liveexport

Development of welfare indicators for cattle & sheep transported by ship

Stage 2: The effect of gaseous ammonia on the health and welfare of sheep & cattle

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Abstract

Exposing livestock to high ammonia concentrations was perceived by stakeholders in the live export industry to be compromising their welfare. Ammonia and other potentially noxious gases were monitored on two sheep voyages on the same ship from Australia to the Middle East and in one sheep assembly depot. In 20% of the sites on board the vessel, the ammonia concentration was above 25 ppm, a maximum exposure limit recently proposed for live export and also a threshold limit for humans. In some vessel sites ammonia was above the normal Short Term Exposure limit for humans, 35 ppm. CO_2 and H_2S were at low levels on the ship and in the depot, and all three gases were at low levels in the depot. The physiological and behavioural responses to 15-45 ppm ammonia were examined in sheep and cattle in a simulated 12 d ship voyage. Macrophage activity was increased at 45 ppm in cattle but physiological responses had disappeared 28 d after the simulated voyage. Increased lacrimation and nasal discharge occurred in a high proportion of steers on exposure to 45 ppm, but not 15-30 ppm. It is therefore recommended that the maximum exposure limit for steers is 30 ppm. Most sheep showed a partial avoidance of ammonia at 45 ppm, and it is therefore recommended that the maximum exposure limit for sheep should also be 30 ppm.

Executive summary

A programme of research to investigate the levels and impact of ammonia during live export on the welfare of cattle and sheep was undertaken by researchers at the Centre for Animal Welfare and Ethics, University of Queensland. A previous questionnaire to stakeholders in the industry had identified that ammonia was a possible welfare indicator during the live export process (Pines *et al*., 2007).

Initially, two voyages of the same ship were attended that were transporting sheep from Australia to the Middle East, with the purpose of mapping the ships and depot for ammonia as a potential noxious gas, and comparing the behaviour of sheep in high and low ammonia parts of the ship on one voyage. Daily measurements were made at 20 representative sites throughout the vessel on each voyage. The concentration of atmospheric ammonia in 20 % of the sites was above 25 ppm, the level set for the Time Weighted Average (TWA) maximum human exposure during an 8 hour period (NOHSC, 1995) and the maximum limit previously recommended for the live export industry (Costa, 2003). Some locations were also above 35 ppm, the Short Term Exposure Limit for humans, which is determined from the mean concentration in any 15 minute period (NOHSC, 1995). Ammonia occurred at higher concentrations and was more widespread and persistent on closed than on open decks. On the open decks, ammonia concentration was negatively correlated with air movement, and positively correlated with dew point and wet bulb temperatures and faecal pad depth. It was highest behind the bulkhead at the front of the vessel and near the engine room. On the closed decks, ammonia concentration was most closely related to dew point and wet bulb temperatures. Air speed varied considerably within many of the open deck pens, while dew point and wet and dry bulb temperatures were all relatively uniform throughout each pen. Sheep behaviour and the prevalence of conjunctivitis were not affected by the level of ammonia. There was considerable variation in ammonia concentration within pens, so sheep movement in the pens was examined and found to be localised within regions of the pens. Sheep made less use of the available pen space during the second half of the voyage compared to the first, with more time spent lying down. Carbon dioxide and hydrogen sulphide were also monitored as potentially noxious agents, but the atmospheric concentrations (hydrogen sulphide <1.5 ppm; carbon dioxide <1900 ppm) were considerably below the TWA limit for humans (NOHSC, 1995) and were thought unlikely to pose a risk to livestock.

Concentrations of potentially noxious gases and the potential influences of environmental variables were also measured in eight sheds at a sheep assembly depot. Daily measurements were made at 12 randomly selected sites in each shed on 4 separate days. The mean ammonia concentration in all sheds was < 2 ppm, and was positively associated with dew point and wet and dry bulb temperatures. Concentrations of hydrogen sulphide (<1.7 ppm) and carbon dioxide (<1500 ppm) were also low.

The effects of ammonia concentration (0, 15, 30 and 45 ppm) on the physiology and behaviour of steers and wethers held for 12 days under micro-climate and stocking density conditions which approximately simulated a voyage from Australia to the Middle East during the northern summer were recorded. Ammonia increased macrophage activity in bronchial alveolar lavages of the cattle but not the sheep, in proportion to concentration. Ammonia also increased lacrimation, nasal secretions and coughing in cattle, particularly at 45 ppm, suggesting that under simulated shipboard conditions, ammonia irritates the eyes and nasal passage of cattle.

Sheep, but not cattle, exposed to 30 and 45 ppm ammonia lost 6-8 % of their live weight. Ammonia had no effect on cattle behaviour and little consistent effect on sheep behaviour, and there was no effect of ammonia on the haematological parameters of either species. Twenty-eight days after exposure the animals were removed from the ammonia and macrophage activity in cattle had decreased and sheep live weight increased; suggesting the effects of ammonia were temporary.

The avoidance of ammonia was tested using operant conditioning techniques by training 12 sheep to operate a lever to open a door that would allow them to traverse a chamber (with or without ammonia) and obtain a food reward. One half of the sheep had been prior exposed to ammonia in a simulated ship voyage, and the other half were kept in identical conditions but without ammonia. The maximum number of lever presses performed (breakpoint) when the chamber contained 45 ppm ammonia was compared with the number when the chamber contained no ammonia. There was a significantly lower mean breakpoint when sheep traversed the chamber filled with ammonia (mean 5.5 presses), compared with the chamber filled with fresh air (mean 7.6 presses), demonstrating that sheep exhibited a partial aversion to 45 ppm ammonia. There was no evidence that prior exposure influenced this aversion. The proportion of sheep apparently exhibiting this aversion (75%) was greater than the recommended maximum proportion of humans exposed to noxious odours that report unpleasant sensory stimulation (5-20%; Paustenbach and Gaffney, 2006).

This research will increase industry understanding of noxious gases, and in particular ammonia, that have potential risks to animal and human safety on live export ships, and it has generated information on variables which with further research could be used to manage ammonia.

In considering the establishment of critical limits for ammonia concentration on live export vessels and pre-export assembly depots, it is recommended that a No Observable Adverse Effect Level is inappropriate because exposure is not toxic. Establishing a Maximum Exposure Limit that is relevant for ships should take into account the proportion of animals experiencing prolonged physiological disturbances, irritation and the proportion experiencing unpleasant sensory stimulation. Physiological disturbances and irritation were investigated in the exposure of sheep and cattle to 0-45 ppm in the simulated voyage, and unpleasant sensory stimulation was investigated in the operant conditioning test. Physiological disturbances were short-lived and mainly confined to macrophage activity in bronchiole lavages of steers, which was elevated at 45 ppm. On exposure to 15-30 ppm ammonia, the proportion of steers with nasal discharge and lacrimation (10-20 %) was acceptable by proposed criteria for humans (Paustenbach and Gaffney, 2006), whereas it was unacceptably high at 45 ppm (35-40%). Hence it is proposed that the critical exposure limit for steers should be 30 ppm. Irritation of the nasal or ocular mucosa was not recorded with sheep in this study, but the partial avoidance of ammonia by most animals in the operant conditioning study is evidence of unpleasant sensory stimulation, and we therefore recommend the same critical exposure limit for sheep.

The critical duration of exposure is normally that sufficient to cause the irritation, but this is less relevant to animals that are permanently penned than humans, who have transient exposure, and it is also difficult to measure, compared with an instantaneous recording of ammonia concentration. We therefore recommend that the maximum exposure level should not be time dependent. Evidence from the initial monitoring of live export shipments suggests that there will be more variation in ammonia concentrations in vessels with open than closed decks, and that the highest concentrations will be found where airflow is diminished.

We identified that some ammonia concentrations on the vessels that we monitored were in excess of the levels permitted for human attendants, and action to reduce ammonia is required to safeguard human health, as well as animal welfare in some circumstances. We further identified the optimum methods of measuring ammonia on live export vessels, comparing several different commercially-available systems.

Contents

1 Background

1.1 Ammonia gas

Gaseous ammonia is generated by urease activity breaking down urea in urine, manure and bedding (Dawes and Goodall 1995; James *et al*., 1999; MAMIC 2000). Ammonia is an irritant pollutant within livestock buildings and ships, and has comfort and health impacts on both cattle and people (Dawes and Goodall 1995; MAMIC 2000). The level at which ammonia concentration causes respiratory problems in sheep and cattle has not yet been determined. However, Dawes and Goodall (1995) reported that calves exposed to high (non-specified) levels of ammonia had significantly higher respiratory disease and higher death rates than those exposed to low (non-specified) levels.

Published ammonia values for housed cattle range from less than 2 ppm to 29 ppm (Groot Koekamp *et al.,* 1998). Much higher values (50 ppm) have been reported for pig and poultry sheds (Groot Koerkamp *et al*., 1998; Hinz and Linke 1998).

The published allowable atmospheric ammonia concentrations for humans vary from 10 (National Swedish Board of Occupational Safety and Health, 1990) to 50 ppm (Occupational Safety & Health Administration, 1989) with 25 ppm being a common threshold value (Groot Koerkamp *et al.,* 1998; MAMIC 2000). One suggested occupational exposure limits for swine workers is 7.5 ppm (Reynolds *et al*., 1996). A level of 25 ppm is the Threshold Limit Value used by the American Conference of Governmental Industrial Hygienists (ACGIH), which reassesses its limit values annually. The American National Institute for Occupational Safety and Health (NIOSH) and Occupational Safety and Health Administration (OSHA) limits are both 50 ppm, but these are not updated annually. The ACGIH level is set to avoid irritation in individuals who have not been exposed to ammonia before, which reduces their sensitivity. Australian exposure standards for humans have two critical ammonia levels (NOHSC, 1995): The threshold limit value Time Weighted Average[1] (TWA) is 25 ppm over an eight-hour period. The threshold limit value for Short-term Exposure Limit[2] is 15 minutes exposure to 35 ppm, which can be repeated up to four times a day. This is supported by work by Luttrell (2002), who reported throat and nose irritation of humans at 24 ppm. Humans also report a reduction in well-being in environments with ammonia above 50 ppm, although this is less severe for humans regularly exposed (Ihrig *et al*., 2006).

Values in excess of 25 ppm have been reported on live export vessels (MAMIC 2001; Costa *et al*., 2003). In a recent LiveCorp (LIVE.219) study, Gaughan *et al*. (2005) reported that levels above 20

[1] **Time-weighted average (TWA)**' means the average airborne concentration of a particular substance when calculated over a normal eight-hour working day, for a five-day working week

[2] **Short term exposure limit (STEL)**' means a 15 minute exposure which should not be exceeded at any time during a working day even if the eight-hour TWA average is within the TWA exposure standard. Exposures at the STEL should not be longer than 15 minutes and should not be repeated more than four times per day. There should be at least 60 minutes between successive exposures at the STEL.

ppm were uncomfortable i.e. made breathing somewhat difficult and caused throat irritation for humans working on the project. Gaughan *et al*. (2005) observed that as ammonia levels increased above 20 ppm so did the amount of coughing and rapid respiration in cattle. Long term exposure to ammonia may lead to respiratory damage in cattle (Dawes and Goodall 1995; Costa *et al.*, 2003), which will have a negative impact on their ability to deal with heat stress. In the study of Gaughan *et al*. (2005), wetting the bedding resulted in a substantial increase in ammonia concentration. Lambs exposed to 75 ppm atmospheric ammonia for 28 days showed clear signs of irritation of the mucous membranes, with profuse lacrimation, severe coughing and sneezing, and profuse nasal discharge that at times was bloody (Drummond *et al.,* 1976).

In penned animals, the increase in ammonia concentration is a function of increasing ambient temperature, increasing urine concentration of the bedding and pH levels (increasing alkaline) of the bedding (Dawes and Goodall 1995; Argo *et al.*, 2001; Costa *et al*., 2003; Zhao and Chen 2003).

1.2 Measuring ammonia on ships

Long-term measurement of ammonia levels on ships is difficult. Attempts to measure the effects, rather than the concentrations of ammonia have not been successful. For example, a cough sound indicator (Van Hirtum and Berckmans, 2003) has been developed in response to ammonia concerns in pigs, which presumably relates more directly to animal distress, but it is of inadequate accuracy (92% correct positives, 21% misclassified).

Three different types of meters were used to measure ammonia concentrations on ships in LIVE.223 (McCarthy, 2005), Neotox Mk % Ammonia Meter, ToxiRae II Toxic Gas Monitor and QRae Plus Multigas meter. These are all personal alarm monitors designed to alert people in hazardous situations that they have entered a toxic level of the gas (Lou Farro, App-Tek Safety Pty Ltd, personal communication). They were not designed for regular monitoring in a challenging environment, where the requirement was to determine levels accurately over many days (Lou Farro, App-Tek Safety Pty Ltd, personal communication). The latter two instruments proved unable to cope with the temperature and humidity conditions, but readings were obtained from the first instrument (summarised in Table 1). The mean readings for the five voyages was approximately 10 ppm, with minimum and maximum readings on the voyage of c. 2 and 31 ppm, respectively. However, the industry standard (Australian Standard 2865, 1995, Safe Working in a Confined Space) emphasises that gas detection instruments should be regularly challenged, using a standard dose of ammonia from a cylinder with a regulator. This should be undertaken each day before measurements are taken (Lou Farro, App-Tek Safety Pty Ltd, personal communication). The Neotox meter used in LIVE.223 was not challenged or calibrated and may have been subject to drift in readings both at the top and bottom end of the scale. Despite this there is a reasonable consistency in readings between voyages, which may indicate a greater reliability than indicated by the company with whom we have liaised on instrumentation (App-Tek Safety Pty Ltd). They advise that in addition to a regular daily challenge, the machine should be overhauled every 6 months, including returning to base standard, with an accuracy of $\pm$ 10%.

Table 1 Summary of ammonia readings obtained using the Neotox Ammonia Meter in project LIVE.223

Voyage number	Number of readings	Mean	Median	Standard Deviation	Standard Error of Mean	Minimum	Maximum
1	26	11.21	10	4.1	0.81	5	19
2	0						
3	24	11.4	8	8.4	1.7	2	31
4	0						
5	28	4.0	0	6.1	1.2	0	22
6	25	9.0	8	3.5	0.69	4	17
7	25	12.8	13	3.9	0.78	5	21
8	0						
9	0						

1.3 Setting a maximum level for cattle

Information for ammonia has been almost exclusively derived from cattle, and there is considerable variation in the levels at which effects have been recorded. Tudor *et al*. (2003) in LIVE.202 concluded that a TWA of 20 ppm ammonia should be the target maximum during live export, which was based on preliminary lung studies using bronchial alveolar lavages. However, they observed significant increases in white blood cell count and mononucleated cell counts after just 9 days exposure to levels of c. 20 ppm, obtained after treatment of the bedding with gypsum.

A literature review by Costa *et al.* (2003) (LIVE.218) concluded that a TWA of 25 ppm would be an appropriate maximum level to be implemented on ship, however, they also conclude from their investigations of haematological immunocompetence that 'there are initial clinical signs of inflammation at 22 ppm atmospheric ammonia'. They summarised their findings by stating that there is insufficient information on the impact of long-term (> a few days) exposure to establish standards. Stacey (2003) found that typical levels below decks were 15 ppm, with readings commonly reaching 20 – 30 ppm. There are also known to be small increases in ammonia concentration with height in cattle buildings (EA Systems, 2003).

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1.4 Other farm animals

Although there is limited and somewhat conflicting evidence with livestock, considerable literature with pigs and poultry exists due to their being normally housed in high density farming operations.

With poultry, there is evidence that welfare is adversely affected below 25 ppm (Kristensen *et al.,* 2000; Kristensen and Wathes, 2000), and both pigs and poultry show preference for fresh air compared with 10 ppm ammonia (Wathes *et al*., 2002.

1.5 Desensitisation to ammonia

Continuous exposure of animals to ammonia could reduce their aversion to the gas (adaption), and it is known that humans working daily in highly odorous environments, such as those with ammonia, can show evidence of a persistent reduction in responsiveness to the odour (Harada *et al*. 1983; Schiffman, 1998). Reduced olfactory acuity has also been reported to occur in some pigs exposed to 40 ppm ammonia (Jones *et al*., 2001). However, the effects of ammonia on olfaction are not conclusive, with Holness *et al*. (1989) reporting no change in odour sensitivity in humans chronically exposed to moderate levels of ammonia (12.5 ppm).

2 Project objectives

1. Describe the effects of gaseous ammonia on sheep and cattle exposed long-term, under simulated shipboard conditions, to ammonia and the preferences of sheep and cattle for critical ammonia concentrations.

2. Make accurate and repeated measurements of ammonia concentration at a pre-export assembly depot and exported by sea.

3. Recommend critical ammonia levels for sheep and cattle held in pre-export assembly depots and exported by sea.

3 Experiments

3.1 Experiment 1: Atmospheric concentrations of ammonia, hydrogen sulphide and carbon dioxide on a live export vessel

3.1.1 Experiment aim

The aim of this experiment was to make accurate and repeated measurements of atmospheric ammonia, hydrogen sulphide and carbon dioxide on a live export vessel carrying sheep from Australia to the Middle East.

3.1.2 Methodology

Two voyages were undertaken on a vessel with nine (four open and five closed) single tier decks. The first voyage sailed from Fremantle to Muscat and then to Kuwait during May/June 2005. Wethers (46-55 kg), rams (80 kg), lambs (40 kg), ewes (53 kg) and hoggets (55-60 kg) were shipped

on voyage one. The second voyage sailed from Fremantle to Muscat, at which point the researchers disembarked, and then to Kuwait in July 2005. Wethers (44-58 kg), rams (70 kg), lambs (38 kg), ewes (53 kg) and ram hoggets (50-52 kg) were transported on the second voyage. On both voyages, the majority of the animals were merino or merino cross breeds. The timing of the voyages corresponded with summer in the Middle-East when temperatures were at their highest.

One researcher (Mathew Pines) accompanied the first voyage, and two researchers (Mathew Pines and Clive Phillips) accompanied the second voyage.

On voyage one, ammonia and hydrogen sulphide were measured at 20 study sites; 10 where high concentrations of ammonia were predicted and 10 where low ammonia was predicted (see Table 2 for description). Selection of the high ammonia sites was based on communications with a previous researcher that had measured ammonia levels on this ship (Jeisane Accioly) and the ship boatswain. Selection of the low ammonia sites was based on finding sites located on the same deck and with the same class of sheep and, where possible, similar pen size as the high ammonia sites, but with better ventilation. Eight of the study sites were located on closed decks and 12 on open decks. As there were only enough Gastec gas detection tubes to collect samples on 11 days, day 3 was randomly selected to be the day that ammonia data was not collected; i.e. samples were collected on days 2, and 4 to 13. Samples were collected between 09.00–16.00 h with individual sites visited at approximately the same time each sampling day. Carbon dioxide was not measured on the first voyage as the CO_2 meter was not available.

On days 6-9 of the second voyage, ammonia and carbon dioxide concentrations were mapped using longitudinal and latitudinal transects on two open decks (decks nine and six) and two closed decks (decks five and one). These sites were selected to give adequate coverage of the ship both in terms of the decks sampled and the parts of each deck. Deck five was split into three discrete subsections and deck one split into two discrete subsections, i.e. each subsection was completely independent from the next.

Line transects were conducted along the length of each deck in the middle of the vessel and across the breadth of the ship at the front and middle of the open decks (except deck nine for carbon dioxide) and across the front of each closed deck subsection. Where access was possible, transects were also conducted across the breadth of the deck at the back of each deck or subsection. Additional transects along the length (one on the port and one on the starboard side) of decks nine and one were conducted for carbon dioxide. Samples were collected every 2.2 m.

Ammonia, hydrogen sulphide and carbon dioxide concentrations were also mapped in 20 open deck pens (Table 3). As behaviour measurements (discussed in section 3.3.2) were also made in the same 20 pens, all study sites were restricted to a small geographical area for logistical reasons; the front half of the vessel on decks six to nine. Samples were collected every 2.2 m in both a fore/aft direction and port/starboard. Thus in a 4.4 m x 17.7 m pen, 16 samples were collected, while in the smaller 4.4 m x 13.2 m pen, 12 samples were collected. The 20 pens were mapped over seven consecutive days (with 1-5 pens mapped a day; see Table 3 for details). Direct comparisons between pens were potentially inaccurate as the pens were mapped on different days.

Therefore, for each of the 20 mapped pens, the section of the pen with the highest concentration of ammonia and the section of the pen with the lowest concentration of ammonia were re-sampled a second time on day 9 of the voyage and these data were later used to rank pens in order from lowest to highest ammonia; required for the analysis of the effects of ammonia on sheep behaviour in Experiment 3.

Table 2: Description of study sites on voyage one.

Site	Predicted ammonia	Deck (top-bottom	Deck type (Open/ Closed)	Row (Starboard-Port)	Pen (Fore-Aft)	Length wise position (Fore/Middle/Aft)	Breadth wise position (Port/Starboard)	Nearest vent (m)	Sheep type	Stocking density (m^2/head)	Pen area (m^2)
1a	High	9	Open	4	1-4	Fore	Port	12	55 kg wether	0.351	71
1b	Low	9	Open	4	12-15	Middle	Port	4	55 kg wether	0.351	74
2a	High	9	Open	3	1-4	Fore	Starboard	12	40 kg lamb	0.29	71
2b	Low	9	Open	3	12-16	Middle	Starboard	4	40 kg lamb	0.29	70
3	High	8	Open	2	1-2	Fore	Starboard	12	55 kg wether	0.351	71
3	Low	8	Open	2	6-9	Middle	Starboard	4	55 kg wether	0.351	71
4	High	8	Open	4	32-35	Middle	Port	9	53 kg ewe	0.337	71
4	Low	8	Open	2	35-38	Aft	Starboard	2	53 kg ewe	0.337	70
5	High	7	Open	3	1-5	Fore	Starboard	9	60 kg wether	0.425	89
5	Low	7	Open	3	8-11	Middle	Starboard	4	60 kg wether	0.425	71
6	High	6	Open	1	21-29	Middle	Starboard	7	55 kg wether	0.351	121
6	Low	6	Open	2	13-18	Middle	Starboard	4	55 kg wether	0.351	95
7	High	2	Closed	3	A1-A6	Aft	Starboard	6	46 kg wether	0.305	106
7	Low	2	Closed	4-5	A5-A9	Aft	Port	4	46 kg wether	0.305	167
8	High	1	Closed	1-2	A1-A3	Aft	Starboard	4	46 kg wether	0.305	106
8	Low	1	Closed	1-2	A7-A10	Aft	Starboard	2	46 kg wether	0.305	106
9	High	2	Closed	3	F1-F5	Fore	Port	4	46 kg wether	0.305	84
9	Low	2	Closed	4	F4-F9	Fore	Port	4	46 kg wether	0.305	89
10	High	1	Closed	2	F1-F5	Fore	Starboard	9	46 kg wether	0.305	78
10	Low	1	Closed	3	F3-F8	Fore	Port	4	46 kg wether	0.305	89

Table 3: Description of study sites on voyage two. Sites numbered differently to voyage one.

Site	Deck (top-bottom	Deck type (Open/ Closed)	Row (Starboard -Port)	Pen (Fore-Aft)	Length wise position (Fore/Middle)	Breadth wise position (Port/Starboard)	Ammonia ranking (low-high)	Sheep type	Stocking density (m^2/head)	Pen area (m^2)	Date mapped
1	9	Open	3	1-4	Fore	Starboard	8.0	38 kg wether lambs	0.271	71	20/07/05
2	9	Open	5	1-4	Fore	Port	9.5	38 kg wether lambs	0.344	70	15/07/05
3	9	Open	6	1-4	Fore	Port	15.0	38 kg wether lambs	0.261	77	18/07/05
4	9	Open	5	5-8	Fore	Port	2.5	38 kg wether lambs	0.359	71	20/07/05
5	9	Open	6	5-8	Fore	Port	7.0	38 kg wether lambs	0.256	77	19/07/05
6	9	Open	6	9-12	Middle	Port	17.5	38 kg wether lambs	0.287	74	15/07/05
7	8	Open	3	1-4	Fore	Starboard	9.5	44 kg young wethers	0.322	71	17/07/05
8	8	Open	4	1-4	Fore	Port	4.5	44 kg young wethers	0.273	71	21/07/05
9	8	Open	4	13-16	Middle	Port	4.5	44 kg young wethers	0.343	70	19/07/05
10	8	Open	1	11-14	Middle	Starboard	2.5	44 kg young wethers	0.263	72	18/07/05
11	7	Open	3	1-4	Fore	Starboard	13.0	53 kg ewes	0.298	71	16/07/05
12	7	Open	4	1-3	Fore	Port	11.5	53 kg ewes	0.391	53	20/07/05
13	7	Open	5	1-4	Fore	Port	15.0	44 kg young wethers	0.322	71	18/07/05
14	7	Open	1	14-17	Middle	Starboard	6.0	53 kg ewes	0.381	74	18/07/05
15	7	Open	2	5-9	Fore	Starboard	11.5	53 kg ewes	0.324	53	19/07/05
16	7	Open	5	17-20	Middle	Port	1.0	44 kg young wethers	0.319	70	18/07/05
17	6	Open	3	1-4	Fore	Starboard	20.0	50 kg wether	0.310	70	19/07/05
18	6	Open	4	1-4	Fore	Port	17.5	50 kg wether	0.279	70	15/07/05
19	6	Open	3	9-12	Middle	Starboard	19.0	50 kg wether	0.335	68	19/07/05
20	6	Open	3	F4	Fore	Port	15.0	50 kg wether	0.305	89	17/07/05

All gas samples were collected within the pen and at standing sheep head height, i.e. approximately 60 cm from pen floor.

A vertical transect was conducted at five randomly selected sites (Deck 9, Row 4, Pen 17; Deck 9, Row 2, Pen 1; Deck 2, Row 3, Pen 2; Deck 8, Row 1, Pen 7; Deck 8, Row 4, Pen 18) to measure changes in ammonia and carbon dioxide concentration with increasing height. The first sample was collected 5 cm above the faecal pad and the rest at 30 cm intervals (i.e. at 35 cm, 65 cm, 95 cm etc).

3.1.3 Equipment

Ammonia was measured on the first voyage using Gastec gas detection tubes (Gastec Corporation, Fukaya, Japan) (coefficient of variation 5-10%; resolution: 1 ppm) and a Neotox Mk Ammonia Meter (Nutech Australia, Western Australia, Australia) (accuracy ±5 ppm; resolution: 1 ppm) and on the second voyage using the same Neotox meter, a limited number of QRae gas detection tubes (RAE Systems, California, USA) (Relative standard deviation <12%; resolution: 2 ppm) and a QRae Plus Multigas meter (RAE Systems, California, USA) (drift 10%; resolution: 1 ppm). Readings obtained from the two brands of tubes were corrected for dry bulb temperature. The Neotox meter and the QRae plus meter were calibrated prior to the voyage and fresh air calibrations (i.e. calibration of the meter in a zero ammonia environment) were conducted each time the instruments were used. Four days into the second voyage, readings from the QRae plus meter began to “drift” when compared to both the Neotox meter and the QRae tubes, and therefore no further readings were made from the QRae plus meter.

Carbon dioxide was measured on the second voyage only using a MultiRAE carbon dioxide meter (drift: <5%; resolution: 10 ppm). The MultiRAE meter had been calibrated prior to the second voyage and was fresh air calibrated prior to each use.

An OdaLog gas data logger (accuracy: ±2 ppm; resolution: 0.1 ppm) was used to measure hydrogen sulphide. This instrument was calibrated before the first voyage and was fresh air calibrated before each use.

3.1.4 Statistical analysis

Before analysis, the Anderson-Darling test was applied to check whether the data were normally distributed. The vertical transect ammonia data was square-transformed and the vertical transect carbon dioxide data was square root-transformed in order to achieve normal distribution.

Pearson’s correlation was used to examine the relationship between the Neotox meter and the Gastec tubes, QRae plus meter and the QRae tubes and all linear relationships were described using regression analysis.

Sites where high ammonia concentrations were predicted were compared to the corresponding sites at which low ammonia was predicted using a two sample *t*-test.

The scores for ammonia, on the open and closed decks were analysed using a General Linear Model (GLM) to test for an interaction between the open and closed decks over days 2-13 of the

voyage. In order to partition the interaction, 'days of voyage' was considered as a continuous variable. When no significant interaction was found, the interaction was removed from the model and the scores were reanalysed to determine whether there was any main effect of day of voyage and any difference between the open and closed decks. This model assumes parallelism in the morning and afternoon conditions over days of sampling. Model adequacy was assessed using a pure error lack of fit test.

Spearman's correlation was used to analyse the relationship between vertical distance from faecal pad and ammonia and carbon dioxide concentration.

Hydrogen sulphide was analysed with day of voyage used as a repeated measure to determine whether there was any variation of scores over voyage one.

3.2 Experiment 2: Factors affecting ammonia concentrations on a live export vessel

3.2.1 Experiment Aim

The aim of this experiment was to examine the effects of wet and dry bulb temperature, dew point temperature, air speed and faecal pad depth on atmospheric ammonia concentration on a live export vessel. Carbon dioxide and hydrogen sulphide were not considered as shipboard concentrations were low and unlikely to pose a health and welfare risk to livestock.

3.2.2 Methodology

Wet and dry bulb temperature, dew point temperature, air speed and faecal pad depth were measured daily at the same 20 sites as ammonia on the first voyage (see section 3.1.2 for a description of the sampling procedure). Similarly, on the second voyage, wet and dry bulb temperature, dew point temperature and air speed were mapped every 2.2 m in a grid using the same 20 pens in which ammonia was measured. Faecal pad depth was not measured on voyage two. Pens were mapped over seven consecutive days (with 2-4 pens mapped a day: see Table 3 for details).

All samples were collected within the pen and, aside from faecal pad depth, were collected at standing sheep head height, i.e. approx 60 cm from pen floor. Two digital anemometers were used to measure air speed: a CFM Master 8901 meter (accuracy: ±2%; resolution: 0.01 m/s) and a Kestrel 4000 Pocket weather station (accuracy: ±3%; resolution: 0.3 m/s) (Nielsen Kellerman Australia, ACT, Australia). The Kestrel 4000 was also used to measure wet (accuracy: ±2%; resolution: 0.1^{o}C) and dry bulb temperature (accuracy: ±2%; resolution: 0.1^{o}C) and dew point temperature (accuracy: ±2%; resolution: 0.1^{o}C). Readings from the Kestrel 4000 were routinely compared to those of a second hygrometer (PCWI Model 8705 Hygrometer, PCWI Precision Instrumentation, NSW, Australia) to ensure accuracy. Faecal pad depth was measured by insertion of a ruler through the pad to the floor and measuring the average depth of faeces at that point.

Bridge wind speed and direction were recorded by the ship's duty officer every four hours. Wind speed is presented as a cumulative value.

3.2.3 Statistical analysis

The statistical analysis used here to analyse air speed and faecal pad depth was the same as that used in Experiment One. Wet and dry bulb temperature and dew point temperature were not analysed using a GLM as the data were not normally distributed; two sample *t*-test were instead used to look for main effects within the normally distributed mean data.

Forward and backward stepwise regressions were used to examine which variables (air speed in the pen, wet bulb temperature, dry bulb temperature, dew point temperature, faecal pad depth and cumulative wind speed at the bridge) were associated with ammonia concentration in order to evaluate the order of importance of variables. The results from the two voyages were analysed separately to see if there was any difference between them. Data from the first voyage were further divided based on whether it was collected from an open deck or a closed deck (all voyage two data were collected on an open deck).

Prior to conducting the stepwise regression, variables were examined for co-linearity. Variables were also screened for those that did not have an unconditional association with ammonia (Dohoo ***et al.***, 2003). All two-way interactions between the remaining terms were included in the regression. Log 10 transformation of ammonia concentration for open deck on voyage one and voyage two was necessary to ensure the residuals from the regression analysis were normally distributed.

3.3 Experiment 3: The effects of ammonia on a live export vessel on sheep behaviour.

3.3.1 Experiment Aim

The aim of this experiment was to monitor changes in sheep behaviour over the course of a voyage and to examine the effects of atmospheric ammonia on sheep behaviour and prevalence of conjunctivitis.

3.3.2 Methodology

The behaviour and pen space usage of 20 marked sheep (one sheep per pen) were scored on the second voyage. Focal animals were marked with a line on the nape of the neck using a red water-based paint to distinguish them from the rest of the animals in the pen (group size in pens ranged between 131-299 animals).

The behaviour and location of the focal animals was scored at 30-minute intervals for 4.5 h/day for four days during days 2-5 (the first half of the voyage) and again during days 6-9 (second half of the voyage). Sampling occurred between 03.30 h and 21.30 h with a different time period sampled on each of the four days, i.e. after four days there was data for every 30 minute interval between 03.30 h and 21.30 h. The two researchers each scored the behaviour of 10 sheep (20 sheep in total). Due to the practicalities of visiting 10 pens in 30 minutes, all pens were restricted to the front half of the vessel and on the open decks. Based on the results of voyage one, 10 of the focal animals were housed in pens in which high concentrations of ammonia were likely to occur and 10 animals in pens where low concentrations of ammonia were likely to occur.

Focal animals were selected at random. As it happened, the division between pens with high and low ammonia was not clear cut and so it became necessary for statistical purposes to rank the 20 pens in order from highest to lowest ammonia.

Using a scan sampling technique (i.e. the group is scanned for the location of each focal sheep and the behaviour of that sheep at that instant is recorded), a number of behaviours of the focal sheep were scored: rumination, the vertical position of the head (i.e. whether the head was above or below the shoulders; Hall *et al*., 1998) and whether the sheep was standing, lying or feeding/drinking. A measure of pen space usage was obtained by dividing the pen into 2.2 m x 2.2 m virtual segments (depending on pen size there were either 12 or 16 virtual segments per pen) and noting which segment the head of the focal animal was in at each 30-minute interval.

As a crude indication of eye irritation, the proportion of sheep within the 20 focal pens with one or both eyes more than half closed was scored. Using a scan sampling technique, each focal pen was scored daily during days 4-10 of the second voyage.

3.3.3 Statistical analysis

Spearman's correlation was used to examine whether there was any relationship between ammonia and ruminating, head position, feeding, lying and standing.

Chi square analysis was used to examine whether sheep behaviour (feeding, lying, standing, head position and ruminating) was influenced by voyage duration and whether the position of individual sheep within the pen was random.

Eye irritation was analysed with day of voyage used as a repeated measure to determine whether there was any variation of scores between days. Spearman's correlation was also used to examine whether there was any association between eye irritation and ammonia and air speed.

3.4 Experiment 4: Concentrations of potentially noxious gases at a pre-export assembly depot

3.4.1 Description of the assembly depot

The study was conducted between the 19th and 27th of November 2005 at a sheep pre-export assembly depot located approximately 60 km southeast of Perth, Western Australia. Samples were collected in eight holding sheds (approx. 100 m L x 20 m W x 5 m H) at the assembly depot. The walls of each shed were open at the top and lined with 1 m high galvanised iron at the base. All sheds had a roof made from galvanised iron and a floor constructed of 15 mm mesh. There was a 2 m gap between the mesh floor and the ground in order to allow faeces and urine to drop through the floor and accumulate beneath the shed. The accumulated faeces beneath the sheds were removed on a rotational basis every 2-3 months. This meant that sheds differed in the amount of faeces accumulated beneath them, with some sheds having very little faeces below them (i.e. there was a 2 m gap between the floor of the shed and the ground) while in others the accumulated faeces was flush with the floor in some sections of the shed.

Feed troughs ran down the centre of each shed while water troughs were built into the walls. Each shed was divided into eight pens; four either side of the feed trough.

At the time of the study there was considerable variation in the number of sheep housed in each shed. Some sheds were full (approx. 6000 sheep) while others had sheep in four to seven of the eight pens.

3.4.2 Procedure

Ammonia, carbon dioxide, hydrogen sulphide, wet and dry bulb temperature, dew point temperature, wind speed and distance between the mesh floor of the shed and the accumulated faeces (i.e. distance from faeces) were measured between 09.00 h and 12.00 h at 12 randomly selected sites in each shed on four separate days. Four sheds were sampled each day.

The abovementioned environmental variables were also mapped using longitudinal and latitudinal transects in four of the eight sheds. One shed was mapped each day between 14.00 h and 16.00 h. Each shed was mapped twice with the mapping occurring 4 days apart. Two longitudinal transects (one down the side of the shed and one down the middle of the shed) and five latitudinal transects (one across the side of each pen) were conducted with samples collected every 2 m. All measurements were collected at sheep head height (i.e. approx 60 cm from shed floor).

3.4.3 Equipment

Ammonia was measured using an OdaLog gas data logger (App-tek Australia, Queensland, Australia) (accuracy ± 5 ppm; resolution: 1 ppm). This instrument was factory calibrated 2 months before the study. For comparative purposes, a second set of ammonia readings were collected using a Neotox Mk Ammonia Meter (Nutech Australia, Western Australia, Australia) (accuracy ± 5 ppm; resolution: 1 ppm). Results for the sheds are presented from the OdaLog data.

Carbon dioxide was measured using a MultiRAE carbon dioxide meter (RAE Systems, California, USA) (drift: <5%; resolution: 10 ppm). The MultiRAE meter was factory calibrated 4 months prior to the study.

An OdaLog gas data logger (App-tek Australia, Queensland, Australia) (accuracy: ± 2 ppm; resolution: 0.1 ppm) was used to measure hydrogen sulphide. This instrument was factory calibrated 5 months before the study. All three instruments were fresh air calibrated (i.e. calibration of the meter in a zero ammonia environment) each day before use.

A Kestrel 4000 Pocket weather station (Nielsen Kellerman Australia, ACT, Australia). was used to measure wind speed (accuracy: ± 3%; resolution: 0.3 m/s), wet (accuracy: ± 2%; resolution: 0.1 ^{o}C) and dry bulb temperature (accuracy: ± 2%; resolution: 0.1 ^{o}C) and dew point temperature (accuracy: ± 2%; resolution: 0.1 ^{o}C).

3.4.4 Statistical analysis

Pearson's correlation was used to examine the relationship between the Neotox meter and the Odalog meter and the linear relationship was described using regression analysis.

Pearson's correlation was also used to analyse the relationship between vertical distance from shed floor and ammonia.

Forward and backward stepwise regressions were used to examine which variables (wind speed, wet bulb temperature, dry bulb temperature, dew point temperature and distance from faeces) were associated with ammonia concentration in order to evaluate the order of importance of variables.

Prior to conducting the stepwise regressions, variables were examined for colinearity. Variables were also screened for those that did not have an unconditional association with ammonia (Dohoo *et al.*, 2003). Two-way interactions between the remaining terms were included in the regression. Log 10 transformation of ammonia concentration was necessary to ensure the residuals from the regression analysis were normally distributed.

3.5 Experiment 5: The effect of atmospheric ammonia on the physiology and behaviour of cattle and sheep

3.5.1 Subjects and housing

Cattle – Seventy-five Brahman x Charolais steers, approximately 18 months of age and weighing (mean ± SEM) 413 ± 5 kg (full gut fill), were leased from a breeder in Rockhampton, QLD. Fifty steers were delivered to CSIRO Livestock Industries, Rockhampton, in late January, 2006 and the remaining 25 steers were delivered mid April, 2006. The Meat Standards Australia grading system (based on photographs of shape of head, sized of hump) was used to assess tropical breed content of each animal.

Before and after the experiment the steers were held in a 0.6 ha paddock and fed lucerne hay (offered at 2% of mean bodyweight) and had ad libitum access to water. Due to feral dog attacks in late January and again in early February (causing minor injuries to three of the steers), the steers were housed at night in covered yards with dog-proof fencing. After three incident-free months had passed, the steers were again held in the paddock at night time.

Sheep - One hundred and fifty merino-cross wethers, approximately 4 years old and weighing (mean ± SEM) 51 ± 1 kg (full gut fill), were purchased from a breeder in Tenterfield, NSW. All sheep were delivered to CSIRO Livestock Industries, Rockhampton, in late January.

Before and after the experiment the sheep were housed in a 300 m^2 pen and fed lucerne hay (offered at 2% of mean bodyweight) and had ad libitum access to water. The above-mentioned dog attacks led to three sheep being destroyed, 14 requiring veterinary treatment and leaving many others with minor puncture wounds. Following the first dog attack, chicken wire and an electric wire was placed around the pen. The sheep were provided with access to undercover pens during periods of heavy rain as their outdoor pen quickly became deep in mud.

3.5.2 Description of the climate chambers

Two climate chambers at CSIRO Livestock Industries, Rockhampton, were utilised for the experiment. These were designed to provide constant pre-programmed levels of lighting, oxygen, carbon dioxide and wet and dry bulb temperature. The latter four variables were recorded. Upper and lower set points for each variable were established and the levels could be kept within a set range. An ongoing fault with new ammonia sensors meant that atmospheric ammonia was not able to be monitored by the control system. Atmospheric ammonia was instead monitored twice daily at five locations within each chamber (two at cattle head height, two at sheep head height and one at

ground level) using a hand-held electrochemical meter (OdaLog gas data logger, App-tek Australia) and levels were controlled manually as required by the removal or addition of faeces, urine and urea, and by using manually controlled fresh air fans.

Each chamber (6.4 m W x 9.0 m L x 2.3 m H) had its own air conditioning unit located in a plant room adjacent to the second climate room. The unit servicing the first climate chamber comprised a 109.3 kW a/c unit using R22 gas plus 53.8 kW reheat capacity and air emitted through four vents. Chamber Two had a 68.8 kW a/c unit using R12 gas plus 22.8 kW reheat capacity and air emitted through two vents. Each chamber had two fresh air fans. Air was expelled from the chambers via three vents in chamber one and two vents in chamber two, while two vents in chamber one and one vent in chamber two took in air for recycling. The pen air turnover (PAT) in the first chamber was 468 m/h and in the second chamber 128 m/h. Pressurised water fed through humidifiers provided humidification. Six fluorescent tube fixtures lit each chamber.

The walls and roof of each climate chamber were constructed from insulated panelling, as used for refrigerated facilities. The concrete floor was sloped so that fluid ran into the grated draining system positioned at the centre of each chamber. Waste build-up within the drainage system was removed by manual flushing.

Within each climate chamber were four adjacent pens made from galvanised steel. Three sides of each pen were fixed, while the forth side was adjustable allowing specific pen sizes to be set. Bolts and gate brackets protruding into the pens were padded during the experiment to prevent injury to animals. A food trough and an automated water trough were fixed to the inside of each pen and made to be adjustable to two heights. The troughs occupied approximately 0.9 m^2 of the available pen space. Cattle were housed at a stocking density of 1.45 m^2/head equating to 1.8 x 2.4 m of available pen space for three steers. Sheep were housed at a stocking density of 0.315 m^2/head and this equated to 1.8 x 1.1 m of available pen space for six sheep. These stocking densities were determined using HS software, which based the calculation on a predicted PAT of the climate chambers, animal type and weight, and predicted mean wet and dry bulb temperature for the experiment.

3.5.3 Equipment

An Innotech GENII Modem Interface control system (Mass Electronics, Queensland, Australia) logged climatic data and controlled the climate chambers. Temperature and humidity were measured using a HMW61Y Vaisala humidity and temperature transmitter (Vaisala Oyj, Finland) (accuracy ± 2% R.H. and ± 0.1 ^oC respectively). Carbon dioxide was measured using a Vaisala GMT220 CO_2 transmitter (Vaisala Oyj, Finland; accuracy ± 20 ppm). Oxygen was measured using an AST Oxygen transmitter (Critical Environment Technologies, Canada; accuracy ± 0.2%).

Ammonia was measured during the experiment using an OdaLog gas data logger (App-tek Australia, Queensland, Australia; accuracy ± 5 ppm; resolution: 1 ppm). This instrument was factory calibrated 4 months before the study and again during the second month of the study.

3.5.4 Experimental Procedure

The experiment was conducted between the 25th of February and the 18th of May 2006. Seventy-two steers and 144 wethers were utilised for the study in a replicated design with six replicates each of

12 days. The total length of time that the animals were held for was: four (trials 3-6) 19 days and two (trials 1-2) 47 days. Sheep were shorn within 3 weeks of the commencement of their trial.

Twelve steers (four pens of three steers) and 24 wethers (four pens of six sheep) were tested in each replicate. Animals were marked on their rear and shoulders with large individual numbers using livestock-specific paint.

In order to simulate pre-export assembly depot conditions, sheep and cattle were held in separate undercover pens (cattle pen: 7 m x 12 m; sheep pen: 5 m x 12 m) during the 5 days before their entry into the climate chambers. During this period, the cattle were provided with ad libitum water and pellets. The pellets, made from sorghum (20 %), copra meal (9 %), chick pea offal (20 %) and millrun (40 %). The nutrient content was: 15 % fibre, 12 % protein, 18 % acid detergent fibre, 35 % neutral detergent fibre, 4 % undegradable fibre and 9.9 MJ/kg ME (DM). This conformed to nutritional specifications outlined in the Australian Standards for the Export of Livestock (ASEL: Department of Agriculture, Fisheries and Forestry 2006; see Appendix 2 for analysis of pellets).

Unlike the cattle, the sheep did not readily consume the pellets and were therefore gradually introduced to them over the 5 days in the undercover yards. Using a mixture of pellets, lucerne chaff and water, the sheep were fed at a chaff:pellet: ratio of 2:1 on day 1; 1:1 on day 2; and' 1:2 on day 3 and pellets on days 4 and 5. Pellets were crushed on days 1 and 2 to aid acceptance. The concrete floors of the undercover yards were hosed daily to remove faeces and urine.

On the day prior to the animals entering the climate chambers, two, 2 ml blood samples (one placed into an EDTA vacutainer and the other into an SST vacutainer) and one blood smear were collected via jugular venepuncture from eight steers and eight sheep (two animals from each of the eight pens into which they would later be divided). Cattle were restrained in a cattle crush for the procedure while sheep were restrained by hand. The blood samples and corresponding blood smears were analysed by the School of Veterinary Science (UQ) for plasma cortisol, blood urea and full blood count.

Prior to entering the climate chambers, all animals were weighed once, which for the cattle was when they first arrived at CSIRO and for the sheep it was following shearing (as followed in other experimental studies: Bruns *et al*., 2004, Sáncheza *et al*., 2006) and divided into four groups of three cattle and four groups of six sheep. Determination of the groups was based on an individual's weight when first weighed so that each group of cattle weighed 1239 ± 11 kg and each group of sheep 306 ± 3 kg. In each replicate, two groups of cattle and two groups of sheep were randomly assigned to the four pens within each climate chamber. Animals were moved into the climate chambers at midday and provided with ad libitum access to water and feed (pellets). The animals were left undisturbed for the remainder of the day to provide them with an opportunity to become accustomed to the chambers.

For the next 12 days, the animals were exposed to one of four ammonia treatments: C-control, with animals exposed to <8 ppm atmospheric ammonia; L – Low, in which animals were exposed to approximately 15 ppm atmospheric ammonia; M – Medium, in which animals were exposed to approximately 30 ppm atmospheric ammonia; H – High, in which animals were exposed to approximately 45 ppm atmospheric ammonia (actual ammonia levels are shown in Appendix 3). A concentration of 45 ppm represents the highest mean level recorded in a single pen on the first voyage on a well-ventilated ship in Experiment 1 and that could be legally entered according to

National Occupational Health and Safety guidelines (NOHSC:1003 1995). The replicate schedule was designed so that all pair-wise combinations of ammonia treatments were tested, as shown in Table 4.

In the Control treatment ammonia levels were minimised by hosing the pen floors three times a day. In the 15, 30 and 45 ppm treatments, the faeces and urine were allowed to accumulate and, where necessary, further control of the ammonia levels was achieved through the occasional hosing of the pen floor, the addition of urea to manure (in a separate section of the chamber floor, inaccessible to animals) and by manual manipulation of the fresh air fans.

Table 4: Sampling schedule for the six trials

Trial	Chamber 1	Chamber 2
1	L	C
2	M	L
3	H	C
4	M	H
5	H	L
6	C	M

The wet and dry bulb temperature conditions within the climate rooms were based on shipboard levels recorded on four voyages: two voyages during Stage 1A from Fremantle to the Middle East and one voyage from Darwin to the Middle East during high summer. As shown in Table 5, wet bulb temperature was adjusted once daily at 05.00 h while dry bulb temperature was adjusted twice daily at 05.00 h and again at 17.00 h to take into account the daily fluctuations in the shipboard data.

Table 5: Wet and dry bulb settings within the climate rooms during days 1-12 of each trial

Day of Trial	Wet bulb temperature at 05.00 h (^{o}C)	Dry bulb temperature at 05.00 h (^{o}C)	Dry bulb temperature at 17.00 h (^{o}C)
entry	23	26	25
1	23	26	25
2	24	27	26
3	25	28	27
4	25	29	28
5	26	30	29
6	26	31	30
7	27	31	30
8	27	31	30
9	27	32	31
10	28	33	32
11	28	34	33
12	29	35	34

The climate rooms were lit 24 h/day in order to simulate shipboard lighting conditions.

Behavioural data were collected twice daily between 05.00-06.15 h and between 17.00-18.15 h. Prior to data collection, the observer would enter the climate chamber and sit quietly for 10 minutes in order to allow the animals to adjust to the observer's presence. During each sampling session, each pen was observed in random order for 15 minutes using a continuous sampling technique. Using The Observer® v5.0 software (Noldus Information Technology, Wageningen, Netherlands) on handheld iPaq computers, the behaviours scored were standing, lying, ruminating, feeding, drinking, coughing, teeth grinding, pawing, foot stomping, head butting, self-licking, scratching, panting and

the vertical position of the head. Mounting, biting, swaying, pacing, vocalising and sneezing were also scored, but not reported on as they were rarely observed.

The presence of nasal secretion, lacrimation and any skin breaks were noted at each sampling session. The measurement of lacrimation by scoring the darkened area below the eye proved inaccurate and results are not reported.

Food and water consumption by each pen was measured once daily at 06.30 h. Food troughs were removed temporarily for weighing, while water was measured using a flow meter positioned in the pipe. The flow meters were calibrated in July 2006 and the readings were adjusted accordingly.

After 12 full days in the climate chambers, the animals were removed from the chambers one pen at a time and weighed. A second set of two, 2-ml blood samples were collected from the two animals in each pen from which pre-experiment blood samples were collected. Those cattle from which blood samples were collected then had a bronchial alveolar lavage (BAL) conducted on them (see Appendix 4 for methodology). Similarly, the sheep from which blood samples were collected had a Transtracheal Aspiration (TA) conducted on them (see Appendix 5 for methodology). Smears were made of all blood, BAL and TA samples. Following these procedures all animals were returned to their respective paddock or outside pen and placed on a lucerne hay diet.

Those animals in Replicates Two and Three that underwent physiological sampling were recaptured 28 days later. These animals were weighed and had a third set of blood samples and a second set of BAL (cattle only) and TA (sheep only) samples collected. These samples were used in a pilot study examining recovery in livestock following exposure to atmospheric ammonia.

3.5.5 Statistical analysis

Before analysis, all data were checked for equal variance using a Levene's test and normal distribution using an Anderson-Darling test. For those data not satisfying the Levene's test, transformations were made in order to achieve equal variance. Coughing data were not normally distributed and were therefore analysed using nonparametric tests (Mood's Median test and Mann-Whitney test).

Day 13, the day on which the animals were removed from the chamber, and Day 41 post-experiment data for haematology, BAL, TA and live weight were analysed using a General Linear Model (GLM) to test for an effect of ammonia treatment, trial, and chamber. Where no significant effects of ammonia were found, the data were collapsed and a paired *t*-test was used to determine whether there were any overall differences between pre-experiment, Day 13 post-experiment and Day 41 post-experiment levels.

The qualitative macrophage activity data from the BAL and TA procedures were analysed using Chi square analysis.

All behaviour data were collapsed to pen level and, along with data for food and water intake, analysed using a GLM to test for an effect of ammonia treatment, day of experiment, trial, pen and chamber. Tukey's simultaneous test was used for post-hoc analysis of effects of ammonia.

3.6 Experiment 6. The avoidance of ammonia by sheep.

3.6.1 Subjects and experimental apparatus

Fourteen merino cross lambs (three ewes and 11 wethers), approximately 10 months old and weighing (mean ± SEM) 33 ± 1 kg, were purchased from a breeder in Dalby, Queensland, Australia. They were used for a split plot experimental design, with prior exposure to ammonia in one half of the sheep, simulating a ship journey, followed by testing their willingness to enter chambers filled with ammonia or just fresh air to obtain a food reward. During training, the lambs were housed in a 12 m^2 covered pen at the Gatton Campus of the University of Queensland and fed, during training and testing, ad libitum wheaten chaff and provided with water.

3.6.2 Simulated ship journey

Two metabolic rooms within the animal house facilities of the Gatton Campus were used to house the sheep during their exposure to ammonia. The 6 m^2 pens within each room were raised so that faecal material and urine fell through the slatted floor boards into collection trays beneath the pen. The remaining waste was removed during daily cleaning. There were similar dry bulb (26.7 ± 1.2^oC) and wet bulb (20.1 ± 0.9^oC) temperatures in both metabolic rooms.

3.6.3 Apparatus for testing their response to ammonia

It was planned to initially train the subjects to enter a Y-maze and to associate different sized stimuli (circles) located outside the Y-maze to five sized food rewards located within the maze, and later to associate different coloured stimuli located outside the Y-maze to two ammonia concentrations (0 and 45 ppm) located within the maze. However, after four weeks of training none of the sheep were able to discriminate between the different sized stimuli and the experimental procedure was altered.

The experimental apparatus for the new procedure, built in a semi-enclosed shed with a concrete floor, was a modified version of the Y-maze. The experimental apparatus consisted of a 0.9 m^2 holding cage, covered in black plastic and opening into a 1 m long foyer beyond which was a 6 m long raceway enclosed in transparent plastic (Figure 1). The enclosed race and the holding cage were connected by a passageway, enabling the sheep to complete a circuit and return to the holding cage. Transparent plastic vertical curtains covered the entrance and exit to the enclosed raceway, which meant that the enclosed area could be filled with ammonia gas, released from containers of clouded ammonia and containers of cattle faeces mixed with urea. A food bowl containing sultanas was positioned 1 m from the exit of the enclosed raceway. A lockable mesh gate, which opened outwards and upwards, was attached to the exit of the holding cage to restrain the sheep. The lock and the gate were operated by the experimenter, out of sight of the sheep in the cage. Attached at a height of 0.3 m to the inside of the holding cage exit gate was a wooden lever that could be pressed onto a screw attached to the cage, thereby making an audible click when pressed by the sheep.

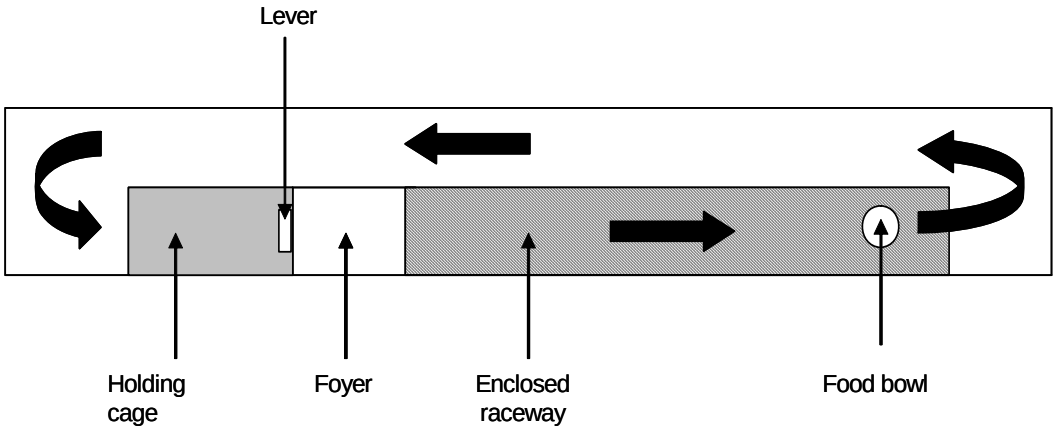


Figure 1: Experimental apparatus. Sheep were trained to press the lever to exit the holding cage and enter an enclosed raceway, filled either with fresh air or ammonia gas, to feed on a reward of sultanas. A connecting raceway allowed the sheep to return to the holding cage to complete the circuit.

3.6.4 Training and testing procedure

The first stage of training was conducted in a pen nearby to that in which they were housed to allow the sheep to maintain visual contact with one another. The sheep were initially trained once per day over a 12 day period to press the lever, attached to the side of the training pen, an increasing number of times in order to receive a food reward (eight sultanas, determined so as to minimise any risk of gastrointestinal disturbance). Starting at one and finishing with nine presses, the number of times the lever had to be pressed in order to receive the sultanas increased by one after every second successful sequence of pressing (i.e. the sequence was 1, 1, 2, 2, 3, 3, ….9, 9). Repeated testing at the same lever pressing schedule was chosen so that the subjects had their successful responses reinforced. Subjects were considered to have successfully completed the training process when they had completed the sequence of pressing on three consecutive days. Twelve of the 14 sheep passed the initial training.

The second stage of the training was conducted in the experimental apparatus. Here the sheep were required to press the lever, now mounted on the mesh door of the holding pen, in order to exit the pen and enter the enclosed raceway to access the sultanas. If the sheep did not leave the enclosed raceway within 30 sec. an experimenter drove the sheep from it and back into the holding pen. Fresh sultanas were replaced after the sheep exited the raceway. The sheep were already familiar with the experimental apparatus, having previously been trained in the Y-maze for 2 weeks during our earlier unsuccessful attempts at training. Consequently, the second stage of training took just 6 days for the 12 sheep to be able to successfully press the lever in the required sequence for three consecutive days. There was no ammonia in the enclosed runway during training.

All 14 sheep were then weighed and semi-randomly split into two groups of seven (with one unsuccessfully trained sheep and six successfully trained sheep in each group) and rehoused in the two metabolic rooms. Ammonia was measured once daily at 05.00 h using the Neotox Mk Ammonia Meter (Nutech Australia, Western Australia, Australia) (accuracy ±5 ppm; resolution: 1 ppm).

One group was exposed to 45 ppm atmospheric ammonia for 9 days and the other acted as a control by being exposed to < 2 ppm ammonia for the same amount of time. A concentration of 45 ppm was achieved by mixing urea with cattle manure in the collection trays underneath the sheep pen and controlled via manipulation of the air flow into the room. The collection trays in the control were cleaned daily and external doors and windows were left open to allow fresh air into the room.

Following the 9 days in the metabolic rooms, the sheep were tested once daily for 6 days in the experimental apparatus. Half the trained sheep (three previously exposed to 45 ppm ammonia and three previously exposed to < 2 ppm) were tested when there was 43 ± 4 ppm ammonia in the enclosed raceway of the experimental apparatus and the other half when there was 0 ppm ammonia in the experimental apparatus. The concentration of ammonia in which the sheep were tested was rotated daily so that each sheep was tested three times in 0 ppm and three times in 45 ppm. During testing, each sheep was required to press the lever to exit the holding cage and enter the enclosed runway to gain access to the food reward. Starting with one press, the number of times the lever had to be pressed in order to gain access to the reward increased by one after each successful circuit. As during their training, the sheep had 30 sec to exit the enclosed runway before being driven out by the experimenter. The process continued until one of two end points was achieved: 1) 1 minute had passed without the sheep pressing the lever in the holding cage, or 2) the sheep had passed through the enclosed raceway five consecutive times and not consumed the sultanas. Once an end point was achieved, the subject was deemed to have lost motivation to feed on sultanas and was returned to its metabolic room. Both the maximum number of lever presses (the breakpoint) and the amount of time spent in the enclosed runway were recorded. On the last day of testing, all 14 sheep were reweighed and a Schirmer Tear Test (Schering Plough Animal Health, New Jersey, USA) was used to test the rate of tear production over a 1-minute period as an indicator of lacrimation.

3.6.5 Equipment

Ammonia was measured during the experiment using an OdaLog gas data logger (App-tek Australia, Queensland, Australia) (accuracy ± 5 ppm; resolution: 1 ppm). This instrument was factory calibrated 1 month prior to the commencement of the study.

3.6.6 Statistical analysis

A split plot analysis of variance was conducted using the statistical package Minitab (1995), with prior exposure to ammonia (the simulated ship journey) as one main factor and current exposure to ammonia (the preference test) as the second. Before analysis, all breakpoint and time in the enclosed raceway data were checked for normal distribution using an Anderson-Darling test. Time in chamber data were normally distributed, but the breakpoint responses were not. These were therefore transformed by $\log_{10} + 1$, after which they approximated a normal distribution. The model was a mixed hierarchical model with subjects nested within prior exposure, and repeated observations nested within each subject-current exposure combination.

The linear model describing the design was:

$$x_{ijkl} = \mu + \beta_j + \varepsilon''_{ij} + \gamma_k + (\beta\gamma)_{jk} + \varepsilon'_{ijk} + \Delta_l + (\beta\Delta)_{jl} + (\gamma\Delta)_{kl} + (\beta\gamma\Delta)_{jkl} + \varepsilon_{ijkl}$$

where:

x_{ijkl} = the observed value for subject *i* with prior exposure *j* and current exposure k during period l.
μ = the overall mean
β_j = the effects of prior exposure
ε''_{ij} = the random error associated with subjects within prior exposure treatments
γ_k = the effects of current exposure
ε'_{ijk} = the random error associated with different trials within each subject
Δ_l = the effects of period
ε_{ijkl} = the random error associated with repeated observations

Interactions between factors are represented by combinations of factors in brackets.

Two-sample, two-tailed *t*-tests were used to compare differences between the control and ammonia-exposed group in lacrimation and the time spent in the ammonia treatment. Weight change was analysed using a single sample, two tailed *t*-test.

4 Results

4.1 Experiment 1: Atmospheric concentrations of ammonia, hydrogen sulphide and carbon dioxide

4.1.1 Correlations between readings from ammonia meters and tubes

A Pearson's correlation was conducted between the readings from the Neotox meter and the Gastec tubes and a significant linear correlation was found ($r^2 = 0.67$, $p = 0.001$). A regression analysis was used to describe the relationship between the Neotox meter and the Gastec tubes [$NH_{3Gastec\ tubes} = -6.5(\pm 1.28) + 0.86(\pm 0.04)\ NH_{3Neotox\ meter}$, where NH_3 is ammonia concentration measured in ppm] and it was discovered that the readings from the Gastec tubes were lower than those from the Neotox meter (Figure 2). According to the supplier of the Gastec tubes, the tubes are the more accurate of the two instruments (Lou Farro, App-Tek Safety Pty Ltd, personal communication) and, as such, are presented here.

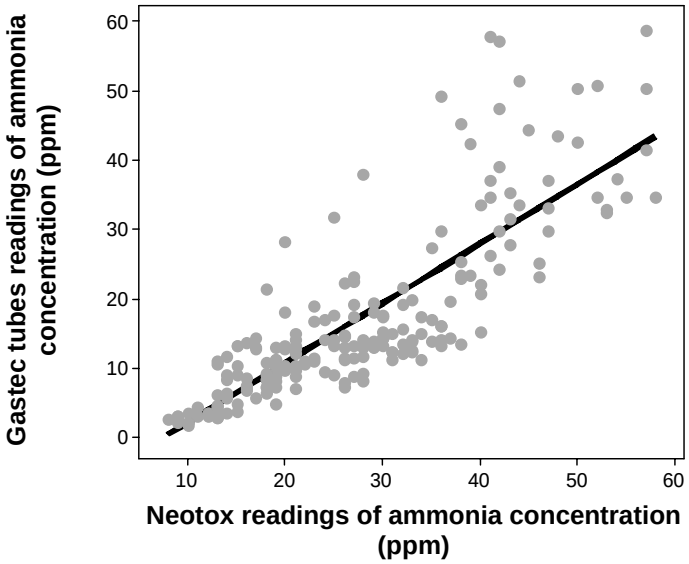


Figure 2: Correlation between the Gastec tubes and the Neotox meter on the first voyage.

A strong linear correlation was found between the between the Neotox meter and the Qrae plus meter ($r^2 = 0.79$, $p = 0.001$) (Figure 3). Regression analysis revealed the readings from the QRae plus meter were higher than those from the Neotox meter [$NH_{3QRae\ plus\ meter} = 6.653(\pm 1.31) + 0.8339(\pm 0.05)\ NH_{3Neotox\ meter}$].

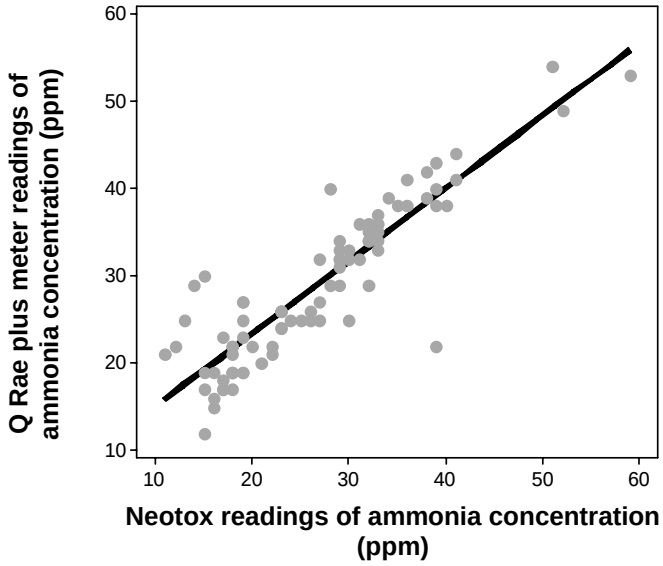


Figure 3: Correlation between the QRae plus meter and the Neotox meter on the second voyage.

There was also a strong linear correlation between the Neotox meter and the Qrae tubes (r^2 = 0.59, p = 0.001) (Figure 4). The QRae tubes were found to provide lower ammonia readings than the Neotox meter [$NH_{3Qrae\ tubes}$ = 0.285(± 1.27) + 0.7553(± 0.06) $NH_{3Neotox\ meter}$]. Given the early failure of the QRae plus meter and the limited number of QRae tubes, the voyage two ammonia data are based on readings from the Neotox (which provided readings less than the QRae plus meter, but greater than the QRae tubes).

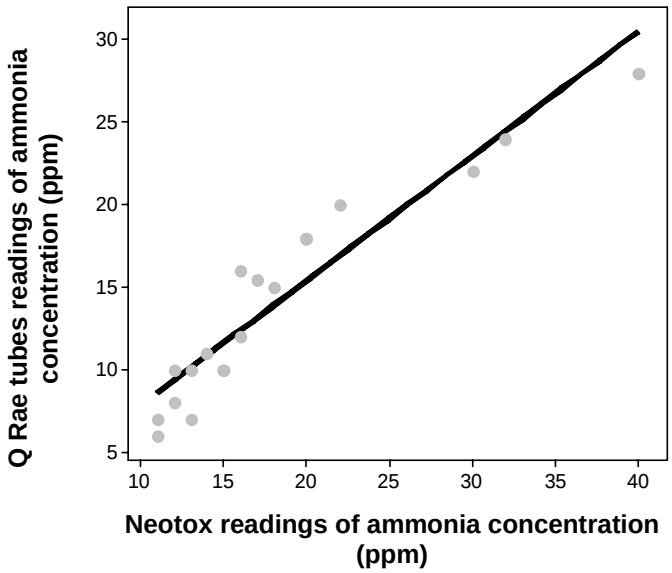


Figure 4: Correlation between the QRae tubes and the Neotox meter on the second voyage.

4.1.2 Atmospheric ammonia concentration - Voyage one

Seven of the 10 paired sites where high ammonia concentrations were predicted had a higher concentration than the corresponding predicted low ammonia sites (two sample *t*-test: *t* values ranged from 2.11-4.83, *p* values from 0.001-0.05) (Figure 5). Of the four paired closed deck study sites, two had a mean ammonia concentration for the voyage above the short-term exposure limit (STEL) for humans (35 ppm; NOHSC, 1995) and, on 3 days, above the maximum concentration (50 ppm) that humans are permitted to enter. At two other closed deck sites, the mean ammonia concentration for the voyage was above 25 ppm; the recommended maximum limit for live export by Costa *et al* (2003) and the time weighted average (TWA) limit that humans are permitted to be exposed to in a 40-h week. The remaining four closed deck sites had a mean ammonia concentration within the safe exposure limit for humans (<25 ppm). The minimum ammonia concentration measured on a closed deck was 8.9 ppm and the maximum 58.8 ppm.

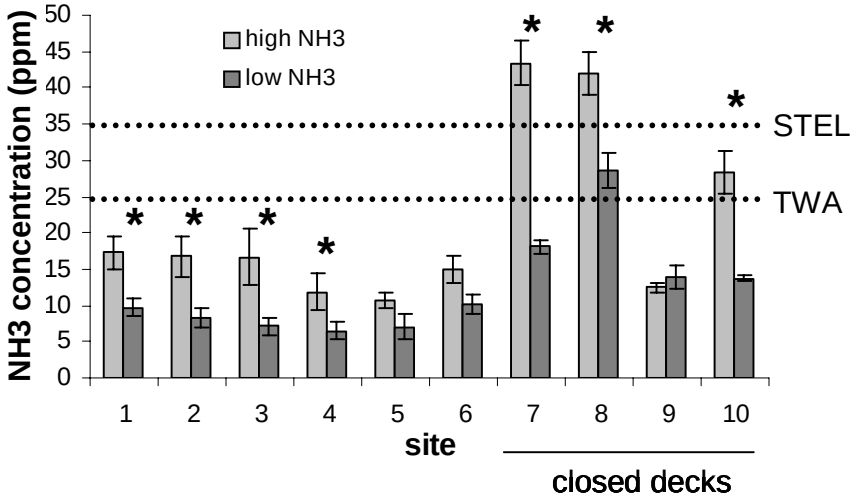


Figure 5: Ammonia concentration. Mean (± SEM) concentration of ammonia for the first voyage at 10 sites where high ammonia was predicted (light grey columns: n =11) and 10 sites with predicted low ammonia (dark grey columns: n = 11) are plotted. Sites 1-6 were located on open decks and sites 7-10 on closed decks. An asterisk indicates a difference ($p < 0.05$) between high and low ammonia sites. STEL = Short-term exposure limit. TWA = Time weighted average.

All six paired open deck study sites had a mean ammonia concentration for the voyage below 18 ppm; well below the TWA exposure limit for humans. However, it should be noted that on seven occasions ammonia levels at certain open deck sites exceeded the TWA limit of 25 ppm by as much as 19.5 ppm. Ammonia concentrations on the open decks ranged between 1.2 and 44.5 ppm.

There was a main effect of deck type (ANOVA: $F_{1,189}$ = 81.19; p = 0.001), the ammonia concentration on the closed decks was higher than on the open (Figure 6). However, there was no main effect of days of voyage ($F_{1,189}$ = 0.01; p = 0.93): i.e. ammonia concentrations did not change over the course of the voyage. No interaction was found between the factors deck type (open vs closed) and days of voyage (deck type vs days of voyage: $F_{1,188}$ = 2.18; p = 0.14).

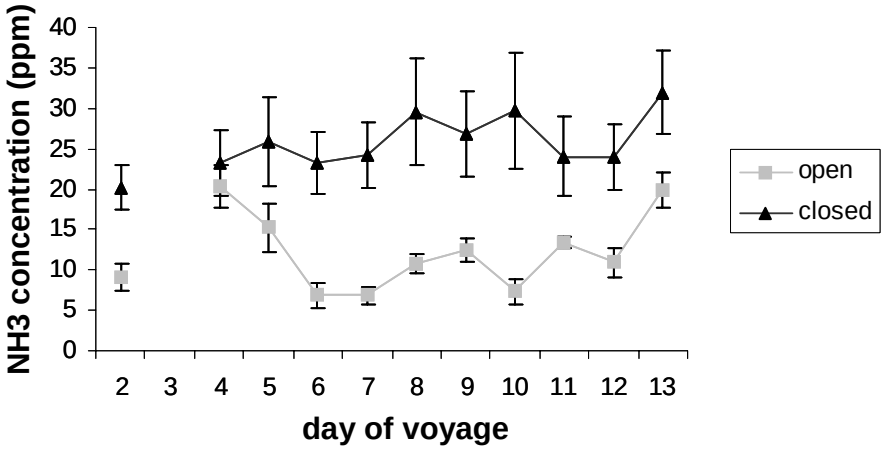


Figure 6: Ammonia concentration over voyage one. Mean (± SEM) concentration of ammonia on the open decks (grey square: n = 12) and on the closed decks (black triangle: n = 8) over the course of the voyage. No ammonia data were collected on day 3.

4.1.3 Mapping of shipboard ammonia - Voyage two

Transects of two open decks and two closed decks revealed differences both within and between decks (Figure 7). On deck nine (open), high ammonia concentrations were found at the front on the ship and near the engine block. A similar but weaker pattern to that on deck nine was found on deck six (open), i.e. higher ammonia levels at the front of the ship and near the engine block. Both locations represent areas where air movement may be disrupted by walls. There were no clear patterns in the transects conducted along the breadth of the open deck. In some instances ammonia levels decreased towards the edge of the ship (e.g. deck nine fore), while in others, ammonia concentrations increased near the edge of the ship (e.g. deck nine aft). In one instance (deck six fore) ammonia levels were higher on the port side of the ship than on the starboard side.

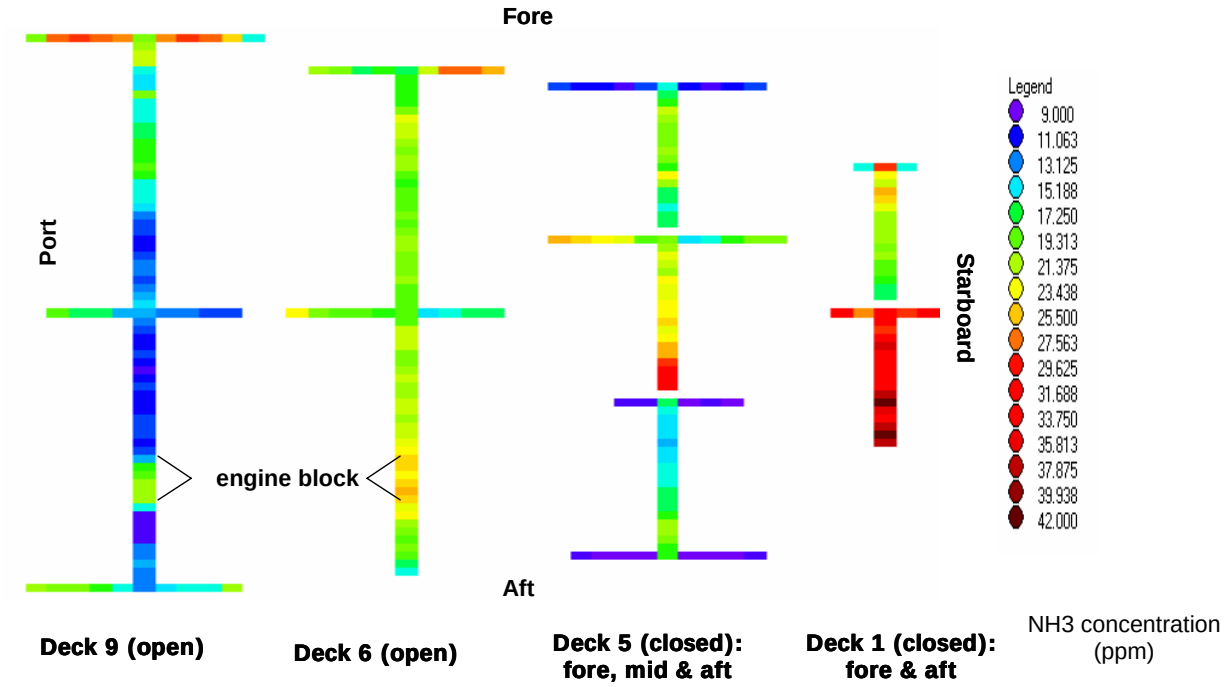


Figure 7: Mapping of ammonia on four decks. Transects along the length and breadth of two open decks (nine and six) and two closed decks (five and one). Deck five is divided into three discrete subsections and deck one into two discrete subsections.

In the fore and aft subsections of deck five and the aft section of deck one there were no clear patterns of ammonia concentrations, with levels relatively uniform across each subsection. By contrast in the middle subsection of deck five there was a tendency for ammonia concentration to increase towards the rear, while in the fore subsection of deck one, ammonia levels increased towards the front. The concentration of ammonia in deck one aft was almost double that of the other subsections.

Just as there was variation in ammonia concentration between and across each deck, ammonia levels also varied considerably within some pens (Figure 8). The mean (±SEM) range (maximum – minimum concentration) in ammonia concentration within a pen was 12.6 ± 2.4 ppm. The minimum range in the ammonia concentration within a pen was 4 ppm while the maximum range was 37 ppm. Changes in the concentration can happen over a short distance; in one instance the level of ammonia increased by 29 ppm (deck six, row 1, pen 1-4) in the distance of 2.2 m. These readings indicate that pockets of ammonia (hotspots) do occur and that they can be localised. Moreover, preliminary evidence suggests that predicting where these hotspots may occur could prove difficult. Of the 20 pens that were re-sampled a second time, in five pens the section of the pen that had previously had the highest concentration of ammonia then had the lowest.
In three other pens there was no longer a difference in ammonia levels between the site that had previously had the highest concentrations of ammonia and the site which had the lowest.

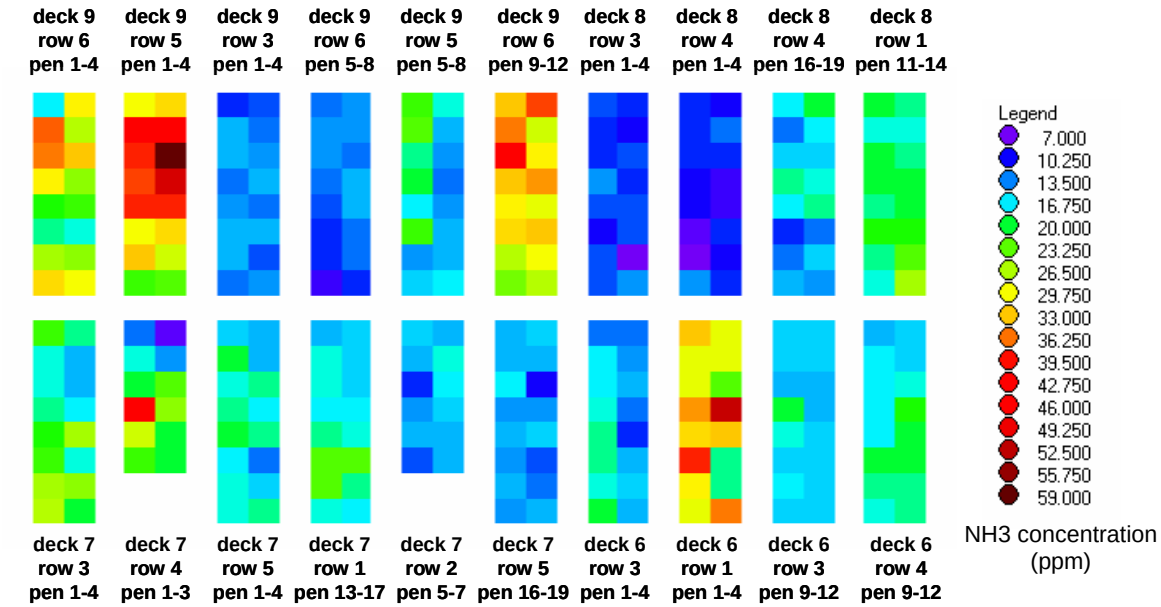


Figure 8: Mapping of ammonia within 20 pens. Ammonia concentrations were mapped within 20 open decks at 2.2 m intervals. Ammonia was relatively stable within 15 of the 20 pens mapped.

4.1.4 Ammonia concentration vs vertical distance from faecal pad - Voyage two

There was a weak significant cubic relationship (r^2 = 0.025, p = 0.006: square NH_3 = 7.23 – 0.02_{height}+ $7.4E^{-05}{}_{height}$ – $1.0E^{-07}{}_{height}$) between ammonia concentration and height from faecal pad; i.e. ammonia concentration decreased with vertical distance from the faecal pad (Figure 9).

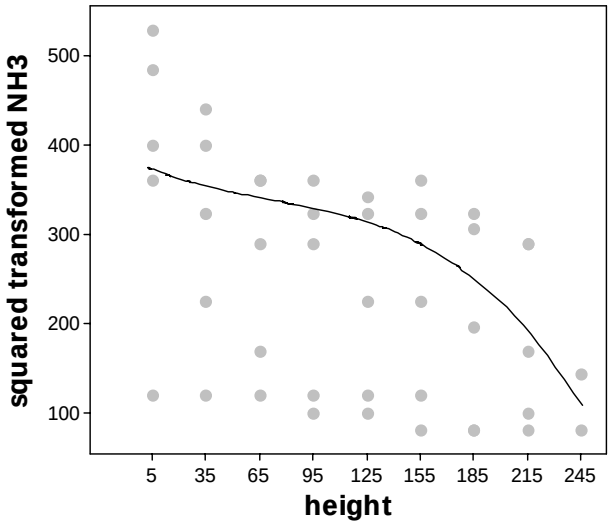


Figure 9: Correlation between ammonia concentration and vertical distance from faecal pad. Correlation between ammonia concentration (on the Y-axis) and vertical distance from faecal pad (on the X-axis) on the second voyage.

4.1.5 Atmospheric hydrogen sulphide concentration - Voyage one

The concentration of hydrogen sulphide at 20 study sites is shown in Figure 10. At all 20 sites, the mean hydrogen sulphide concentration for the voyage was below the TWA limit of 10 ppm that humans are permitted to be exposed to in a 40-hour week (NOHSC, 1995). At eight sites hydrogen sulphide was not detected at all. The maximum concentration of hydrogen sulphide detected was 1.8 ppm.

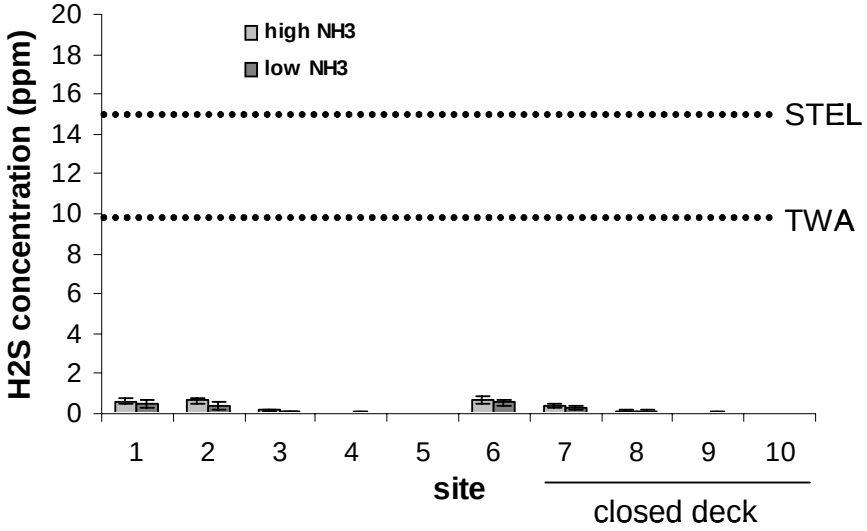


Figure 10: Hydrogen sulphide concentration. Mean (± SEM) concentration of hydrogen sulphide (H_2S) for voyage one at 10 sites where high ammonia was predicted (light grey columns: n =12) and 10 sites with predicted low ammonia (dark grey columns: n = 12) are plotted. Sites 1-6 were located on open decks and sites 7-10 on closed decks. STEL = Short-term exposure limit. TWA = Time weighted average.

Despite overall low concentrations of hydrogen sulphide, gaseous concentrations increased over the course of the voyage (Figure 11). Analysed using a repeated measures ANOVA, with day as a repeated measure, the change in hydrogen sulphide concentration with time was found to be significant ($F_{11,197} = 5.12$, $p = 0.001$).

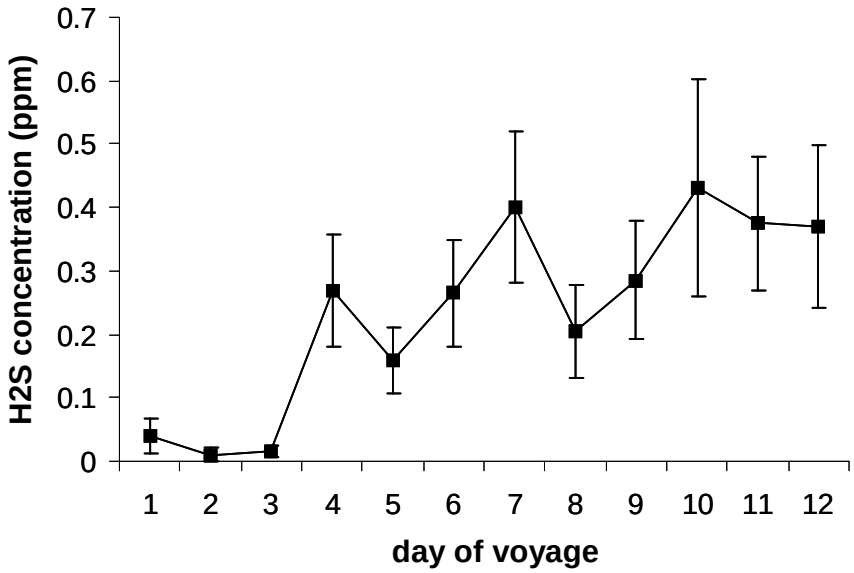


Figure 11: Hydrogen sulphide concentration over voyage one. Mean (± SEM) concentration of hydrogen sulphide (H_2S) over the 12 days of the voyage are plotted.

4.1.6 Mapping of shipboard hydrogen sulphide - Voyage two

Hydrogen sulphide was mapped in 20 pens during voyage 2 (Figure 12), but was not detected in seven of these. In most pens where hydrogen sulphide was present, the concentration was relatively constant throughout the pen. The greatest range (highest – lowest) in hydrogen sulphide concentrations in any one pen was 0.9 ppm (deck nine, row 6, pen 1-4). The mean (±SEM) range in hydrogen sulphide concentration within the 20 pens was 0.2 ± 0.1 ppm.

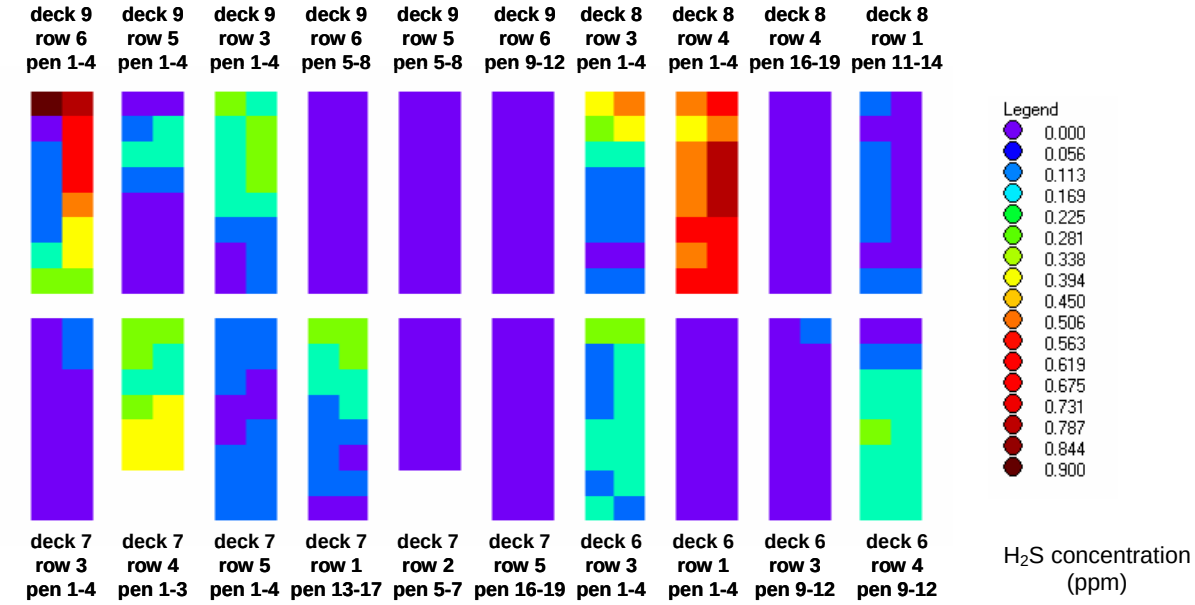


Figure 12: Mapping of hydrogen sulphide within 20 pens. Hydrogen sulphide concentrations were mapped within 20 open decks at 2.2 m intervals.

4.1.7 Mapping of shipboard carbon dioxide - Voyage two

Carbon dioxide was mapped on two open and two closed decks (Figure 13) and within 20 pens (Figure 14) during voyage two. The highest concentration of carbon dioxide recorded was 1890 ppm, well below the human TWA limit of 12500 ppm. The concentration of carbon dioxide varied between decks with higher concentrations on deck six and, to a lesser extent, deck five compared to decks nine and one. On deck nine, carbon dioxide levels were higher at the front of the vessel and on the port side. A peak in carbon dioxide also occurred near the engine block. Carbon dioxide levels were relatively high across deck six, although there was a decrease in levels towards the ship aft. The concentration of carbon dioxide was relatively constant across deck five, while there was a slight increasing gradient towards the front of deck one.

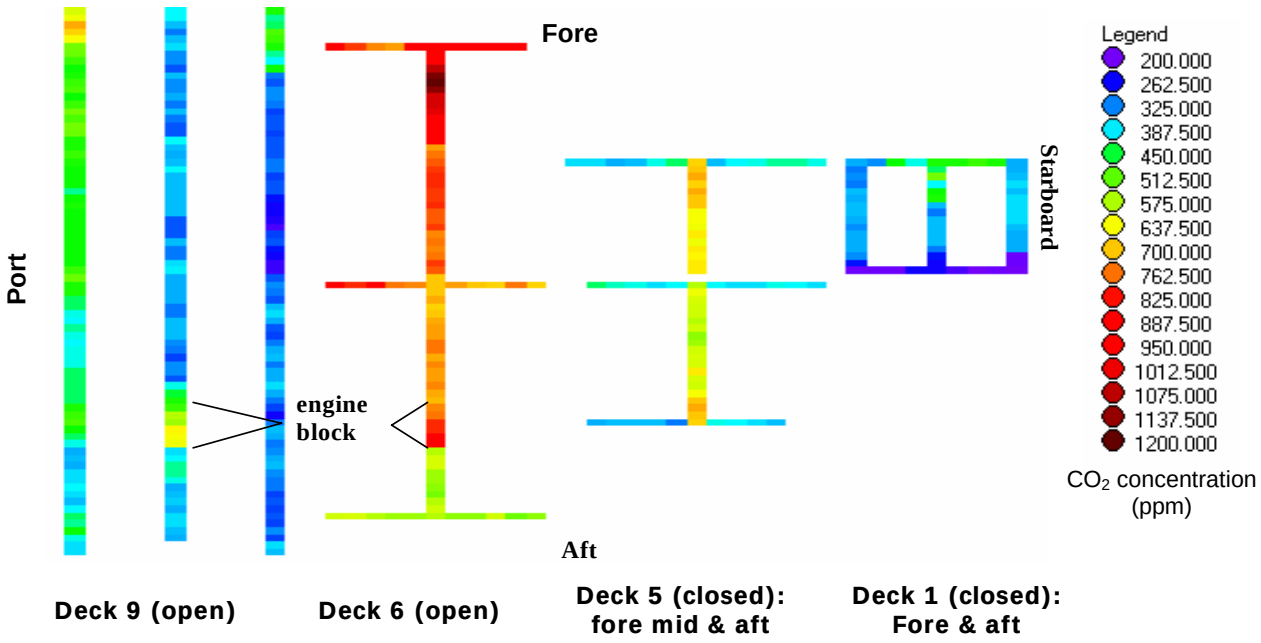


Figure 13 Mapping of carbon dioxide on four decks. Transects were run along the length and breadth of two open decks (nine and six) and two closed decks (five and one). Deck five is divided into three discrete subsections and deck one into two subsections.

The mean (±SEM) range in carbon dioxide concentration within the 20 mapped pens was 250 ± 53 ppm. Of the 20 pens mapped, only three pens had pockets of relatively high carbon dioxide. The remaining 17 pens mapped had relatively constant levels of carbon dioxide throughout the pen.

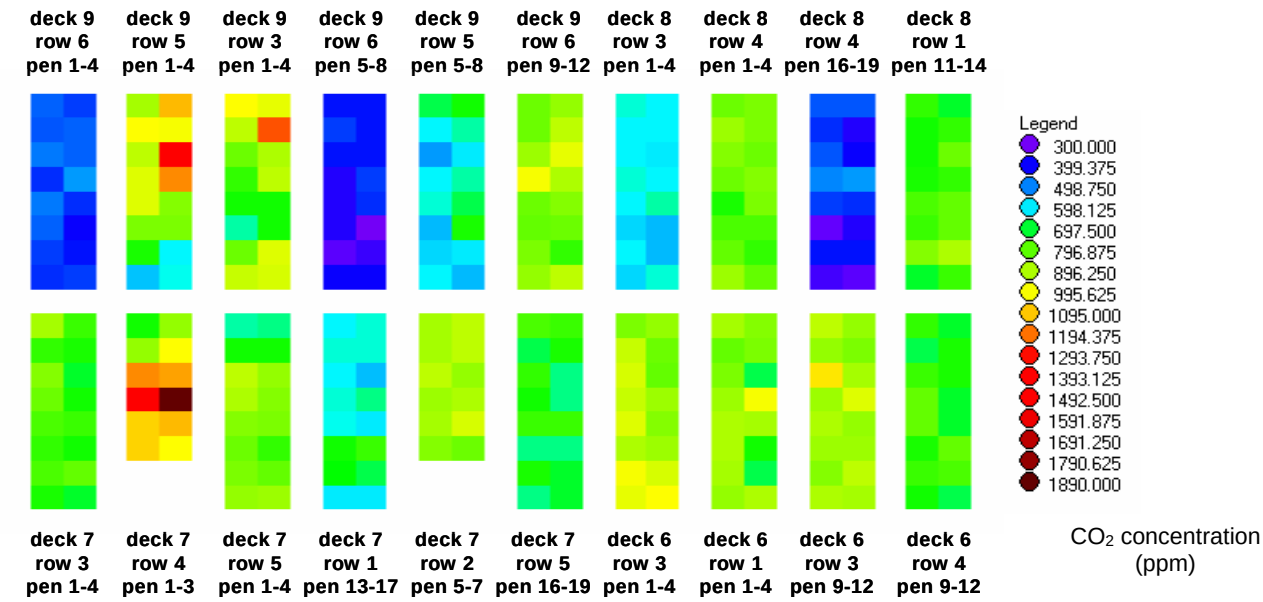


Figure 14: Mapping of carbon dioxide within 20 pens. Carbon dioxide concentrations were mapped within 20 open deck pens at 2.2 m intervals.

4.1.8 Carbon dioxide concentration vs vertical distance from faecal pad - Voyage two

There was no correlation (r^2 = 0.0000003, p = 0.64) between carbon dioxide concentration and height from faecal pad; i.e. carbon dioxide concentration was not associated with vertical distance from the faecal pad (Figure 15).

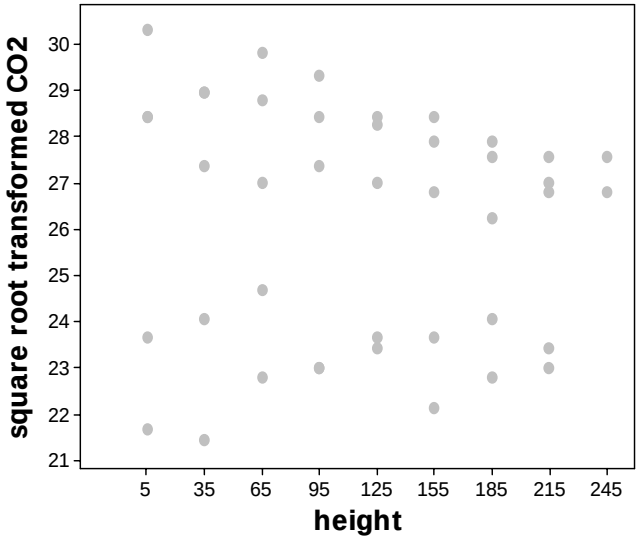


Figure 15: Correlation between carbon dioxide and vertical distance from faecal pad. Correlation between carbon dioxide concentration (on the Y-axis) and vertical distance from faecal pad (on the X-axis) on the second voyage.

4.2 Experiment 2: Factors affecting ammonia concentrations on a live export vessel

4.2.1 Air speed in pens

For voyage one, no interaction was found between the factors deck type (open vs closed) and days of sampling (deck type vs days of sampling: ANOVA: $F_{1,277} = 0.13$; $p = 0.72$). There was also no main effect of deck type ($F_{1,228} = 0.02$; $p = 0.89$) or days of sampling ($F_{1,228} = 0.11$; $p = 0.74$): i.e. there was no change in air speed over the course of the voyage nor was there a difference in air speed between the open and closed decks (Figure 16).

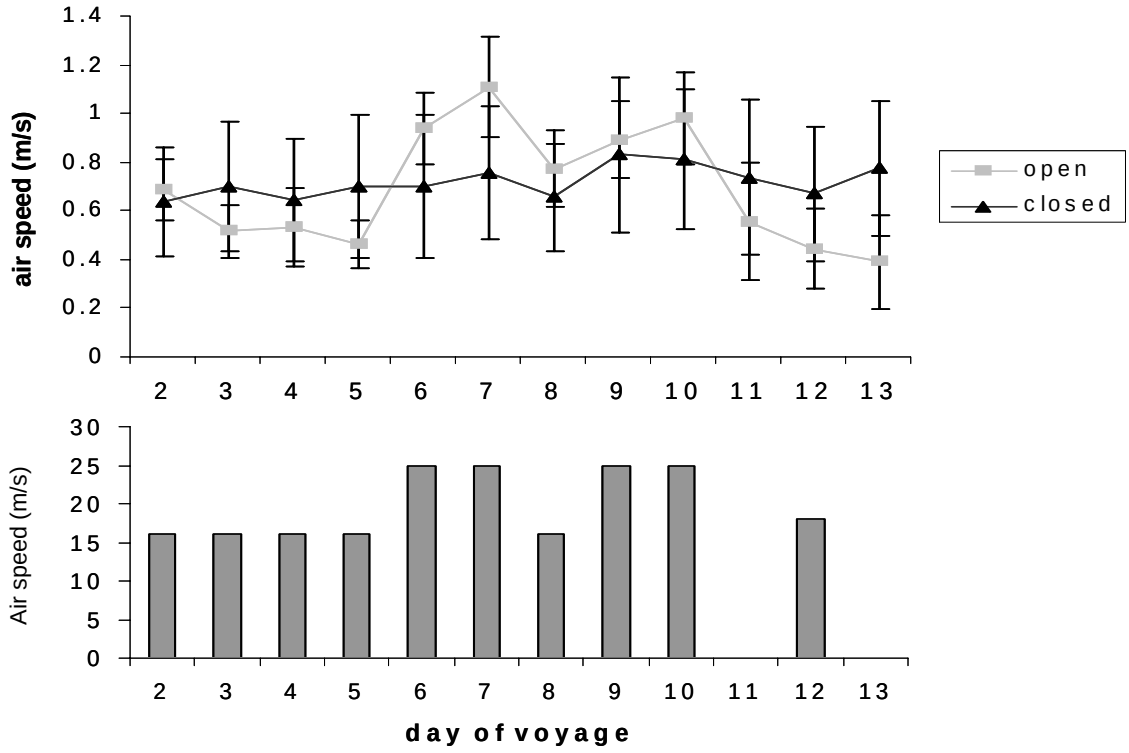


Figure 16: Voyage 1 air and wind speed. Upper graph. Air speed. Lower graph Wind speed A. Mean (± SEM) air speed on the open decks (grey square: n = 12) and on the closed decks (black triangle: n = 8), and B. cumulative daily wind speed as measured on the ship bridge, over days 2-13 of the voyage (ship was in port at Muscat on day 11 and Kuwait on day 13 of the voyage).

A Pearson's correlation was used to examine whether there was any relationship between daily air speed on the open decks and the cumulative wind, as measured by the ship. Air speed correlated strongly and positively ($r^2 = 0.72$, $p = 0.002$) to cumulative wind speed. The cumulative wind speed for the first and second voyage is shown in Table 6, and tended on both occasions to increase with voyage duration.

During the second voyage, air speed was mapped in 20 pens. As Figure 17 shows, there was considerable variation (mean range ± sem: 1.44 ± 0.16 m/s) in air speed within many of the pens. It is also worth noting that in 12 of the 20 pens there were sections in which there was no recordable air speed. In some pens, such as deck nine, row 6, pen 9-12, the absence of recordable air speed was widespread across the pen. The maximum air speed measured was 3.4 m/s.

Table 6: Cumulative wind speed and direction, as measured on the ship bridge, on the first and second voyage.

Voyage One				Voyage Two			
Date	**Wind speed (knots/day)**	**Wind direction**	**Ship's course (degrees)**	**Date**	**Wind speed (knots/day)**	**Wind direction**	**Ship's course (degrees)**
28/05/05			Fremantle	13/07/05			Fremantle
29/05/05	16	NE	315	14/07/05	15	SW	315
30/05/05	16	NNE	315	15/07/05	12	SE	315
31/05/05	16	SSE	315	16/07/05	12	SE	315
1/06/05	16	SE	315	17/07/05	20	SE	315
2/06/05	16	NE	315	18/07/05	20	SE	315
3/06/05	25	SW	315	19/07/05	18	W	315
4/06/05	25	WSW	315	20/07/05	20	NW	315
5/06/05	16	NNW	310	21/07/05	21	NW	315
6/06/05	25	NW	310	22/07/05	24	NNW	315
7/06/05	25	W		23/07/05	30	NNW	315
8/06/05			Muscat	24/07/05			Muscat
9/06/05	18	W	277				
10/06/05			Kuwait				

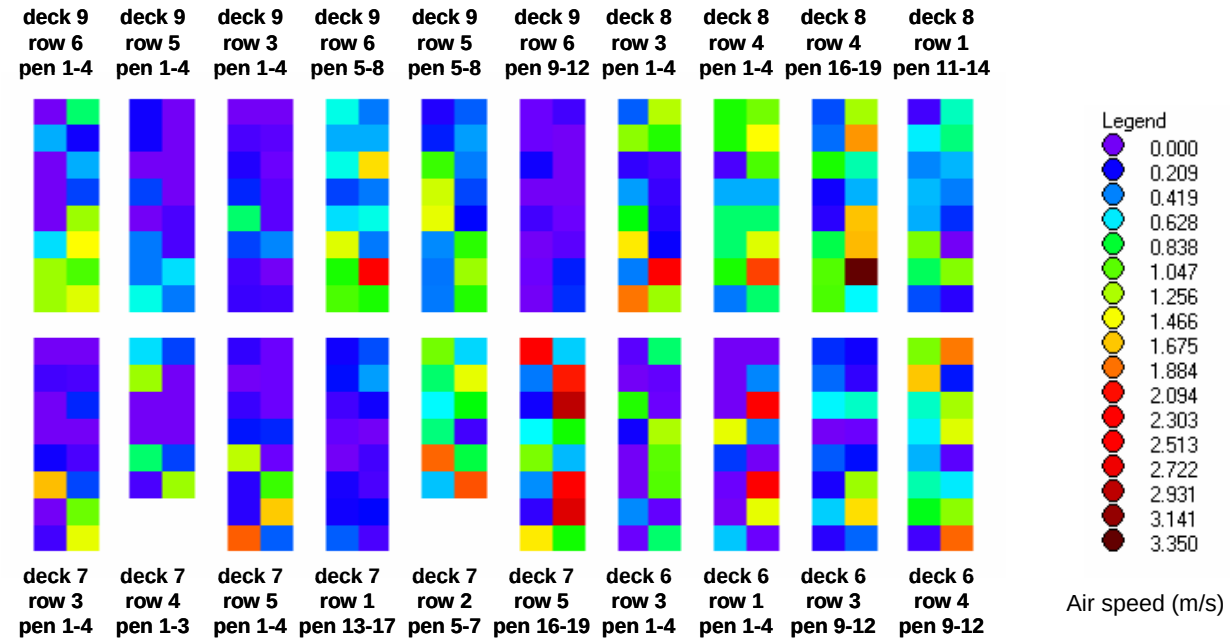


Figure 17: Mapping of air speed on voyage two. Air speed mapped within 20 open deck pens at 2.2 m intervals. Air speed was highly variable within most pens.

4.2.2 Dry bulb temperature

Voyage one daily dry bulb temperature on the open and closed decks is shown on Figure 18. Dry bulb temperature increased rapidly over the first 4 days of the voyage before levelling off. There was a peak in dry bulb temperature on the day that the ship was in the port at Muscat (day 11) and again when the ship was in port at Kuwait (day 13). Overall, dry bulb temperature was higher on the closed decks (mean ± sem: 32.2 ± 0.8°C) than the open decks (mean ± sem: 31.0 ± 0.8°C) (two sample *t*-tests: $t = -10.78$, df = 16, $p = 0.01$).

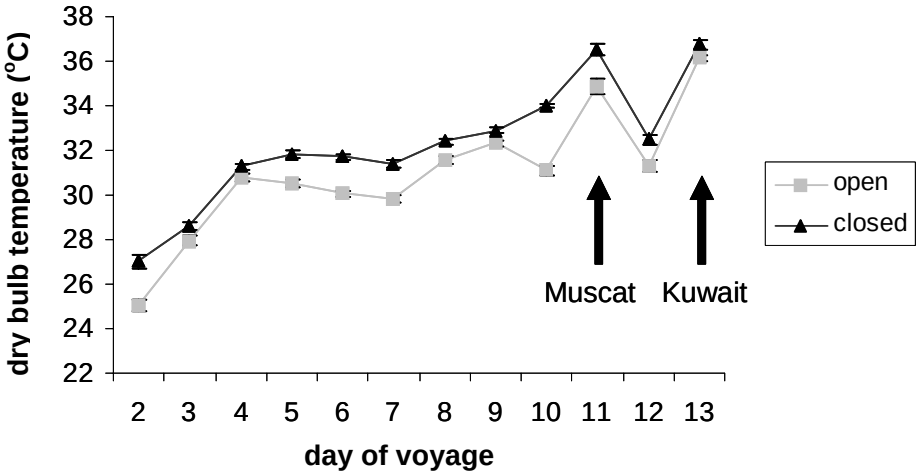


Figure 18: Dry bulb temperature over voyage one. Mean (± SEM) dry bulb temperature on the open decks (grey square: n = 12) and on the closed decks (black triangle: n = 8), over days 2-13 of the voyage.

Dry bulb temperature, as mapped in 20 pens during the second voyage, is shown in Figure 19. Dry bulb temperature was relatively stable within each pen (mean range ± sem: 0.95 ± 0.12°C).

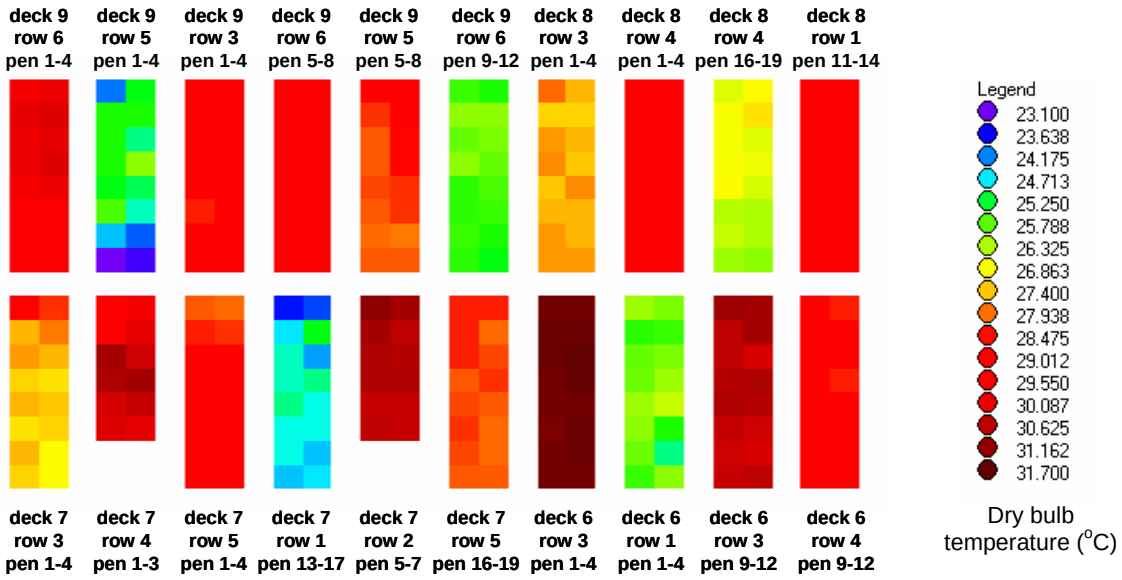


Figure 19: Mapping of dry bulb temperature on voyage two. Dry bulb temperature mapped within 20 open deck pens at 2.2 m intervals.

4.2.3 Wet bulb temperature

Voyage one wet bulb temperature increased rapidly on the fourth day of the voyage before levelling off (Figure 20). There was a temporary decrease in wet bulb temperature on the closed decks when the ship was in the port at Muscat (day 11). Wet bulb temperature also decreased when the ship was in port at Kuwait (day 13); this time on both the open and closed decks. Overall, wet bulb temperature was higher on the closed decks (mean ± sem: 27.5 ± 0.7°C) than the open decks (mean ± sem: 26.7 ± 0.7°C) (two sample *t*-tests: $t = -3.41$, df = 10, $p = 0.01$).

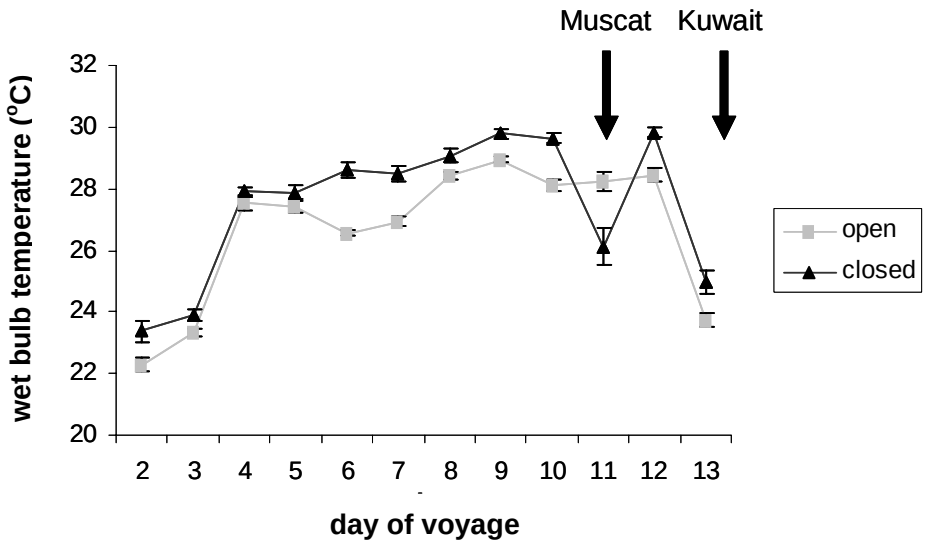


Figure 20: Wet bulb temperature over voyage one. Mean (± SEM) wet bulb temperature on the open decks (grey square: n = 12) and on the closed decks (black triangle: n = 8), over days 2-13 days of the voyage.

Wet bulb temperature was relatively stable within each of the 20 pens mapped on voyage two (mean range ± sem: 1.10 ± 0.20°C), as shown in Figure 21. There was no significant difference between in the variability of wet and dry bulb temperature (paired *t*-tests: $t = 1.26$, df = 20, $p = 0.22$). The largest range in wet bulb temperature within a pen was 2.6 °C (deck nine, row 5, pen 1-4).

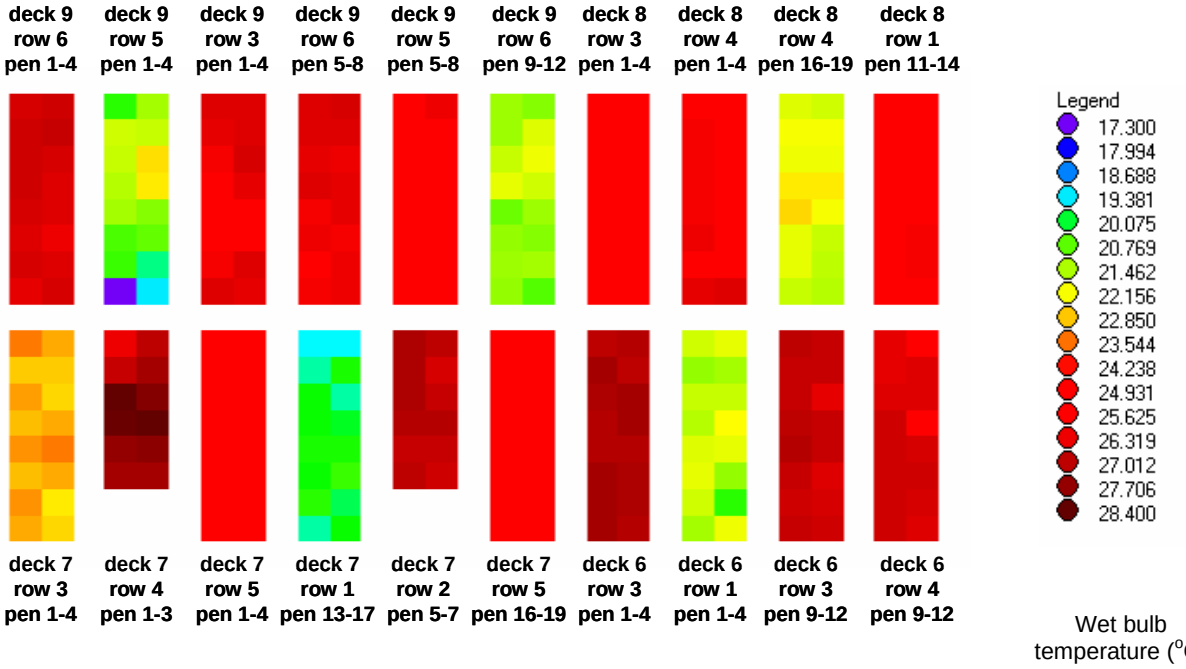


Figure 21: Mapping of wet bulb temperature on voyage two. Wet bulb temperature mapped within 20 open deck pens at 2.2 m intervals.

4.2.4 Dew point temperature

There was a sharp increase in dew point temperature on the fourth day of the voyage, followed by six days of relative stability (Figure 22). Dew point temperature on the closed decks decreased temporarily by 6°C when the ship was in port at Muscat on day 11. On day 13, when the ship was in port at Kuwait, dew point temperature on both the open and closed decks decreased by 8°C. Overall, there was no significant difference in dew point temperature between the open decks (mean ± sem: 25.2 ± 0.9°C) and the closed decks (mean ± sem: 25.9 ± 0.9°C) (two sample *t*-tests: $t = -1.68$, df = 10, $p = 0.12$).

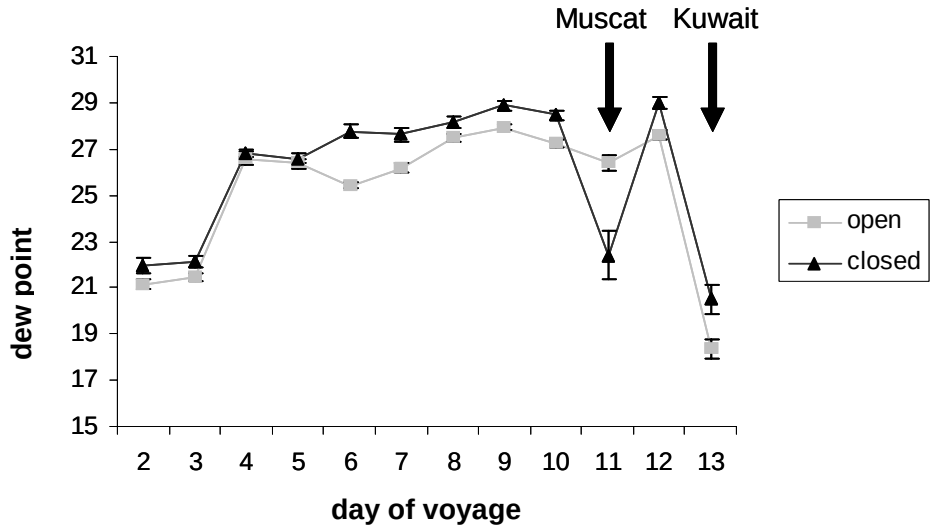


Figure 22: Dew point temperature over voyage one. Mean (± SEM) dew point temperature on the open decks (grey square: n = 12) and on the closed decks (black triangle: n = 8), over days 2-13 days of the voyage.

Dew point temperature was relatively stable within each of the 20 pens mapped on voyage two (mean range ± sem: 1.2 ± 0.2°C), as shown in Figure 23. The largest range in dew point temperature within a pen was 2.8°C (deck nine, row 5, pen 1-4).

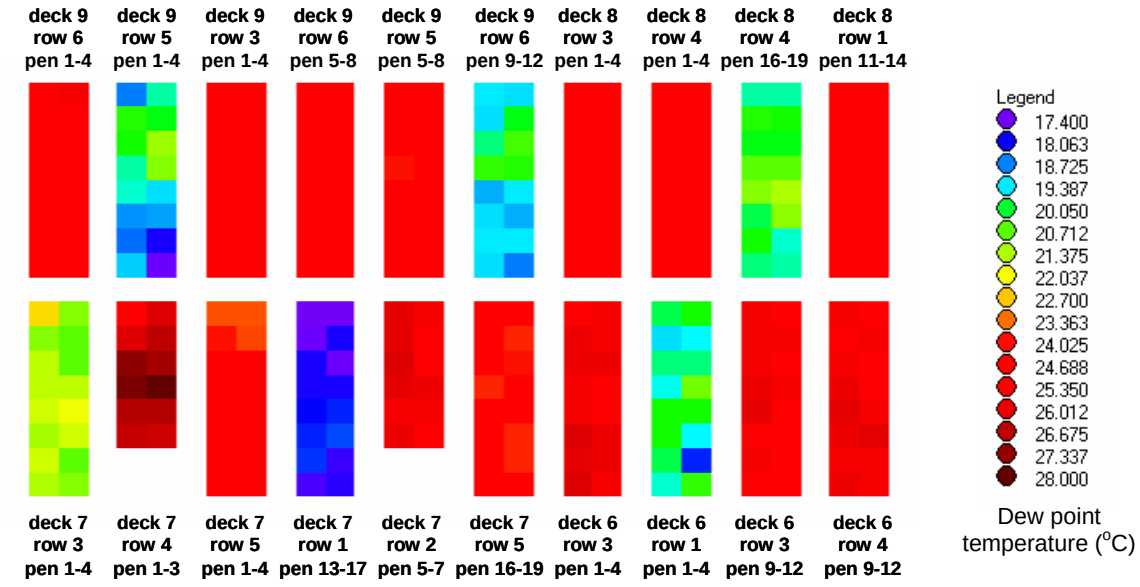


Figure 23: Mapping of dew point temperature on voyage two. Dew point temperature (°C) mapped within 20 open deck pens at 2.2 m intervals.

4.2.5 Faecal pad depth

Faecal pad depth increased consistently in both the open and closed decks over the course of the voyage (Figure 24). Mean faecal pad depth was greater on the closed decks (mean ± sem: 45.2 ± 6.3 mm) than the open decks (mean ± sem: 39.2 ± 5.3 mm)($F_{1,218}$ = 4.72; p = 0.03). The rate of accumulation was greater for the closed decks than the open decks.

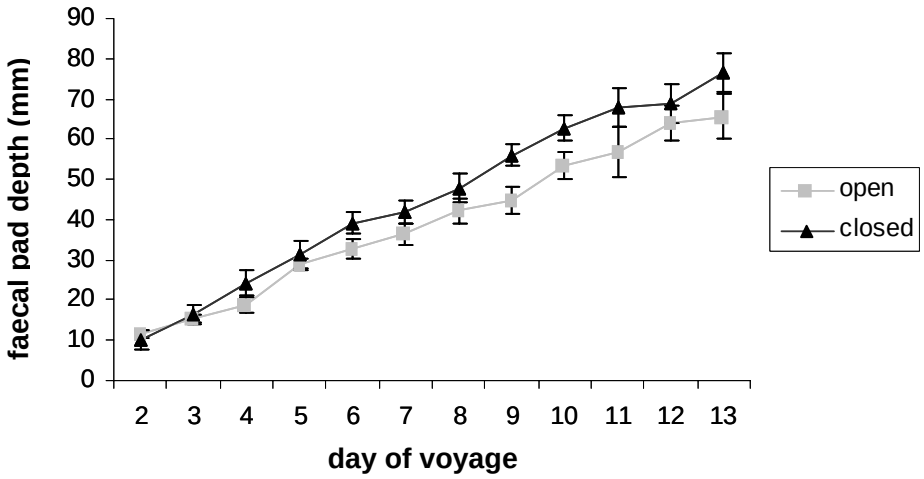


Figure 24: Faecal pad depth over voyage one. Mean (± SEM) faecal pad depth on the open decks (grey square: n = 12) and on the closed decks (black triangle: n = 8), over days 2-13 days of the voyage.

4.2.6 Regression analysis of potential factors affecting gas concentrations

Wet bulb temperature was highly correlated with dew point temperature on the open (r^2 = 0.88, p = 0.001) and closed (r^2 = 0.90, p = 0.001) decks during voyage one and with dew point temperature (r^2 = 0.90, p = 0.001) and dry bulb temperature (r^2 = 0.85, p = 0.001) during voyage two. In all instances the latter variable was removed from the respective analysis to prevent multi-co-linearity. Dry bulb temperature was not associated with ammonia on the open decks of voyage one and was removed from the stepwise regression.

Log ammonia concentration on the open decks on the first voyage was negatively correlated with faecal pad depth and the interaction between cumulative wind and air speed and positively with the interaction between wet bulb temperature and faecal pad depth and between air speed and faecal pad depth (r^2 = 0.55: Log 10 $NH_{3\ \text{voyage one open deck}}$ = 1.63 - $0.012_{\text{cumulative wind*air speed}}$ – $0.045_{\text{faecal pad depth}}$ + $0.001_{\text{wet bulb temperature*faecal pad depth}}$ + $0.003_{\text{air speed*faecal pad depth}}$). The interaction between cumulative wind and air speed was the first variable to be included in the model and therefore had the strongest association with log ammonia concentration (stepwise regression: t: = -5.88, p = 0.01), followed by faecal pad depth (t: = -4.36, p = 0.01), the interaction between wet bulb temperature and faecal pad depth (t: = 3.51, p = 0.01) and finally air speed and faecal pad depth (t: = 2.67, p = 0.01) (Table 7).

Table 7: Results of a stepwise regression of 10 variables associated with log 10 ammonia concentration on the open decks of voyage one (n = 99, Constant =1.63).

Explanatory Variable	Order of incorporation into model	Coefficient	*p* - value
Cumulative wind*air speed	1	-0.012	0.01
Faecal pad depth	2	-0.045	0.01
Wet bulb temperature*faecal pad depth	3	0.001	0.01
Air speed*faecal pad depth	4	0.003	0.01

The concentration of ammonia on the closed decks on the first voyage was negatively correlated with the interaction between wet bulb temperature and air speed, and between wet bulb temperature and faecal pad depth, and positively with wet bulb temperature (r^2 = 0.60: $NH_{3\ voyage\ one\ closed\ deck}$ = -10.57 + 2.15 $_{wet\ bulb\ temperature}$ – 0.193 $_{wet\ bulb\ temperature*air\ speed}$ - 0.005 $_{wet\ bulb\ temperature*faecal\ pad\ depth}$). Wet bulb temperature was the first variable to be included in the model and had the strongest correlation with ammonia concentration (*t*: = 8.04, *p* = 0.01), followed by the interaction between wet bulb temperature and air speed (*t*: = -4.46, *p* = 0.01) and finally between wet bulb temperature and faecal pad depth (*t*: = -2.59, *p* = 0.01) (Table 8). Dry bulb temperature was not associated with ammonia concentrations.

Table 8: Results of a stepwise regression of 10 variables associated with ammonia concentration on the closed decks of voyage one (n = 96, Constant = -10.57).

Explanatory Variable	Order of incorporation into model	Coefficient	*p* - value
Wet bulb temperature	1	2.15	0.01
Wet bulb temperature*air speed	2	-0.193	0.01
Wet bulb temperature*faecal pad depth	3	-0.005	0.01

Log 10 ammonia concentration on the second voyage (open decks) correlated negatively with cumulative wind and air speed, and positively with the interaction between wet bulb temperature and air speed (r^2 = 0.41: Log 10 $NH_{3\ voyage\ two\ open\ deck}$ = 1.79 - 0.087$_{air\ speed}$ – 0.048$_{cummulative\ wind}$ + 0.001$_{wet\ bulb\ temperature*cumulative\ wind}$). Air speed was the second variable included in the model, but had the strongest correlation with log ammonia concentration (*t*: = -7.43, *p* = 0.01), followed by cumulative wind, which was the first variable included (*t*: = -3.59, *p* = 0.01), and lastly by the interaction between wet bulb temperature and cumulative wind (*t*: = 2.03, *p* = 0.04) (Table 9). Faecal pad depth was not included in the analysis as it was not measured on the second voyage.

Table 9: Results of a stepwise regression of 6 variables associated with log 10 ammonia concentration on the open decks of voyage two (n = 312, Constant = 1.79).

Explanatory Variable	Order of incorporation into model	Coefficient	*p* - value
Air speed	2	-0.087	0.01
Cumulative wind	1	-0.048	0.01
Wet bulb temperature *cumulative wind	3	0.001	0.04

4.3 Experiment 3: The effects of ammonia on a live export vessel on sheep behaviour.

4.3.1 The effect of ammonia on sheep behaviour- Voyage two

As shown in Table 10, there was no correlation ($p > 0.05$) between ammonia and any of the measured sheep behaviours during days 2-5 or days 6-9, i.e. ammonia had no affect on the measured behaviours.

Table 10: Correlation between ammonia and sheep behaviour during days 2-5 and 6-9 of voyage two.

Behaviour	Days 2-5	Days 6-9
Feeding	$r^2 = 0.01$, $p = 0.64$	$r^2 = 0.02$, $p = 0.60$
Lying	$r^2 = 0.004$, $p = 0.93$	$r^2 = 0.012$, $p = 0.66$
Standing	$r^2 = 0.009$, $p = 0.90$	$r^2 = 0.0025$, $p = 0.83$
Head down	$r^2 = 0.05$, $p = 0.33$	$r^2 = 0.0036$, $p = 0.81$
Ruminating	$r^2 = 0.06$, $p = 0.30$	$r^2 = 0.0064$, $p = 0.74$

4.3.2 Changes in sheep behaviour over the course of the voyage - Voyage two

Sheep behaviour during the first half of the voyage (days 2-5) was compared to behaviour during the second half of the voyage (days 6-9). As shown in Figure 25, there was a significant change in the activity cycle, i.e. time spent standing, lying down and feeding (Chi square: $\chi^2 = 14.94$, df = 2, $p = 0.001$). The proportion of time spent lying down increased from 24.0 ± 3.1% during the first half of the voyage to 33.2 ± 3.5 % during the second half of the voyage. Associated with the increased time lying down was the decrease in time spent standing; down from 68.8 ± 3.4% during days 2-5 to 60.0 ± 4.0 % during days 6-9. No change was found in time spent feeding (approx. 7 %). There was also no change in the amount of time spent ruminating (approx. 24 %) ($\chi^2 = 0.001$, df = 1, $p = 0.95$). There was however a significant increase in the amount of time the sheep spent with their head down ($\chi^2 = 22.52$, df = 1, $p = 0.001$); time spent with the head down increased from 17.9 ± 2.2% to 28.5 ± 2.7%.

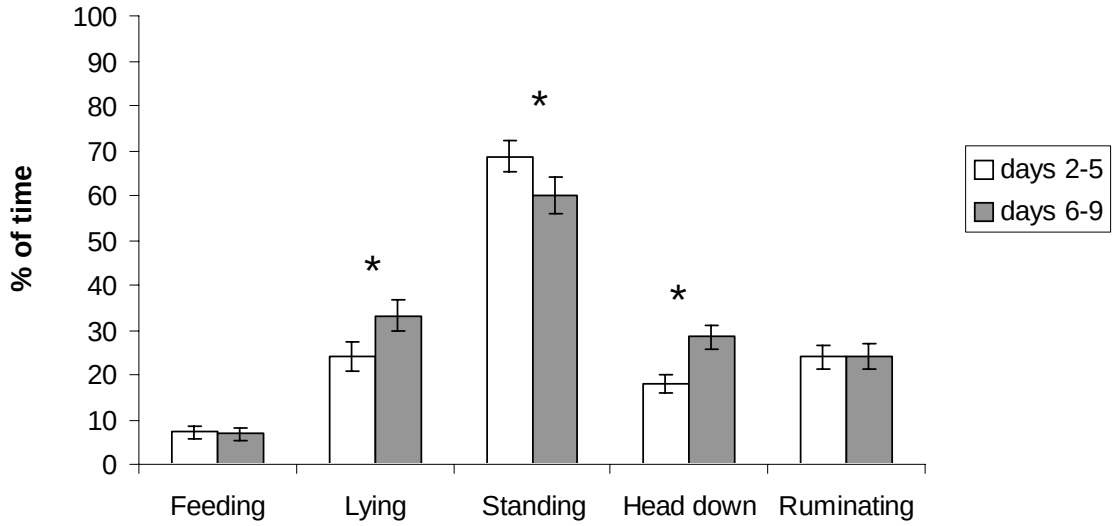


Figure 25: Behaviour changes with voyage duration. Mean (± SEM) time spent feeding, lying down, standing, with the head down and ruminating during days 2-5 (white columns, n = 20) and days 6-9 (grey columns, n = 20) is plotted. Asterisk indicates a significant ($p < 0.05$) difference.

4.3.2.1 Activity cycle

There appeared to be a number of subtle differences in the activity cycle of the sheep over the duration of the voyage, as shown in Figure 26. During days 2-5 sheep tended to feed sporadically throughout the day. By contrast, feeding was more cyclic during days 6-9, with a peak in feeding between 5.30 h and 9.30 h and again between 14.30 h and 18.30 h. Time of the day sheep spent lying down also differed between the start and end of the voyage. During days 6-9, the peaks in time spent lying down tended to occur outside of the times during which there were peaks in feeding. This pattern was less evident during days 2-5. Moreover, before 06.30 h and after 17.30 h, the sheep spent almost as much time lying down as they did standing up during days 6-9 and this did not happen during days 2-5; sheep spent most of the early morning and late at night standing throughout the first half of the voyage.

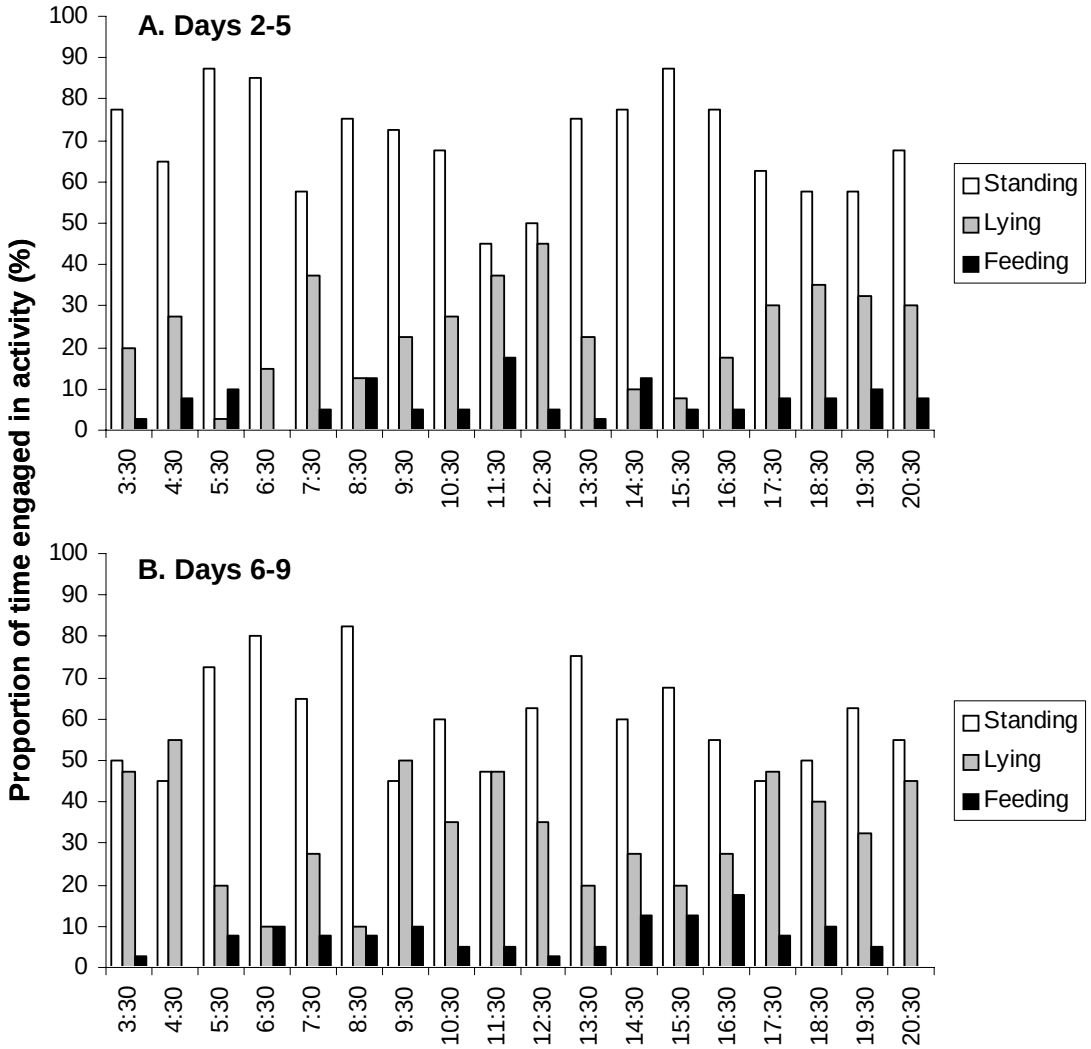


Figure 26: Activity cycle. The proportion of time spent standing, feeding and lying from 3.30 h to 20.30h during A. days 2-5 and B. days 6-9 pf the voyage.

4.3.2.2 Pen usage

The positioning of the sheep within the pen was, for most animals, not random (Figure 27). Despite sheep occupying all sections of the pen, individuals spent most of their time in specific parts of the pen and very little or none in other parts of the pen. During the first half of the voyage, 75 % of the focal animals were not randomly distributed throughout the pen (Figure 27) (pens in which the focal animal was randomly distributed as determined by Chi Square analysis: deck nine, row 6, pen 5-8; deck nine, row 5, pen 5-8; deck eight, row 3, pen 1-4; deck six, row 3, pen 1-4; deck six, row 3, pen 9-12), while during the second half of the voyage 100 % were not randomly distributed.

The proportion of the pen utilised by the focal animals decreased from 74.1 ± 3.9% during the first half of the voyage to 63.1 ± 2.6% in the second half of the voyage (Paired *t*-test: $t_{19} = 3.9$, $p = 0.001$).

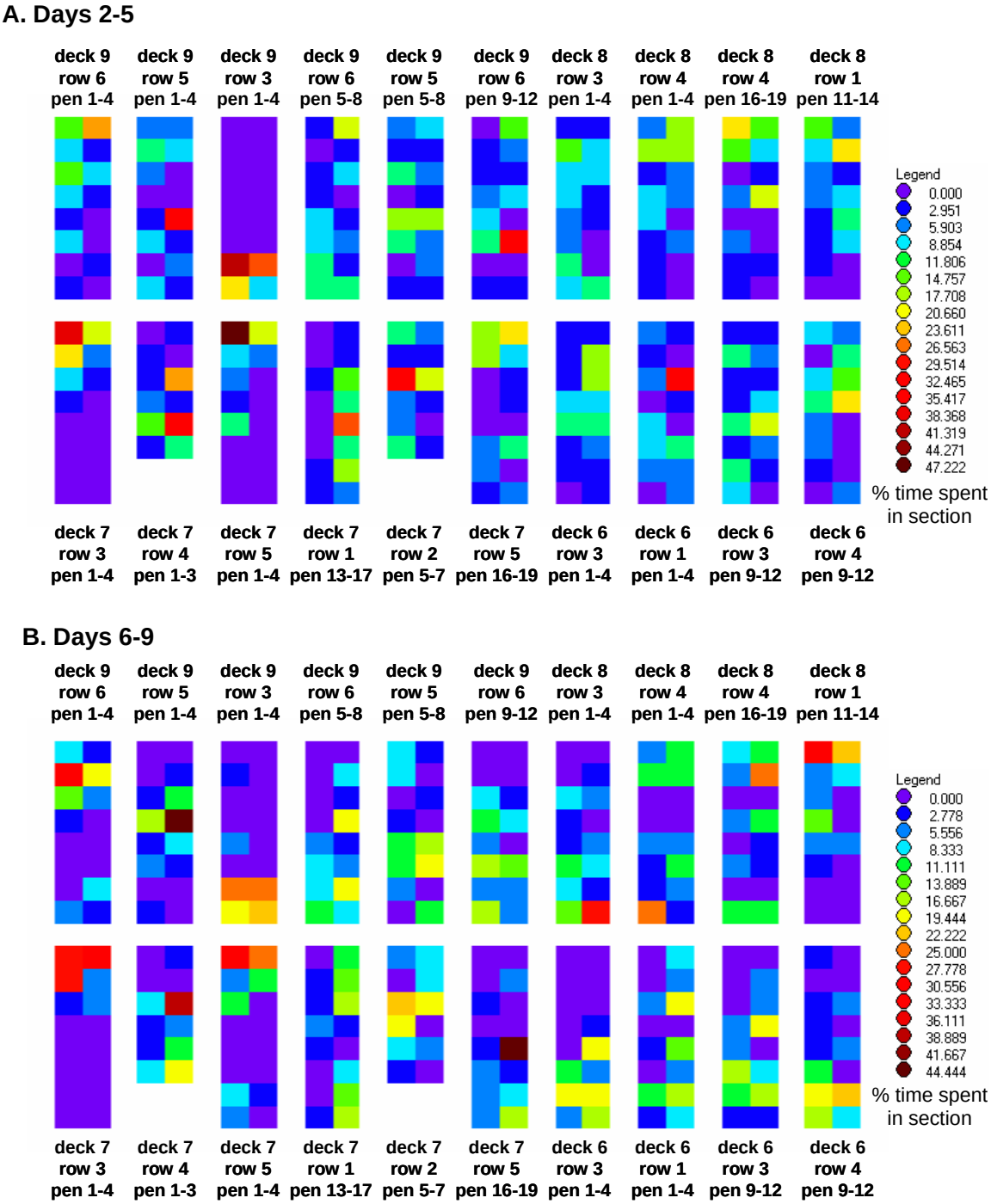


Figure 27: Pen usage. Percentage of time 20 focal animals spent in each section of the pen during A. days 2-5 and B. days 6-9 of the second voyage.

4.3.3 Conjunctivitis prevalence (eye irritation)

As shown in Figure 28, the proportion of the pen with possible eye irritation did not differ between days 4-10 of the second voyage ($F_{6,114} = 1.85$, $p = 0.10$). The mean (± SEM) proportion of the pen with possible eye irritation was 1.8 (± 0.1) %. Eye irritation was not correlated with ammonia ($r^2 = 0.05$, $p = 0.36$) or air speed ($r^2 = 0.04$, $p = 0.43$).

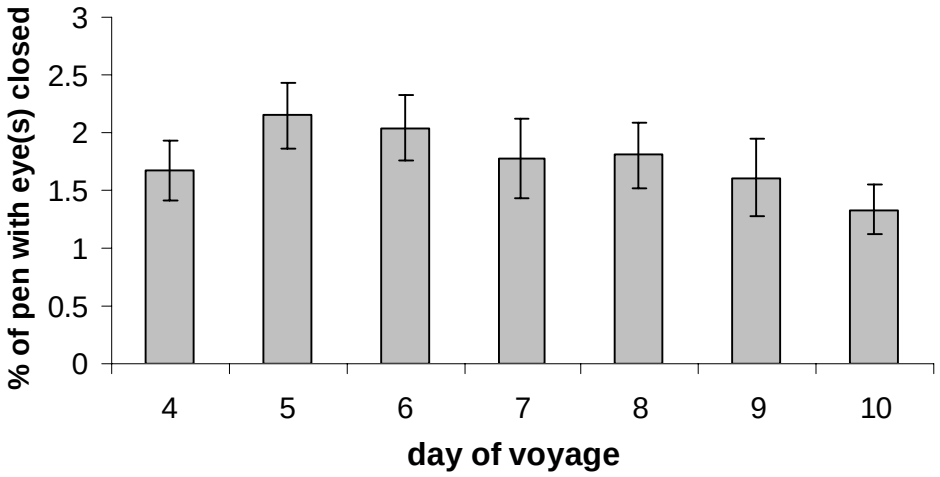


Figure 28: Eye irritation: Percentage of sheep in pen with eye(s) closed, as an indication of eye irritation, during days 4-10 of the second voyage.

4.4 Experiment 4: Concentrations of potentially noxious gases at a pre-export assembly depot

4.4.1 Correlation between readings from the Odalog and Neotox ammonia meters

A Pearson's correlation was conducted between the 48 readings from the Odalog meter and Neotox meter and a significant linear correlation was found ($r^2 = 0.49$, $p = 0.001$). A regression analysis was used to describe the relationship between the Odalog meter and the Neotox meter [$NH_{3Odalog\ meter} = 0.54$ (± 0.008) + 0.55 (± 0.08) $NH_{3Neotox\ meter}$, where NH_3 is ammonia concentration measured in ppm]. The readings from the Odalog meter were therefore higher than those from the Neotox meter (Figure 29).

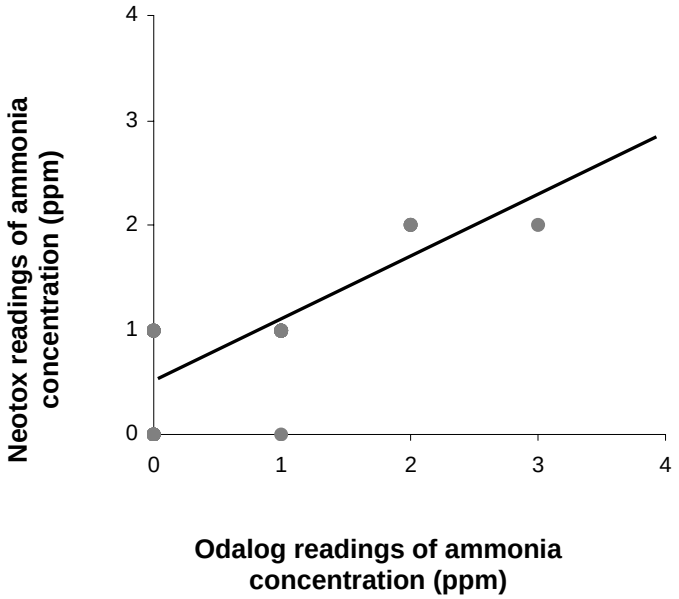


Figure 29: Correlation between the Odalog meter and the Neotox meter (n = 48)

4.4.2 Atmospheric ammonia concentration

The concentration of ammonia in the eight sheds is shown in Figure 30. In all eight sheds, the mean ammonia concentration was below the Time Weighted Average (TWA) limit of 25 ppm that humans are permitted to be exposed to in an 8-h day. The maximum concentration of ammonia detected was 13 ppm.

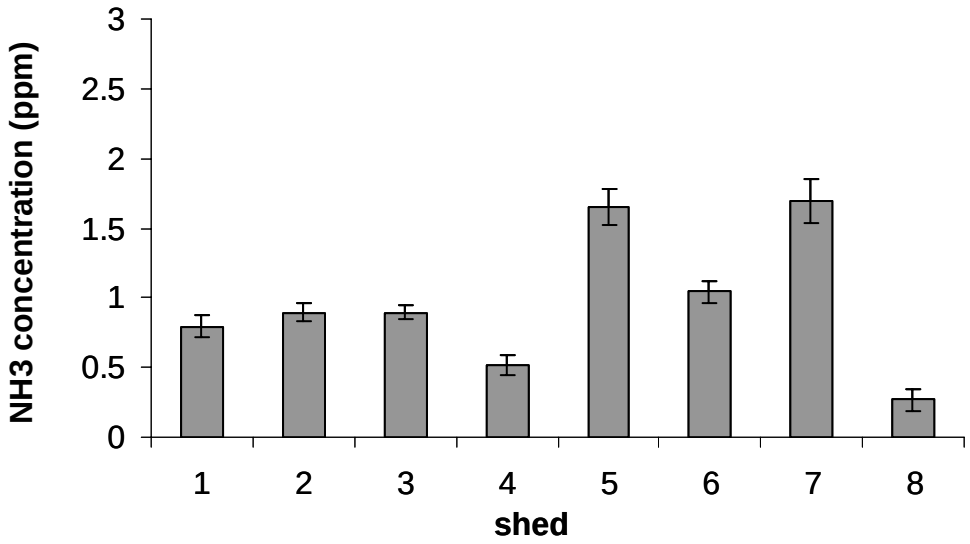


Figure 30: Ammonia concentration. Mean (± SEM) concentration of ammonia (NH_3) in the eight assembly depot sheds (n = 48).

Ammonia was mapped in four sheds (Figure 31). The concentration of ammonia was relatively constant throughout sheds two, six and eight. In shed five there was a section in which ammonia was as high as 9.5 ppm. The greatest range (highest – lowest) in ammonia concentrations in any one shed was 8 ppm (shed five). The mean (± SEM) range in ammonia concentration within the eight sheds was 4.3 ± 1.2 ppm.

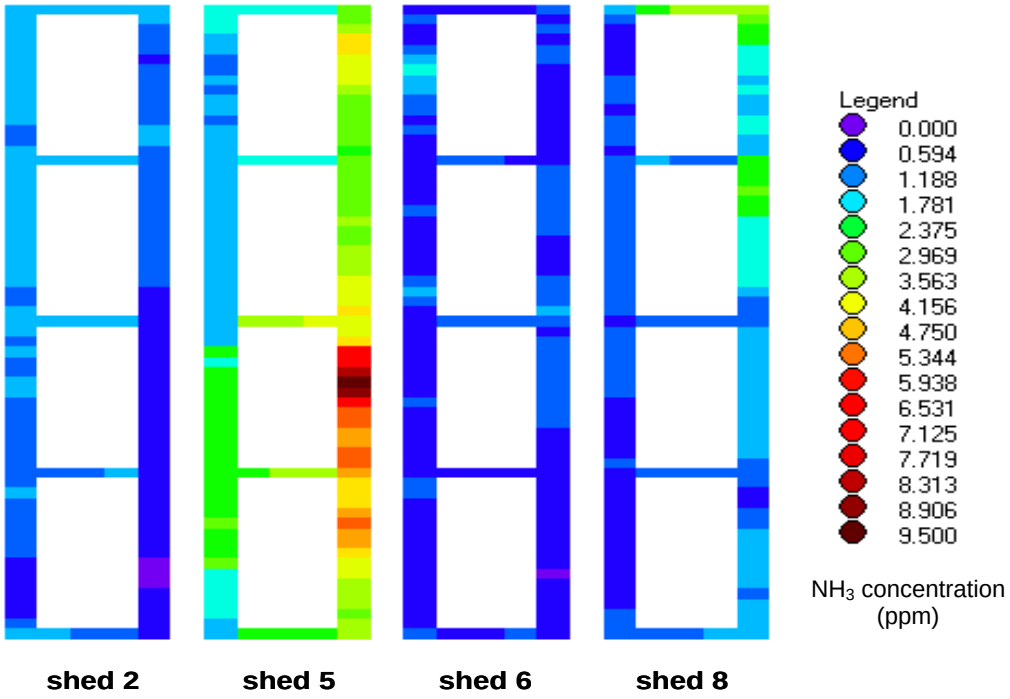


Figure 31: Mapping of ammonia within four sheds. Mean ammonia concentrations mapped within four sheds at 2 m intervals (n =48).

The relationship between ammonia concentration and vertical distance from the shed floor (excluding faecal pad) is shown in Figure 32. There was a weak linear relationship (r^2 = 0.12, *p* = 0.001: $NH_3 = 1.94 - 0.005_{height}$) between ammonia concentration and height from shed floor; i.e. ammonia concentration decreased with vertical distance from the shed floor.

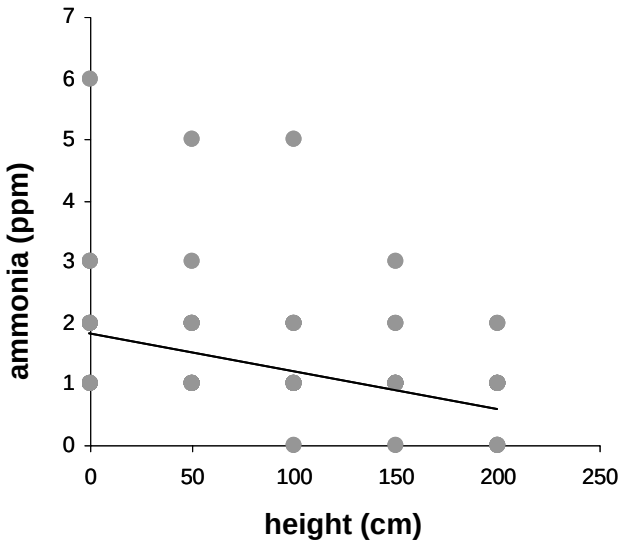


Figure 32: Correlation between ammonia concentration and vertical distance from shed floor. Correlation between ammonia concentration (on the Y-axis) and vertical distance from the shed floor (on the X-axis) in the assembly depot (n = 80).

4.4.3 Other environmental variables

Descriptive statistics for carbon dioxide, hydrogen sulphide, dry and wet bulb temperature, dew point temperature, wind speed and distance from faeces are shown in Table 11. The mean concentration of carbon dioxide of 738 ppm was well below the TWA limit (12500 ppm) for humans. The maximum concentration of carbon dioxide detected was 1460 ppm.

Table 11: Other environmental variables. Mean levels of carbon dioxide, hydrogen sulphide, dry and wet bulb temperature, dew point temperature, wind speed and distance from faeces within eight assembly depot sheds (n = 384).

Environmental Variable	Shed (mean ± SEM)								
	One	Two	Three	Four	Five	Six	Seven	Eight	Overall mean
Carbon dioxide (ppm)	696.9 ± 1.4	754.8 ±15.0	664.2 ± 21.2	749.2 ± 2.1	813.5 ± 3.4	760.8 ± 3.8	722.9 ± 20.9	730.2 ± 8.4	738.9 ± 7.1
Hydrogen sulphide (ppm)	0.2 ± 0.1	0.0 ± 0.0	0.3 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.1
Dry bulb temperature (^{o}C)	25.9 ± 0.3	25.7 ± 0.4	25.5 ± 0.4	26.2 ± 0.5	27.5 ± 0.3	26.3 ± 0.4	26.4 ± 0.4	25.5 ± 0.3	26.1 ± 0.1
Wet bulb temperature (^{o}C)	16.9 ± 0.3	16.9 ± 0.3	16.9 ± 0.4	17.2 ± 0.2	18.0 ± 0.2	17.5 ± 0.4	17.4 ± 0.4	16.8 ± 0.2	17.2 ± 0.1
Dew point temperature (^{o}C)	11.4 ± 0.4	11.6 ± 0.3	11.5 ± 0.5	11.7 ± 0.3	12.6 ± 0.3	12.3 ± 0.5	12.2 ± 0.5	11.4 ± 0.3	11.9 ± 0.1
Wind speed (m/s)	0.5 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.0	0.3 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.4 ± 0.1
Distance from faeces (m)	1.9 ± 0.1	2.0 ± 0.1	2.0 ± 0.0	1.8 ± 0.1	1.2 ± 0.1	1.4 ± 0.1	1.5 ± 0.1	1.5 ± 0.1	1.7 ± 0.1

Hydrogen sulphide was not detected in two of the eight sheds. The mean (0.13 ppm) concentration of hydrogen sulphide was below the TWA limit (10 ppm) for humans. The maximum concentration of hydrogen sulphide detected was 1.7 ppm.

There was little difference between the eight sheds in dry and wet bulb temperature, dew point temperature and wind speed. The dry bulb temperature within the sheds tended to be 2-4^{o}C cooler than the outdoor temperature (daily maximum outdoor dry bulb temperature during the study ranged from 27.3^{o}C to 34.0^{o}C). Wet bulb temperature within the sheds was comparable to that outdoors (daily maximum outdoor wet bulb temperature during the study ranged from 13.4^{o}C to 19.9^{o}C).

Distance from the faeces varied considerably between sheds with some sheds having little faecal matter beneath them (i.e. there was a 2 m gap between the shed floor and the faeces) while in others the accumulated faeces was flush with the shed floor. On average, the faecal pad was less than 1.2 m below the shed floor.

Each of the major environmental variables was mapped in four sheds (Appendix 1). Dry and wet bulb temperature and dew point temperature levels were relatively constant within each shed, but did differ between sheds. Shed two, where carbon dioxide and dry bulb temperature were highest, was the only shed mapped that had hydrogen sulphide present.

Wind speed and distance from faeces were highly variable within each shed. In Appendix 1) it can be seen that in some areas there was no space between the accumulated faeces and the shed floor.

4.4.4 Regression analysis of potential factors affecting ammonia concentrations

Wet bulb temperature was highly correlated with dry bulb temperature (r^2 = 0.81, p = 0.001) and dew point temperature (r^2 = 0.86, p = 0.001) and so the latter two variables were removed from the stepwise regression analysis to prevent multi-co-linearity.

Log ammonia concentration was positively correlated with wet bulb temperature, and negatively with the interaction between distance from the faeces and wet bulb temperature (r^2 = 0.25: Log 10 NH_3 = -3.71 + $0.508_{\text{wet bulb temperature}}$ – $0.221_{\text{distance from faeces*wet bulb temperature}}$). Wet bulb temperature was the first variable to be included in the model and therefore had the strongest association with log ammonia concentration (stepwise regression: t: = 9.65, p = 0.01), while the interaction between wet bulb temperature and distance from faeces (t: = -4.19, p = 0.01) was the second variable included (Table 12).

Table 12: Results of a stepwise regression of five variables associated with log 10 ammonia concentration (N = 384, Constant = -3.71).

Explanatory Variable	Order of incorporation into model	Coefficient	p – value
Wet bulb temperature	1	0.508	0.01
Distance from faeces*Wet bulb temperature	2	-0.221	0.01

4.5 Experiment 5: The effect of atmospheric ammonia on the physiology and behaviour of cattle and sheep

4.5.1 Cattle

Haematology – As presented in Table 13, ammonia treatment had no significant effect on any of the haematological parameters measured in the steers immediately after they left the climate chambers (i.e. day 13). As there was no treatment effect, the results were aggregated and analysed for differences between pre-experiment levels and day 13 (post-experiment) levels and significant effects were found for eight haematological parameters. Compared to pre-experiment levels, there was a significant decrease in day 13 post-experiment levels of haemoglobin (t-test: t_{47} = -3.28, p = 0.002), mean cell volume (MCV: t_{47} = -4.10, p = 0.001) and mean cell haemoglobin (MHb: t_{47} = -2.63, p = 0.03), and a significant increase in post-experiment levels of mean platelet volume (MPV: t_{47} = 4.12, p = 0.001), eosinophils (t_{47} = 4.33, p = 0.001), neutrophils (t_{47} = 2.42, p = 0.02), white cell counts (t_{47} = 4.22, p = 0.001) and monocytes (t_{47} = 2.19, p = 0.03) as shown in Figure 33. It was also found that pre- and day 13 post-experiment levels of neutrophils, white cell count and monocytes were above the normal range. All other parameters were within the normal range specified by The University of Queensland's Clinical Pathology Laboratory.

Table 13: Haematological tests conducted on steers and effect of ammonia pre-experiment and immediately after they left the climate chambers (day 13)

Test	Pre-experiment	Control	15 ppm	30 ppm	45 ppm	Normal range[1]	Effect of ammonia
Haemoglobin (g/dl)	14.8 ± 0.2	14.5 ± 0.5	14.3 ± 0.4	14.2 ± 0.3	13.2 ± 0.7	8.0-15.0	F = 1.38, *p* = 0.26
Red cell count (x10E12/1)	9.4 ± 0.1	9.8 ± 0.4	9.3 ± 0.2	9.9 ± 0.2	9.7 ± 0.2	5.0-10.0	F = 1.47, *p* = 0.24
Packed cell volume (l/l)	0.44 ± 0.01	0.43 ± 0.02	0.42 ± 0.01	0.44 ± 0.01	0.41 ± 0.01	0.24-0.46	F = 0.69, *p* = 0.57
MCHC (g/dl)	33.0 ± 0.3	33.6 ± 0.7	33.9 ± 0.6	32.4 ± 0.5	33.9 ± 0.6	30.0-36.0	F = 0.37, *p* = 0.78
MCH (pg)	15.7 ± 0.3	15.0 ± 0.6	15.4 ± 0.6	14.3 ± 0.3	14.7 ± 0.4	11.0-17.0	F = 0.29, *p* = 0.83
MCV (fl)	47.6 ± 0.9	43.8 ± 1.4	45.1 ± 1.3	44.7 ± 0.9	42.7 ± 1.0	40.0-60.0	F = 0.72, *p* = 0.55
Platelet count (x10E9/l)	284.9 ± 19.0	224.6 ± 30.5	277.3 ± 39.7	265.4 ± 31.1	301.4 ± 27.5	100.0-800.0	F = 1.69, *p* = 0.19
MPV (fl)	9.7 ± 0.1	10.0 ± 0.3	9.9 ± 0.2	9.8 ± 0.2	9.7 ± 0.1	unknown	F = 0.51, *p* = 0.68
PCT (fl)	0.6 ± 0.3	0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	unknown	F = 2.01, *p* = 0.13
White cell count (x10E9/l)	14.8 ± 1.0	18.4 ± 0.9	21.8 ± 3.1	17.5 ± 1.7	15.5 ± 1.7	4.0-12.0	F = 0.49, *p* = 0.69
Neutrophils (x10E9/l)	4.0 ± 0.4	7.6 ± 1.0	6.2 ±1.0	4.0 ± 0.4	4.3 ± 0.9	0.6-4.0	F = 1.57, *p* = 0.21
Lymphocytes (x10E9/l)	9.3 ± 0.6	8.3 ± 0.8	13.1 ± 2.1	10.8 ± 1.4	8.8 ± 1.2	2.5-7.5	F = 0.97, *p* = 0.42
Monocytes (x10E9/l)	0.9 ± 0.1	1.0 ± 0.1	1.0 ± 0.2	1.5 ± 0.2	1.1 ± 0.3	0.3-0.8	F = 0.01, *p* = 0.99
Eosinophils (x10E9/l)	0.8 ± 0.1	1.5 ± 0.4	1.5 ± 0.4	1.1 ± 0.2	1.3 ± 0.2	0.0-2.4	F = 0.18, *p* = 0.91
Cortisol (nmol/l)	192.7 ± 11.0	184.6 ± 25.6	170.3 ± 8.8	199.0 ± 12.2	207.3 ± 20.3	unknown	F = 0.50, *p* = 0.69
Urea (mmol/l)	5.8 ± 0.1	6.3 ± 0.3	5.5 ± 0.4	5.8 ± 0.3	6.1 ± 0.4	3.0-10.7	F = 1.94, *p* = 0.14

1. Determined from The University of Queensland's Clinical Pathology Laboratory

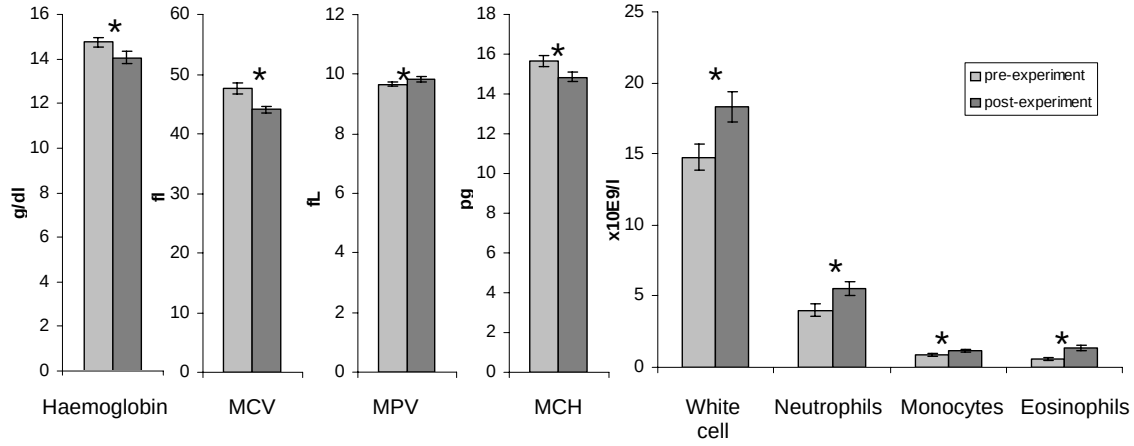


Figure 33: Pre-experiment and day 13 (post-experiment) haematological parameters of the steers. Compared to pre-experiment levels (light grey columns), there was a significant decrease in day 13 post-experiment levels (dark grey columns) of haemoglobin, MCV and MCH, and a significant increase in post-experiment levels of MPV, white cell counts, neutrophil, monocytes and eosinophils as indicated by the asterisk. Neutrophil, white cell count and monocyte levels were above the normal range.

Haematology day 41 (28 days post-experiment) – The results of the blood sampling conducted 28 days after the cattle left the climate rooms are shown in Table 14. Of the various haematological parameters measured, three were found to be significantly effected by the earlier exposure to ammonia: platelet count (ANOVA: $F_{3,11} = 3.80$, $p = 0.043$), plateletcrit (PCT: $F_{3,11} = 3.88$, $p = 0.041$) and urea ($F_{3,11} = 4.35$, $p = 0.030$). Post-hoc analysis was unable to reveal where the specific differences between the various ammonia treatments lay for platelet count or PCT (t values ranged from -2.61 to 0.13, p values ranged from 0.10 to 0.99). For urea, levels in the 15 ppm ammonia treatment were significantly higher than those in the 45 ppm treatment (t-test: $t_{15} = -3.61$, $p = 0.02$). No other significant differences were found between the blood samples collected immediately after the steers left the climate chamber and those collected 28 days later (t values ranged from -2.07 to -0.69, p values ranged from 0.06 to 0.93).

Table 14: Haematological tests conducted on steers 28 days post-experiment (day 41)

Test	Control	15 ppm	30 ppm	45 ppm	Effect of ammonia
Haemoglobin (g/dl)	14.5 ± 0.7	14.1 ± 0.3	13.3 ± 0.9	14.5 ± 0.2	F = 0.70, *p* = 0.57
Red cell count (x10E12/1)	9.0 ± 0.8	10.1 ± 0.3	9.9 ± 0.2	9.5 ± 0.2	F = 2.65, *p* = 0.10
Packed cell volume (l/l)	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	F = 0.59, *p* = 0.63
MCHC (g/dl)	32.7 ± 2.0	35.0 ± 0.7	31.5 ± 1.0	35.3 ± 0.5	F = 0.81, *p* = 0.51
MCH (pg)	16.3 ± 0.9	13.8 ± 0.6	13.5 ± 0.5	15.3 ± 0.5	F = 1.55, *p* = 0.25
MCV (fl)	49.7 ± 5.5	39.5 ± 2.7	42.5 ± 2.5	43.0 ± 1.6	F = 1.30, *p* = 0.32
Platelet count (x10E9/l)	156.7 ± 38.7	362.8 ± 46.5	185.5 ± 25.8	353.0 ± 76.1	F = 3.80, *p* = 0.04
MPV (fl)	9.8 ± 0.3	10.0 ± 0.4	9.9 ± 0.5	9.5 ± 0.2	F = 0.05, *p* = 0.98
PCT (fl)	0.15 ± 0.03	0.36 ± 0.05	0.18 ± 0.02	0.33 ± 0.07	F = 3.88, *p* = 0.04
White cell count (x10E9/l)	18.0 ± 4.6	16.8 ± 2.9	15.9 ± 2.2	13.9 ± 0.7	F = 0.60, *p* = 0.62
Neutrophil (x10E9/l)	4.8 ± 1.3	4.7 ± 1.7	3.5 ± 0.5	4.6 ± 0.5	F = 1.27, *p* = 0.33
Lymphocytes (x10E9/l)	11.1 ± 3.3	10.2 ± 1.3	9.9 ± 1.9	7.2 ± 1.4	F = 0.71, *p* = 0.56
Monocytes (x10E9/l)	0.8 ± 0.4	0.9 ± 0.5	1.6 ± 0.2	0.9 ± 0.2	F = 0.06, *p* = 0.98
Eosinophils (x10E9/l)	1.2 ± 0.2	1.1 ± 0.3	1.1 ± 0.2	1.2 ± 0.5	F = 0.13, *p* = 0.93
Cortisol (nmol/l)	145.0 ± 31.2	172.3 ± 35.1	171.0 ± 7.2	219.5 ± 16.4	F = 1.40, *p* = 0.29
Urea (mmol/l)	7.8 ± 1.2	8.3 ± 0.6	7.0 ± 0.6	5.4 ± 0.3	F = 4.35, *p* = 0.03

Bronchial alveolar lavage (day 13, 0 days post-experiment) – There was a significant effect of ammonia treatment on the percentage of neutrophils in the alveolar lavages (ANOVA: $F_{3,43}$ = 4.33, p = 0.009). As shown in Figure 34, there was a significantly higher percentage of neutrophils in the 30 ppm ammonia than in all other ammonia treatments (t values ranged from 2.60 to 3.31, p values ranged from 0.01 to 0.06).

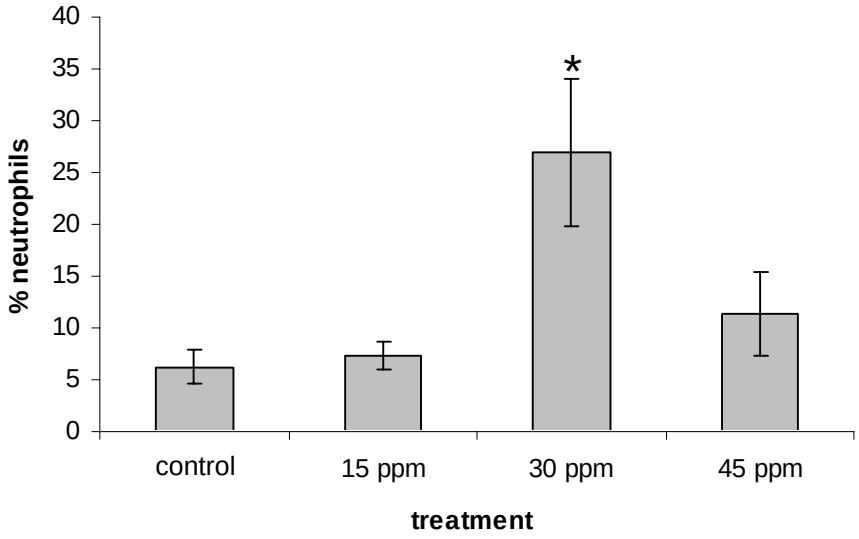


Figure 34: Percentage of neutrophils in the steers' bronchial alveolar lavage in the four ammonia treatments. There were significantly more neutrophils in the 30 ppm ammonia treatments than in all other ammonia treatments, as denoted by the asterisk.

Chi-square analysis revealed a significant (p = 0.04) difference in macrophage activity between the four ammonia treatments (Fig. 35). More steers had "very high" macrophage activity in the 45 ppm ammonia treatment (83 %) than in the 30 ppm treatment (50 %), 15 ppm treatment (45 %) or control (8 %). The control had the highest percentage of steers (25 %) with "low" macrophage activity while the 45 ppm treatment had the lowest (0 %). There was little difference in macrophage activity between the 15 and the 30 ppm ammonia treatments.

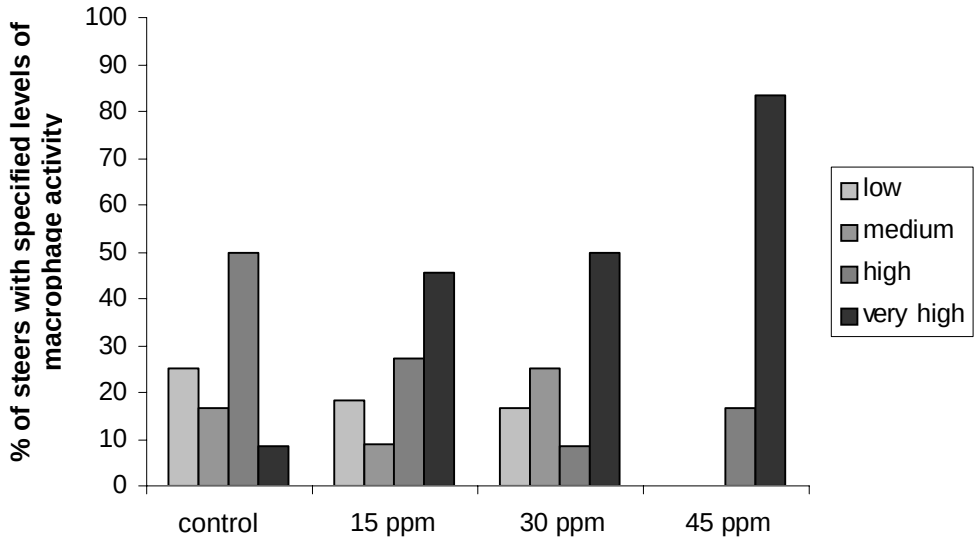


Figure 35: Percentage of steers with specified levels of macrophage activity in the bronchial alveolar lavage. The 45 ppm ammonia treatment had the highest percentage of steers with very high activity macrophages while the 0 ppm had the lowest.

Table 15: Results of the bronchial alveolar lavages.

Test	control	15 ppm	30 ppm	45 ppm	Effect of ammonia	†LIVE.218 pre-experiment	†LIVE.218 post exposure to NH3 ranging from 22-42 ppm
Red cells (x10E6/l)	43.3 ± 29.6	77.9 ± 65.0	155.8 ± 68.6	214.2 ± 188.9	F = 0.52, p = 0.67		
Total white cells (x10E6/l)	271.7 ± 34.9	170.8 ± 28.6	286.7 ± 81.6	170.0 ± 28.5	F = 1.67, p = 0.19	700	1000-1400
Lymphocytes (x10E6/l)	1.7 ± 0.7	1.8 ± 0.9	0.8 ± 0.3	2.6 ± 1.0	F = 0.67, p = 0.58	2.6	10-13
Segmented leukocytes (x10E6/l)	4.4 ± 3.0	2.6 ± 0.8	1.3 ± 0.6	4.1 ± 2.1	F = 0.56, p = 0.64	1.5	12-26
Macrophages (x10E6/l)	87.9 ± 4.2	88.2 ± 2.4	71.3 ± 7.5	82.5 ± 4.1	F = 2.48, p = 0.07	62	80-100

† Data from Costa, N., Accioly, J. & Cake, M. (2003) LIVE.218: Determining critical atmospheric ammonia levels for cattle, sheep and goats - a literature review.

Ammonia treatment had no significant affect on red cells, white cells, lymphocytes or segmented leukocytes (Table 15). There was a trend for fewer macrophages in the 30 ppm ammonia treatment (p = 0.07).

For comparison, the pre- and post- exposure white cell, lymphocytes, segmented leukocytes and macrophage levels reported in Costa *et al*. (2004) for steers exposed to ammonia are presented in Table 15. The white cell, lymphocytes, segmented leukocytes levels reported here are lower than the post-exposure levels reported by Costa *et al*. (2004), while the macrophage levels are comparable.

Bronchial alveolar lavage (day 41, 28 days post-experiment) – Macrophage activity in the cattle 28 days following their departure from the climate room are shown in Figure 36. Due to a small sample size (n = 3-4 per ammonia treatment), statistical analyses was not possible. However all steers in the control, 30 ppm and 45 ppm, and three of the four steers in the 15 ppm ammonia treatment, had "medium" macrophage activity suggesting no real difference between the various ammonia treatments (Fig. 36).

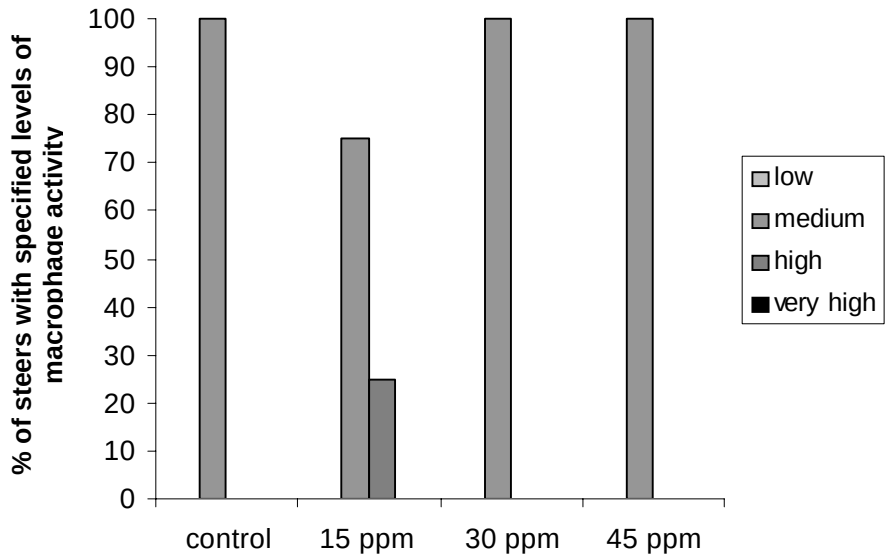


Figure 36: Percentage of steers with specified levels of macrophage activity 28 days post-experiment. Macrophage activity was similar in all four ammonia treatments.

There was no difference between ammonia treatments in the 28 day post-experiment levels of red cells, white cells, neutrophils, lymphocytes, segmented leukocytes and macrophages (Table 16). There was also no significant difference between the lavage samples collected immediately after the steers left the climate chamber and those collected 28 days later (t values ranged from 1.36 to 0.94, p values ranged from 0.19 to 0.77).

Table 16: Results of the bronchial alveolar lavages 28 days post-experiment

Test	control	15 ppm	30 ppm	45 ppm	Effect of ammonia
Red cells (x10E6/l)	17.5 ± 17.5	0.0 ± 0.0	5.0 ± 2.9	0.0 ± 0.0	F = 0.85, *p* = 0.49
Total white cells (x10E6/l)	307.5 ± 75.9	267.5 ± 72.3	95.0 ± 24.0	172.5 ± 39.0	F = 0.85, *p* = 0.49
Neutrophils (%)	7.0 ± 1.8	2.5 ± 0.7	2.3 ± 0.6	9.8 ± 5.0	F = 1.74, *p* = 0.21
Lymphocytes (x10E6/l)	2.0 ± 0.7	4.5 ± 2.5	1.5 ± 0.4	1.0 ± 0.6	F = 0.50, *p* = 0.69
Segmented leukocytes (x10E6/l)	4.3 ± 3.3	2.5 ± 1.9	0.5 ± 0.3	5.0 ± 1.8	F = 0.19, *p* = 0.89
Macrophages (x10E6/l)	88.8 ± 3.1	95.0 ± 2.4	97.5 ± 0.9	85.3 ± 6.5	F = 0.34, *p* = 0.79

Weight (0 days post experiment) – As shown in Figure 37, there was no significant difference in the pre-experiment and day 13 post-experiment weights of the steers for all four ammonia treatments ($F_{3,20}$ = 1.16, p = 0.35). There was no significant change in live weight following the 12 days in the climate chamber (t_{23} = -0.48, p = 0.64).

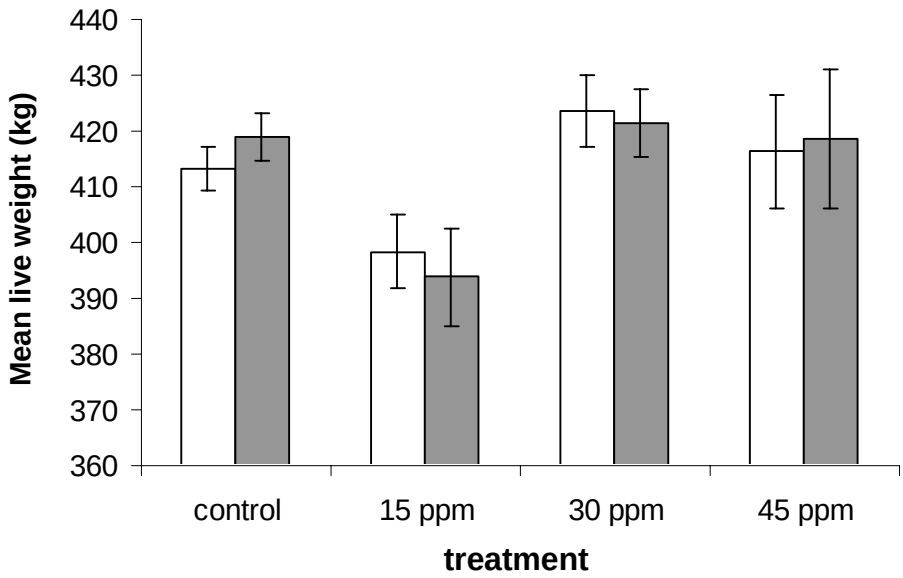


Figure 37: Steer live weight in the four ammonia treatments. Steer live weights did not differ significantly between pre-experiment (white columns) and day 13 (grey columns) measurements. Ammonia concentration also had no significant effect on steer live weight.

Weight (28 days post-experiment) – As shown in Figure 38, there was no significant difference in steer live weight change between the four ammonia treatments on day 41 (28 days post-experiment) ($F_{3,12}$ = 0.18, p = 0.91). Therefore, the results were combined and analysed for overall differences between pre-experiment, day 13 and day 41 post-experiment weights.

Steer live weight 28 days after their departure from the climate room (411 ± 7 kg) was significantly higher than their pre-experiment (390 ± 7 kg) (t_{15} = 4.53, p = 0.01) and day 0 post experiment weight (392 ± 6 kg) (t_{15} = 5.04, p = 0.01).

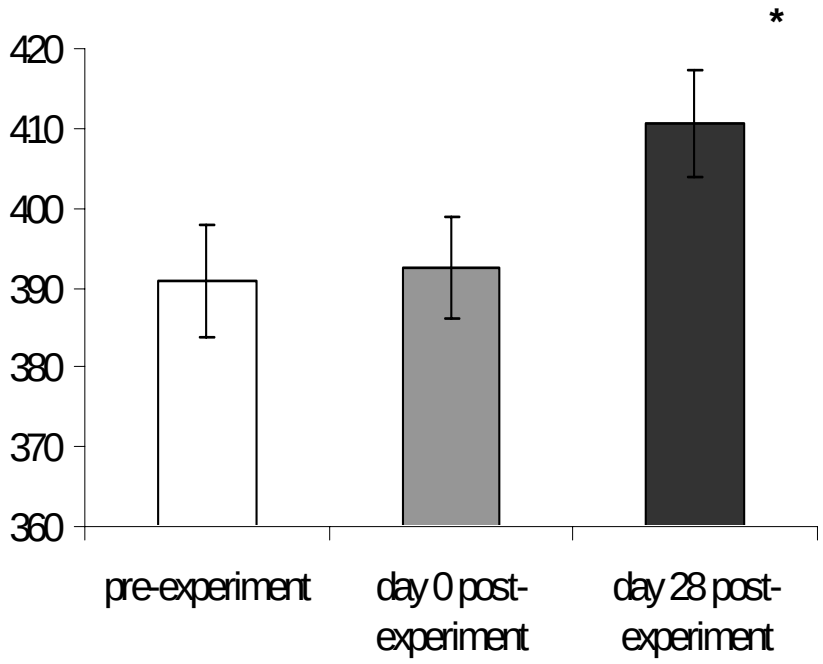


Figure 38: Steer live weight in the four ammonia treatments 28 days post-experiment. There was no effect of ammonia on live weight, but there was an overall increase in live weight on day 28 post-experiment (dark grey column) compared to pre-experiment (white column) and day 0 post-experiment (light grey) live weights as indicated by the asterisk (p < 0.05).

Feed consumption – There was a significant effect of ammonia on feed consumption ($F_{3,234}$ = 4.25, p = 0.006). Post hoc analysis revealed that the steers in the 15 ppm (5.5 ± 0.2 kg) ammonia treatment consumed less feed than those in the 45 ppm (6.5 ± 0.1 kg) (t_{71} = -4.60, p = 0.003) ammonia treatments (Figure 39). Feed consumption in 15 ppm did not differ significantly from the control (6.4 ± 0.1 kg) (t_{71} = -1.87, p = 0.242) or 30 ppm (6.4 ± 0.1 kg) (t_{71} = 2.56, p = 0.111). Feed ratio equated to 1.55 % of the body weight in the control, 1.38 % in 15 ppm, 1.52 % in 30 ppm and 1.56 % in 45 ppm.

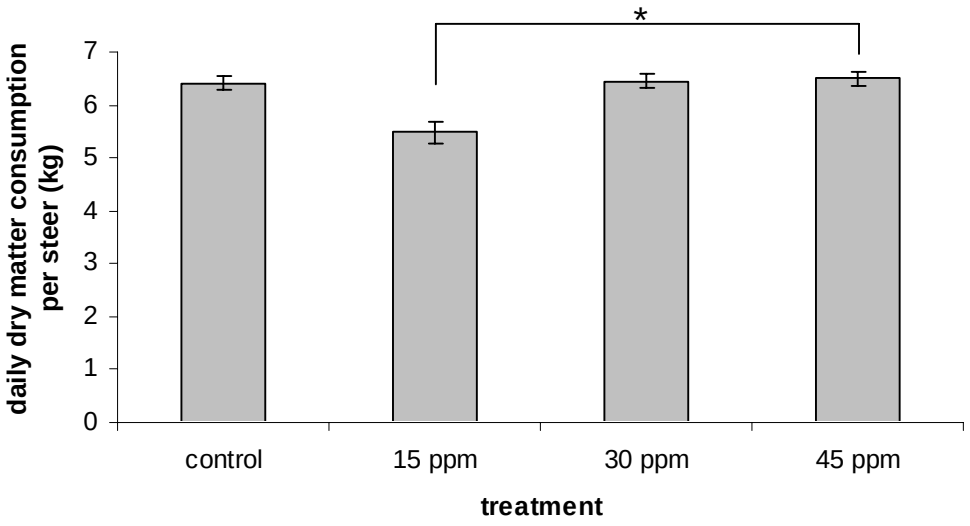


Figure 39: Mean dry matter consumed daily per steer in the four ammonia treatments. More dry matter was consumed in the 45 ppm ammonia treatment than in the 15 ppm ammonia treatment, as indicated by the asterisk ($p < 0.05$).

The level of feed consumption did not change during the 12 days in the climate chambers ($F_{11,234} = 1.48$, $p = 0.14$).

Water consumption – There was a significant change in water consumption during the 12 days the steers were in the climate chambers ($F_{11,273} = 2.12$, $p = 0.02$) (Fig. 40).

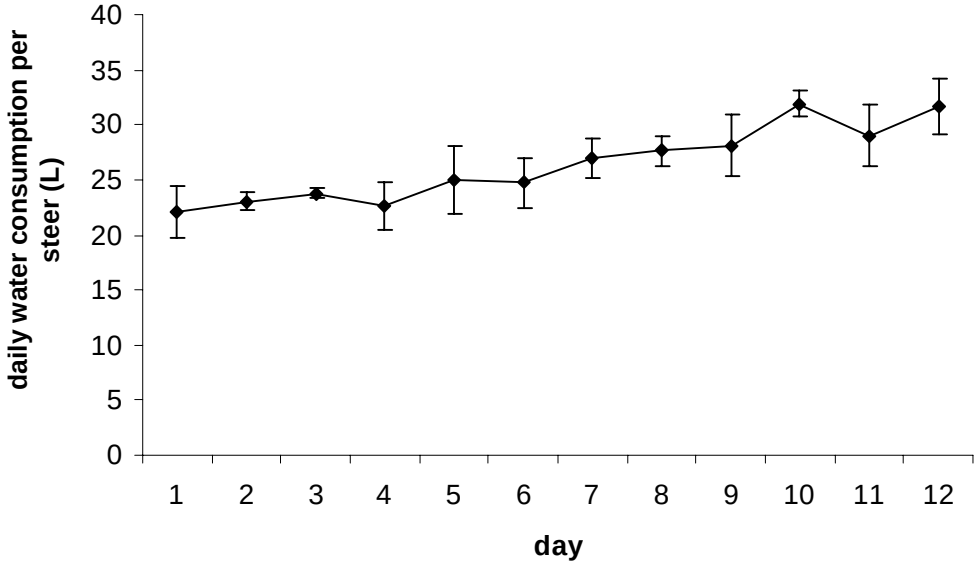


Figure 40: Water consumed by the steers during the 12 days in the climate chambers. Water consumption increased over time.

Ammonia had no significant effect on water consumption (control: 27.5 ± 1.8 L; 15 ppm: 24.7 ± 0.9 L; 30 ppm: 27.9 ± 1.6 L; 45 ppm: 26.4 ± 1.0 L) ($F_{3,273} = 1.60$, $p = 0.19$).

Standing/Lying – There was a significant difference between the four ammonia treatments in the proportion of time the cattle spent standing and, conversely, lying (ANOVA: $F_{3,234} = 3.87$, $p =$

0.03) (Fig. 41). Cattle spent less time standing in the control (64.2 ± 3.2 %) as compared to the 15 ppm (74.5 ± 3.6 %) (*t*-test: t_{14} = -2.69, p = 0.02) and the 30 ppm (76.5 ± 2.6 %) (t_{14} = -3.00, p = 0.01) treatment. There was, however, no significant difference between the control and the 45 ppm (74.4 ± 3.0 %) (t_{14} = -1.39, p = 0.19) treatments.

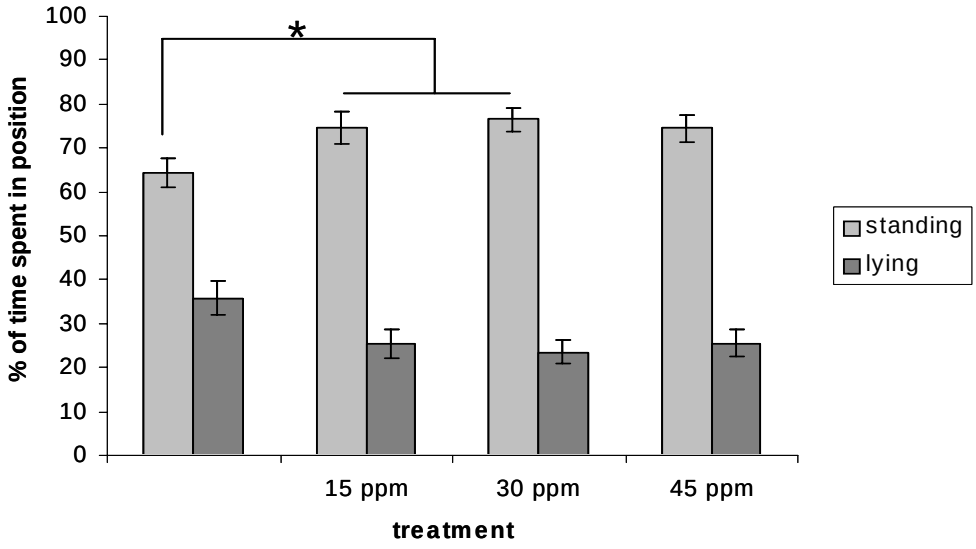


Figure 41: Percentage of time the steers spent standing and lying spent in the four ammonia treatments. More time was spent standing and, conversely less time lying, in the 15 and 30 ppm ammonia than in the control, as indicated by the asterisk (p < 0.05), but not in the 45 ppm treatment. Faecal material was present on the floor of the 15, 30 and 45 ppm treatments.

The percentage of time spent standing changed during the 12 days the steers were in the climate chamber ($F_{11,234}$ = 1.99, p = 0.03). As shown in Figure 42, time spent standing decreased from 87 % on day 1 to approximately 70 % on day 12. Conversely, time spent lying increased from 13 % to 30 % over the same period.

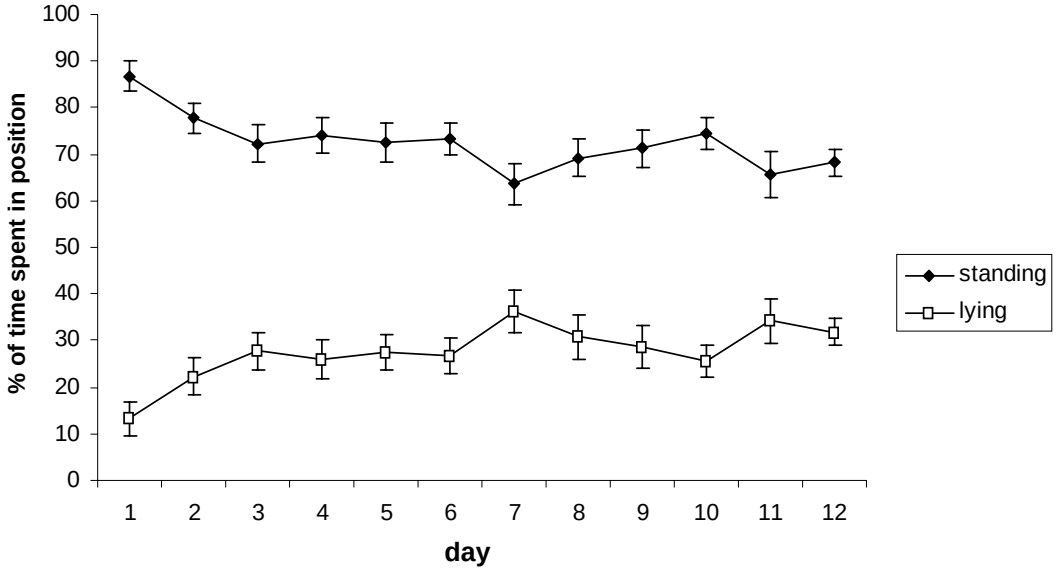
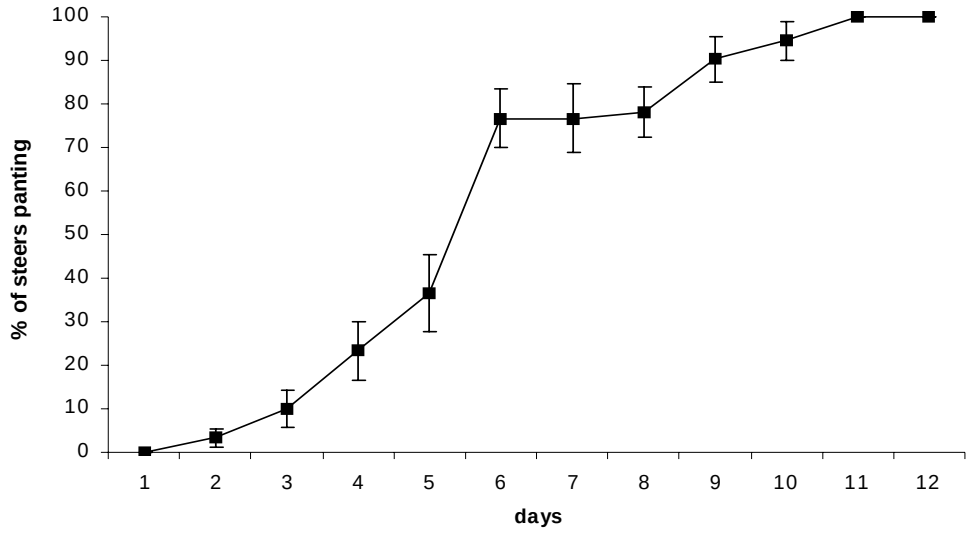


Figure 42: Percentage of time the steers spent standing and lying during the 12 days in the climate chamber. Time spent standing decreased and, conversely, time spent lying increased during the 12 days the steers were in the climate chambers.

Panting – The different ammonia treatments had no significant effect on the proportion of steers panting (control: 60.8 ± 3.5 %; 15 ppm: 53.7 ± 4.2 %; 30 ppm: 59.7 ± 6.3 %; 45 ppm: 55.1 ± 3.7 %) ($F_{3,234}$ = 0.89, p = 0.47). The percentage of steers panting increased significantly during the 12 days they were in the climate chamber ($F_{11,234}$ = 1.99, p = 0.03). As shown in Figure 43, the percentage of steers panting increased two-fold on day 6 when the dry bulb temperature increased from 30°C to 31°C and the wet bulb temperature remained at 26°C. All steers were panting by day 11.



DBT °C	26	27	28	29	30	31	31	31	32	33	34	35
WBT °C	23	24	25	25	26	26	27	27	27	28	28	29

Figure 43: Percentage of steers panting and the corresponding dry (DBT) and wet bulb temperatures (WBT) during the 12 days in the climate chamber. The percentage of steers panting increased significantly during the 12 days in the climate chamber. The greatest increase in panting occurred on day 6, when the DBT increased from 30 to 31°C and the WBT remained at 26°C.

Coughing – As shown in Figure 44, the frequency of coughing in all ammonia treatments was very low. Nonetheless, there were significant differences between the various ammonia treatments (Moods' median test: Chi square =12, df = 3, p = 0.007). Post hoc analysis revealed that there was significantly more coughing in the 30 ppm (0.38 ± 0.15) and 45 ppm (0.42 ± 0.21) ammonia treatments than in the control (0.05 ± 0.02) and 15 ppm (0.08 ± 0.02) ammonia treatments (Mann-Whitney test: W ranged from 22.0 to 26.0, p from 0.045 to 0.008).

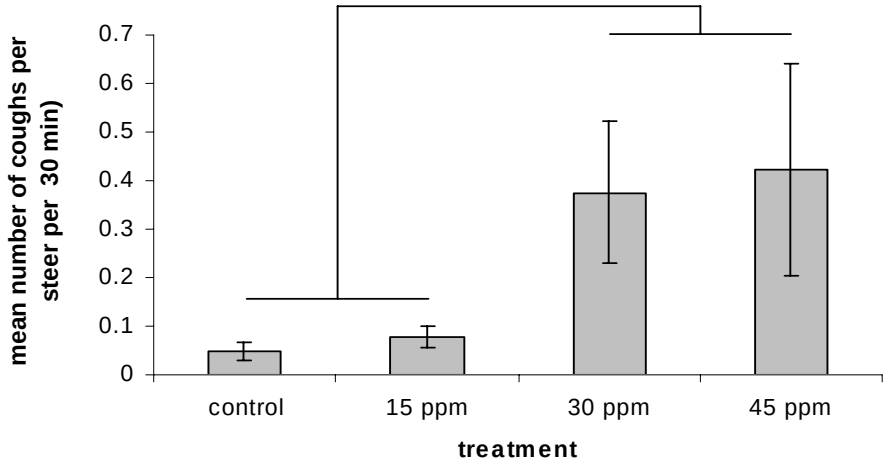


Figure 44: Coughing in the four ammonia treatments. There was significantly more coughing in the 30 ppm and 45 ppm ammonia than in the control and 15 ppm ammonia as indicated by the asterisk (p<0.05).

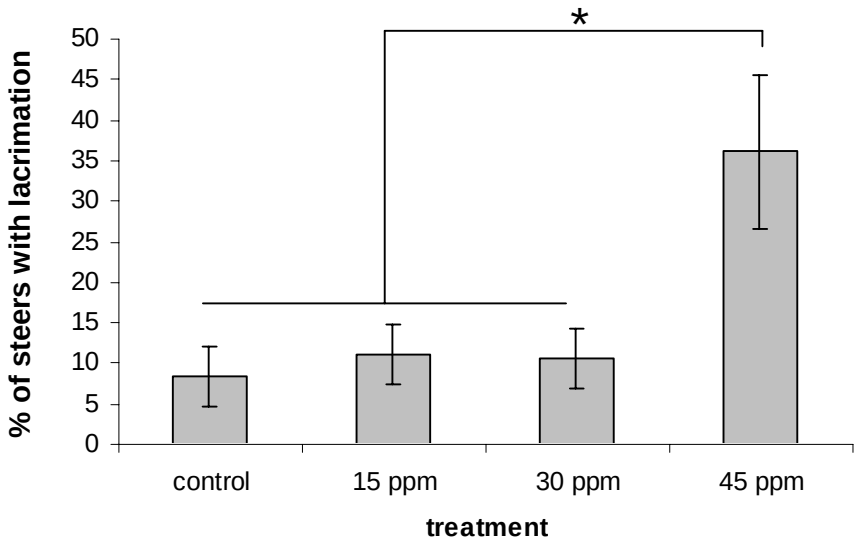


Figure 45: Percentage of steers with lacrimation in the four ammonia treatments. As indicated by the asterisk ($p < 0.05$), the 45 ppm ammonia had the highest percentage of steers with lacrimation. There was no difference between the control, 15 and 30 ppm NH_3.

Lacrimation – Ammonia concentration had a significant effect on lacrimation ($F_{3,238} = 22.74$, $p = 0.001$). As shown in Figure 45, significantly more steers had lacrimation in the 45 ppm (36.1 ± 9.5 %) ammonia treatment than in any other ammonia treatment (control: 8.3 ± 3.7 %; 15 ppm: 11.1 ± 3.7 %; 30 ppm: 10.6 ± 3.8 %) (*t*-test: *t* values ranged from -7.40 to -6.24, $p = 0.001$).

There was no change in lacrimation over the 12 days of the trial ($F_{11,238} = 1.31$, $p = 0.22$).

Nasal secretion - There was a significant effect of ammonia on the percentage of steers with nasal secretions ($F_{3,238}$ = 21.69, p = 0.001) (Figure 46). The percentage of steers with nasal secretions was significantly higher in the 45 ppm (41.2 ± 5.8 %) ammonia than all other ammonia treatments (*t*-test: t values ranged from -7.92 to -6.24, p = 0.001). No difference was found between the 15 ppm (22.7 ± 2.7 %) and the 30 ppm ammonia (20.8 ± 6.4 %) (*t*-test = t_{14} = 0.60, p = 0.93); however, both treatments had a significantly higher percentage of steers nasal secretions than the control (8.3 ± 2.7 %) (t values ranged from -2.90 to -3.61, p values ranged from 0.02 to 0.002).

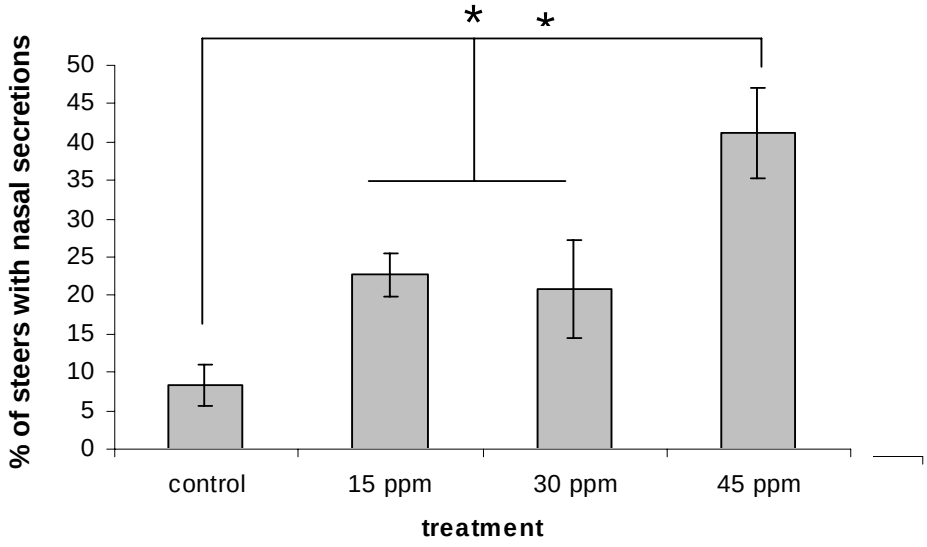


Figure 46: Percentage steers with nasal secretions in the four ammonia treatments. As indicated by the asterisk (p < 0.05), the 45 ppm ammonia had the greatest percentage of steers with nasal secretions, while the control had the lowest percentage of steers with nasal secretions. There was no difference between the 15 and 30 ppm ammonia.

The percentage of steers with nasal secretions increased significantly during the 12 days the steers were in the climate chamber from 8 % to 40 % ($F_{11,238}$ = 2.36, p = 0.01) (Figure 47).

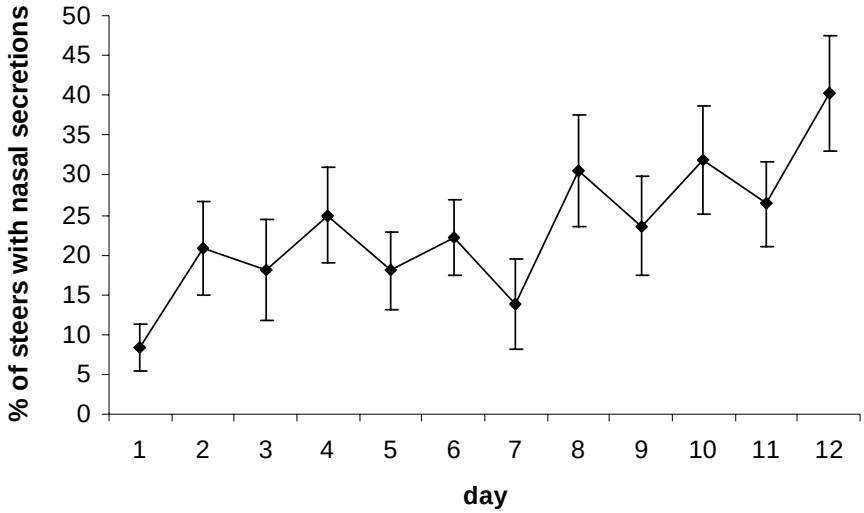


Figure 47: Percentage of steers with nasal secretions during the 12 days in the climate chamber. The percentage of steers with nasal secretions increased significantly during the 12 days the steers were in the climate chamber.

Other Behaviours – Neither ammonia treatment nor time spent in the climate chamber significantly affected the number of bouts of licking, scratching, locomotion and time spent ruminating and with the head down (Table 17).

Table 17. Statistical values of behaviours where no significant effect of ammonia or time in the chamber were found.

Behaviour	Control	15 ppm	30 ppm	45 ppm	Ammonia treatment	Trial Duration
Licking	3.8 ± 0.6	3.4 ± 0.7	4.3 ± 0.5	3.5 ± 1.0	F = 1.82, p = 0.190	F = 1.58, p = 0.110
Scratching	1.8 ± 0.3	2.2 ± 0.3	2.3 ± 0.2	2.1 ± 0.4	F = 0.07, p = 0.970	F = 1.20, p = 0.290
Locomotion	7.8 ± 1.4	11.6 ± 1.5	8.8 ± 1.0	8.1 ± 1.3	F = 0.51, p = 0.680	F = 0.84, p = 0.600
Ruminating (%)	2.3 ± 0.1	2.1 ± 0.7	2.6 ± 0.1	3.3 ± 0.9	F = 0.39, p = 0.760	F =1.02, p = 0.430
Head down (%)	26.6 ± 3.1	26.3 ± 2.6	24.9 ± 6.0	26.3 ± 2.6	F = 2.73, p = 0.080	F = 0.82, p = 0.620

Tropical breed content – A Pearson correlation was used to determine whether there was any relationship between the measured haematological and BAL parameters and the tropical breed content. Of the various parameters measured, tropical breed content was positively correlated with blood haemoglobin (r^2 = 0.05, p = 0.03) and blood neutrophils (r^2 = 0.10, p = 0.002); however, in both instances the strength of the relationship was weak (Figure 48). No other correlations were found (r^2 values ranged from -0.000001 to 0.07, p values ranged from 0.07 to 0.99).

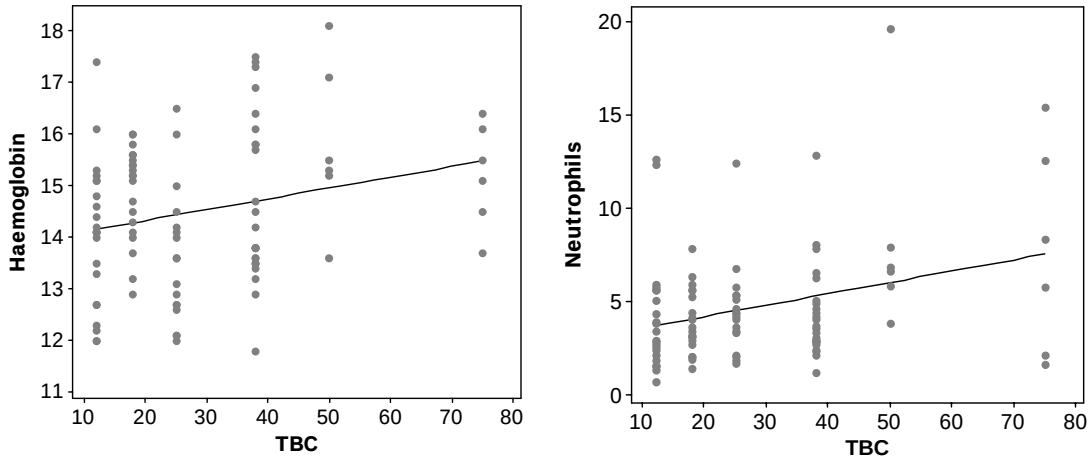


Figure 48: Correlation between tropical breed content (TBC %) and haemoglobin (left hand graph) and neutrophils (right hand graph)

4.5.2 Sheep

Haematology (0 days post-experiment) – As presented in Table 18, ammonia treatment had no significant effect on any of the haematological parameters measured in the wethers. There was however a significant effect of the experiment on four haematological parameters. Compared to pre-experiment levels, there was a significant decrease in post-experiment levels of neutrophil (t_{45} = -2.22, p = 0.03) and mean cell haemoglobin concentration (MCHC: t_{45} = -2.55, p = 0.01), and a significant increase in post-experiment levels of haemoglobin (t-test: t_{45} = 2.72, p = 0.009) and packed cell volume (t_{45} = 4.26, p = 0.001) as shown in Figure 49. All parameters were within the normal range.

Table 18: Haematological tests conducted on wethers and effect of ammonia pre-experiment and on day 13

Test	Pre-experiment	control	15 ppm	30 ppm	45 ppm	Normal range	Effect of ammonia
Haemoglobin (g/dl)	10.6 ± 0.3	10.9 ± 0.4	11.7 ± 0.6	11.9 ± 0.4	10.7 ± 0.6	9.0-15.0	F = 1.71, p = 0.18
Red cell count (x10E12/1)	11.4 ± 2.1	9.9 ± 0.4	9.6 ± 0.6	11.0 ± 0.4	10.2 ± 0.6	9.0-15.0	F = 2.55, p = 0.07
Packed cell volume (l/l)	0.32 ± 0.01	0.36 ± 0.02	0.36 ± 0.02	0.39 ± 0.01	0.35 ± 0.02	0.27-0.45	F = 2.28, p = 0.10
MCHC (g/dl)	32.7 ± 0.3	31.2 ± 1.0	33.0 ± 0.6	30.8 ± 0.8	30.8 ± 0.7	31.0-36.0	F = 0.83, p = 0.49
MCH (pg)	11.3 ± 0.2	11.1 ± 0.3	12.2 ± 0.4	10.8 ± 0.4	10.6 ± 0.3	8.0-12.0	F = 1.92, p = 0.15
MCV (fl)	34.8 ± 0.8	35.9 ± 1.0	38.1 ± 1.2	35.4 ± 1.1	34.4 ± 0.9	28.0-40.0	F = 0.82, p = 0.49
Platelet count (x10E9/l)	268.9 ± 14.2	291.8 ± 46.9	296.2 ± 30.4	314.8 ± 62.8	300.4 ± 28.9	250.0-750.0	F = 0.54, p = 0.66
MPV (fl)	9.1 ± 0.1	9.3 ± 0.1	9.0 ± 0.2	9.2 ± 0.1	9.0 ± 0.1	unknown	F = 2.37, p = 0.09
PCT (fl)	0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	unknown	F = 0.40, p = 0.76
White cell count (x10E9/l)	6.1 ± 0.4	6.1 ± 0.4	5.4 ± 0.4	6.4 ± 0.4	5.3 ± 0.4	4.0-12.0	F = 1.17, p = 0.33
Neutrophil (x10E9/l)	3.4 ± 0.2	2.9 ± 0.2	2.5 ± 0.4	3.2 ± 0.4	2.7 ± 0.4	0.7-6.0	F = 0.19, p = 0.90
Lymphocytes (x10E9/l)	2.3 ± 0.2	2.6 ± 0.3	2.6 ± 0.4	2.8 ± 0.2	2.2 ± 0.3	2.0-9.0	F = 0.78, p = 0.51
Monocytes (x10E9/l)	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.0-0.8	F = 0.84, p = 0.48
Eosinophils (x10E9/l)	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.0-1.0	F = 0.44, p = 0.72
Cortisol (nmol/l)	112.4 ± 7.3	93.4 ± 9.7	94.7 ± 19.0	143.4 ± 11.8	117.5 ± 66.6	unknown	F = 0.73, p = 0.54
Urea (mmol/l)	7.5 ± 0.2	8.0 ± 0.3	7.5 ± 0.5	7.9 ± 0.5	7.4 ± 0.4	3.0-8.0	F = 0.43, p = 0.74

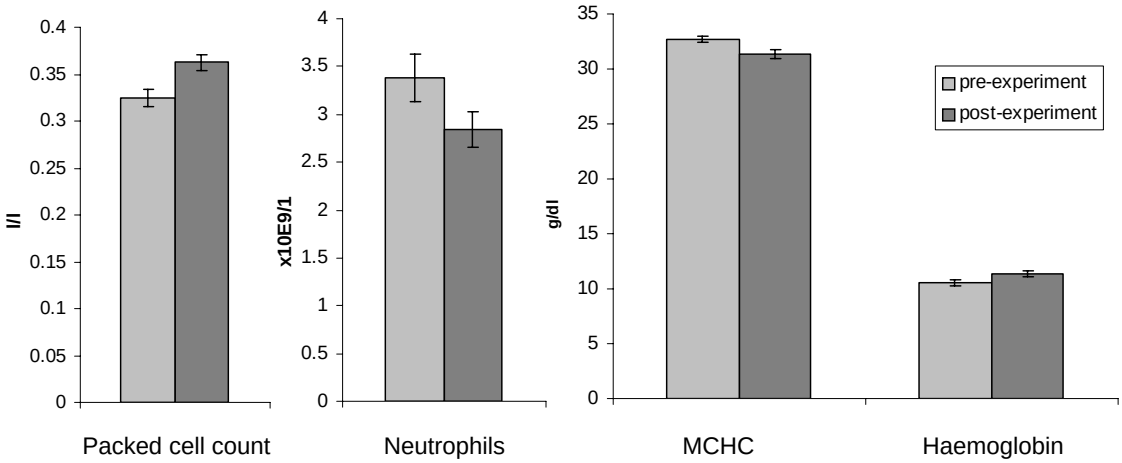


Figure 49: Changes in post-experiment haematological parameters of the wethers. Compared to pre-experiment levels, there was a significant decrease in post-experiment levels of neutrophil and MCHC, and a significant increase in post-experiment levels of haemoglobin and packed cell count (p<0.05). All parameters were within the normal range.

Haematology (28 days post-experiment) – There was a significant difference between ammonia treatments in levels of MCV ($F_{3,11}$ = 4.21, p = 0.046) and urea ($F_{3,11}$ = 6.65, p = 0.014) (Table 19). However, post-hoc analyses were unable to reveal where the specific differences between the ammonia treatments lay for MCV (t values ranged from -2.82 to 0.12, p values ranged from 0.09 to 0.99). Post-hoc analysis were able to identify specific differences for urea, with levels in the 15 ppm and 30 ppm ammonia treatment being significantly higher than those in the control and 45 ppm treatment (t values ranged from 3.36 to 3.15, p values ranged from 0.040 to 0.054). No other significant effects of ammonia were found.

Table 19: Haematological tests conducted on wethers 28 days post-experiment

Test	Control	15 ppm	30 ppm	45 ppm	Effect of ammonia
Haemoglobin (g/dl)	10.0 ± 1.2	10.9 ± 1.4	13.2 ± 1.6	10.4 ± 0.1	F = 0.29, *p* = 0.83
Red cell count (x10E12/1)	10.5 ± 0.6	8.8 ± 0.6	10.8 ± 1.2	10.2 ± 0.5	F = 0.83, *p* =0.51
Packed cell volume (l/l)	0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	F = 0.53, *p* =0.67
MCHC (g/dl)	28.0 ± 0.1	34.7 ± 0.9	34.0 ± 1.0	30.3 ± 1.5	F = 3.66, *p* = 0.06
MCH (pg)	9.5 ± 0.5	12.3 ± 0.9	12.3 ± 0.3	10.0 ± 0.6	F = 3.08, *p* = 0.09
MCV (fl)	33.5 ± 2.5	35.3 ± 2.4	35.8 ± 0.5	33.7 ± 0.3	F = 4.21, *p* = 0.05
Platelet count (x10E9/l)	331.5 ± 109.5	263.7 ± 72.9	222.3 ± 52.8	337.0 ± 4.2	F = 1.03, *p* = 0.42
MPV (fl)	9.1 ± 0.1	8.9 ± 0.2	9.3 ± 0.2	9.1 ± 0.3	F = 0.20, *p* = 0.89
PCT (fl)	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	F = 1.17, *p* = 0.38
White cell count (x10E9/l)	5.9 ± 2.8	5.0 ± 0.2	8.9 ± 1.9	4.3 ± 0.9	F =2.75, *p* = 0.11
Neutrophil (x10E9/l)	2.6 ± 1.6	1.9 ± 0.4	5.7 ± 1.6	1.7 ± 0.2	F =2.21, *p* = 0.16
Lymphocytes (x10E9/l)	2.5 ± 0.7	2.5 ± 0.3	2.8 ± 0.4	2.0 ± 0.5	F =0.54, *p* = 0.66
Monocytes (x10E9/l)	1.9 ± 1.7	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	F =1.95, *p* = 0.20
Eosinophils (x10E9/l)	0.5 ± 0.4	0.1 ± 0.1	0.1 ± 0.1	0.3 ± 0.1	F = 0.71, *p* = 0.57
Cortisol (nmol/l)	153.5 ± 0.24.5	167.7 ± 19.2	166.0 ± 28.5	137.0 ± 9.5	F =0.38, *p* = 0.77
Urea (mmol/l)	5.4 ± 0.3	8.3 ± 0.8	9.7 ± 0.4	6.4 ± 0.4	F = 6.65, *p* = 0.01

There was no significant difference between the blood samples collected immediately after the wethers left the climate chamber and those collected 28 days later (*t* values ranged from -1.48 to 0.05, *p* values ranged from 0.16 to 0.96).

Transtracheal aspiration (28 days post-experiment) - Chi-square analysis revealed a significant (*p* = 0.001) difference in macrophage activity between the four ammonia treatments. As shown in Figure 50, the 15 ppm ammonia treatment had the highest percentage of wethers with “very high” macrophage activity, while the 45 ppm ammonia treatment had the highest percentage of wethers with “high” activity macrophages. There was a relatively even spread of all levels of macrophage activity in the 30 ppm ammonia treatment, while in the control, macrophage activity tended to be lowest.

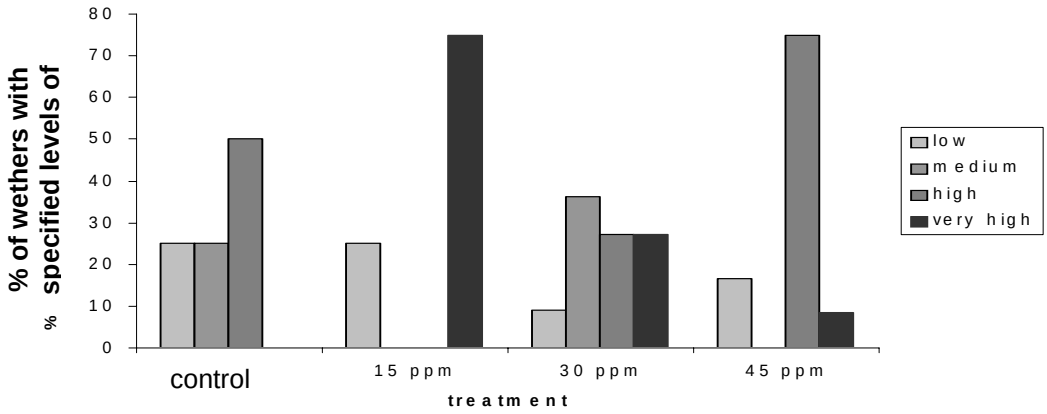


Figure 50: Percentage of wethers with specified levels of macrophage activity. Macrophage activity was variable and showed no clear trend between treatments

Ammonia treatment had no significant affect on the red cell, white cell, neutrophils, lymphocytes, segmented leukocytes or macrophage counts (Table 20).

Table 20: Results of the day 28 post-experiment transtracheal aspirations.

Test	control	15 ppm	30 ppm	45 ppm	Effect of ammonia
Red cells	2026.7 ± 1675.8	2390.0 ± 1242	4230 ± 3399	7225 ± 5170	F = 0.43, *p* = 0.73
Total white cells	75.6 ± 16.1	90.0 ± 27.4	102.5 ± 19.1	98.3 ± 24.8	F = 0.27, *p* = 0.85
Neutrophils	2.9 ± 0.7	5.57 ± 1.97	3.9 ± 1.1	4.1 ± 1.4	F = 0.33, *p* = 0.80
Lymphocytes	3.0 ± 1.7	4.7 ± 2.3	1.2 ± 0.5	2.4 ± 0.7	F = 0.89, *p* = 0.46
Segmented leukocytes	2.1 ± 0.9	0.9 ± 0.4	3.6 ± 1.7	0.9 ± 0.3	F = 1.61, *p* = 0.20
Macrophages	92.0 ± 2.2	87.7 ± 2.1	90.1 ± 1.4	92.7 ±1.5	F = 1.40, *p* = 0.26

Transtracheal aspiration (28 days post-experiment) – Macrophage activity was “medium” to “low” in all four ammonia treatments. Statistical analysis was not possible due to the small sample size (n = 2-4 per ammonia treatment) (Fig. 51).

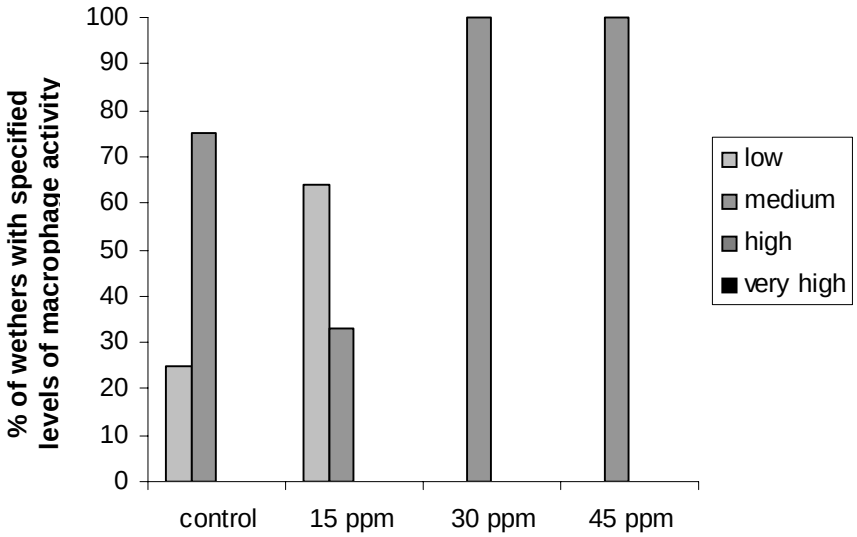


Figure 51: Percentage of wethers with specified levels of macrophage activity 28 days post-experiment. Macrophage activity was medium to low in all ammonia treatments.

There was no difference between ammonia treatments in the 28 day post-experiment levels of red cells, white cells, neutrophils, lymphocytes, segmented leukocytes or macrophages (Table 21). There was also no significant difference between the lavage samples collected immediately after the wethers left the climate chamber and those collected 28 days later (*t* values ranged from 1.57 to 0.07, *p* values ranged from 0.15 to 0.95).

Table 21: Results of the transtracheal aspirations 28 days post-experiment.

Test	control	15 ppm	30 ppm	45 ppm	Effect of ammonia
Red cells (x10E6/l)	2383.3 ± 1448.1	2323.3 ± 1748.5	400.0 ± 50.0	2812.5 ± 1504.8	F = 0.39, *p* = 0.76
Total white cells (x10E6/l)	80.0 ± 36.1	150.0 ± 25.2	205.0 ± 75.0	80.0 ± 24.8	F = 2.16, *p* = 0.17
Neutrophils (%)	5.0 ± 2.1	4.3 ± 2.8	1.0 ± 1.0	5.7 ± 1.4	F = 1.29, *p* = 0.35
Lymphocytes (x10E6/l)	4.3 ± 0.9	3.3 ± 1.8	3.0 ± 1.0	2.0 ± 0.5	F = 0.37, *p* = 0.77
Segmented leukocytes (x10E6/l)	94.0 ± 1.3	2.0 ± 1.9	0.5 ± 0.5	0.3 ± 0.3	F = 1.18, *p* = 0.38
Macrophages (x10E6/l)	95.0 ± 2.1	93.0 ± 2.6	98.5 ± 0.5	94.0 ± 1.3	F = 0.75, *p* = 0.55

Weight (0 days post-experiment) – Live weight differed significantly between the ammonia treatments ($F_{3,20}$ = 3.47, *p* = 0.036) (Fig. 52). Post-hoc analysis revealed a decrease in the live weight of the wethers following exposure to the 30 ppm (t_5 = -3.80, *p* = 0.010) and the 45 ppm ammonia treatments (t_5 = -6.33, *p* = 0.001); the decreases equated to an 8 % loss and a 6 % loss

in body weight respectively. Body weight did not change significantly in the control (t_5 = -1.42, p = 0.210) or the 15 ppm (t_5 = -1.09, p = 0.330) ammonia treatments.

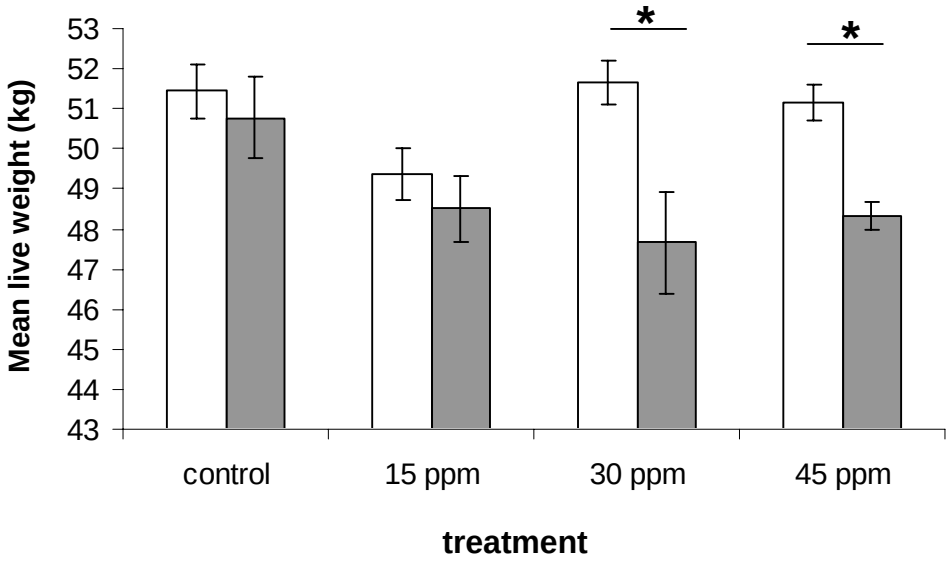


Figure 52: Wether live weight in the four ammonia treatments. As indicated by the asterisk (p < 0.05), live weight was significant lower at 0 day post-experiment (grey column) compared to pre-experiment (white column) live weight in the 30 ppm and 45 ppm ammonia treatments. Live weight change was not significant in the control and 15 ppm ammonia treatments.

Weight (28 days post-experiment) –There was no significant difference in wether live weight between the four ammonia treatments measured 28 days post-experiment ($F_{3,12}$ = 1.62, p = 0.24) (Fig. 53). Therefore, scores were collapsed across treatment and analysed for overall differences and live weight 28 days post-experiment (50.7 ± 1.4 kg) was found to be significantly higher than weights collected pre-experiment (47.3 ± 1.4 kg) (t_{15} = 4.53, p = 0.01) and 0 days post-experiment (45.9 ± 1.5 kg) (t_{15} = 5.37, p = 0.01).

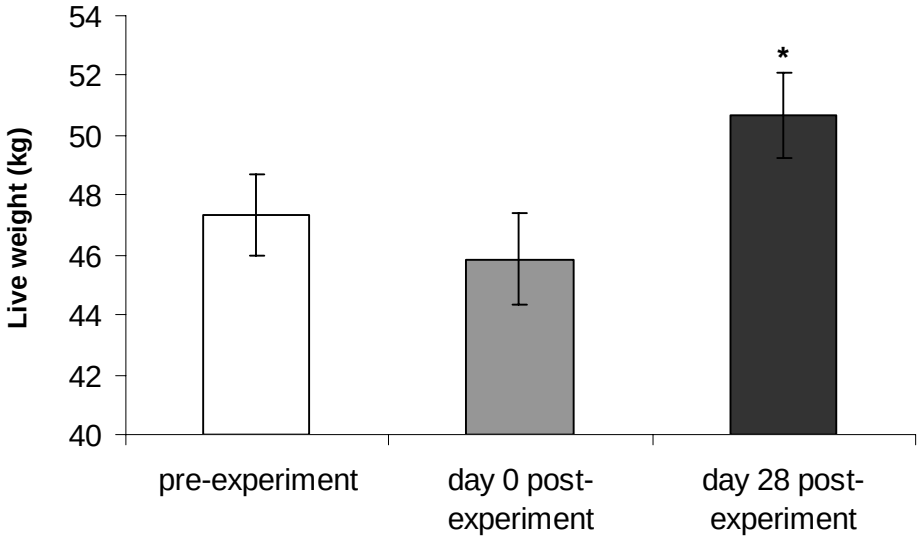


Figure 53: Wether live weight in the four ammonia treatments 28 days post-experiment. There was no effect of ammonia on live weight, but there was an overall increase in live weight on day 28 post-experiment (dark grey column) compared to pre-experiment (white column) and day 0 post-experiment (light grey) live weights

Feed consumption – There was a significant effect of ammonia on feed consumption (control: 0.99 ± 0.02 kg; 15 ppm: 0.82 ± 0.03 %; 30 ppm: 0.71 ± 0.03 %; 45 ppm: 0.67 ± 0.03 %) ($F_{3,273}$ = 2.59, p = 0.05). Post hoc analysis revealed that the wethers in the 45 ppm ammonia treatment consumed less dry matter than those in the control (t_{71} = -2.56, p = 0.05) ammonia treatment (Figure 54). Feed ratio equated to 1.93 % of the body weight in the control, 1.66 % in 15 ppm, 1.38 % in 30 ppm and 1.32 % in 45 ppm.

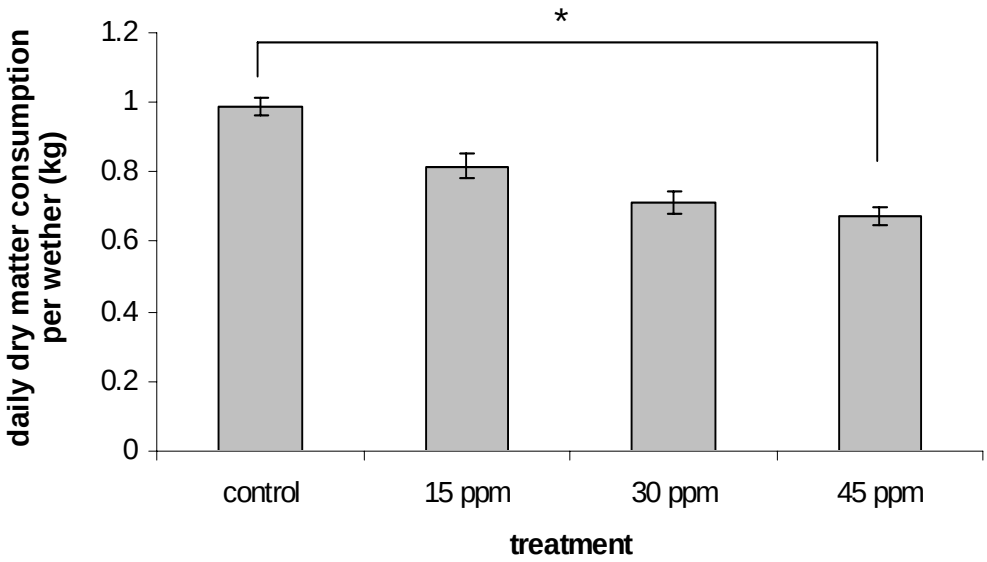


Figure 54: Mean dry matter consumed daily per wether in the four ammonia treatments. Less feed was consumed in the 45 ppm ammonia treatment than in the control treatment, as indicated by the asterisk (p < 0.05).

The level of feed consumption did not change during the 12 days in the climate chambers ($F_{11,234}$ = 1.48, p = 0.14).

Water consumption – There was a significant change in water consumption during the 12 days the wethers were in the climate chambers ($F_{11,273}$ = 2.13, p = 0.019). As shown in Figure 55, there was an overall increase in water consumption over time.

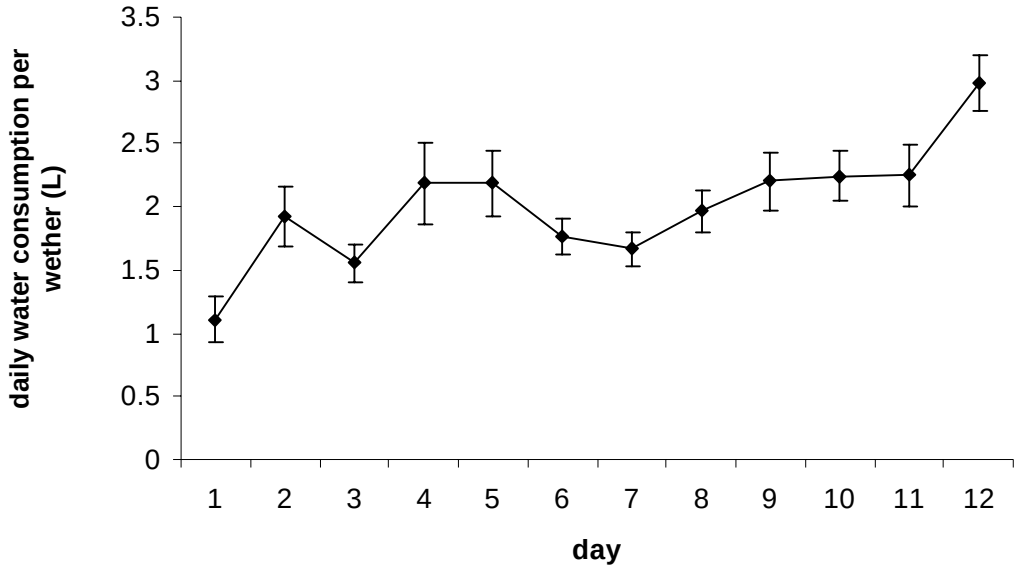


Figure 55: Water consumed by the wethers during the 12 days in the climate chambers. Water consumption increased over time.

There was no significant effect of ammonia on water consumption (control: 2.22 ± 0.16 L; 15 ppm: 2.24 ± 0.12 L; 30 ppm: 2.18 ± 0.16 L; 45 ppm: 1.80 ± 0.21 L) ($F_{3,273}$ = 1.75, p = 0.16).

Ruminating – Ammonia had no significant effect on ruminating (control: 2.46 ± 1.01 %; 15 ppm: 21.22 ± 0.86 %; 30 ppm: 12.52 ± 1.10%; 45 ppm: 14.13 ± 0.68 %) ($F_{3,234}$ = 0.60, p = 0.619). There was a significant change in time spent ruminating during the 12 days the wethers were in the climate chamber ($F_{11,234}$ = 3.31, p = 0.001). As Figure 56 shows, time spent ruminating increased from 12 % to 19 % on the second day in the climate chamber before gradually decreasing to 6 % over the remaining 10 days.

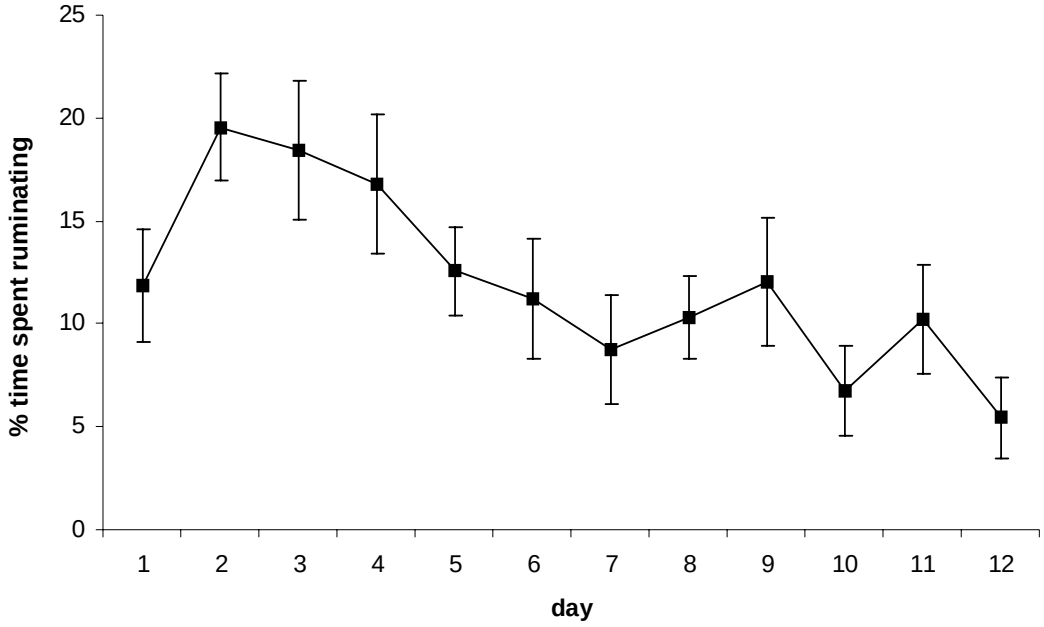


Figure 56: Ruminating during the 12 days the wethers were in the climate chamber. Time spent ruminating peaked on the second day the wethers were in the climate chamber then decreased during the remaining 10 days.

Head lowered - Ammonia treatment had no significant effect on head lowered (control: 15.3 ± 3.6 %; 15 ppm: 17.1 ± 2.7 %; 30 ppm: 12.2 ± 4.0 %; 45 ppm: 17.0 ± 2.4 %) ($F_{3,227}$ = 0.21, p = 0.648).There was a significant increase over the experiment in the percentage of time the wethers spent with their head lowered ($F_{11,227}$ = 4.02, p = 0.001). As Figure 57 shows, time spent with the head lowered increased from 6 % to 17 % during the 12 days in the climate chamber.

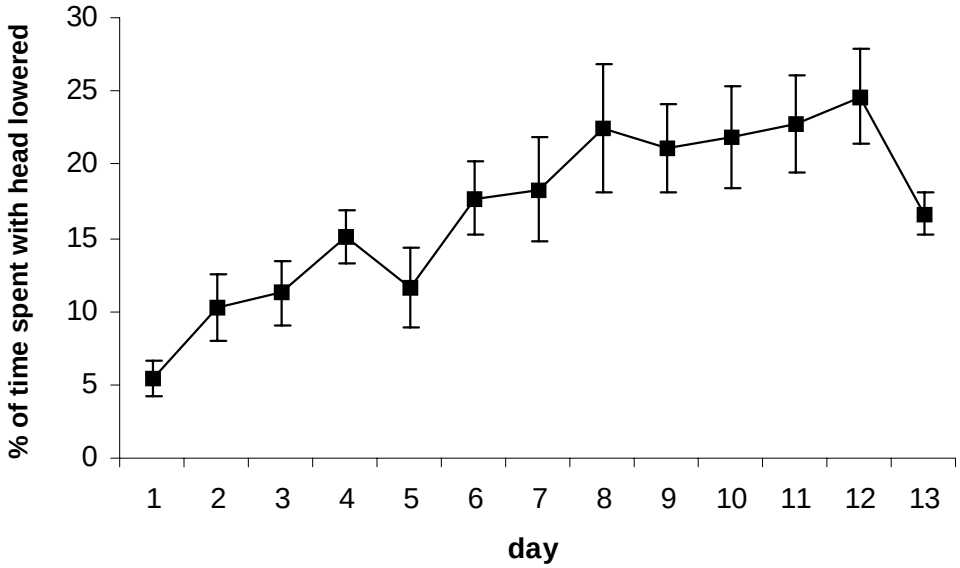


Figure 57: Head lowered during the 12 days the wethers were in the climate chamber. Time spent with the head lowered increased during the 12 days in the climate chamber.

Standing/lying – There was no significant difference in the percentage of time the wethers spent standing (and lying) between the four ammonia treatments (control: 83.4 ± 0.3 %; 15 ppm: 85.1 ± 0.6 %; 30 ppm: 82.7 ± 0.7 %; 45 ppm: 87.4 ± 0.3 %) ($F_{3,227}$ = 0.21, p = 0.648) ($F_{3,234}$ = 1.71, p = 0.21).

There was a significant change in the percentage of time the wethers spent standing and lying during the 12 days in the climate chambers ($F_{11,234}$ = 3.47, p = 0.002). Time spent standing decreased from 93 % to 80 % over the 12 days while time spent lying increased by the same margin (Fig 58).

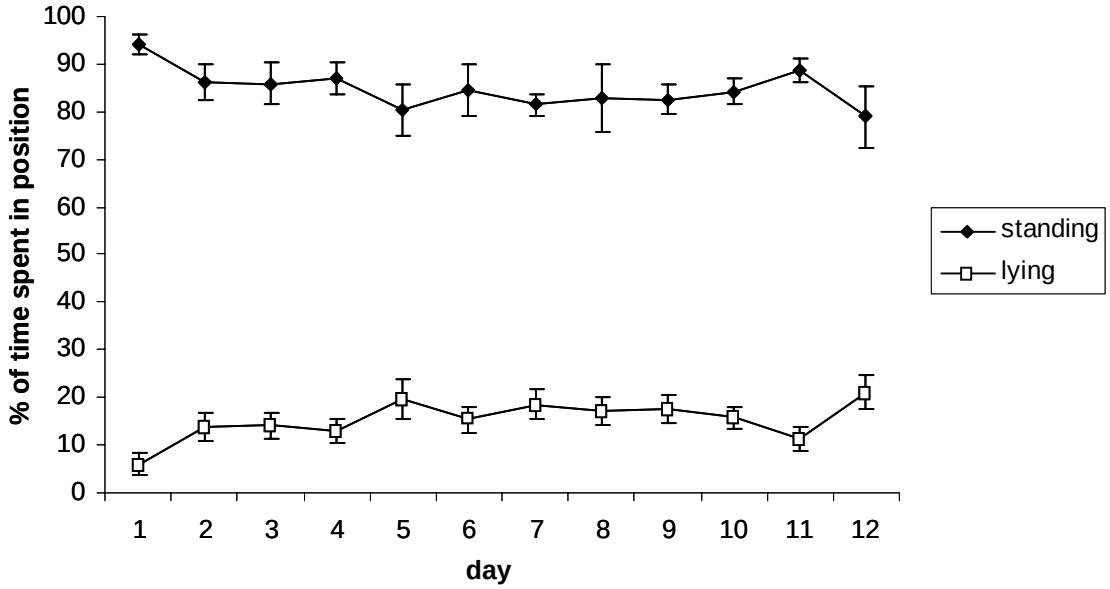


Figure 58: Percentage of time the wethers spent standing and lying during the 12 days in the climate chamber. Time spent standing decreased and, conversely, time spent lying increased during the 12 days the wethers were in the climate chambers.

Panting – The percentage of wethers panting differed between the four ammonia treatments ($F_{3,234}$ = 3.24, p = 0.045). There was a trend for more wethers panting in the 30 ppm (74.9 ± 5.4 %) ammonia treatment than in the 45 ppm treatment (57.9 ± 3.4 %) (Tukey's: t_5 = -2.71, p = 0.061) (Fig 59). No other significant differences were found between the four ammonia treatments control (58.9 ± 4.4 %) 15 ppm: 68.6 ± 4.4 %; 45 ppm: (t values ranged from -1.91 to 0.24, p values ranged from 0.10 to 0.99).

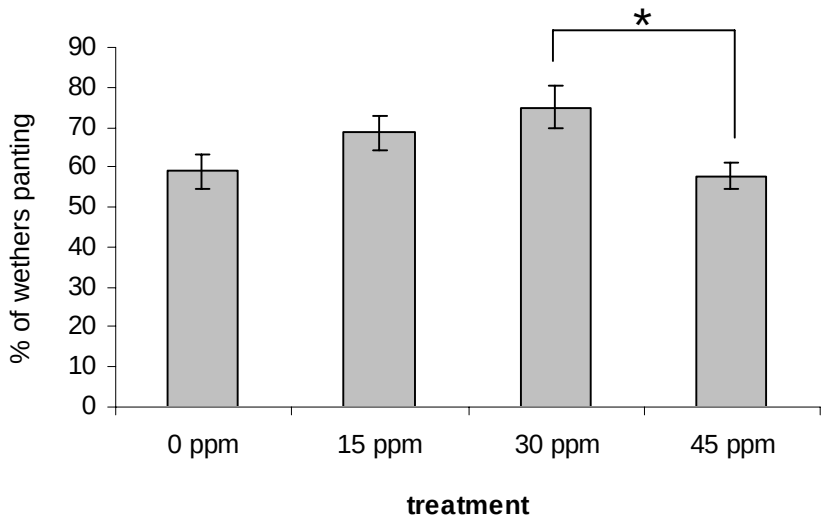
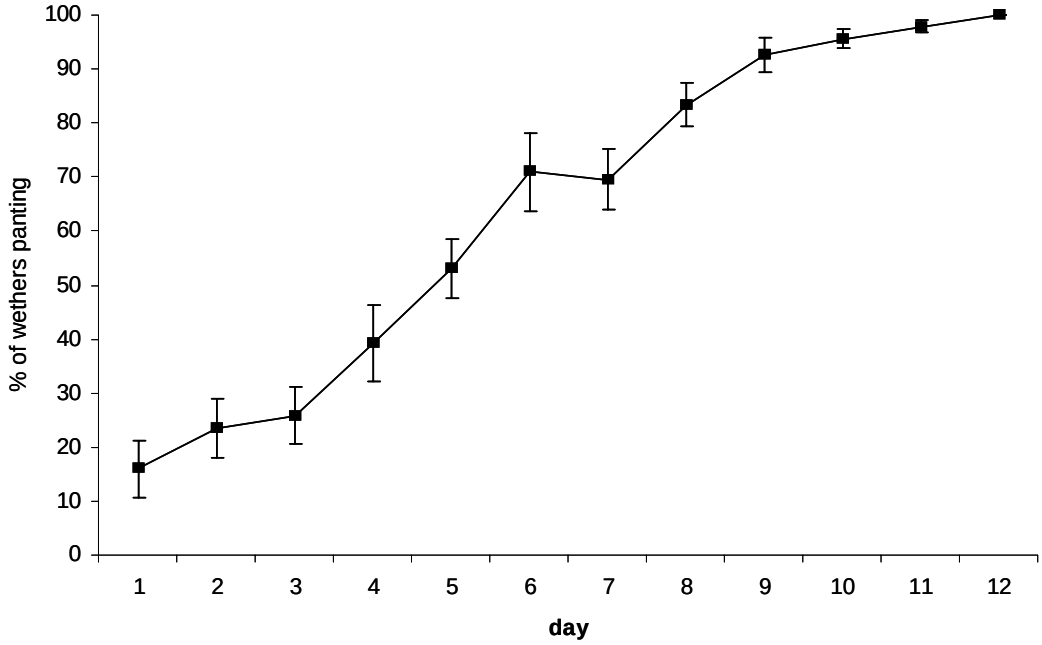


Figure 59: Percentage wethers panting in the four ammonia treatments. As indicated by the asterisk (p < 0.05), there were significantly more wethers panting in the 30 ppm ammonia treatment than in the 45 ppm treatment.

The percentage of wethers panting increased significantly during the 12 days in the climate chamber ($F_{11,234}$ = 52.81, p = 0.001). As shown in Figure 60, the percentage of wethers panting increased from 16 % on day one to 100 % on day 12.



DBT ^{o}C	26	27	28	29	30	31	31	31	32	33	34	35
WBT ^{o}C	23	24	25	25	26	26	27	27	27	28	28	29

Figure 60:
Percentage of wethers panting and the corresponding dry (DBT) and wet bulb temperatures (WBT) during the 12 days in the climate chamber. The percentage of wethers panting increased significantly during the twelve days in the climate chamber.

Locomotion – As shown in Figure 61, there was a significant difference in locomotor activity between the four ammonia treatments (control: 7.9 ± 2.0; 15 ppm: 5.4 ± 0.8; 30 ppm: 5.3 ± 1.0; 45 ppm: 4.6 ± 0.9) ($F_{3,234}$ = 4.42, p = 0.02). Post hoc analysis showed that there was significantly more locomotion in the control compared to the 45 ppm treatment (t-test = t_{14} = 2.96, p = 0.01).

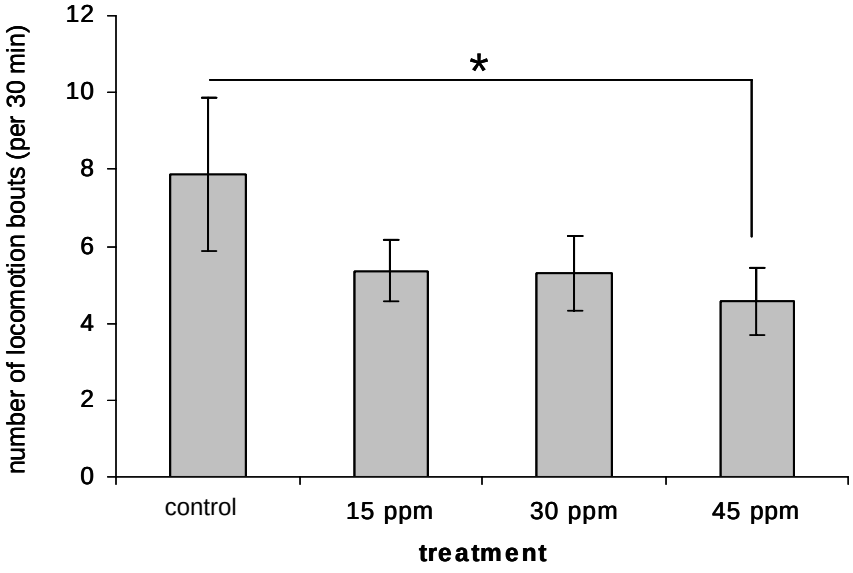


Figure 61: Locomotion in the four ammonia treatments. As indicated by the asterisk (p < 0.05), the sheep were significantly more active in the control than in the 45 ppm ammonia.

There was a significant difference in locomotion during the 12 days the wethers were in the climate chambers ($F_{11,234}$ = 1.94, p = 0.04). As shown in Figure 62, there was a peak in locomotor activity during days 5 and 6 and again on day 11.

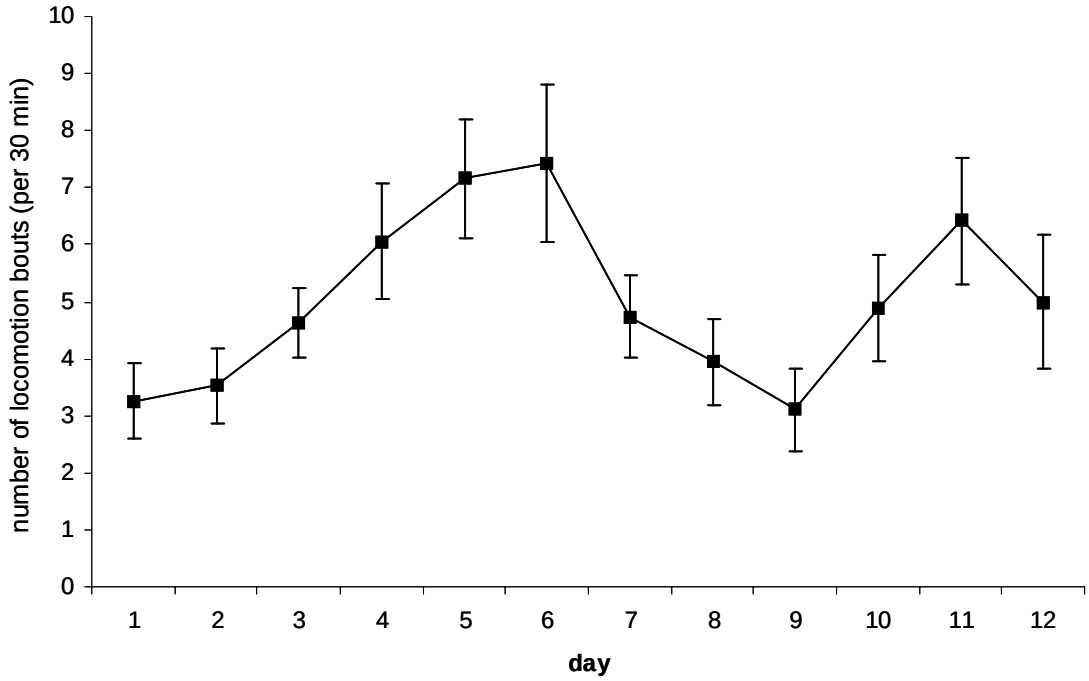


Figure 62: Locomotion during the 12 days the wethers were in the climate chamber. Locomotor activity fluctuated during the 12 days in the climate chambers with peaks in locomotion on days 6 and 7 and again on day 11.

Head butting – Ammonia treatment did not significantly affect head butting bouts by the wethers (control: 2.03 ± 0.20; 15 ppm: 1.81 ± 0.18; 30 ppm: 1.15 ± 0.17 ; 45 ppm: 0.93 ± 0.12) ($F_{3,234}$ = 0.62, p = 0.61).

As shown in Figure 63, the number of bouts of head butting changed significantly during the 12 days the wethers were in the climate chambers ($F_{11,234}$ = 2.15, p = 0.02). Similar to locomotion, there was a peak in head butting on days 6 to 8.

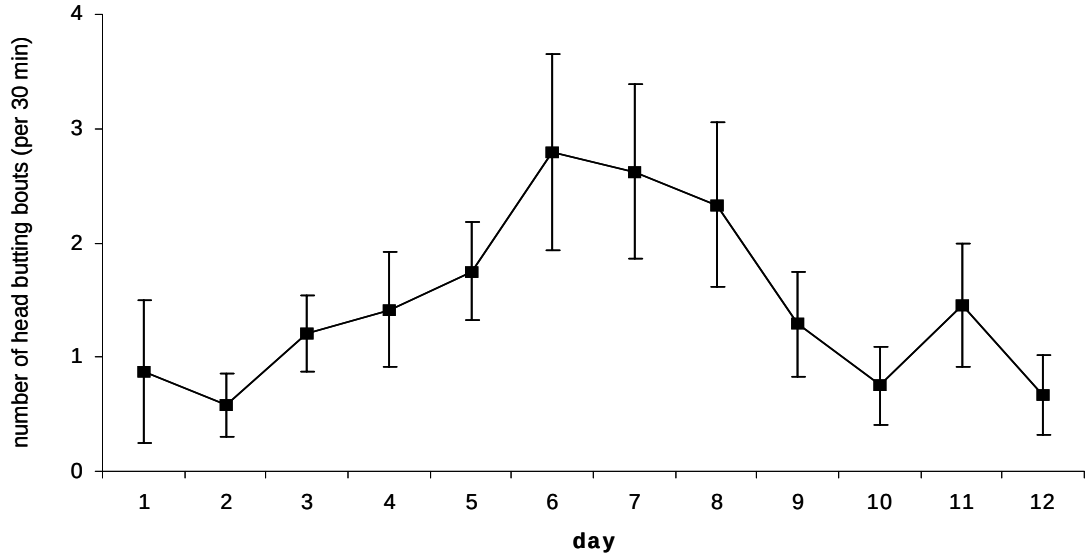


Figure 63: Head butting during the 12 days the wethers were in the climate chamber. Head butting peaked during days 6, 7 and 8 in the climate chambers and to a lesser extent on day 11.

Self-Licking - There was no effect of ammonia treatment on the number of self-licking bouts (control: 3.46 ± 0.55; 15 ppm: 2.86 ± 0.42; 30 ppm: 3.14 ± 0.48; 45 ppm: 4.14 ± 0.58) ($F_{3,234}$ = 2.85, p = 0.08).

As shown in Figure 64, the number of bouts of self-licking themselves increased significantly during the 12 days the wethers were in the climate chambers from one bout per 30 minutes to approximately four bouts per 30 minutes ($F_{11,234}$ = 3.67, p = 0.001).

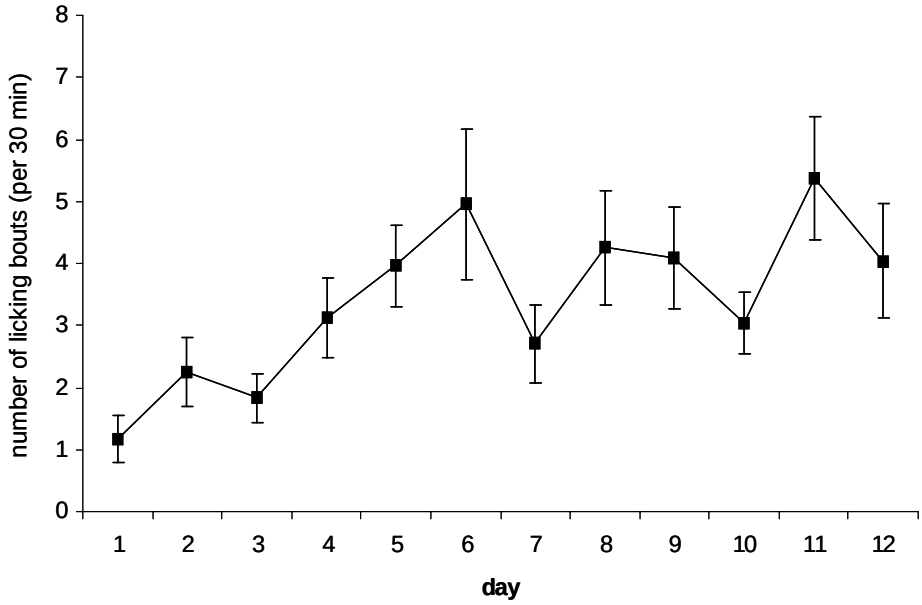


Figure 64: Self-licking during the 12 days the wethers were in the climate chamber. There was an overall increase in self-licking during the 12 days in the climate rooms.

Correlations - There was a significant and positive correlation between locomotion and head butting (Pearsons correlation: $r^2 = 0.34$, $p = 0.05$) and between locomotion and licking ($r^2 = 0.36$, $p = 0.03$) (Figure 65).

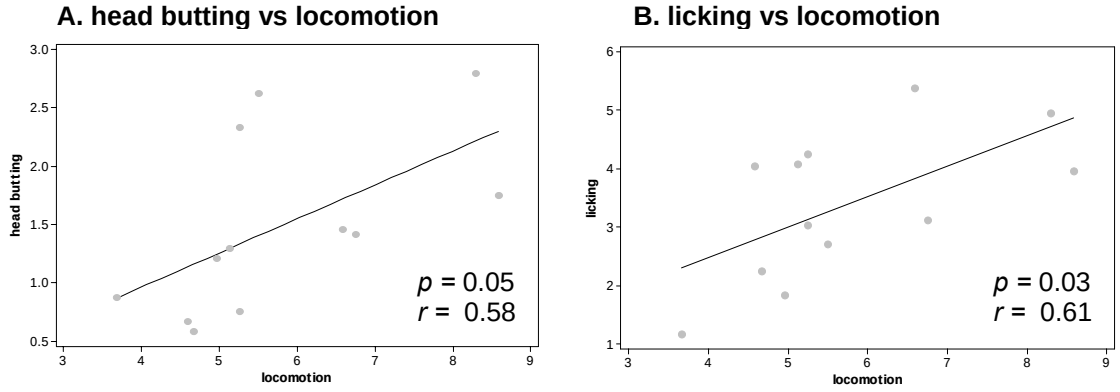


Figure 65. Correlation between locomotion and A. head butting and B. licking. There was a significant and positive relationship between locomotion and both head butting and licking.

Other measures – No effect of ammonia treatment was found on coughing in sheep (control: 0.025 ± 0.012; 15 ppm: 0.014 ± 0.007; 30 ppm: 0.014 ± 0.007; 45 ppm: 0.016 ± 0.004) (Mood's median test: Chi square =0.53, df = 3, $p = 0.91$). Neither ammonia treatment nor time spent in the climate chamber significantly affected the number of bouts of scratching (control: 1.36 ± 0.25; 15 ppm: 1.22 ± 0.08; 30 ppm: 1.04 ± 0.09; 45 ppm: 1.18 ± 0.09) (F values ranged from 0.83 to 1.37 and p values from 0.50 to 0.19) and pawing (control: 2.99 ± 0.15; 15 ppm: 2.36 ± 0.31; 30 ppm: 2.78 ± 0.17; 45 ppm: 2.18 ± 0.27) (F values ranged from 2.01 to 1.55 and p values from 0.16 to 0.11).

4.6 Experiment 6 the avoidance of ammonia by sheep

4.6.1 Maximum number of lever presses (Breakpoint)

The maximum number of lever presses (hereafter termed the breakpoint) decreased after the first test (mean breakpoints 1.06, 0.84, 0.72 $\log_{10}$ presses, i.e. 10.5, 5.9 and 4.2 presses, for the first, second and third test, respectively, SED = 0.0897 $\log_{10}$ presses, $p < 0.001$). There was a significantly lower mean breakpoint when sheep traversed the chamber filled with ammonia (mean breakpoint = 0.81 $\log_{10}$ presses +1, i.e. 5.5 presses), compared with the chamber filled with fresh air (mean breakpoint = 0.94 $\log_{10}$ presses +1, i.e. 7.6 presses) (SED = 0.0925 $\log_{10}$ presses +1, $p = 0.04$). Although the sheep as a group significantly avoided the ammonia chamber, it is not possible to state with any certainty the proportion of sheep demonstrating this aversion. The proportion of sheep preferring the Control lane was nine out of 12, with three preferring the ammonia lane, when the number of times they entered each chamber is considered. Although this proportion is of considerable interest in relation to the establishment of limits, the experiment was not designed to determine the significance of responses in individuals, only in the group, which is assumed to be representative of the sheep population. It can therefore be stated categorically that the sheep as a group demonstrated a partial avoidance of the chamber with ammonia, but not the proportion of animals that were acting in this way. To determine this accurately would require a much greater number of tests on each animal.

There was no effect of prior exposure to ammonia on the breakpoint (mean breakpoint = 0.83 $\log_{10}$ presses +1, i.e. 5.7 presses for Control sheep, mean breakpoint = 0.92 $\log_{10}$ presses +1, i.e. 8.3 presses for Prior Exposed sheep, SED = 0.131 $\log_{10}$ presses +1, p = 0.49). There were no significant interactions between period and current or prior exposure, or between prior and current exposure (p > 0.25). However, there was a significant effect of subject (SED = 0.0897 $\log_{10}$ presses +1, p < 0.01).

4.6.2 Time spent in the chamber

The time in the chamber did not change from the first to the third trial (mean time 14.0, 14.1, 13.7 sec for the first, second and third test, respectively, SED = 5.34 sec, P = 0.97). It took a significantly shorter time for sheep to traverse the chamber filled with ammonia (mean = 11.9 sec), compared with the chamber filled with fresh air (mean 15.9 sec) (SED = 2.70 s, p = 0.03). There was no effect of prior exposure to ammonia on the mean time spent in the chamber (mean 13.8 sec for Control sheep, mean for Prior Exposed sheep 14.0 sec, SED = 3.82 sec, p = 0.96). There were no significant interactions between trial number and current or prior exposure, or between prior and current exposure (p > 0.25). However, there was a significant effect of subject (SED = 5.34 sec, p = 0.05).

There was no significant difference between the liveweight change of the ammonia-exposed group of sheep (+1.76 kg) and the Control group (+1.03 kg) (SED = 1.80 kg, p = 0.67). The mean ± SEM weights for the ammonia exposed group were Day 1: 32.3 ± 1.8 kg; Day 15: 34.4 ± 1.5 kg, and the control group: Day 1: 33.6 ± 1.7 kg; Day 15: 34.1 ± 1.3 kg.

No significant difference was found in the rate of tear production between the control (18.9 ± 1.4 mm/min) and the ammonia-exposed group of sheep (21.0 ± 1.6 mm/min) (two sample t test: t_{11} = 1.03, P = 0.324). Water consumption by both groups was similar (approximately 2.7 L/sheep/day).

5 Discussion

5.1 Experiment 1: Atmospheric concentration of ammonia, hydrogen sulphide and carbon dioxide

The concentration of atmospheric ammonia in some locations on board a live export vessel transporting sheep were above the TWA and STEL limits for humans (NOHSC, 1995) and the levels recommended for live export by Tudor *et al*. (2003) and Costa *et al*. (2003). Ammonia concentration reported here also tended to be higher than levels previously reported on live export vessels (Stacey, 2003).

Ammonia generally occurred at higher concentrations on closed decks than on open decks. Moreover, high concentrations of ammonia were generally more widespread, as evidenced by 25-42 ppm ammonia throughout the aft subsection of deck one, and persisted for longer on closed decks than on open decks. Thus sheep housed in some areas of the closed decks may be exposed to high concentrations of ammonia for most of the voyage suggesting ammonia to be more of an issue on closed decks than on open. Concentrations of ammonia could still be high on open decks, indeed the highest concentrations of any were recorded on open decks behind the bulkhead at the front of the vessel (15-29 ppm) and near the engine block (15-27 ppm).

Small pockets of very high ammonia concentrations (39-59 ppm) were also found in a quarter of the open deck pens mapped. However, unlike the closed decks, high concentrations of ammonia on the open decks tended to be transient; which is probably due to regular through drafts, at least when the vessel was out of port.

It is not known if the ammonia concentrations reported here are potentially harmful to sheep. There is evidence to suggest that exposure of lambs to 75 ppm ammonia for 28 days resulted in decreased feed conversion efficiency and clear signs of ammonia toxicosis (Drummond *et al*., 1976). However, these results represent the extremes of ammonia exposure and it would appear unlikely that exported sheep on this vessel would be exposed to such a high level of ammonia as the highest concentration of ammonia measured on the two voyages was 59 ppm over a short time-frame.

Studies on other animals exposed to ammonia concentrations similar to that on board ship have produced mixed results. Tudor *et al*. (2003) reported increases in white cell count and mononucleated cell counts in blood of cattle exposed to 20 ppm ammonia for 9 days. Exposure to 20 ppm for 72 h increased the infection rate of chickens exposed to Newcastle disease (Anderson *et al*., 1964). Anderson and colleagues also showed that guinea pigs and chickens exposed to 20 ppm ammonia for 6 weeks displayed pulmonary edema, congestion and haemorrhage. However, it should be noted that no measurable effects were seen at two weeks - a timeframe comparable to the length of a voyage to the Middle East. Coon *et al*. (1970) found no lung effects in rats and guinea pigs exposed to 28 ppm ammonia for 114 days.

The two other potentially noxious gases measured were hydrogen sulphide and carbon dioxide. Despite the increase in hydrogen sulphide over the course of the voyage, the maximum concentration measured was 1.8 ppm - well below the TWA limit of 10 ppm for humans (NOHSC, 1995). Carbon dioxide was found to accumulate in small pockets onboard the vessel; however, concentrations (max. 1890 ppm) were well below the TWA limit of 12500 ppm for humans (NOHSC, 1995). Given the low concentrations of both hydrogen sulphide and carbon dioxide on board the vessel, it is unlikely that either gas will pose a health and welfare risk to livestock.

5.2 Experiment 2: Factors affecting ammonia concentrations on a live export vessel

Air movement (air speed and/or cumulative wind) had the strongest correlation with atmospheric ammonia concentration on the open decks. The negative association between ammonia levels and air movement supports the finding that ammonia concentration tended to be higher in those sections of the open decks where air flow was disrupted; i.e. behind the bulkhead at the front of the vessel and near the engine room. This finding is consistent with research by McCarthy (2005) who found a correlation between ammonia and poor ventilation or poor airflow on open decks. Wet bulb temperature, dew point temperature, dry bulb temperature (voyage two only) and, where measured, faecal pad depth were also associated with atmospheric ammonia concentration on the open deck. Similar correlations between ammonia and air movement, humidity and manure (the size of area exposed) have been reported in poultry houses (Nimmermark and Gustafsson, 2005).

The relationship between ammonia concentration and wet bulb temperature, air movement and faecal pad depth was not the same on the closed decks as it was on the open decks. On the closed decks, the concentration of ammonia was most strongly related to wet bulb temperature and dew point temperature and, to a lesser extent, faecal pad depth and air movement. Reduced variability of wind on the closed decks may be the reason why air movement is of less

importance there. Although we found no significant overall difference between open and closed decks in air speed the variability in closed decks tends to be less than on open decks.
This is consistent with McCarthy's (2005) study which found that poor air movement was correlated with high ammonia on open decks more so than on closed decks. The absence of a relationship between ammonia and dry bulb temperature on the closed decks is surprising given that the volatilisation of ammonia within the faecal pad increases with ambient temperature (McCarthy, 2005).

The correlation between ammonia and air movement, wet bulb temperature and faecal pad depth on both voyages and on open and closed decks was only moderate (r ranged from 0.64-0.77), suggesting other variables may be influencing ammonia concentrations. Other variables that have been shown to influence ammonia concentration include animal weight, stocking density (the open and closed decks had similar stocking density on voyage one) and pH and moisture content of the manure (Nimmermark and Gustafsson, 2005). Further research would be required to examine if these variables influence ammonia concentrations on live export vessels.

A number of two-way interactions were correlated with ammonia concentration. Where these interactions were negative (cumulative wind*air speed, dew point temperature*faecal pad depth and dew point temperature*air speed: as indicated by the coefficient) an increase in the level of one variable corresponds to an associated decrease in ammonia concentration. The magnitude of the associated decrease in ammonia increases with an increase in the levels of both interacting variables. Conversely, where positive interactions occurred (dew point temperature*cumulative wind, dew point temperature*faecal pad depth and air speed*faecal pad depth) an increase in one or both variables indicates an associated increase in ammonia concentration. The interaction between dew point temperature and faecal pad depth was positively correlated with ammonia on the open decks and negatively correlated with ammonia on the closed decks suggesting that the relationship between these variables may be context dependent. At first glance, the relationship between some of the abovementioned interactions and ammonia concentration may conflict with what is known in the literature (e.g. an increase in dew point temperature*faecal pad depth is associated with a decrease in ammonia). However, it is important to realise that each of these interactions is only one part of the overall model describing the association of ship board variables with ammonia. All parts of the model need to be considered for a more complete understanding of the relationship between ammonia and shipboard conditions.

Despite ventilation shafts being spread evenly throughout the vessel, air speed varied considerably within many of the pens. Given the variability of air speed within the pen and the negative correlation between air movement and ammonia, it is not surprising that the concentration of ammonia was also highly variable within many pens.. By contrast, dew point temperature, wet bulb temperature and dry bulb temperature were all relatively uniform throughout each pen.

Dew point temperature, wet bulb temperature and dry bulb temperature increased sharply during the first 4 days of the first voyage as the vessel moved towards the equator. After day 4, changes in dew point temperature, wet bulb temperature and dry bulb temperature were more gradual. When the vessel was in port at Muscat (day 11) and Kuwait (day 13), wet bulb temperature and dew point temperature decreased by 4-8^{o}C while dry bulb temperature increased by 4^{o}C; presumably due to local climatic conditions (note that meteorological records show dew point temperature and wet bulb temperature at Muscat and Kuwait to be as much as 15^{o}C higher onboard the ship than on land). The change in conditions at Muscat, and presumably Kuwait, were only temporary with conditions reverting back to levels as measured prior to the arrival at port.

Faecal pad depth increased steadily over the course of the voyage, albeit at a faster rate on the closed decks than on the open decks. The reason for the difference between the open and closed decks in faecal build-up is not known. One possible explanation may be that, unlike closed decks, the open decks are, as the name suggests, an open environment in which faeces may be removed from the system by wind or ventilation. If true, the faecal pad may have reduced moisture content on the open decks and potentially reduced ammonia generating potential. This is worthy of further investigation. The negative correlation between faecal pad depth and ammonia on the open decks may relate to the existence of dry elements of the pad in the higher regions, which prevented ammonia either being produced or released to the atmosphere.

5.3 Experiment 3: The effects of ammonia on a live export vessel on sheep behaviour.

There was no effect of ammonia concentration on sheep behaviour. However, these results should be regarded as preliminary. As was shown in Experiment 1, the concentration of ammonia within the pen can vary considerably and so the actual concentration of ammonia that the focal animals were being exposed to is not known. Moreover, when the ammonia concentration at the 20 sites was remeasured, the range in concentration was a mere 9.5 ppm and the maximum concentration was 14.5 ppm; 10 ppm below the maximum recommended limit for the live export industry. It seems unlikely that small increments in ammonia would result in detectable behaviour changes. Further research under controlled ammonia conditions is required to determine the effects of ammonia on sheep behaviour.

Sheep behaviour did change with voyage duration. During the second half of the voyage there was an increase in the amount of time the sheep spent with their head lowered down; behaviour considered by some to be an indication of discomfort in sheep (Hall *et al*., 1998). However, with sheep spending more time lying down and less time standing up during the second half of the voyage compared to the first, the lowered head may simply be an indication of increased resting. Resting, as indicated by time spent lying down, has also been shown to increase with journey duration in road transport (Cockram *et al*., 2004).

There was some evidence to suggest that feeding and lying behaviour became more cyclic during the second half of the voyage compared to first half of the voyage. Increased cyclic behaviour may be an indication of the sheep adjusting to shipboard conditions such as feeding times and crew movements. Further monitoring of voyages is required to confirm such a suggestion.

The sheep made less use of the available pen space during the second half of the voyage. This may be a reflection of stocking density (ranging between 0.26-0.39 m^2/head) preventing the animals moving through the pen. However, this seems unlikely as sheep were observed moving around the pen (Personal Observation). An alternative explanation for the sheep not making full use of the pen may be that they prefer to remain amongst familiar conspecifics or within a section of the pen with which they are familiar. Support for this latter argument can be found in the response of the sheep following the stockman moving through the pen. Every day, the stockmen passed through each pen looking for sick and injured animals. This action invariably forced the sheep to move *en masse* to the other end of the pen. Yet, despite this daily “stirring”, once the stockman had left the pen, individuals were often found to return to sections of the pen they had previously occupied, suggesting sheep do prefer certain sections of the pen. This could increase actual stocking density in some parts of the pen, reducing it in others.

Two behaviours that did not change with voyage duration were the amount of time spent feeding and the amount of time spent ruminating. This result is in contrast to a study by Black *et al*. (1994), who reported a decrease in the amount of time lambs spent feeding and ruminating while on a 24 day voyage from New Zealand to the Middle East. However, the actual time spent feeding in this study (7 %) is comparable with the amount of time the lambs spent feeding at the end of the voyage when in >30^{o}C temperatures in the study by Black and colleagues. The amount of time spent ruminating in this study (24 %) was double that reported by Black and colleagues (13.5 % in one pen and 10.8 % in another). However, the absence of night recording in our study may have influenced this figure.

Less than 2 % of sheep showed signs [i.e. eye(s) closed] of eye irritation. However, it should be recognised that this figure is only an estimate as it was not possible to see the eyes of those sheep with their head down or facing away from the researcher. Discussions with the veterinarian on voyage two suggest that ~5 % of shipped sheep usually show signs of eye irritation. Amongst other reasons, eye irritation is thought to be associated with dusty pre-export assembly depots and easily fragmented feed pellets used in the assembly depot and/or shipboard (Brightling and Lightfoot, 2003). Given that the pellets used on voyage two fragmented easily, it was surprising that there was no association between air speed and eye irritation. Another variable which eye irritation was not associated with was ammonia. However, for the same potential reason that no correlation was found between ammonia concentration and behaviour (i.e. highly variable ammonia concentrations and air speed within the pen), these results should be regarded as preliminary.

5.4 Experiment 4: Concentrations of potentially noxious gases at a pre-export assembly depot

The concentration of atmospheric ammonia within the holding sheds of the pre-export assembly depot were well below the TWA limits for humans and the levels recommended for live export by Tudor *et al*. (2003) and Costa *et al*. (2003). Ammonia concentrations reported here were also lower than levels previously reported in Australian feedlots (peak feedlot ammonia levels ranged from 25 to 30 ppm: FLOT.317, 2003) and onboard live export vessels (peak shipboard levels ranged form 42 to 59 ppm: LIVE.222 Stage 1A , 2005). The results of this study suggest that the atmospheric ammonia levels at this assembly depot would not pose a health and welfare risk to the sheep or humans.

Dry and wet bulb temperature and dew point temperature had the strongest correlation with atmospheric ammonia concentration at the assembly depot. This finding is consistent with the research conducted in Experiment 2, in which it was shown that dry and wet bulb temperature and dew point temperature were correlated with ammonia on a live export vessel. Ammonia concentration has been shown to be directly affected by dry bulb temperature in Australian feedlots, with higher ammonia levels during the warmer parts of the day (FLOT.317, 2003).

The two-way interaction - distance from faeces*dry bulb temperature – was negatively correlated with ammonia concentration. An increase in the distance from the accumulated faeces, combined with dry bulb temperature, corresponded to an associated decrease in ammonia concentration.

No relationship was found between wind speed and ammonia. This is inconsistent with the results of Experiment 2, in which air movement (air speed and/or cumulative wind) had a strong correlation with ammonia concentration on the open decks of a live export vessel. The lack of a relationship between wind speed and ammonia at the assembly depot may be explained by the fact that the mean wind speed at the assembly depot (0.4 m/s) was almost half that on board the live export vessel (mean shipboard air movement: 0.7 m/s).

There was a linear relationship between ammonia concentration and height of measurements from the floor. The concentration of ammonia tended to be highest near the floor of the holding shed and decreased with height. The relationship between ammonia concentration and height from shed floor was weak; this may have been due to differences between sheds in the depth of the accumulated faeces. Nonetheless, the results here are consistent with Experiment 2 and at an Australian feedlot (EA Systems, 2001), which reported higher ammonia concentration closer to the floor/ground. It would be advisable to standardise measurements for future experiments to say 1 m above ground level, which would approximate to the mean standing/lying height for cattle and sheep. This would enable comparison between experiments to be facilitated.

Measurements of hydrogen sulphide and carbon dioxide, two potentially noxious gases, were also measured at the assembly depot. The maximum concentration of hydrogen sulphide, which was present in just one of four sheds mapped, was 1.7 ppm - well below the TWA limit of 10 ppm for humans. Similarly, the maximum concentration of carbon dioxide (max. 1460 ppm) was well below the TWA limit of 12500 ppm for humans. Given the low concentrations of both hydrogen sulphide and carbon dioxide at the assembly depot, it is unlikely that either gas poses a health and welfare risk.

5.5 Experiment 5: The effect of atmospheric ammonia on the physiology and behaviour of cattle and sheep

The effects of different atmospheric ammonia concentrations on steers and wethers kept for 12 days under conditions which simulated a voyage from Australia to the Middle East during the northern summer was examined. A range of behavioural, clinical, haematological and pathological measures were used to assess the effects of ammonia.

The distal airway mucus absorbs most inhaled ammonia, thereby protecting the lungs (Gustin *et al*., 1994; Schaerdel *et al*., 1983). However, a fraction of inhaled ammonia can still reach the lungs and, dependent on the concentration, can cause damage (World Health Organisation, 1986). Macrophages and neutrophils are white blood cells that form part of the lung immune response by engulfing and destroying cellular debris and pathogens (Quinn *et al*., 2002). Neutrophils are short lived, but respond rapidly to invading micro-organisms. Macrophages are long lived and slow acting, but are able to initiate specific immune responses and secrete cytokines to activate lymphocytes and promote inflammatory responses (Quinn *et al*., 2002). It is therefore likely that measurable changes to these macrophages may occur in an irritated lung; as was indeed the case following the exposure of cattle to ammonia. The results of the BAL in the cattle and the TA in the sheep showed that macrophage activity and neutrophil count (steers only) were influenced by the different ammonia treatments. In the cattle, macrophage activity was highest in the 45 ppm ammonia treatment, high in the 30 ppm and 15 ppm ammonia treatments, and lowest in the control ammonia treatments but control neutrophils were no different to 45 ppm. The pattern for the sheep was not as clear cut as that for the cattle with the highest macrophage activity occurring in the 15 ppm ammonia, and the 0, 30 ppm and 45 ppm ammonia treatments being less. The occurrence of high to very high macrophage activity in the 15, 30 and 45 ppm ammonia treatment in the cattle suggests that these ammonia concentrations were irritating their lungs (M. Latter, Personal Communication).

BALs and TAs conducted 4 weeks after the animals left the climate chambers were used to assess recovery in the animals. Results showed no difference between the ammonia treatments for any of the cell counts. More importantly, macrophage activity was medium to low indicating that the ammonia was no longer irritating the lungs and had no measured long-term effect.

There was no evidence that ammonia caused eye irritation to sheep in Experiment 3, but it was noted that ammonia concentrations were transient and varied considerably across each pen. However, in experiment 5, where ammonia concentrations were more consistent, the increased neutrophil levels in the BAL of cattle exposed to 30 ppm ammonia suggest inflammation secondary to irritation by the ammonia.

Neutrophil count has been shown to have a positive linear relationship with ammonia concentration (25, 50, 75 and 100 ppm) in pigs (Urbain *et al*., 1994). This finding is in contrast to that of the present study in which cattle neutrophil count was higher in the 30 ppm ammonia treatment than in the 45 ppm ammonia treatment. The raised neutrophils in the BALs would suggest increased recruitment / inflammation secondary to irritation by ammonia. The drop at 45 ppm is hard to interpret, but there is a school of thought that irritant gases such as cigarette smoke can cause local immuno-suppression and therefore a reduction in inflammatory cell number (M. Latter, Personal Communication). Li *et al*.'s (1997) research on the dose-dependent necrosis of human alveolar macrophages by acrolein, an irritant gas, supports this suggestion. This is a possible interpretation although more work would obviously need to be done to prove such a claim.

There were clear clinical signs that irritation to the eyes, nose and lungs of the steers increased with ammonia concentration. Forty percent of the steers exposed to 45 ppm had nasal secretions compared to 20 % in the 15 and 30 ppm treatments and 8 % in the control. Similarly, 35 % of the steers in 45 ppm ammonia had lacrimation compared to approximately 10 % in the three lower concentrations. Steers exposed to the 30 and 45 ppm ammonia coughed more, albeit in low numbers, than those in the control and 15 ppm ammonia treatment. Clearly the 45 ppm treatment is more noxious to mucous membranes than the lower ammonia concentrations.

Increased eye, nose and throat irritation with ammonia concentration has also been reported in humans exposed to ammonia (Ferguson *et al*., 1977). Exposure was for 6 h/day over a 6-week period. Irritation at 50 and 100 ppm ammonia was transient with acclimation occurring after 2 to 3 weeks; too long a period to be of relevance to the live export industry.

Severe coughing and profuse lacrimation and nasal discharge has been reported in lambs exposed to 75 ppm ammonia for 28 days (Drummond *et al*., 1976). Despite this, there was no evidence to suggest that ammonia concentration influenced coughing or nasal discharge by the wethers in the study reported here. Indeed, both coughing and nasal secretions were rarely observed in all ammonia treatments. However, this finding is not unique, with Gustin *et al* (1994) reporting no coughing, nasal discharge or sneezing in pigs exposed for 6 days to 0, 25, 50 and 100 ppm ammonia. The prevalence of lacrimation in the wethers examined here is not known, as the method of scoring proved inaccurate. The only comment that can be made is that lacrimation was observed to occur in at least some wethers, but not profusely.

In the wethers, but not the steers, there was an effect of ammonia on live weight. The wethers exposed to 30 ppm ammonia lost 8 % of their body weight over the 12 days in the climate chamber, while those exposed to 45 ppm lost 6 % of their live weight. This result is consistent with Drummond *et al*. (1976) who found that lambs exposed to an atmosphere of 75 ppm ammonia for 28 days had lower feed conversion efficiency than lambs in a control atmosphere. In pigs, reduced body weight gain has previously been attributed to a decrease in food consumption (Stombauch *et al* 1969), which fits with the reduction in dry matter intake by the wethers in the 45 ppm ammonia in the present study. Indeed, the feed intake:body weight ratio of the wethers exposed to ammonia (1.66 % in 15 ppm, 1.38 % in 30 ppm and 1.32 % in 45 ppm) was well below the 2 % recommended by ASEL (DFAT, 2006) and below that required for maintenance (Dawson and Steen, 1998).

However, later work by Gustin *et al* (1994), in which pigs exposed to 50 and 100 ppm ammonia levels showed significant decreases in body weight, suggest that loss of body weight may be associated with an adverse effect on the central nervous system following pulmonary absorption of ammonia. Either way, the results suggest that concentrations of 30 ppm or higher reduce live weight.

The feed:body weight ratio in cattle in all four ammonia treatments (1.55 % in the control, 1.38 % in 15 ppm, 1.52 % in 30 ppm and 1.56 % in 45 ppm) was below that required for maintenance (Dawson and Steen, 1998). With no significant change in live weight, the results suggest that the simulated shipboard conditions were not conducive to increasing steer body condition, but this may not be a priority during transport.

Live weight measurements made 28 days after the animals left the climate chambers revealed significant changes. Wether live weight increased by 5 to 16 %, while for steers, it was a modest 2 to 5 % increase. There was no effect of ammonia on the weight gain for either species. This increase in body weight is likely to be due to the longer time frame over which these measurements were made and the Lucerne diet which the animals were fed.

One unexpected effect of ammonia was the higher percentage of wethers panting in the 30 ppm ammonia as compared to the other ammonia treatments, particularly the 45 ppm ammonia treatment. The experimental methodology employed in the study reported here was extremely robust and it is unlikely that the result was due to a bias in a climate chamber, pen or trial. It is therefore difficult to explain why the observed anomaly may have occurred.

The percentage of sheep and cattle panting did increase over the 12 days in the climate room. This result is to be expected given that wet and dry bulb temperature also increased over the 12 days. Similarly, the increase in water consumption over the 12 days is likely to be due to the increasing temperature, as has recently been reported by Beatty *et al*. (2006) in their study on the response of cattle to heat stress.

Ammonia concentration had no effect on any of the blood parameters measured immediately after the animals left the climate chamber. However, there were some differences between the pre- and day 0 post-experiment levels of particular blood parameters. In the cattle, haemoglobin, MCV and MCH decreased, and MPV, white cell count, neutrophil, monocytes and eosinophils increased, while for sheep neutrophil and MCHC decreased and haemoglobin and packed cell volume increased. Of these, only the white blood cells: neutrophils and monocytes occurred outside of the normal range and only in cattle. In the week prior to entering the climate chamber, cattle were vaccinated for bovine ephemeral fever (3-day sickness). It is likely that the increase in the white blood cells may be associated with the animal's immune response to the vaccination.

Blood samples collected 4 weeks after the animals left the climate chamber revealed significant treatment effects on certain parameters. PCT, platelet count and urea varied in the cattle, while in the sheep there were differences in MCV and urea. Specific differences between the ammonia treatments could be determined for urea only in both species: urea levels were higher in those wethers previously exposed to 15 and 30 ppm, and in the steers, those previously exposed to 15 ppm had higher urea levels than those exposed to 45 ppm ammonia. However, as the urea levels for all ammonia treatments were within the normal range, the results cannot be be interpreted as being significant from a clinical pathology point of view (M. Latter, Personal Communication)

The steers, but not the wethers, spent more time standing and, conversely, less time lying in the 15 and 30 ppm ammonia treatments compared to controls. These two treatments (along with the 45 ppm treatment, in which time spent standing and lying did not differ significantly to all other treatments) differed from the control in that they all had excreta covering the floor and the control did not (the latter was cleaned three times/day to reduce ammonia). Research by Phillips and Morris (2002) has shown that cattle avoid floors covered in faecal matter which could explain the observed differences in standing and lying.

Ammonia had no measurable effect on the behaviour of steers and little effect on the behaviour of the wethers. Aside from the previously mentioned elevated panting in 30 ppm ammonia, the only other sheep behaviour to be influenced by ammonia was locomotion; wethers in the 45 ppm ammonia had lower scores of locomotion than those in the control. Similar to standing and lying in the cattle, the difference may be due to presence of faeces in the 45 ppm, but not the control. If this was true then one might expect lower locomotion scores in the 15 and 30 ppm ammonia where faeces were also present; however, this was not the case. In any case, the results indicate that ammonia up to 45 ppm has no effect on steer behaviour and barely alters the behaviour of wethers.

Locomotor activity by the wethers was positively correlated with head butting and licking. Stocking density in the pens was such that movement (either licking or locomotion) by an individual often led to the disturbance of other individuals within the pen (Personal Observation); as evidenced by pen-mates standing, head butting or performing locomotion. Why this disturbance was greater on some days than others is not known.

The percentage of time the wethers spent with their head lowered increased from 6 % to approximately 20 % during the 12 days in the climate chamber. A lowered head is considered by some to be an indication of discomfort in sheep (Hall *et al*., 1998); however, further research is needed for this to be confirmed.

5.6 Experiment 6: The avoidance of ammonia by sheep

The effects of atmospheric ammonia concentrations on the motivation of sheep, previously exposed to <2 or 45 ppm ammonia to enter a raceway filled with gaseous ammonia and containing a food reward was examined. A lever, pressed by the sheep to gain access to the raceway, was used to measure the "price" they were prepared to pay to gain access to the raceway. The price provides an index of how the sheep "feels" about the situation (Dawkins, 1990).

The aversion to ammonia was only partial, i.e. the sheep still entered the raceway filled with ammonia, but were not prepared to work so hard to get the food reward. There was no interaction between period and treatment, so this difference did not change over time. Other animals have been shown to avoid ammonia when given a choice: Smith *et al*. (1996) reported that pigs showed a clear preference for fresh air instead of 100 ppm ammonia, and Kristensen *et al* (2000) demonstrated that laying hens would avoid concentrations above 25 ppm. This aversion is unlikely to be due to trigeminal nociception (a perception of pain mediated by the nasal trigeminal nerves, which detect irritating substances) because this does not occur until 200 ppm (McKeegan *et al*., 2002).

The absence of any effect of the prior exposure to ammonia on the avoidance behaviour of the sheep suggests that this is a consistent response, independent of their short-term experience of ammonia. Desensitisation following prolonged exposure to odours has sometimes been reported in humans (Schiffman, 1998), but usually this is in response to prolonged exposure, for example at the work-place. It has been reported in the literature that persons working daily in highly odorous environments, including ammonia, can show evidence of persistent reduction in responsiveness to the odour and that this can last for days (Harada *et al*. 1983; Schiffman, 1998; Ihrig *et al*., 2006). For example, Dalton (1997) showed that workers exposed to acetone were still able to detect the gas, but found the intensity to be weak, whereas, unexposed subjects rated the odour as very strong. However, some research with humans suggested that reduced odour sensitivity may be evident up to two weeks following exposure to a test odorant for as little as two weeks (Dalton and Wysocki, 1996). Reduced olfactory acuity has also been reported to occur in some pigs previously exposed to 40 ppm ammonia (Jones *et al*., 2001). However, the effects of ammonia on olfaction are not conclusive, with Holness *et al*. (1989) reporting no change in odour sensitivity in humans chronically exposed to moderate levels of ammonia (12.5 ppm). Clearly habituation (desensitisation) does not occur in all situations, as Jones *et al*. (2005) reported that boiler fowl exposed to 19 ppm ammonia for the first 26 days of their life will, when tested immediately after their exposure, avoid ammonia when given the opportunity.

There was no evidence to suggest that 45 ppm ammonia irritated the eyes of sheep, with both the control and the ammonia exposed group having a similar tear production rate. While a larger sample size would be required to confirm this suggestion, the results may help explain our difficulty in measuring lacrimation in Experiment 5.

6 Success in achieving objectives

6.1 Experiments 1-3

All objectives have been met and final report delivered in a timely manner.

The specific objectives for Stage 1a of LIVE.222 were:

1. To make accurate and repeated measurements of potentially hazardous gases, including ammonia, hydrogen sulphide and carbon dioxide on two ship voyages carrying sheep and cattle[3] from Australia to the Middle East.

2. To determine potential influences on these gaseous concentrations (temperature, faecal presence, ventilation rate and humidity).

3. If time permits, to make measurements of the impact of these variables on animal behaviour.

Two voyages were made on a vessel transporting sheep from Australia to the Middle-East on which accurate measurements of ammonia, hydrogen sulphide and carbon dioxide were made and gaseous concentrations mapped.

Changes in wet and dry bulb temperature, dew point temperature, air movement and faecal pad depth over the course of the voyage were measured and the effects of influencing variables on ammonia were noted. Hydrogen sulphide and carbon dioxide were not considered as shipboard concentrations were low.

The effect of shipboard concentrations of ammonia on sheep behaviour was examined. Additional measurements of behaviour change over the course of the voyage were also made.

6.2 Experiments 4-6

The final three experiments did not have specific objectives but were aimed at meeting the overall objectives, which were:

1) Produce a report that describes the effects of gaseous ammonia on sheep and cattle exposed long-term, under simulated shipboard conditions, to ammonia and the preferences of sheep and cattle for critical ammonia concentrations.

2) Make accurate and repeated measurements of ammonia concentration at a pre-export assembly depot and exported by sea.

3) Recommend critical ammonia levels for sheep and cattle held in pre-export assembly depots and exported by sea

6.2.1 Experiment 4

Ammonia and other noxious gases were monitored at a pre-assembly depot, fulfilling Objective 2. These contribute to the overall recommendations, fulfilling objective 3.

[3] It was decided in the Advisory Committee meeting that because no data was available for sheep, only cattle, that only sheep voyages would be attended.

6.2.2 Experiment 5

Detailed measurements of physiological and behavioural responses to different ammonia levels were recorded in a simulated live export voyage for cattle and sheep, fulfilling Objective 1.

6.2.3 Experiment 6

Avoidance of ammonia was recorded for sheep, that had, or had not been exposed to ammonia, fulfilling Objective 1. It was decided by the Project Advisory Committee not to test the avoidance of ammonia by cattle, in view of the additional resources required.

7 Impact on meat and livestock industry – now and in five years time

7.1 Experiments 1-3

The research which examined atmospheric concentrations of ammonia, hydrogen sulphide and carbon dioxide on a live export vessel transporting sheep, enabled us to establish methods of recording concentrations of these gases, and their variation over time and location on live export vessels. We established that ammonia, but not the other two gases, could reach levels hazardous to human health on some ships[4], and the effects on animals were later examined experimentally. Ammonia concentrations above the limit for 8 h exposure for humans on live export vessels (25 ppm) were found in 20% of locations. Our earlier research had indicated that industry stakeholders believed ammonia was one of the top welfare problems for the animals on board a vessel (Pines *et al*., 2007), and our later research indicated that ammonia does cause irritation to the respiratory tract and eyes and some localised, transient immune responses. Therefore controlling the high ammonia concentrations will improve welfare standards for both humans and animals on the vessels.

There are various strategies that could be used to overcome the problem, and the most suitable combination will be specific to each vessel and even voyage. First, it is possible to include feed additives that reduce ammonia release from the faeces. However, these would probably have to be added to the feed for the whole ship, whereas the second strategy, to reduce stocking density, could be targeted to critical areas of the vessel. These areas were identified as those with minimum air flow and high wet bulb temperature, and our regression equations could be used to predict ammonia risk at specific locations on different vessels. The possibility exists to feed small amounts of a suitable additive just to sheep in the high risk areas, but adequate uptake by all animals and avoidance of overdosing some animals would both be difficult to achieve. Adding it to the water could be an acceptable alternative. Third, ventilation could be increased in the high risk areas, but it was observed on the second voyage that the introduction of floor mounted fans in particularly hot periods had only a localised effect. The large variation in ventilation rate within a pen and the limited opportunities to site additional fans in locations where they can function without hindrance to the ship's operations make this option problematic. Fourth, high risk animals could be sited away from the high risk areas. We did not make any comparisons between types of animals, and different effects were observed in the two species monitored, but it is conceivable

[4] We did not establish whether the voyages that we monitored were better, worse or the same as other vessels engaged in live export, in terms of their ventilation performance and stocking density, but the possibility exists that other vessels are more prone to ammonia accumulation.

that animals more susceptible to other sorts of stress, such as heat stress, could also be more susceptible to ammonia stress. Fifth, the recognition of ammonia being a potential health hazard for workers on the ships could lead to shifts in high ammonia areas being limited. This already happens on some closed decks, where attendance by crew is limited by the high ammonia content, but results in less effective monitoring of the stock on these decks.

Accurate and repeated measurements showed that two potentially noxious gases, hydrogen sulphide and carbon dioxide, were well below critical levels likely to impact on animal or human welfare.

7.2 Experiment 4

The absence of potentially hazardous concentrations of gases at the one pre-export assembly depot monitored will benefit the industry by assuaging any public or industry concern about the quality of the air in these facilities.

7.3 Experiment 5

The establishment of responses to a range of ammonia concentrations in cattle and sheep will allow the industry to identify the welfare impact accurately and address the concerns of experts and the public in a measured and scientific manner. The physiological responses were limited and transient and there were no major haematological responses. The monitoring of effects over the range of concentrations to which the stock are likely to be exposed has allowed a maximum exposure limit to be determined with reasonable certainty, at least in cattle. The inability to measure irritation responses in sheep led to less certainty with the exposure limit in that species. However, identifying significant loss of live weight in exposed sheep should enable weight to be preserved during a shipment if the ammonia can be controlled. When exposed to the higher two levels, 30 and 45 ppm, wethers lost on average 7 % of their live weight during the voyage, or 3.5 kg in a 50 kg wether, whereas control wethers and those exposed to 15 ppm did not lose or gain any weight. This loss is worth approximately $5/animal to the trade, if animals were routinely sold on a weight basis, as opposed to the headage basis at present. If 20% of a 50,000 head shipment were affected by high ammonia and lost weight, then the problem could cost the industry approximately $ 50,000 per shipment.

7.4 Experiment 6

The recognition that sheep partially avoid 45 ppm ammonia, and that this response is not affected by prior exposure, enabled a maximum exposure limit to be established for this species as it provides evidence of unpleasant sensory responses at this level. Establishment of a limit for sheep would otherwise have been difficult due to the variable information provided in Experiment 5. Establishment and application of critical exposure limits to all vessels will facilitate reasoned responses to any public criticism of the gaseous environment for livestock on export shipments.

8 Conclusions and recommendations

8.1 Conclusions – Experiments 1-3

Concentrations of atmospheric ammonia above the TWA and STEL limits for humans and the levels recommended for live export animals occurred in some locations on board a live export vessel. Atmospheric concentrations of hydrogen sulphide and carbon dioxide were low and are unlikely to pose a health and welfare risk to livestock.

8.2 Conclusions - Experiment 4: Concentrations of potentially noxious gases at a pre-export assembly depot

Concentrations of atmospheric ammonia at a pre-export assembly depot were low and were unlikely to pose a health and welfare risk to livestock and humans.

8.3 Conclusions - Experiment 5: The effect of atmospheric ammonia on the physiology and behaviour of cattle and sheep

Ammonia concentrations typical of those measured on board a live export vessel had a measurable influence on the physiology of both cattle and sheep, but there was no evidence of long-term responses. Haematological parameters were not affected by ammonia. There was evidence that ammonia irritated the respiratory surfaces of both cattle and sheep and the eyes of cattle. The proportion of cattle experiencing irritation at the highest level (45 ppm) was greater than the recommended maximum proportion of humans experiencing irritation as a result of exposure to noxious odours (5-20%), but this proportion was acceptable in sheep exposed to the lower levels of ammonia.

8.4 Conclusions - Experiment 6: The avoidance of ammonia by sheep.

Most sheep exhibited a partial aversion to 45 ppm ammonia, and there was no evidence that prior exposure influenced this aversion. The proportion of sheep exhibiting this aversion (75%) was greater than the recommended maximum proportion of humans that report unpleasant sensory stimulation upon exposure to noxious odours (5-20%).

8.5 Recommendations for critical ammonia levels

The Australian exposure standards for humans have three critical ammonia levels (NOHSC:1003, 1995): The threshold limit value time-weighted average (TWA) is 25 ppm over an 8-h period. The threshold limit value for short-term exposure limit is 15 minutes exposure to 35 ppm, which can be repeated four times a day. The final level is the permissible exposure limit time-weighted average which is set at 50 ppm. This standard represents atmospheric concentrations of ammonia that should neither impair the health of nor cause undue discomfort to humans (NOHSC:1003, 1995). It is important to note that the NOHSC exposure standards are, as stated above, applicable to intermittent exposure only. Livestock aboard live export vessels present a different problem since they are constantly exposed to ammonia without respite. Therefore it is not entirely appropriate to set critical levels for livestock based on human standards.

The European standard for pig housing systems is 20 ppm (Commission Internationale de Génie Rural, 1984). However, Urbain *et al*. (1994) recommended 15 ppm to be the No Observable Adverse Effect Level (NOAEL)[5] of ammonia and to be the maximal ammonia concentration tolerated in pig buildings. However, the authors did not actually test 15 ppm; indeed 25 ppm was the lowest level tested. Their recommendations are based on observed inflammatory responses in pigs at 25 ppm.

Costa *et al*. (2003) recommend the live export industry adopt 25 ppm as a critical level for atmospheric ammonia on the basis that this level is in line with the abovementioned standards for humans and is an achievable level for sea transport systems and feedlots. However, Costa *et al*. (2003) reported increased indicators of inflammation in cattle exposed to 22 ppm ammonia for 6 days which suggests that their recommended critical level could be lower than 25 ppm if it is based on the NOAEL.

Should a NOAEL be applied to our study then, based on macrophage activity, it would be necessary to establish a critical level below 15 ppm for both sheep and cattle. However, as stated above, current exposure standards do not represent 'no-effect' levels that guarantee protection to every individual, rather they are indicative of where and when appropriate control measures are required (Costa *et al*., 2003). Protection of every individual in a NOAEL involves building in cumulative uncertainty factors, which in our case would include differences between individuals (factor of x 10); sub-chronic rather than chronic exposure (factor of x 3); and knowledge of the lowest observed adverse effect level, rather than NOAEL (factor of x 10), giving a cumulative uncertainty factor of perhaps 300. For example, the US Agency for Toxic Substances and Disease Registry has derived an acute-duration inhalation Minimal Risk Level for ammonia of 1.7 ppm for ammonia based on a Lowest Observable Adverse Effect Level (LOAEL) of 50 ppm for eye, nose, and throat irritation in a study with volunteers (Anon, 2006). No NOAEL was identified in that study. An uncertainty factor of 30 (3 for the use of a minimal LOAEL and 10 to protect sensitive individuals) was applied to the LOAEL. However, NOAELs are usually established to protect humans from acute exposure, not the chronic exposure to which livestock are exposed on live export vessels (Collins *et al*., 2004), and the rationale for implementing them to protect animals has not yet been established.

Our results demonstrate that the effects are transitory, but there can be no doubt that elevated ammonia can cause temporary discomfort (irritation), as evidenced by elevated lacrimation, nasal discharge and coughing. Elevated macrophage activity in cattle and reduced growth in sheep are also evidence of reduced welfare.

We recognise that the physiological effects of the examined levels of ammonia on sheep and cattle were not severe and were reversible after 28 days. Given the differences in the irritation responses of sheep and cattle to ammonia, it is necessary to consider each species separately. For the cattle, there were significant clinical and pathological differences between the control and 15 ppm and between 30 and 45 ppm. However, the magnitude of the differences in macrophage activity, lacrimation and nasal discharge were much greater for the latter than for the former. Aside from increased coughing at 30 ppm, we found that the steer response to 15 ppm is similar to that at 30 ppm. Therefore our results support the adoption of 30 ppm, which is similar to the 25 ppm recommended by Costa *et al*. (2003), as a critical exposure limit (not a NOAEL).

[5] No Observable Adverse Effect Level : An exposure level at which there are no statistically or biologically significant increases in the frequency or severity of adverse effects between the exposed population and its appropriate control; some effects may be produced at this level, but they are not considered as adverse, or as precursors to adverse effects.

The response of the cattle to the different ammonia levels was only on a proportion of the animals. For example, approximately 35-40 % of the steers exposed to 45 ppm ammonia had lacrimation and nasal discharge as opposed to 10-20 % at 15/30 ppm and 8 % in the control. At what percentage of animals affected does a limit become critical? Paustenbach and Gaffney (2006) have considered this question in relation to human exposure to odours and suggest that the majority (which they suggest might be 80-95%) should be protected from irritation or unpleasant sensory stimulation. By this criterion, the percentage of steers with nasal secretions was just acceptable at 15 and 30 ppm, but was unacceptable at 45 ppm, being 41%. Similarly the percentage of steers lacrimating was too high on exposure to 45 ppm ammonia at 36%, but was acceptable at the other ammonia concentrations. Hence in relation to irritation, 45 ppm exposure is too high, but 30 ppm acceptable. In the absence of any information between these two values, it is recommended that a value of 30 ppm is the most appropriate critical exposure limit for ammonia exposure for cattle.

For sheep, there was a difference in macrophage activity between 15 ppm ammonia and the control; however, weaker differences between 30 ppm ammonia and the control negate using macrophage activity as a basis to set a critical ammonia level at or below 15 ppm. The other factor to differ significantly between ammonia concentrations was live weight. Here the difference was between 15 and 30 ppm ammonia. The inability to monitor lacrimation and nasal secretions in the sheep in Experiment 5 leaves some doubt as to whether they respond in a similar way to cattle. There was no significant increase in coughing following ammonia exposure in the sheep, but there was in the cattle. The absence of evidence that ammonia caused irritation of sheep's eyes in Experiment 3 suggests that the ammonia to which they were exposed was insufficient to cause irritation, but this is likely to have been below 30 ppm on nearly all occasions. The exposure to 45 ppm in Experiment 6 did not increase lacrimation in the sheep, but numbers on each treatment were low and the experiment was not designed to test this hypothesis rigorously. The most important piece of evidence for sheep is the avoidance of 45 ppm ammonia, demonstrated in Experiment 6 by reduced lever pressing to obtain a reward and reduced time spent in the ammonia filled chamber, which indicate the presence of an unpleasant sensory stimulus. Although we cannot be certain about the proportion of sheep preferring the Control lane, it was likely to be more than the 5-20% which Paustenbach and Gaffney (2006) propose is the maximum proportion of humans that can acceptably indicate that they perceive unpleasant sensory stimulation. As we have no evidence of the aversion shown to levels less than 45 ppm, we recommend that the next highest level tested in Experiment 5 (30 ppm) should be the maximum exposure limit for voyages.

On the basis of this evidence, which is by no means totally conclusive, it is recommended that the critical level for exposure of both cattle and sheep should be 30 ppm. The critical period of exposure necessary to cause irritation or unpleasant sensory stimulation is unclear. Animals were continuously exposed in experiment 5, and in experiment 3 and where evidence of irritation due to ammonia was not detected, the exposure was lower and variable. On a live export vessel, exposure is likely to be variable over time, and we determined in Experiment 2 that it is mainly affected by such factors as wind speed, faecal pad depth etc. We have only comparisons of ammonia concentrations over days from the first attended voyages, which suggest that the closed decks showed little variation, but there was considerable between-day variation in the open decks.

It is therefore not possible to justify any maximum time period for exposure, such as is currently in place for human exposure to ammonia. However, as the livestock are continuously housed in the atmosphere, in comparison with the humans who are only transiently exposed, a time weighted mean is less appropriate unless there are regular and predictable cycles of exposure concentrations. We therefore recommend that the proposed limit of 30 ppm for cattle and sheep

should be the critical exposure limit for any period of time. This also acknowledges the difficulties that would be posed by attempting to measure cumulative ammonia exposure on live export vessels, rather than an instantaneous ammonia concentration that could be checked by the veterinarian or stockman on ship.

As well as a maximum concentration on ships (critical exposure level), we recommend that ammonia concentration be incorporated into a welfare index, in conjunction with other high priority welfare indicators established by expert opinion in Part 1 of this project, LIVE.222 (Pines ***et al***., 2007). This should be a mathematical index, which acknowledges the possibility that ship owners might find it easy to provide for some aspects of welfare, whereas others may involve considerable expense or disadvantage.

We recommend that further research should focus on:

1) avoidance of ammonia by cattle; using animals exposed or not exposed to ammonia prior to the study.
2) the time dependence of the onset of irritation in mucosal surfaces in cattle and sheep, to assist in devising a time-weighted exposure limit. Following exposure to ammonia, measurements of the time taken for irritation to develop would be made using infra-red sensors to detect vascular enlargement, and callipers to detect swelling of the region. The team has a potential collaborator in Ass. Prof. Paul Mills in the UQ Vet School, who specialises in dermatology.
3) the effects of feed additives on ammonia accumulation in addition to the existing research done under LIVE.202.
4) the impact of stocking density on ammonia accumulation. We have determined that faecal pad depth has a direct relationship with ammonia accumulation, therefore knowing how this relates to stocking density should enable small reductions in critical areas (e.g. behind the bulkhead and by the engine room) to bring the ammonia concentrations to within the critical exposure limit, should this be applied to the industry. This could best be done by a modelling approach, enabling ship owners to calculate the stocking density alterations required to alleviate any ammonia problems, in conjunction with the Heat Stress Model currently in use.
5) repeating macrophage measurements with a larger sample size.
6) exploring the reasons for the reduction in live weight of sheep by conducting a standard metabolism study.
7) utilising quantifiable measurements of lacrimation for livestock exposed to ammonia.
8) determining the relationship between pad depth, air flow and ammonia production.

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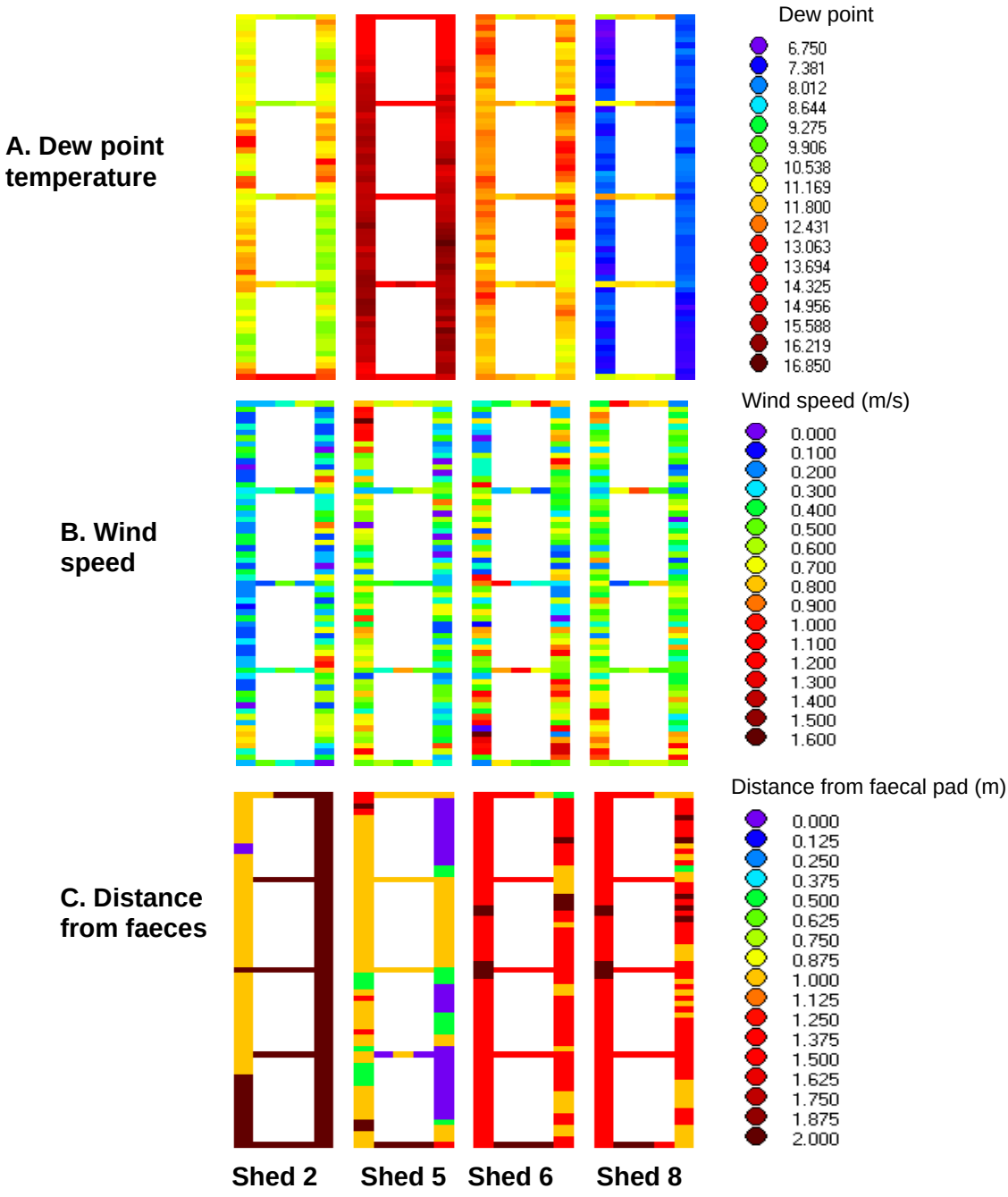
10 Appendices

10.1 Appendix 1- Experiment 4: Concentrations of potentially noxious gases at a pre-export assembly depot : Mapping of dew point temperature, wind speed and distance from faeces within four sheds.



10.2 Appendix 2: Analysis of pelleted diet

RIDLEY (4182)

Single-Mix (PR) ROCKHAMPTON MILL 82 {12} DEC 2005 ALL DATA 13:24 08/12/05 0003
12-December-2003/408.3 (82) QUEENSLAND CTWEED

Formula basic data

Code : TEST Name : HIGH ADF PELLET V3

Sell price: 0.0 Batch [Kg]: 1000.0 Group code:
Cost : 0.0 Created : 08/12/05 Version :
Margin : 0.0 Updated : 08/12/05 FM origin : 80510

Analysis

[VOLUME] % : 100.0	NA % : 0.232072	BIOTIN mg/kg : 0.163564
DRY MATTER % : 88.91875	CL % : 0.417746	FE mg/kg : 160.035
PROTEIN % : 12.0126	MG % : 0.319223	ZN mg/kg : 90.98
FIBRE % : 14.762325	S % : 0.18297	MN mg/kg : 90.0085
ADF % : 18.0335	VIT A IU/g : 6.0	CU mg/kg : 22.7453
NDF % : 35.3846	VIT E mg/kg : 19.9205	SE mg/kg : 0.430645
UDP % : 3.725648	VIT B1 mg/kg : 6.12055	MO mg/kg : 0.706
ME_RUM MJ MJ/kg : 9.892145	VIT B2 mg/kg : 2.1194	CO mg/kg : 2.061935
CALCIUM % : 1.191529	VIT B6 mg/kg : 4.3788	I mg/kg : 0.621
PHOSPHORUS % : 0.605615	NIACIN mg/kg : 63.0055	STARCH % : 21.692
CAL:PHOS NIL : 1.967469	PANTO mg/kg : 11.08875	MONENSIN mg/kg : 25.0

Raw material	Available	%	[Kg]	Tonnes
1655 SORGHUM FINE 10.5	[X]	20.0	200.0	6.0
2671 COPRA MEAL 22.0	[X]	9.0	90.0	2.7
2750 MILLRUN 16.0	[X]	40.235	402.35	12.0705
2864 CHICK PEA OFFAL	[X]	20.0	200.0	6.0
2940 MOLASSES CANE	[X]	3.0	30.0	0.9
3179 LIMESTONE FINE	[X]	2.74	27.4	0.822
3215 KYNOFOS/BIOFOS MDCP	[X]	0.3	3.0	0.09
3225 SALT	[X]	0.5	5.0	0.15
3254 BENTONITE FINE	[X]	4.0	40.0	1.2
3340 RAP QLD DAIRY/BEEF PMX (2KG/TON)	[X]	0.2	2.0	0.06
4653 RUMENSIN 10%	[X]	0.025	0.25	0.0075
		100.0	1000.0	30.0

10.3 Appendix 3: Mean ammonia concentrations for four treatments in 8 trials in Experiment 5

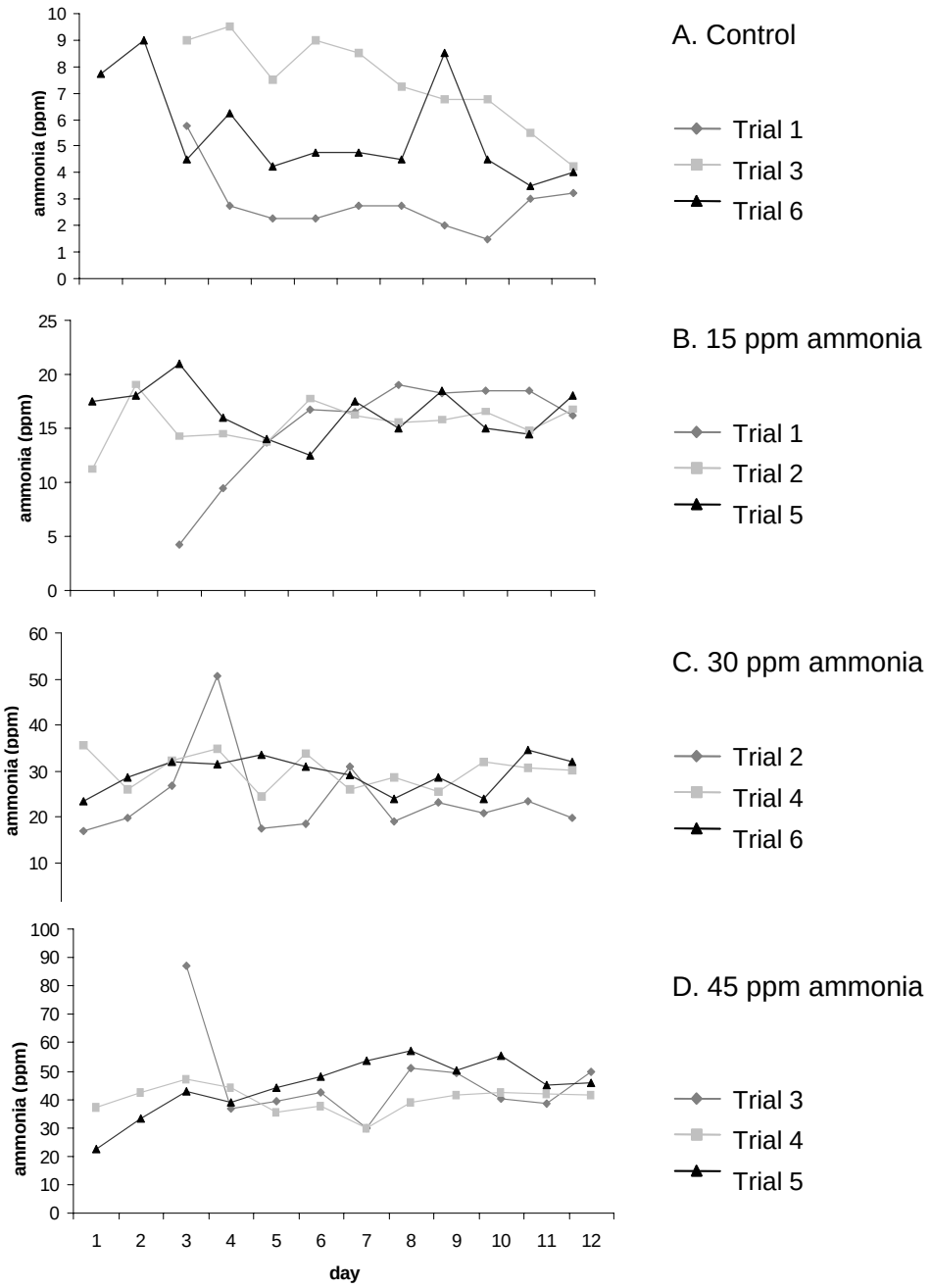


Figure. A3.1: Actual ammonia levels during each trial. The mean (± SEM) ammonia levels for A. the control were 2.8 ± 0.4, 5.0 ± 0.4 and 7.4 ± 0.5; B. 15 ppm were 15.1 ± 1.5, 15.6 ± 0.4 and 16.2 ± 0.7; C. 30 ppm were 25.0 ± 2.9, 29.8 ± 1.0 and 30.0 ± 1.0 and D. 45 ppm were 46.5 ± 5.0, 40.1 ± 1.4 and 48.1 ± 1.7.

10.4 Appendix 4: Bronchio-alveolar Lavage in Cattle:

Applies to: **Cattle, Respiratory Disease Diagnosis**
Author: **Scott Norman**

Introduction:
Broncho-alveolar lavage can be used as an aid to the diagnosis of inflammation in the lower respiratory tract. Inflammation can result from infectious agents, or through irritant atmospheric chemicals. BAL can be assessed for the presence of WBC's and cultured for bacterial identification. Note: Ideally, samples should be processed within 30 minutes of collection. This may involve centrifugation of the sample in order to harvest the cells to make a smear. Smears can be stained using the Diff-Quick procedure, or alternatively with Wright's stain for long-term preservation. If samples need to be stored, they can be placed in either plain or EDTA tubes and chilled to 4 degrees Celsius.

Procedure:
1. Light sedation should be administered to the animal either with xylazine (0.04mg/kg), or acepromazine (0.04mg/kg) via intravenous injection. A one or two mL syringe will be needed to accurately measure this dose.
2. Catch the animal in a head-bail and apply a halter. Tie the head so that it is in full extension with the nose pointing upwards.
3. Prepare sampling equipment including: 1 x 10mL syringe containing 2% lignocaine, 1 x 10mL syringe for filling the cuff, 2 x 50mL syringes containing normal saline, 1 x 20mL syringe for collecting the return fluid and a 5mL yellow top bottle for placing the sample in.
4. Apply a small amount of xylocaine gel to the end of a 10mm outside diameter, 240cm long equine BAL silicone catheter (BAL240, Cook Pty Ltd), pass it into the ventral meatus of the left nostril and advance it toward the pharynx.
5. Once the BAL tube is felt to touch the pharynx, instil 10mL of 2% lignocaine followed by a small amount of air to ensure the lignocaine is expelled from the tube.
6. After a few seconds, advance the tube while attempting to time the forward movement of the tube with inspiratory breaths. This will help to ensure the larynx is open when the tube is advanced.
7. When the tube advances, vigorously shake the trachea to confirm the presence of the tube within. If the tube is not felt, partially withdraw and repeat step 6.
8. Once within the trachea, advance the tube until it can no longer be pushed forward with gentle pressure.
9. Fill the tube cuff with between 5 and 10 mL air to seal the bronchi.
10. Instil 100mL sterile normal saline into the tube and follow through with 10 to 15mL air to ensure all of the fluid has entered the lungs.

11. Remove the instillation syringe so that it doesn't become contaminated and attach a new 20mL syringe. Commence suction to recover as much of the infused fluid as possible. If greater than 20mL is recovered, place the first syringe-full into a 50mL plain yellow-top bottle for storage while the next syringe-full is collected.
12. Once no more fluid can be recovered, mix the full volume of aspirated fluid together and save a 5mL sample of the well mixed aspirate in a 5mL yellow-top bottle. Label the sample and store at 4 degrees Celsius.
13. Deflate the cuff on the BAL tube and slowly withdraw the tube.
14. Remove the halter and release the animal from the crush after ensuring the exit path is not too slippery for a sedated animal to negotiate.
15. Flush the bore of the BAL tube with water and rinse the whole tube in chlorhexidine solution. Then rinse the tube in fresh water and flush the bore of the tube with sterile normal saline prior to the next animal.

You have completed the procedure.

Suggested equipment check-list

- BAL catheters – Reference BAL240, Cook veterinary products
- Tuberculin syringes for xylazine
- 22g 1.5 inch needles
- 18g 1.5 inch needles
- 10mL syringes
- 20mL syringes
- 50mL syringes
- 5mL yellow-top bottles
- 50mL yellow-top bottles
- One-litre bags of normal saline
- Chlorhexidine concentrate
- 2 buckets
- Halters and ropes
- 2% Lignocaine
- Xylazine 20mg/mL or Acepromazine 10mg/mL
- Xylocaine gel
- Sharps container and rubbish bin

Questions or problems regarding this Fact Sheet should be directed to s.norman@.uq.edu.au.

10.5 Appendix 5.Transtracheal aspiration in sheep:

Applies to: Respiratory Disease, BAL, Sheep
Author: Scott Norman

Introduction:

Transtracheal aspiration can be used as an aid to the diagnosis of inflammation in the lower respiratory tract. Inflammation can result from infectious agents, or through irritant atmospheric chemicals. The aspirated fluid can be assessed for the presence of WBC's and cultured for bacterial identification. This factsheet will describe a procedure for use when cell identification is all that is required and bacterial culture is not necessary.

Note: Ideally, samples should be processed within 30 minutes of collection. This may involve centrifugation of the sample in order to harvest the cells to make a smear. Smears can be stained using the Diff-Quick procedure, or alternatively with Wright's stain for long-term preservation. If samples need to be stored, they can be placed in either plain or EDTA tubes and chilled to 4 degrees Celsius.

Procedure

Sedate the sheep using xylazine at 0.1mg/kg (~ 0.2mL xylazine 20/40kg sheep).

1. Once sedated, clip the mid-tracheal area and prepare for surgical intervention. This should include a scrub, prep and alcohol application.
2. Infuse 3mL lignocaine 2% subcutaneously over the entry site.
3. Prepare equipment including: 1 x 2mL syringe for the cuff, 1 x 50mL syringe filled with sterile normal saline, 1 x 20mL syringe for the aspirate, 1 x 5mL yellow-top bottle for the sample, Indoplas foley catheter adaptor, refrigeration equipment for sample storage.
4. Restrain the sheep in sternal recumbency with the head pulled in extension.
5. Insert a 10g angiocath cannula in between the cartilaginous rings of the mid to lower trachea. Initially insert at 90 degrees until the cannula has entered the tracheal lumen, then angle the cannula ventrally into the trachea.
6. Remove the trochar from the cannula and insert a 55cm Cook U20 6g foley catheter which has had a small amount of xylocaine applied to the tip for lubrication.
7. Pass the foley catheter down into the bronchi until resistance is felt. Inflate the cuff with one to two mL of air.
8. Attach an Indoplas foley catheter adaptor to the end of the foley catheter, attach the 50mL syringe containing the normal saline and instill the saline into the bronchi and alveoli. Follow through with approximately 3mL air to expel all of the saline from the catheter.

9. Remove the instillation syringe so that it doesn't become contaminated and attach a new 20mL syringe. Commence suction to recover as much of the infused fluid as possible. If greater than 20mL is recovered, place the first syringe-full into a 50mL plain yellow-top bottle for storage while the next syringe-full is collected.
10. Once no more fluid can be recovered, mix the full volume of aspirated fluid together and save a 5mL sample of the well-mixed aspirate in a 5mL yellow-top bottle. Label the sample and store at 4 degrees Celsius.
11. Deflate the cuff on the BAL tube and slowly withdraw the foley catheter. Once the catheter is removed, extract the cannula from the trachea.
12. Apply cetrigen spray to the wound and allow the sheep to stand and recover.
13. Flush the bore of the foley catheter with water and rinse the whole tube in chlorhexidine solution. Then rinse the tube in fresh water and flush the bore of the tube with sterile normal saline prior to the next animal.

You have completed the procedure.

Suggested equipment check-list

- 6g x 55cm foley catheters – Reference U20, Cook veterinary products
- Indoplas foley catheter adaptor
- 10g angiocath cannula
- Tuberculin syringes for xylazine
- 22g 1.5 inch needles
- 18g 1.5 inch needles
- 10mL syringes
- 20mL syringes
- 50mL syringes
- 5mL yellow-top bottles
- 50mL yellow-top bottles
- One-litre bags of normal saline
- Chlorhexidine concentrate
- 2 buckets
- 2% Lignocaine
- Xylazine 20mg/mL or Acepromazine 10mg/mL
- Xylocaine gel
- Cetrigen spray or similar
- #22 and #15 scalpel blades
- Sharps container and rubbish bin

Questions or problems regarding this Fact Sheet should be directed to s.norman@mailbox.uq.edu.au.