pellets some days after loading even though they had not eaten at previous stages (Norris, 2005). In this sense, inappetence is a multi-factorial problem in which the different animal factors associated to the several stressors that sheep have to face, from mustering of the animals until the animal finishes at the country of destination abattoir, may interact, produce stress and reduce feed intake (Norris et al., 1989; Bernier, 2006; Phillips, 2008). One factor that could also be affecting food intake, at least in susceptible individuals is ship motion. As reported in humans, heave is the most relevant motion that produces motion sickness responses (Shupak and Gordon, 2006), and hence digestive disorders and appetite reduction (Muth, 2006; Hasler, 2013). During this study, the results support the hypothesis that rumination reduction could have resulted from motion sickness, however, only behavior and heart rate and its variability measurements were carried out and therefore it was not possible to differentiate from stress a physiological response to the motion. Future research may include additional measurements such as gastric myoelectric activity, rumen physiology, metabolic and stress hormones, central nervous system intake regulation, among others, and increase the duration of the experimental trials to measure medium and long term responses.

Heave and roll were the most relevant motions that affect body posture and balance. During this study and the rest (Chapter 5 to 7), sheep continuously positioned their head under/above the companion when experiencing different stressful procedures (e.g. Irregular sequences, heave motion, without division). To my knowledge this behavior has not been reported during road or sea transport conditions and may reflect the gregarious nature of sheep and the need to restore body stability. It is worthwhile to mention that the sheep were part of the university's flock and they were already an established male (castrated) group before the start of experimental trials. Therefore, this may discard the submissive behavior interpretation, although as reported more research should be done to understand this behavior better.

More time spent standing supporting the body against the crate and less lying during heave motion were interesting results as well. An important aspect that may aggravate these responses were the vibrations produced when the platform and crate were raised by the forklift. Future experiments should incorporate a heave equipment that could fluently raise the platform.

As a result of the first study's rumination results, the next study aimed to test if a combination of antiemetic drugs would reduce the impact of motion on digestive behaviors and improve sheep body position and balance. One challenge of this study was the interpretation of the results produced by the antiemetic combination. This combination was previously used by Provenza et al. (1994) to study food aversions by sheep caused by a toxicant, and the doses used by these authors were taken from recommendations to help humans under quimiotherapy treatment (Harris and Cantwell, 1986). Future research that incorporates the use of antiemetic drugs should review beforehand the impact that these drugs have on different systems (e.g. digestive, cardiovascular, autonomous nervous) in sheep. During this study, there were some problems with the heart rate monitors and we could not measure every heart beat and therefore the variability was not obtained. Future research that incorporates antiemetic drugs should also include heart rate measurements to determine changes on vagal activity. On the other hand if future research determines that some antiemetic drugs reduce significantly the impact of motion on feed intake, I do not see the feasibility to provide this to every sheep transported to the Middle East because not every sheep experiences inappetence (Phillips, 2008), and as reported in humans it is likely that the degree of motion sickness symptoms, if experienced, would vary among individuals (Buyuklu et al., 2009).

Nonetheless, antiemetic drugs could be incorporated into the veterinary drugs portfolio[10] used during transport overseas if they do not impact food safety.

Prehension and mastication recording was time-consuming but relevant to obtain a better analysis of motion impact on feeding behaviors. The video footage had to be checked in slow motion as these behaviors were performed very fast. One possible solution for future research to reduce video recording time are the automatic jaw movement recorders which detect several feeding behaviors such as mastication, chewing, rumination, among others (Gregorini et al., 2013).

One of the aspects discussed from the second study was the importance of studying the effects that motion combinations have on sheep welfare as this has been demonstrated in humans and other animals to aggravate motion sickness and stress responses. In this sense, the third study aimed to evaluate the impact that the combination of roll and pitch and the regularity of them have on sheep welfare. Irregularity and the combination of roll and pitch motions resulted in a reduction of sheep welfare. This is an important result because the combination of these motions have been described to occur during potentially dangerous situations for ships such as parametric rolling, a phenomenon that affects large containers ships, in particular during irregular waves and head seas[11] (Carmel, 2006; Belenky et al., 2011). Roll and pitch combination has also been described to produced motion sickness responses in humans (Wertheim et al., 1998). As discussed with heave motion, roll and pitch motions are monitored on ships for the reasons mentioned previously, and therefore data obtained in laboratory conditions such as the present study could be compared to commercial overseas transport. Research could also be done during a commercial livestock sea transport, obtaining sea transport motion data and correlating with behavioral and physiological ones. During the present study irregular sequences were created using thirty amplitude and period values. Future research could investigate the minimum number of random sequences that sheep cannot predict and produce physiological and behavioral stress responses.

The first three studies used a barrier to divide the crate and prevent sheep from turning around. Conversely, the last study provided the opportunity for sheep to interact with the companion sheep during regular and irregular motions. A main result from this study was the responses from the sheep when they interacted with their conspecific in combination with irregular sequences. Irregular motions as described previously on Chapter 6 represented a challenge for sheep to cope with the motions, as they experienced stress mainly when there was no barrier, and therefore they had the opportunity to turn around and interact with the other sheep, and perhaps as expected, support each other to reduce the impact of motions. This hypothesis was based on previous studies observations where sheep continuously placed their head above or under the sheep

[10] https://www.livecorp.com.au/sites/default/files/publication/file/best_practice_use_of_veterinary_drugs.pdf
[11] Waves running directly against the course of a ship.

during stressful situations, and therefore I thought this behavior would occur again, as well as supporting each other with their body, which did not happen. This coincides with previous observations where sheep prefer to not support each other during transport, and it stresses the importance of carrying out research on the minimum space allowance that sheep and other livestock species need during sea transport conditions, in particular during rough seas (when motion combinations are increased) and irregular waves (which are less predictable). The space allowance used during this study (0.56 m^2), which was significantly higher than the one during live export from Australia (0.28 m^2, DAFF, 2011), was convenient to detect this situation. Future research could investigate if different stocking densities, for example higher ones, would increase stress on sheep by forcing them to be in contact with the other animal, and reduce agonistic behaviors, rumination, stepping, physical exertion, among others. This is an important topic that must be attended by the live export industry considering the length of sea journeys (Petherick and Phillips, 2009) and several other factors involved during live export. Future research could also work on allometric equations for space allowance incorporating sea transport motions and modify if needed the standards for long distances transportation.

Finally, it is important to review the limitations of the project in relation to the behavioural and physiological responses that sheep experienced during the experiments and would experience in relation to sea transport motions on a real commercial transport.

First, the simulator platform establishment, including setting the movement patterns only took into consideration some sea transport elements that affect hydrostatic characteristics of ships such as metacentric height, and some dimensional characteristics of a commercial livestock carrier such as length between perpendiculars and ship´s beam, but it did not include others such as underwater volume, center of gravity, buoyancy and flotation, wave motion effects (restoring forces and moments), hull dimensions, all of which influence ship motion. Our simulation was, therefore, a simplification of the motions to which sheep are subjected, but we attempted to examine whether predictability affected responses in one experiment. The results indicated that predictability of the motion is a major determinant of responses. All these forces, in particular ship and waves characteristics, can be evaluated with ship motion computation simulators, and therefore it would be possible but difficult to develop a more realistic method that could be used with animals. This means that although the experiments included some characteristics and forces that affected ship motion, the results are not likely to be comparable with actual responses in a real commercial sea transport, just indicative and specific to the motions used and the amplitude and period chosen.

The second limitation of the research project was the amount of sea transport motions chosen and the time spent in the platform. In this study it was decided to work with three of the most important motions that have been described to impact human and animal behaviour (Wertheim et al., 1998; Lang et al., 1999; Howarth and Griffin, 2003; Shupak and Gordon, 2006) but it did not include two other displacement motions - sway and surge - and one angular motion - yaw. All these motions are present to some degree when a ship is moving but to replicate these in a platform would have been extremely difficult and the simulators available did not replicate the amplitude and period required for the project. The other limitation was the time spent by sheep experiencing the motions in the crate. It was decided to keep them between 0.5 and 1 hour because of the time required to obtain data for video and heart rate analysis acknowledging the heart rate monitors' limited capacity to store data. In addition concerns of the animal ethics committee about the sheep's welfare limited the time available, which stemmed from the fact that this was the first time this type of experiment had been conducted.

The final and most important limitation was the capacity of the cage that was placed above the platform to keep a limited amount of sheep during experimental trials. During experimental trials, only two sheep were kept inside with the possibility to include one more at a high stocking density and hence very limited space to move. In this sense the motion simulator could not be compared with the conditions of a commercial transport where large numbers of sheep are kept in one pen, usually approximately 100, and have little opportunity for displaying normal behaviour, including interactions between them. The increased space was deliberate as one of the aims was to investigate sheep body posture responses to the motions' amplitude, period and regularity, and second their responses with the possibility to have contact with the other sheep.

Finally it is important to mention that the changes in behaviour and physiology during the research experiments in response to the motion treatments were significant in comparison to other challenges that sheep face when they are transported on long distance transport (Hall et al., 1998; Andronine et al., 2008). For example, heart rate elevation was small in relation to previous work where sheep were transported in trucks. Also there was no evidence of development of deleterious stereotyped behaviours, which does happen in ruminants that are stressed (Redbo, 1993).

CONCLUSIONS

1. The novel method developed to simulate roll, pitch and heave motion contributes to the study of sheep animal welfare transported overseas under laboratory conditions. Research done under laboratory conditions has many advantages such as the control of potential stressor variables, whereas it would be very difficult to evaluate the impact of one stressor during a commercial environment.

2. Heart rate variability was an important tool to measure stress and autonomic nervous system responses. Behavior measurements, although time consuming, were very useful to evaluate changes in body posture and stability, as well.

3. Heave, irregular sequences and their combination with Roll and Pitch motions, and the interaction with the companion sheep were the most stressful procedures that produced a reduction of the parasympathetic system, and continuous adjustments to recover body stability.

4. Livestock carriers should monitor roll, pitch and heave motions and their combination not just for human security reasons but for the probable impact on animal welfare. The livestock industry should carry out research during commercial transport comparing ship motion data with sheep behavioral and physiological values. They could use the comprehensive knowledge that has been obtained for ship security and human health benefit and incorporate it into the livestock industry.

5. Space allowance standards currently used around the world should be evaluated to incorporate other conditions such as sea transport motions impact.

6. Overall the research project provides enough evidence to conclude that sea transport motions represent a potential stressor that must be further investigated by different stakeholders around the world that seek to improve livestock welfare when they are transported by sea.

FUTURE RESEARCH

Future research that uses a ship motion platform should increase the time spent by sheep experiencing the motions, to be more representative of typical journeys, test a combination of the three most important motions: heave, roll and pitch, and investigate the impact of different amplitude and period, and regularity of motions. Below are listed more specific research topics that could be explored:

a) Additional physiological indicators of welfare to measure are: vasopressin (related to motion sickness during road transport in pigs), cortisol, respiration rate, body temperature, creatine kinase and lactate dehydrogenase (to measure physical exertion).

b) Behavioural measurements should also focus on loss of balance and body posture and obtain additional measurements, such as detailed stepping analysis of each leg (with pedometer equipment) and tension of muscles (e.g. passive tension which can be measured with a goniometer). In relation to feed intake behaviours and motion impact on rumen function, additional research could integrate jaw movement recorders and ruminal physiological measurements, such as pH and microbial endocrinology.

c) Inclusion of additional potential stressors such as stocking density, ammonia, noise and ventilation to evaluate the additive stress impact of each of them.

d) Heart rate variability. Describe changes of the parasympathetic and sympathetic nervous systems during the time spent by sheep experiencing the different motions and correlate with behavioural and physiological measurements.

The other option is to measure the impact of sea transport motion during a commercial voyage. In this sense some potential research projects are:

e) Correlation of behavioural and physiological measurements with the different amplitude and period of motions during different sea conditions (e.g. Rough seas) and stages of sea transport (e.g. departure/arrival at port).

f) Using the motion records from different voyages, correlate this data with information obtained at the end of the voyage, such as body condition, mortality and if possible necropsy data.

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APPENDICES

APPENDIX 1. SHEEP WELFARE STANDARDS.

Summary of sheep welfare standards included in those areas of the export chain related to the sea transport live export procedure (from Australian Standards for the Export of Livestock, DAFF, 2011).

<table>
<tr><th>Chain area</th><th>Topic</th><th colspan="3">Standard</th></tr>
<tr><td>Preparation</td><td>a) Body condition
b) Pregnancy (ewes ≥40kg)
c) Lambs
d) Horned sheep

e) Shorn & wool</td><td colspan="3">a) Must be 2 to 4 scores. 1 and 5 must not be exported
b) Certification of not be pregnant (within 30 days prior export)
c) Weaned at least 14 days before export & liveweight > 28 kg
d) Horns not endanger other animals, not turned in to cause damage to head or eyes, not restrict food/water, one full curl or less.
e) Wool < 25mm length, >10 days off shears, shorn 10 day period before export.</td></tr>
<tr><td>Land Transport</td><td>a) Mixing animals
b) Water deprivation
c) Loading densities
d) Mustering & loading
e) Rest periods</td><td colspan="3">a) Sheep with horns up to one curl may be mixed with hornless
b) Mature stock :32-38hrs / Young stock 20-28 hrs.
c) Floor area: from 0.17m^2/head (20kg) to 0.29 m^2/head (60kg)
d) Feeding if remain in yards or travel for > 24 hours
e) 12 hours of rest period for adults after 32 hrs. of combined curfew and travel. After 20 hrs. of transport, animals between weaning and 12 months must rest 12 hours.</td></tr>
<tr><td>Registered premises</td><td>Stocking density</td><td colspan="3">* Sheep (54kg) held in sheds for ≥ 10 days :
• Penned in groups < 8 animals: 0.9m^2 minimum
• Penned in groups 9-15 animals: 0.8m^2 minimum
• Penned in groups 16-30 animals: 0.6m^2 minimum
• Penned in groups ≥ 31 animals: 0.5m^2 minimum
* Sheep (54kg) held in sheds for < 10 days:
• Penned in groups < 8 animals: 0.6 m^2 minimum
• Penned in groups 9-15 animals: 0.53 m^2 minimum
• Penned in groups 16-30 animals: 0.4 m^2 minimum
• Penned in groups >31 animals: 0.33 m^2 minimum</td></tr>
<tr><td rowspan="11">Vessel preparation</td><td rowspan="11">Minimum pen area per head

*Horned rams: add 10%
*Add 10% pen space for Sheep > 25mm wool</td><td>Liveweight (kg)</td><td>Nov-Apr (m^2)</td><td>May-Oct (m^2)</td></tr>
<tr><td>28</td><td>0.261</td><td>0.261</td></tr>
<tr><td>35</td><td>0.278</td><td>0.278</td></tr>
<tr><td>40</td><td>0.290</td><td>0.290</td></tr>
<tr><td>50</td><td>0.315</td><td>0.315</td></tr>
<tr><td>60</td><td>0.360</td><td>0.381</td></tr>
<tr><td>65</td><td>0.394</td><td>0.423</td></tr>
<tr><td>70</td><td>0.429</td><td>0.468</td></tr>
<tr><td>75</td><td>0.465</td><td>0.515</td></tr>
<tr><td>80</td><td>0.502</td><td>0.563</td></tr>
<tr><td>90</td><td>0.575</td><td>0.658</td></tr>
<tr><td>On-board management</td><td>Reportable incident</td><td colspan="3">Incident that has potential to cause serious harm to welfare. Includes:
* Shipboard mortality rate ≥ 2 per cent
* Rejection of livestock at an overseas port
* Ventilation, feeding or watering systems disablement
* Diagnosis or strong suspicion of an emergency disease
* Any other incident that has serious adverse effect on welfare</td></tr>
</table>

APPENDIX 2. PITCH AND ROLL VISUAL STUDIO PROGRAM

Visual studio program used to replicate pitch and roll motion (*includes explanation in italics*). Machine programmable units are included (512 = neutral position), which have been translated into degrees for description of Method.

```
static int pitch=512;
static int roll=512;
static int delay_move=0;
static int i=1;

static void Delay(int ms)
{
    Thread::Sleep(ms);
}
void ThreadProc()
{
    while(1){
        pitch-=2;  //(increment required)
        if(pitch=470){  //(minimum position required minus the increment)
            pitch+=2; //(increment required)
            while(pitch<552){  //(maximum position required)
                pitch+=2; //(increment required)
                Delay(75); //(delay required, in ms)
                Invoke(gcnew
MessageDispatcher(this,&UQvet::Form1::DispatchMessage));
            }
        }
        Delay(75);  //(delay required, in ms)
        count++;
```

APPENDIX 3. REGULAR PITCH AND ROLL COMBINATION VISUAL STUDIO PROGRAM.

Visual studio program used to replicate Regular pitch and roll combination sequence.

```
static int pitch=512;
  static int roll=512;
  static int delay_move=0;
  static int i=1;
  static void Delay(int ms)
  {
        Thread::Sleep(ms);
  }
  void ThreadProc()
  {
        while(1){
              pitch-=5;
              roll-=6;
                    if(pitch==427){
                    roll+=6;
                    pitch+=5;
                    while(pitch<597){
                          roll+=6;
                          pitch+=5;
                          Delay(235);
                          Invoke(gcnew
MessageDispatcher(this,&UQvet::Form1::DispatchMessage));
                    }
              }
              Delay(235);
```

APPENDIX 4. IRREGULAR ROLL AND PITCH VISUAL STUDIO PROGRAM.

Visual studio program used to replicate Irregular roll and pitch sequences (*includes explanation in italics*).

```
static int pitch=512;
static int roll=512;
static int delay_move=0;
static int i=1;
static int j=1;
static int a=1;
static int b=31;//(has to be equal to the number of amplitude sequences +1)
static int c=1;
static int d=31; //(has to be equal to the number of speed sequences +1)
static int k=1;
static int l=1;
static int m=1;
static void Delay(int ms)
{
   Thread::Sleep(ms);
}
void ThreadProc()
{
   srand (time(NULL));
   m= rand()%(d-c)+c; //(random selection of one of the speed sequences)
   while(1){
      srand (time(NULL));
      j= rand()%(b-a)+a;  //(random selection of one of the amplitude sequences)
      if (m==1){ //(Beginning of the sequences for speed)
         k=8;
      }
      if (m==2){
         k=9;
            }
[...] (add as many sequences as needed)
            }
      if (m==30){
         k=714;
      }  //(End of the sequences for speed)
      if (j==1){      //(Beginnning of the sequences for amplitude)
         while (roll>344){
         roll-=3;
         Delay(k);
         Invoke(gcnew MessageDispatcher(this,&UQvet::Form1::DispatchMessage));
      }
      srand (time(NULL));
      m= rand()%(d-c)+c;
      while (roll<680){
         roll+=3;
         Delay(k);
         Invoke(gcnew MessageDispatcher(this,&UQvet::Form1::DispatchMessage));
      }
      while (roll>512){
         roll-=3;
         Delay(k);
         Invoke(gcnew MessageDispatcher(this,&UQvet::Form1::DispatchMessage));
      }
   }
[...] //(add as many sequences as needed)
   if (j==30){
      while (roll>496){
         roll-=3;
         Delay(k);
         Invoke(gcnew MessageDispatcher(this,&UQvet::Form1::DispatchMessage));
      }
      srand (time(NULL));
      m= rand()%(d-c)+c;
      while (roll<528){
         roll+=3;
         Delay(k);
         Invoke(gcnew MessageDispatcher(this,&UQvet::Form1::DispatchMessage));
      }
      while (roll>512){
         roll-=3;
         Delay(k);
         Invoke(gcnew MessageDispatcher(this,&UQvet::Form1::DispatchMessage));
      } //(End of the sequences for amplitude)
   }
count++;
//NOTE: For pitch sequences, change the word 'roll' to 'pitch' and the values needed.
```

APPENDIX 5. EQUIPMENT AND FACILITIES USED DURING EXPERIMENTS.

Photo 7. Crate covered to reduce visual stimulation effect.

Photo 8. Mesh attached to crate division.

Photo 9. Habituation to pellets combined with molasses.

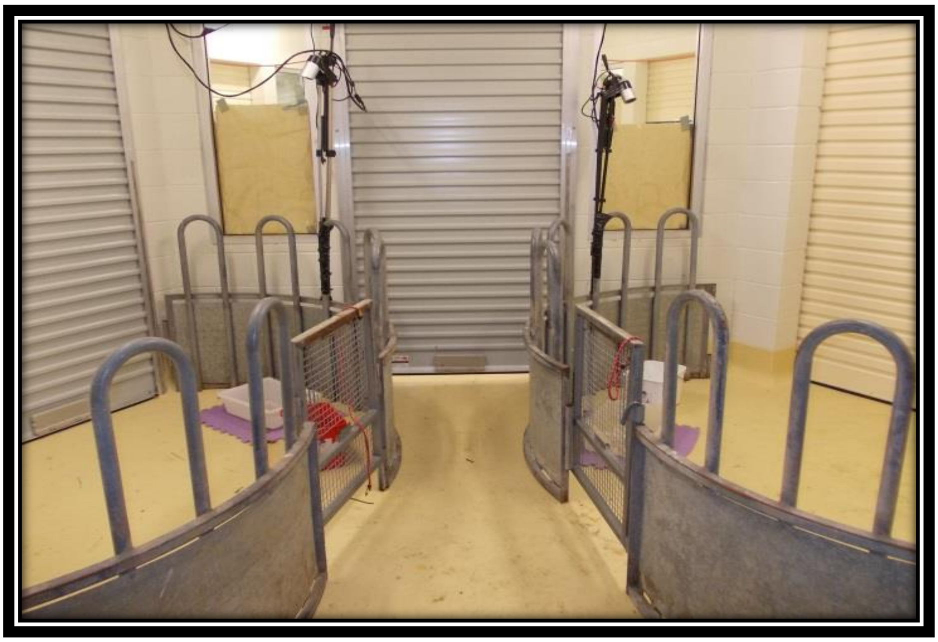

Photo 10. Observational room used to record residual behaviors.

Photo 11. External feed device used during experimental trials.

Photo 12. Mesh attached to external feed device.

Photo 13. Crate tubular structure with a barrier in the middle.

APPENDIX 6. LATIN SQUARE FACTORIAL DESIGN USED FOR EACH EXPERIMENT.

Experiment 1.

	Sheep pairs			
Period (day)	**Sheep 1 & 2**	**Sheep 3 & 4**	**Sheep 2 & 3**	**Sheep 1 &4**
1	Pitch	Roll	Heave	Control
2	Roll	Pitch	Control	Heave
3	Heave	Control	Pitch	Roll
4	Control	Heave	Roll	Pitch
5	Pitch	Roll	Heave	Control
6	Roll	Pitch	Control	Heave
7	Heave	Control	Pitch	Roll
8	Control	Heave	Roll	Pitch

Experiment 2.

			Sheep pairs				
Day	**1 & 6**	**2 & 4**	**3 & 5**	**Day**	**4 & 1**	**5 & 2**	**6 & 3**
1	Control	Roll	Heave	2	Control^{+AE}	Roll^{+AE}	Heave^{+AE}
3	Roll	Heave	Control^{+AE}	4	Roll^{+AE}	Heave^{+AE}	Control
5	Heave+^{AE}	Control	Roll	6	Heave	Control^{+AE}	Roll^{+AE}
7	Heave	Control^{+AE}	Roll^{+AE}	8	Heave^{+AE}	Control	Roll
9	Roll+^{AE}	Heave^{+AE}	Control	10	Roll	Heave	Control^{+AE}
11	Control^{+AE}	Roll^{+AE}	Heave^{+AE}	12	Control	Roll	Heave

AE= Antiemetic drugs

Experiment 3.

Day	1 & 2	3 & 4	5 & 6	Day	1 & 4	3 & 6	2 & 5
1	Reg. Roll	Irreg. Pitch	Reg. Comb.	2	Reg. Pitch	Irreg. Comb.	Irreg. Roll
3	Irreg. Pitch	Reg. Pitch	Reg. Roll	4	Irreg. Roll	Reg. Comb.	Irreg. Comb.
5	Reg. Pitch	Irreg. Roll	Irreg. Pitch	6	Irreg. Comb.	Reg. Roll	Reg. Comb.
7	Irreg. Roll	Irreg. Comb.	Reg. Pitch	8	Reg. Comb.	Irreg. Pitch	Reg. Roll
9	Irreg. Comb.	Reg. Comb.	Irreg. Roll	10	Reg. Roll	Reg. Pitch	Irreg. Pitch
11	Reg. Comb.	Reg. Roll	Irreg. Comb.	12	Irreg. Pitch	Irreg. Roll	Reg. Pitch

Reg. = Regular sequence / Irreg. = Irregular Sequence/ Comb.= Roll and pitch combined.

Experiment 4.

Day	1 & 2	3 & 4	5 & 6	Day	1 & 4	3 & 6	2 & 5
1	Control-Without	Control-With	Irreg.-With	**2**	Reg.-Without	Reg.-With	Irreg.-Without
3	Control-With	Reg.-Without	Control-Without	**4**	Irreg.-Without	Irreg.-With	Reg.-With
5	Reg.-Without	Irreg.-Without	Control-With	**6**	Reg.-With	Control-Without	Irreg.-With
7	Irreg.-Without	Reg.-With	Reg.-Without	**8**	Irreg.-With	Control-With	Control-Without
9	Reg.-With	Irreg.-With	Irreg.-Without	**10**	Control-Without	Reg.-Without	Control-With
11	Irreg.-With	Control-Without	Reg.-With	**12**	Control-With	Irreg.-Without	Reg.-Without

Reg. = Regular sequence / Irreg. = Irregular Sequence/ With= With Barrier / Without= Without Barrier

APPENDIX 7. ETHOGRAMS USED DURING RESEARCH PROJECT.

Experiment 1.

BEHAVIOR	DEFINITION
EVENT (Frequency)	
Stepping	Stepping towards any direction
STATES (Duration)	
Head Position	**Head towards different directions**
Up	Above withers
Middle	At withers level
Down	Below the withers
Under / above	Towards above or under the companion sheep
Looking	
Towards companion sheep	Towards the companion sheep
Towards side bars	Towards side bars (opposite to the other sheep)
Turned around	Towards the back of sheep
Standing	**Standing with the four legs without moving any foot.**
No support	Standing without support
Against crate	Standing with body support on crate
Against barrier	Standing with body support on division
Lying	Lying down in sternal recumbency
Ruminating	Time spent by sheep chewing during rumination.

Experiment 2.

BEHAVIOR	**DEFINITION**
	Feeding
Eating	Time spent by sheep eating
Drinking	Time spent by sheep drinking
Prehension	Time spent & number of times grabbing the pellets with the lips
Mastication	Time spent & number of times crushing the food before swallow it
	Body posture
Head Position	**Head towards different directions**
On mesh	Supporting head on mesh
Up	Above withers
Middle	At withers level
Down	Below the withers
Above	Towards above or under the companion sheep
Looking	
To companion	Towards the companion sheep
To side bars	Towards side bars (opposite to the other sheep)
Standing	Standing with the four legs without moving any foot
Lying	Lying in sternal recumbency
Kneeling	Hind legs erect, front legs on ground from carpal joint to foot

Experiment 3.

BEHAVIOR	**DEFINITION**
	Events (Frequency)
Stepping	Stepping towards any direction
Pawing	Pawing the floor with any front foot
Butting	Butting the other sheep
	Body postures states (Duration)
Head Position	**Head towards different directions**
Up	Above withers
Middle	At withers level
Down	Below the withers
Under / above	Towards above or under the companion sheep
Looking	
To companion sheep	Towards the companion sheep
To side bars	Towards side bars (opposite to the other sheep)
Standing	**Standing with the four legs without moving any foot.**
No support	Standing without support
Against crate	Standing with body support on crate
Lying	Lying down in sternal recumbency
Kneeling	Hind legs erect, front legs on ground from carpal joint to foot
	Feeding
Eating	Time spent by sheep eating
Drinking	Time spent by sheep drinking
	Other states
Licking bowl	Time spent licking the bowl feeder

Experiment 4.

BEHAVIOR	DEFINITION
EVENT (Frequency)	
Stepping	Stepping towards any direction
Pawing	Pawing the floor with any front foot
Butting	Butting the other sheep
Push with body	Pushing the companion sheep with any part of the body
Evading	Forced movement to evade touching the other sheep
STATES (Duration)	
Head Position	**Head towards different directions**
Level	At withers level
Down	Below the withers
Under / above	Towards above or under the companion sheep
Looking	
To companion sheep	Towards the companion sheep head
Away from companion	Towards the opposite of companion sheep head
Standing	**Standing with the four legs without moving any foot.**
No support	Standing without support
Against crate	Standing with body support on crate
Lying	Lying down in sternal recumbency
Kneeling	Hind legs erect, front legs on ground from carpal joint to foot
Ruminating	Time spent by sheep chewing during rumination.