liveexport

Determining critical atmospheric ammonia levels for cattle, sheep and goats - a literature review

Project LIVE.218
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1 Summary

This report is a review of the literature on the issue: “The effects of atmospheric ammonia production on animal health and performance in livestock ships and feedlots.”

The principal findings from the literature are:

- Ammonia gas is volatilised into the atmosphere by the action of bacterial urease enzymes in the bedding or manure pads, breaking down urea in urine and also undigested protein in faeces.
- Volatilisation of ammonia from the bedding or pad increases when pH rises above 7.0 and with increasing ambient temperature.
- Atmospheric ammonia concentration is typically 15 ppm (with a range of 10 to 50 ppm) onboard vessels during transport of cattle and sheep. Common readings below decks reach 20 to 30 ppm. In cattle feedlots in eastern Australia, typical atmospheric ammonia concentrations are 14 to 16 ppm. Thus, the average atmospheric ammonia concentrations are comparable in these two production systems. Of even greater interest for feedlots, is that atmospheric ammonia is 25 to 30 ppm at heights of 0.25 m from the ground. Thus, even the upper ranges of atmospheric ammonia for the two systems are comparable.
- The Australian National Occupational Health and Safety Commission has three standards for exposure to atmospheric contaminants such as ammonia gas in the occupational environment: the time-weight average (TWA) exposure limit for humans working for up to eight-hour shifts on a 40-hour week; the short-term exposure limit (STEL) which is a time-weight average measured over 15 minutes and should not be exceeded in any working day; and the permissible exposure limit which is the maximum concentration that humans are permitted to enter. The time-weighted average (TWA) exposure limit for atmospheric ammonia for humans is 25 ppm. The short-term exposure limit (STEL) is 35 ppm. The permissible exposure limit (PEL) for ammonia is 50 ppm.
- Under Australian legislation and workplace conditions, atmospheric ammonia concentrations should be below the TWA, STEL and PEL concentrations. However, each of these concentration limits could be exceeded under usual conditions recorded on vessels used for live export and in Australian feedlots.
- Atmospheric ammonia can be reduced by a number of atmospheric and nutritional means:
  - Establishing adequate ventilation rates onboard vessels.
  - Feeding diets that contain good quality cereal hay, lower protein (12% or less) or a greater proportion of rumen by-pass (RBP) or undegraded intake protein (UIP) (25% of the ration protein) will decrease the urea-N substrate for ammonia production.

  - Using salts such as calcium chloride or ammonium chloride at 1% in rations to acidify urine and decrease the pH of bedding. The lower pH reduces volatilisation of ammonia.
  - Adding bedding agents that reduce the pH (eg gypsum) to reduce ammonia volatilisation.
- Production indices such as feed intake, average daily gain and feed conversion efficiency were all adversely affected in lambs, calves, and pigs by exposure to ammonia levels of 50 ppm or more. However, there was no significant production effect below this concentration of ammonia. Moreover, the length of exposure is much greater during live export and feedlots. From the literature, it is not possible to clearly deduce the effects of these longer exposures to atmospheric ammonia of 25 ppm or more.
- The respiratory system of cattle is poorly adapted anatomically and physiologically to handle respiratory challenge from ammonia, heat, or exercise.
- High ammonia concentrations can irritate the upper respiratory tract leading to coughing (particularly on hot days) and rapid breathing. The small airways of the lower respiratory tract become inflamed after exposure to ammonia.
- Pneumonia is a significant cause of mortalities in cattle during live export. However, in cattle feedlots, pneumonia is associated with a complex of mycoplasmas and viruses that induce disease in the first week after entry. Most of the literature on respiratory disorders in cattle during their period in feedlot concentrates on this early period and not on chronic effects of 60-day plus exposure.
- The possible role of ammonia in damaging the respiratory tract of cattle and hence providing the opportunity for pneumonia complex of pathogens to invade and infect the lungs should be evaluated.
- The notion of atmospheric ammonia playing a role in the aetiology of pneumonia infections is supported by work in calves, lambs, and pigs. However, the levels of ammonia used in these studies were either not recorded (calves), or were much higher in the pig (often 50ppm and 100 ppm and sometimes 150 ppm) and lamb studies (75 ppm) than commonly observed in vessels during sea transport or in feedlots.
- In contrast to Australian, UK, Canadian and USA authorities, the Commission Internationale de Génie Rural (CIGR) recommended critical value of atmospheric ammonia for pigs is 20 ppm for countries in the European Commission. If this critical value gained wider currency, then cattle feedlots and live export would frequently exceed this limit.
- The no-observed-effect value for atmospheric ammonia in pigs is debatable. Some workers propose 15 ppm as the maximal ammonia concentration to be tolerated in the air of pig buildings, while others suggest 50 ppm. In both systems, feedlot and live export, the average value for atmospheric ammonia happens to be measured at 15 ppm.

- The critical value of atmospheric ammonia above which cattle welfare and production could be adversely affected should be set at 25 ppm. This value is the same as the TWA for humans and 5 ppm above the European standard for pig housing systems. In a practical sense, setting critical ammonia at 25 ppm is achievable under current production systems. It is important to remember that the TWA of 25 ppm for humans is set for regular exposures of 8 to 10 hours maximum, repeated each day for five days. Moreover, pigs tolerate atmospheric ammonia better than cattle probably due to a better-adapted respiratory system.
- The rates of ammonia generated by sheep during transport assessed on either a m^3/s or mg/h basis are the same as cattle when measured on the same units of live weight. It is unlikely that sheep or goats are going to be qualitatively or quantitatively different to cattle in their respiratory responses to ammonia. Therefore the same critical value for atmospheric ammonia of 25 ppm should be applied to sheep and goats.
- Assessment of respiratory damage or extent of loss of lung function is difficult in cattle and sheep. Bronchioalveolar lavage (BAL) could be standardised as a functional test to assess inflammatory response in the bovine lung after prolonged exposure to high atmospheric ammonia levels.
- BAL results in cattle showed that ammonia exposure at 22 ppm and 42 ppm for nine days significantly increased the indicators of inflammation such as white cell count, macrophages, mononucleated cells and segmented cell fragments in cattle. The increases were higher at 42 ppm or more than at 22 ppm. The important aspect here is that there are initial clinical signs of inflammation at 22 ppm atmospheric ammonia. This infers that the no observed effect value for atmospheric ammonia is less than 25 ppm for cattle
- Respiratory functions of cattle are affected at lower atmospheric ammonia concentrations than production functions.
- More work should be done to develop effective tests of respiratory function in ruminants.
- More research, such as dietary acidification, is needed to evaluate methods of reducing atmospheric ammonia near ground level in feedlots.

2 Final Recommendation

The critical value of atmospheric ammonia for cattle, sheep and goats undergoing sea transport and in feedlots in Australia should be set at 25 ppm, in line with the Australian TWA for humans. In this way, action to lower atmospheric ammonia is required at the same concentration point for humans and cattle, sheep or goats.

3 Introduction

Defining a critical concentration for atmospheric ammonia above which health problems develop in cattle and sheep requires review and integration of a number of different concepts. Firstly, there needs to be a consideration of the fundamental chemical and physical properties of ammonia itself and how it is generated in animal production systems. Secondly, since ammonia is affective in the gaseous state, the gaseous exchanges associated with respiration in cattle and sheep must be considered. This review will provide a summary of the literature on the effects of atmospheric ammonia on health in animals, and humans with particular reference to cattle during long haul sea transport and in feedlots. Moreover, nutritional and atmospheric actions that reduce potential atmospheric ammonia will be evaluated. Finally, the significant gaps in our understanding and information will be identified, and possible means of addressing these deficiencies will be presented. All of this data and information will be used as the basis for finally defining a critical level for atmospheric ammonia and the associated constraints in applying that level.

4 Properties of ammonia

Ammonia is a colourless gas that is strongly alkaline, very soluble in water and has a pungent highly irritant odour. It is a colourless liquid under pressure and forms ammonium hydroxide in water. Ammonia gas has a molecular weight of 17.03, a density relative to air of 0.5967 and an absolute density of 0.771 g/L. The Chemical Abstracts Service Registry Number (CAS No.) for ammonia gas is 7664-41-7.

5 Source of atmospheric ammonia

Most of the ammonia in the environment is generated from the action of bacterial urease enzymes acting on urinary urea-N and faecal protein-N. The temperature optimum for bacterial ureases is 49^{o}C and pH optimum is 7.7 - 8.0. Therefore ammonia would be generated from urine and faeces best at high temperature and slightly alkaline pH. At 760 mm of Hg (normal atmospheric pressure), the density of moist air is 1.9 g/L and that of dry air is 1.2 g/L, so ammonia will rise slowly and disperse rapidly at its relative and absolute densities.

The ammonia in manure or litter is liable to volatilization to the surrounding air. The rate and extent of liberation into the air depends on the equilibrium in the liquid and gas phase according to the equation:

$NH_4^+(l) \rightleftharpoons NH_3(l) + H^+$

The ammonium-ammonia equilibrium is influenced by temperature and pH. Nearly all of the ammonia is bound as ammonium below a pH of 7.0, and therefore not liable to volatilization. Higher temperatures favour ammonia concentrations over ammonium, because of the positive influence that temperature has on the dissociation constant Ka which is defined as:

$Ka = [NH_3] [H_3O^+] / [NH_4^+]$

The volatisation equilibrium of ammonia from the liquid to the gas phase follows Henry's law for dilute systems:

NH_3 (l) ⌦ NH_3 (g)
NH_3 (g, manure) ⌦ NH_3 (g, air)

Therefore the partial pressure of gaseous ammonia, NH_3 (g), is proportional to the NH_3 (l) concentration. The volatilization of ammonia from manure to air is defined as the mass flux. This flux is the product of the difference in partial pressure of ammonia between the liquid and gas media and a mass transfer coefficient. Higher partial pressure increases flux. Mass transfer coefficients increase with increasing air velocity. Ventilation brings fresh air into the area while removing ammonia, water vapour, other gases such as carbon dioxide and air contaminants such as dust. The ventilation rate and pattern affects not only the overall composition of the atmosphere around the animals and their manure, but also the microclimate immediately above the manure or litter.

6 Emissions of ammonia in agriculture

The emission of ammonia into the environment is a major political issue in Europe and emission values and ammonia concentrations have been published for European agriculture (Gustafsson 1997; Groot Keorkamp et al. 1998; Misselbrook et al. 2000). To calculate the ammonia losses from farming, the Swedish Boards of Agriculture have established a data model, STANK, which has used the following standards outlined in Table 1 to calculate ammonia losses from the barn, and from the storage and spreading of manure.

From Table 1, pigs and poultry have the greatest potential for ammonia emission compared with cattle, sheep or horses. Moreover, estimates of ammonia emissions from cattle are different for beef cattle, dairy cattle, grazing cattle, feedlot cattle, bulls, heifers, and calves. To make matters worse, measuring or estimating ammonia emissions from livestock is a difficult process since they can be substantially affected by the factors listed below.

Table 1. Ammonia losses from different forms of manure of different livestock classes within the barn (% of total nitrogen content of the manure) (STANK 1999)

Livestock class	Solid manure	Urine	Deep litter manure	Liquid manure	Semi-liquid manure
Cattle	4	4	20	4	4
Sows	10	10	25	14	10
Weaned pigs/ year	10	10	25	14	10
Boars, growing-fattening pigs	10	10	25	14	10
Laying hens	10	0	35	10	10
Pullets	10	0	20	10	10
Broilers	0	0	10	0	0
Horses	4	4	15	0	4
Sheep	4	4	15	0	4

In addition, quite frequently, the livestock emission estimates were performed by different researchers, using different analysis methods and evaluating different animal handling techniques. It is not possible to produce precise measurements of regional livestock emissions because of these many potential uncertainties in the number of animals and in the ammonia emissions per animal. The ammonia estimates for livestock are based on averages and estimates that apply the best available data applicable to the specific situation. These estimates are dynamic, and as additional information becomes available that is relevant obviously they will be included to improve the ammonia emission estimates.

The amounts of ammonia lost from livestock enterprises are influenced by the following factors:

1. protein content of the feed,
2. nitrogen content of the manure, ie faeces and urine,
3. species, age and weight of the animals,
4. the housing system and the system used to manage and remove manure from the housing system,
5. the system used to store the manure,
6. temperatures in the housing system,
7. length of time animals spend in the housing and on the pasture, and
8. factors associated with the spreading of the manure.

Feeding more protein than is needed for optimum production results in increased urea concentrations in the urine. Urea from urine is often rapidly hydrolysed into ammonia in the soil or bedding. This increases the risk of ammonia losses directly from the soil or bedding.

The mean atmospheric ammonia concentrations varied between 0 to 8 ppm in houses for dairy cows, beef cattle and calves and were less than 1.5 ppm in England (Groot Keorkamp et al. 1998). In contrast, the mean atmospheric ammonia values in houses for sows, weaners, and finishers were 5 to 18 ppm, and in houses for laying hens and broilers they varied between 5 and 30 ppm (Groot Keorkamp et al. 1998). These ammonia concentrations were all determined in winter in the Northern Hemisphere with temperatures of 10.1^{o}C for England, 9.8^{o}C for The Netherlands, 8.4^{o}C for Denmark and 10.5^{o}C for Germany (Groot Keorkamp et al. 1998). These temperatures are much lower than those that are common in Australian feedlots or during the transport of cattle and sheep to the Middle East and northern Africa, or transport of cattle and goats to south-east Asia and north-east Asia. The higher temperatures in feedlots or during transport from Australia will favour ammonia concentrations over ammonium, thereby increasing the level of ammonia in the environment. Typical values measured below decks in vessels transporting cattle and sheep from Australia were 15 ppm with readings commonly reaching 20 to 30 ppm (MAMIC Ventilation Study SBMR.002, 2001). These values for ammonia are well above those reported for European production systems. There appears little information however, on atmospheric ammonia levels in open pens or how the factors such as pad depth, diet, and washing down of cattle pens influence these levels.

Feedlots in Australian produce much higher atmospheric ammonia than feedlots in North America. Ammonia emissions from cattle feedlots were monitored in Canada by the University of Lethbridge (McGinn and Janzen 1998, McGinn et al. 2003). Ammonia concentrations were measured in feedlots in the Lethbridge North Irrigation District in southern Alberta, Canada which is one of the most concentrated beef feedlot areas in Canada. Four feedlot sites, with capacities of 6,000, 12,000 and two with 25,000 were monitored for ammonia concentration (McGinn et al. 2003). The peak ammonia concentrations in the 12,000 and 25,000 feedlots were 1488 and 1050 ug/m^3 respectively. There is a method for converting mg/m^3 to ppm via the following expression: ammonia (mg/m^3) = [MW of ammonia x ammonia (ppm)]/24.4 (molar gas constant at 25^{o}C). Thus a value such as 1488 ug/m^3 would be equivalent to (1488/1000) x 24.4/17 = 2.14 ppm ammonia. Previous studies at three feedlots in Canada showed the following values for atmospheric ammonia: adjacent an average of 863 ppb, with a peak value of 5140 ppb; at 200 metres a peak value of 3660 ppb; and at 1 km peak measurements of 416 ppb ammonia (McGinn and Janzen 1998). Further studies reported levels of 400 – 500 ppb ammonia at distances of 5 km downwind from the feedlots, with levels of 40 - 50 ppb as the new

background compared with more usual levels of 5 ppb (McGinn and Janzen 1998). The important point to remember here is that atmospheric ammonia levels were being monitored for concerns about nuisance odour and human health. No direct measures were made of levels likely to affect the cattle themselves. The monitoring stations at these feedlots were either in a tower placed at three metres next to the feedlot or measured miles down wind from the feedlot.

In contrast, measures of atmospheric ammonia in Australian feedlots were made at varying heights above the manure pad within the feedlot itself. Therefore, these Australian values have direct relevance to the cattle themselves. Australian studies (FLOT.317, 2003) found that ammonia generation from pen surfaces is directly affected by temperature, with higher ammonia levels measured during the warmer parts of the day. What was of further interest was the fact that there was little difference noted between ammonia levels within the shaded and unshaded pens, probably due to the dry weather conditions experienced during the ammonia measurements. Most importantly, this Australian study found a distinct concentration profile above the pen surface. Peak concentrations were in the order of 12 to 16 ppm when measured at a height of 1 metre above the surface. However, peak concentrations measured at a height of 0.3 metres increased to 25 to 30 ppm. These concentrations of atmospheric ammonia actually measured in Australian feedlots (as distinct from next feedlots in Canada) place them in the same concentration range as atmospheric ammonia onboard vessels transporting livestock.

Nevertheless, feedlots in general produce much lower atmospheric ammonia concentrations than pig production systems. For instance, in Ohio, levels of atmospheric ammonia were 35 ppm and 176 ppm in ventilated and unventilated piggeries respectively. These sorts of exposure values led to concerns about the occupational health and safety of piggery workers. Consequently, Ohio passed a new legislative limit for atmospheric ammonia exposure by humans of 10 ppm for 8 hours. In a review of workplace health in Canada, workers from high ammonia environments in agriculture had a 4-fold increase in chronic cough, a 7-fold increase in productive cough and a 4-fold increase in wheezing. Reduced pulmonary function was a common finding and the respiratory effects showed a dose-dependent relationship to particulate matter and ammonia. Clearly atmospheric ammonia is an occupational health and safety concern for humans, and some governments e.g. Sweden and the Ohio state legislature have responded to those concerns by lowering the limits for exposure to atmospheric ammonia.

7 Anatomical features of the ruminant respiratory tract

Since atmospheric ammonia influences all animals, not just humans, through their respiratory system, we have reviewed the basic structure and function of the ruminant respiratory system. This is a comparative review with an emphasis on cattle relative to other species of the same size eg horses (Appendix 1). The anatomy of the ruminant respiratory tract highlights some notable differences from that of man and other animals. Some of these factors – including relative narrowness of the upper respiratory tract, a high degree of anatomic compartmentalisation, small lung size, abundance of lymphoid tissue, and frequent exposure to rumen gases – might be predicted to confer an inherent susceptibility to respiratory pathology and dysfunction. It is in examining the physiological capacity of cattle that more obvious functional deficiencies become apparent. In reviewing anatomical and physiological factors predisposing cattle to respiratory disease, Veit and Farrell (1978) noted their small gaseous exchange capability relative to their basal O_2 needs. Using the reference data of Altman & Dittmer (1971), they demonstrated that the bovine ratio of total alveolar surface area: basal O_2 consumption (VO_2) is less than half of the mean mammalian value. As the mean alveolar diameter is only slightly below (and VO_2 only slightly above) that predicted allometrically (Tenney et al. 1963; Altman et al. 1971), this deficiency appears mostly due to the small size of the bovine lung. Gehr et al. (1981) found that the measured volume of a cow lung was only 48% of that predicted by allometric regression (Gehr et al. 1981). The alveolar surface area was also lower (77%) than that predicted allometrically. The lack of a similar discrepancy in wild African bovids suggests that this loss of respiratory capacity is the result of domestication and selection for other traits such as digestive efficiency and muscle mass (Gehr et al. 1981). It is not known if there is a difference in respiratory capacity or efficiency between *Bos taurus* and *Bos indicus* cattle.

The principal functional implications of this reduced respiratory surface are three-fold. Firstly, greater basal ventilatory activity is necessary. The tidal volume of a 400kg dairy cow is approximately 3100ml when lying down, or 3700ml when standing (Altman et al. 1971). This volume is, in itself, appropriate for an animal of this size (Dukes 1955). However, given the small lung volume, the ratio of resting tidal volume: total volume (percent basal use) is high in cattle (29%) when compared to the mammalian mean of 13.9% (Veit et al. 1978). Cattle therefore use a greater proportion of their lung volume for basal respiration. This, in turn, predisposes to a more extensive and deeper (more distal) pulmonary distribution of any inhaled irritants. Secondly, the airflow rate per unit lung volume (minute volume / total lung volume) is also well above the mammalian mean (Veit et al. 1978). This greater airflow rate might be expected to increase epithelial exposure to inhaled irritants per unit area. In addition, the greater airflow velocity increases turbulence and epithelial impingement of

airborne material. Thirdly, the small alveolar surface area reduces the functional reserve of the bovine respiratory system. Thus, the animal's capacity to increase gas exchange during exertion or disease is limited. Again, comparison with wild African bovids demonstrates that domestication has reduced the maximal rate of oxygen consumption (VO_2 max) relative to that predicted allometrically (Taylor et al. 1980). This is shown more dramatically by the observation that despite similar body size, the horse (a species selected for athleticism) has a VO_2 max more than 3 times that of the cow. Interestingly though, domestication did not similarly reduce the VO_2 max of sheep and goats, which is comparable to that of small wild ruminants such as gazelle (Taylor et al. 1980).

Several other features reduce the respiratory efficiency of ruminants. Delivery of air to the lungs is a function of effort by respiratory muscles, elasticity of the lung and thorax, and flow resistance of the airways (Robinson 1982). The poor compliance of the thoracic wall, and small size of the upper airways have already been noted. The rigid structure of the chest results in a high residual volume, further compromising reserve capacity (Dukes 1955). Epling (1964) subjectively noted the sparsity of elastin fibres in the bovine interalveolar septum. He also reported lower numbers of alveolar capillaries than other species, though this qualitative observation has not subsequently been validated (Epling 1964). If true, this would suggest a comparatively higher proportion of alveolar dead space, hence inherent inefficiency of the air-blood interface. It would also infer that a given amount of toxic material would impact on a proportionately smaller endothelial mass, possibly creating greater vascular exposure to toxins (Veit et al. 1978). The same does not appear to be true of the small ruminants, as at least one author has noted the alveolar capillary network is extensive (Atwal et al. 1971). Cattle also have particularly vigorous pulmonary vasoconstrictor response to hypoxia and hypercapnia. This, combined with their small respiratory capacity, makes them prone to pulmonary hypertension at high altitudes (Robinson 1982).

As noted above, the respiratory tract of sheep appears to have fewer anatomical and physiological constraints than that of cattle. However, the thickness of the ovine fleece gives the respiratory tract great importance in thermoregulation, which to some degree compromises its respiratory functions. As ambient temperature rises, respiratory minute volume increases rapidly, principally due to an increase in respiratory rate. A comparison of Egyptian (Rahmani) and Merino sheep showed that breeds adapted to higher native temperatures (hence more efficient respiratory thermoregulation) have greater respiratory dead space and a smaller alveolar surface area, despite a similar lung volume per unit body weight (Shafie et al. 1978). Thus the Merino is forced to effect a greater increase in its respiratory rate, and this has a correspondingly greater impact on respiratory alkalosis, and presumably on exposure to any inhaled irritants.

8 Airway receptors

In addition to differences in the basic anatomy and physiology of the respiratory system, there are many different types of afferent receptors in the airways. The larynx contains at least five types of receptors: pressure, drive, cold, irritant and C-fibre. In the trachea and bronchi there are least four different types: slowly and rapidly adapting stretch receptors (SARs and RARs), C-fibre receptors and those in the neuroepithelial bodies (NEBs) (Widdicombe 2001). Irritant receptors are found in the epithelium of the conducting airways and are supplied by the smaller myelinated fibres than stretch receptors.

Irritant receptors are normally silent and are then activated by a wide range of chemical and mechanical irritants including ammonia and dust respectively (Widdicombe 2001). They are part of the RAR group of receptors where they mediate the coughing reflex and usually act to constrict airways. C-fibres have proved difficult to study but they are known to be weakly responsive to mechanic irritants and stimulated clearly by chemical irritants. In contrast to irritant receptors the reflex actions of C-fibres are not clear, but may include the pronounced apnoeas often seen on laryngeal irritation. SARs are different to both of these types of receptors since they are not very chemosensitive. Thus the RAR receptors, and part of the action of the C-fibre receptors respond to stimulation by ammonia and act to restrict airways and induce the coughing reflex.

9 Physiology of the respiratory system

The respiratory system has one major physiological function, namely gaseous exchange. In achieving this function, the respiratory system also has a significant role in homeostasis of body temperature and regulation of acid-base balance. Generally these functions are not in conflict but if the animal is exposed to excessive heat load or to acid-base challenge a conflict may arise. In particular as panting changes from deep breathing to shallow breathing especially during heat load, the animal's need to alleviate a rise in core body temperature may be compromised by its need to balance blood CO_2. The CO_2 concentration in the blood has a powerful influence on ventilation. The respiratory centre, which is located in the medulla part of the brain stem responds to the amount of CO_2 in the blood. As blood passes through the respiratory centre, sensors stimulated by the level of CO_2 send impulses to rib cage muscles to increase the rate of respiration. The overall control of acid-base is a function of the Henderson-Hasselbalch equation where:

$$pH = pKa + \log [base]/[acid]$$

The specific case of the body's major system for regulating acid-base balance, the bicarbonate system can be represented in Figure 1.

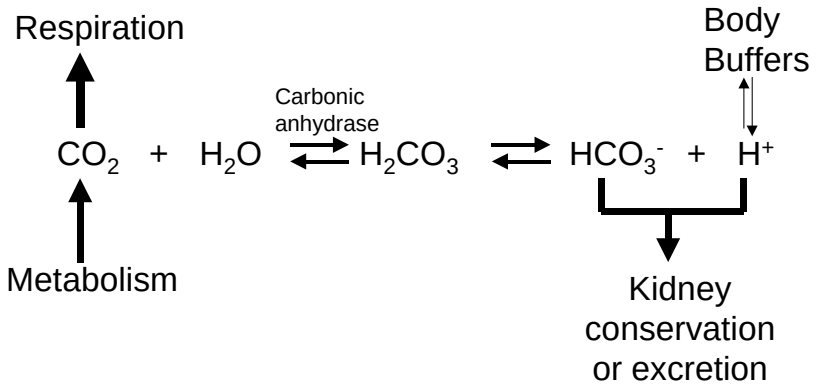


Figure 1. Physiological representation of the bicarbonate buffer system.

Acidity, or H^+ ion concentration of the blood is directly related to CO_2 concentration. A rise in $[H^+]$, ie a decrease in pH, will drive the equation to the left, increasing CO_2. Therefore a decrease in blood pH will stimulate respiration in conjunction with a rise in CO_2. A rise in temperature and humidity will increase the respiratory rate in an attempt to cool the core body temperature. When blood passes through the lung capillaries both CO_2 and heat is removed. If an animal is coping with a given heat load, then the rate of tissue metabolism is modulated by the opposing local influences of temperature and pCO_2 (Bligh 1973).

Barnes et al. (2003) showed that respiratory rates were consistently higher than 100 bpm for *Bos taurus* cattle under heat load of wet bulb temperatures higher than 30^{o}C. Even though respiratory rates were less severely affected by heat load in *Bos indicus* cattle, they nonetheless still showed increases to over 100 bpm when wet bulb temperatures were higher than 32^{o}C (Barnes et al. 2003). Reducing core body temperature is the imperative driving respiratory rates in cattle. Increases in respiratory rate over 75 bpm often cause respiratory alkalosis because of the loss of CO_2 on ventilation. Respiratory alkalosis is a primary acid-base disturbance where pCO_2 falls below normal levels. However, respiratory alkalosis will not always result from increased total ventilation and increased alveolar ventilation will not always result in a respiratory alkalosis. Barnes et al. (2003) found that the respiratory alkalosis arising from a decrease in pCO_2 was compensated by a decrease in bicarbonate with the result that there was no significant change in plasma pH from 7.4. Notwithstanding this, these cattle were severely affected by heat, and the pressure on acid-base balance was also severe. Importantly, this data was collected under conditions where the atmospheric ammonia was kept below an average of 5 ppm by regular cleaning of the room. These types of values for atmospheric ammonia are in line with those reported from the feedlots in Canada. Moreover, the cattle in this study did not develop any coughing or show any gross signs of respiratory inflammation (David Beatty and Anne Barnes, pers comm). Thus, severe heat load per se did not compromise respiratory function when

atmospheric ammonia levels were low. *Bos indicus* have a better capacity to tolerate heat load than *Bos taurus*. Whether there is also a difference in capacity to handle ammonia load during heat load remains unresolved.

10 Effects of ammonia on livestock

Gaseous ammonia is a severe respiratory tract irritant capable of inhibiting the efficiency of the respiratory system at high levels. The health effects of breathing ammonia have been documented in rats and are presented in Table 2. The clinical signs of respiratory compromise developed at atmospheric ammonia from 50 ppm and greater in rats.

The type of effects in rats have been repeated in studies in pigs, poultry, calves and sheep and will be discussed in more detail later in this review. The effects of atmospheric ammonia on livestock have been documented mainly in pigs and poultry. However some studies have been completed in lambs and calves. Respiratory dysfunction is the most frequently observed sign of ammonia exposure in all species.

Drummond et al. (1978) kept young pigs in ammonia-contaminated atmospheres of either 50 ppm or 75 ppm for 2 hours during which they were also challenged with aerosol containing bacteria. Pigs exposed to ammonia harboured 51% more bacteria in their lungs than did control pigs. Notwithstanding the changes in bacterial load in the lungs, short exposures of two hours at these concentrations of ammonia did not affect either production performance or gross histopathology of the respiratory tract. However, when Drummond et al. (1980) kept 27-28 day-old pigs in high atmospheric ammonia of 0, 50, 100 or 150 ppm for longer periods of four weeks, growth rates were significantly reduced by 12, 30, and 29% respectively in the ammonia-exposed groups of pigs. Interestingly, pigs exposed to 50 or 100 ppm atmospheric ammonia were more efficient converters of feed to body weight gain than were control or 150 ppm exposed pigs. So the effect of high ammonia on production parameters is not consistent. Importantly all of these studies had 50 ppm as their lowest value for atmospheric ammonia that is the same as the short-term exposure studies presented in Table 2.

Acute inflammatory reactions were observed in the tracheal epithelium of pigs exposed to atmospheric ammonia of 100 and 150 ppm but not in controls or those pigs subjected to 50 ppm. From these results the inflammatory effects in pigs were not acute at atmospheric ammonia concentrations below 100 ppm. Nevertheless, these reports from Drummond and co-workers (1978 and 1980) should be applied with caution since the experimental design showed serious flaws. Two-hour exposures are too brief to detect acute inflammatory changes below 100 ppm. Moreover, atmospheric ammonia concentrations of 50, 100 and 150 ppm are inappropriate since they begin too high in range and do not extend high enough

into the range for useful comparison to the production conditions observed in the Ohio piggeries. It would have been preferable if these workers included concentrations of 10 ppm (the legislated exposure level for humans in Ohio piggeries) or 25 ppm, the most frequently used legislative limit for humans.

Table 2. Some health effects from breathing ammonia for animals

Short-term Exposure (less than or equal to 14 days)		
Levels in Air (ppm)	Length of Exposure	Description of Effects*
50	3 hours	Slowed breathing rate in rabbits; coughing, eye, mouth, and nose irritation, poor weight gain and food intake in pigs.
100	6 hours	Increased irritability in rats.
500	7 days	Decreased weight gain and food intake in rats. Decreased resistance to disease in mice.
1000	16 hours	Death in rats and mice.
Long-term Exposure (greater than 14 days)		
Levels in Air (ppm)	Length of Exposure	Description of Effects*
653	90 days	Death in rats.

- These effects are listed at the lowest level at which they were first observed. They may also be seen at higher levels.
- Source: Agency for Toxic Substances and Disease Registry, Division of Toxicology, 1600 Clifton Road, E-29, Atlanta, Georgia 30333.

Urbain et al. (1994) investigated the effects of atmospheric ammonia on nasal mucosa and somatic growth and used nasal lavage (NAL) to quantify the inflammatory response by measuring the number of neutrophils and albumin concentration in the NAL liquid. This study used a dose-response design where seven-week old pigs were exposed to atmospheric ammonia concentrations of 0, 25, 75 and 100 ppm until they were 12 to 14 weeks old. This study confirmed that ammonia was an irritant for nasal mucosa and that mucosal permeability to inflammatory cells was a better marker of inflammation than permeability to albumin. Most importantly, a 6-day exposure to ammonia induced nasal irritation and depression of somatic growth at concentrations as low as 25 ppm. Therefore the no-observed value for ammonia-induced somatic growth inhibition in pigs subjected to a 6-day exposure must be lower than 25 ppm. In fact, Urbain et al (1994) proposed 15 ppm as the maximal ammonia concentration to be tolerated in the air in piggeries. This of course did not take into account the exacerbating effect of dust on any ammonia toxicosis. The Commission Internationale de Génie Rural (CIGR) recommendation for pigs is 20 ppm (CIGR 1984). Notwithstanding this

proposal by Urbain et al. (1994), there has been no move to lower the CIGR recommendation of 20 ppm for pigs. The major limitation in the study by Urbain et al. (1994) is the fact that they had no value below 25 ppm for atmospheric ammonia in their dose-response range. Therefore, this proposal for 15 ppm by Urbain et al. (1994) is not grounded in actual data from their study. Nevertheless, this data did show that nasal lavage is a more sensitive technique for evaluation of the effects of atmospheric ammonia than the gross or histo-pathology of previous methods. The fact that previous studies used less sensitive methods may help to explain some of the contradictory findings from previous studies. This is particularly so for the no-observed-effect for atmospheric ammonia of 50ppm in pigs exposed for a period of 6 days (Gustin et al. 1994). Nevertheless, setting a no-observed-effect value for ammonia at 15 ppm for pigs has immediate and important implications. Pigs are often exposed to these levels of atmospheric ammonia for periods much longer than 6 days under normal production circumstance. For instance this concentration of ammonia is already exceeded either as a mean value (17.8 ppm in the Netherlands) in piggeries holding sows on slatted floors or as a frequently measured value in the same type of animal and housing system through Northern Europe (Groot Koerkamp et al. 1998). In addition, ammonia levels reported for piggeries in Ohio were well in excess of 15 ppm. Thus, any broad acceptance of 15ppm as a limit for atmospheric ammonia would present a number of practical difficulties.

Lambs exposed to an atmosphere of 75 ppm ammonia for 28 days had lower feed conversion efficiency than lambs in a control atmosphere (Drummond et al. 1976). Control lambs gained an average of 90 g more liveweight per day and consumed an average of 0.68 kg less feed (4.53 vs 5.21kg) per kg of liveweight gain compared to the ammonia-exposed lambs. Moreover, the ammonia-exposed lambs showed clear signs of ammonia toxicosis such as profuse lacrimation, severe coughing and sneezing, and profuse nasal discharge that at times was bloody (Drummond et al. 1976). Clearly there was a significant effect on respiratory function in these lambs. However, these results represent the extremes of ammonia exposure as it is unlikely that sheep will be exposed to levels of ammonia as high as 75 ppm for the extended 28-day period as in the Drummond study.

Dewes and Goodall (1995) reported some preliminary studies on the possible relationship between ammonia production from soiled bedding in calf rearing sheds and calf illness. This was a limited field study on six farms, where death rates were high (10% and 13.5%) on two properties. Atmospheric ammonia was not measured directly in this study but was determined *in vitro.* The progression of clinical signs in calves continuously exposed to ammonia for a few *days* were languid habit, rapid breathing, noticeable coughing on hot days, shedding of tears, reduced feed consumption, diminished social behaviour and cessation of weight gain. On post-mortem, histological changes showed that small airways were destroyed. Their findings from histopathology supported a diagnosis of subacute purulent

pneumonia with distal necrotising bronchiolitis. Interestingly, these authors concluded that ammonia did not pass in any significant quantity between the aveolar epithelium and the plasma. They reported a plasma ammonia concentration of 29mM, much less than the 50mM recorded for humans. Plasma ammonia concentrations are difficult to interpret as they represent the flux and outflow of ammonia from the rumen, and the hepatic clearance of ammonia through the urea cycle. In some studies an inverse relationship between ruminal ammonia and plasma ammonia has been recorded (Leadbetter 1997). The relationship between atmospheric ammonia and plasma ammonia is even less clear. While it would be worth investigating the relationship between atmospheric and plasma ammonia, it is unlikely that plasma ammonia concentration would be a good indicator of atmospheric ammonia load.

The effects of atmospheric ammonia in feedlots have not been quantified in terms of loss of cattle production. However, the concentrations of ammonia have been measured in feedlots in Eastern Australia (FLOT.317 2003). Ammonia generation from pen surfaces was directly affected by temperature, with higher ammonia levels measured during the warmer parts of the day. Little difference was noted between ammonia levels recorded at a height of 1 metre within the shaded and unshaded pens. This was attributed to the dry weather conditions experienced during the ammonia measurements. Higher ammonia concentrations were noted at lower heights (less than 0.3 m above surface) in the moister areas under shade compared to the unshaded pens. A clear profile of ammonia concentrations existed above the pen surface. Peak concentrations were found to be in the order of 12 to 16 ppm when measured at a height of 1 metre above the surface even a quite high ambient temperatures (FLOT.317 2003). These peak values are less than the human TWA and are of the order of the no-observable effect for piggeries (15 ppm) recommended by Urbain et al (1994). So it is reasonable to conclude that atmospheric ammonia values of 12 to 16 ppm, while pungent, would not present any observable disturbance to the respiratory system of these cattle.

However, peak concentrations measured at a height of 0.3 metres were 25 to 30 ppm for 30 minutes of measurement from 1300 to 1330h in an unshaded pen on January 22nd 2002 (FLOT.317 2003). These concentrations are at or above the human TWA for ammonia concentration but were only sustained for relatively short periods. However, there is no associative study of any potentially harmful effects of these concentrations of ammonia on cattle. What is noteworthy is that this height (0.25m) would be the height of the feed and water troughs. Grazing cattle split a 24-hour day into roughly equal parts of grazing, ruminating and sleeping. If cattle in feedlots follow a pattern where large amounts of time are spent eating and drinking then they would be exposed to relatively higher concentrations of atmospheric ammonia. It is unknown whether the pungency of ammonia affects feed or water intake and, more importantly, whether there is a chronic effect of atmospheric ammonia on the respiratory system of cattle in feedlots.

11 Major health problems during live export and in feedlots

Norris et al. (2000, 2003) surveyed cattle deaths during sea transport from Australia from 1995 to 2000. Although death rates were generally low for cattle during sea transport, the highest rates were recorded from the major southern ports of Fremantle, Adelaide and Portland. Monthly death rates varied with season with rates being much higher from April to July from southern ports. The highest death rates overall occurred on vessels destined for the Middle East and north Africa. A significant proportion of these deaths (17%) occurred on a small number of voyages (2.6%) (Norris et al. 2000, 2003). The main cause of death during sea transport was heat stroke, with *Bos taurus* cattle more susceptible than *Bos indicus* cattle (Norris et al. 2003). Heat stroke could be exacerbated by chronic exposure to atmospheric ammonia at levels such as 15 ppm frequently observed during voyages (SBMR.002, 2001). Trauma was another significant cause of death but this appeared to be confined mostly to one voyage (Norris et al. 2003).

In an earlier report by Norris et al. (2000), a very high proportion of the death rates (32% in voyage 1 and 22% in voyage 2) were associated with the pneumonia that was severe, subacute and characteristic of *Mannheimia (Pasteurella) multocida* and *M. haemolytica.* However, in the later report by Norris et al. (2003), respiratory disease figured less prominently in the causes of death after heat stroke and trauma. Nevertheless, fibrinous pneumonia and pleuro-pneumonia accounted for 28 out of 34 respiratory disease deaths. The gross and histopathology was consistent with typical involvement of *Mannheimia* (*Pasteurella) multocida* and *M. haemolytica. Mannheimia* species are frequently isolated from the upper respiratory tract of cattle (Rheinberger 2000). The presence of these bacteria in the respiratory tract does not of itself induce pneumonia unless the innate defenses of the lung have been compromised. In fact, *Mannheimia multocida* is part of the mycoplasma-induced respiratory disease complex (MIRD) and even though *P. multocida* is often isolated from pneumonia, some damage to the respiratory system is usually required for it to invade and infect. Therefore, development of full pneumonia is often contingent on other factors aggravating the lung. During sea transport of cattle, the prime candidates amongst the other factors would be the level of atmospheric ammonia, and the extent of dust in the environment.

High temperature, humidity, ammonia and dust are all present as factors during sea transport. For instance if air is not conditioned in the upper respiratory tract, then cilia on the lower respiratory tract become dry and cease to function (Rheingberger 2000). The conditions for drying of the upper respiratory tract can occur in cattle under thermal stress for extended periods during sea transport to the Middle East and north Africa. The main function of the

ciliated cells is to propel the tracheobronchial secretions towards the pharynx as part of the "mucociliary escalator". This is part of the non-inflammatory defense directed towards prevention of pathogen adherence. Coughing is one of two components of the physical defense mechanism to eliminate particulate matter such as dust. Ciliated cells reach down to the respiratory bronchioles. At the terminal bronchii and alveoli, macrophages play an important role in clearing inhaled particles. Thus macrophages play an important role in killing and clearing bacteria that have gained entry to the alveoli. Complement and neutrophils make up that second line of defense. Boyd et al. (1944) found that the naso-bucco-pharynx acted as a filter for ammonia by absorbing significant amounts of ammonia thereby protecting the trachea and bronchi but not the small bronchioles and aveoli. If this is so, then ammonia could affect this second line of cell mediated immunity associated with macrophages, neutrophils and complement. Exposure to atmospheric ammonia may be a chronic irritant to airway receptors and may therefore be a key factor in the development of pneumonia during sea transport.

Confirmation that ammonia can act as one of the primary agents for subsequent MIRD infection remains a controversial issue. Andreasen et al. (1994) conducted two experiments in pigs. In the first experiment, pigs were exposed to 5 and 50 ppm ammonia for 59 days during which time they were also challenged with aerosol containing *M. multocida*. In this experiment none of the pigs developed pneumonia. In the second experiment, pigs were exposed to <5, 50 and 100 ppm ammonia for 59 days during which time they were challenged with *M. multocida*. Pigs in the 50 and 100 ppm ammonia-exposed groups did show clinical signs of pneumonia (coughing and dyspnea) but did not show a dose-dependent increase in signs. However, there was no difference in rates that *M. multocida* was re-isolated from the three groups: 5/17 (<5 ppm), 2/10 (50 ppm) and 3/10 (100 ppm) even though the lesions were more extensive in the groups exposed to the high ammonia levels. This is not surprising in the case of pigs exposed at < 5ppm because *M. multicoda* is probably a normal resident of nasal passages. Andreasen et al. concluded that ammonia *per se* was unable to induce the required damage for *M. multocida* to invade and infect the lungs in pigs. On the other hand they also concluded that ammonia increased the extent of pneumonia lesions caused by the MIRD complex but not in a dose-dependent manner. These studies in pigs did not resolve the issue given the poorer capacity of bovine lung anatomically, physiologically and possibly immunologically to meet challenges from ammonia.

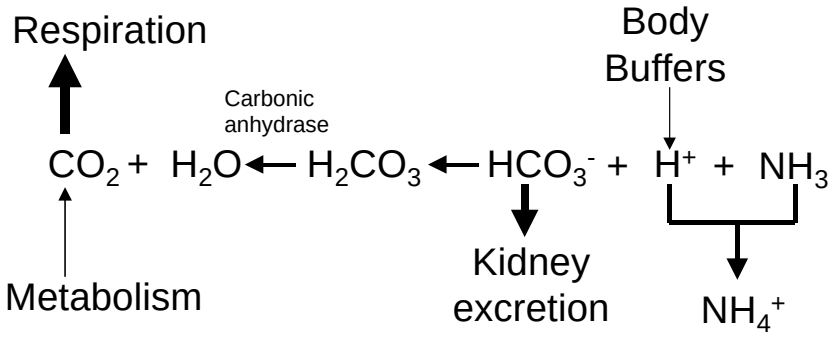
In feedlot production systems, the point of entry of cattle is the critical time for health problems in cattle. Feedlots are a stressful environment for cattle because of transit to the feedlot, mixing of different groups of cattle, admission procedures, introduction to new forms of feed and ingredients in feeds, and new water supplies. Respiratory diseases are the most prevalent health problem in the first three to six weeks in the feedlot (Kelly and Janzen 1986,

Dunn et al., 2000, Sullivan 2000). Bovine respiratory disease (BRD) is the most common diagnosis. The viruses commonly associated with BRD are: bovine herpesvirus 1, bovine parainfluenza 3 virus (PI3), bovine viral diarrhoea virus (BVDV), and bovine respiratory syncytial virus (BRSV). The bacteria associated with BRD in feedlot cattle, including *Mannheimia haemolytica*, *Mannheimia multocida*, (both of these were also isolated from cattle during live export), *Haemophilus somnus* and *Actinomyces pyogenes*, are not often primary pathogens in healthy unstressed cattle. It is important to remember that BRD is a complex, multifactorial and not completely defined disease. Therefore diagnosis, management and prevention are all aimed at controlling BRD in feedlot cattle. However, it is unlikely that atmospheric ammonia would build up to levels that would exacerbate BRD during the entry period. On the other hand, atmospheric ammonia does increase in feedlots with time on feed (FLOT.317, 2003). The specific health effects of atmospheric ammonia in cattle during feedlot are not documented.

Moreover, the effect of chronic exposure to atmospheric ammonia levels of 15 ppm or more commonly observed during voyages must be evaluated in the aetiology of heat stress in cattle during sea transport (Norris et al. 2000, 2003). Ammonia can exacerbate hyperthermia either directly through its direct action on respiration and the airway receptors or indirectly through ammonia disturbing the acid-base balance of animals. Cooling during hyperthermia occurs in significant measure through increased respiratory rate (in addition to sweating through the skin) in cattle, and is almost solely through increased respiratory rate in sheep. Ammonia directly decreases respiratory rate in animals, which will obviously work against respiratory cooling. Moreover, ammonia can induce secondary metabolic alkalosis through the direct effect of NH_4OH on pH. Metabolic alkalosis is a primary acid-base disorder that causes the plasma bicarbonate to rise to a level higher than normal. The effect on respiration is hypoventilation, ie a decrease in respiratory rate. The severity of a metabolic alkalosis is determined by the difference between the actual [HCO_3^-] and the expected [HCO_3^-]. Secondary or compensatory processes that cause an elevation in plasma bicarbonate should not be confused with the primary processes. An elevation in bicarbonate occurring in response to a chronic respiratory acidosis should be referred to as a 'compensatory response' and never as a ‘secondary metabolic alkalosis’. The literature will often refer to a 'compensated metabolic alkalosis' as a 'metabolic alkalosis with a (secondary) respiratory acidosis'. This is wrong as the hypoventilation is a compensatory process and does not indicate any primary respiratory problem. In fact, atmospheric ammonia combining with the moisture in the respiratory tract will further decrease the hydrogen ion concentration by pulling H^+ into NH_4^+ as shown in Figure 2. Removing H^+ from the body buffer system will continue the alkalosis in face of any compensating loss in bicarbonate ions through kidney excretion, and loss of bicarbonate through ventilation. Thus the acid-base system in the animal will not be compensating, and the lower H^+ will act directly through the respiratory control centre in

the brain to decrease the respiratory rate. During hyperthermia, this is precisely the wrong direction for the animal's temperature homeostasis.

Figure 2. Effect of atmospheric ammonia (NH_3) on acid-base compensation during hyperthermia.



Barnes et al. (2003) showed firstly, the increase in respiratory rate during increases in wet bulb temperature and secondly, the decrease in bicarbonate by way of compensation for the respiratory alkalosis. However, there was no confirmation of the effects of ammonia on cattle under severe heat load in these experiments as the cattle were not subjected to high atmospheric ammonia because care was taken to clean the rooms and remove the bedding regularly. Obviously this is a gap in our information about the precise nature and severity of the effects of ammonia on acid-base balance in cattle under high heat load. On the other hand, the studies of Barnes et al. show the primary importance of heat load as the major factor in respiratory compromise.

12 Factors influencing levels of atmospheric ammonia

The major factors influencing atmospheric ammonia during live export include ventilation rate, stocking densities, prior diet, ship-board diet, pad moisture levels, deck washing on ship, and pad depth. In feedlots the major factors are pad depth, rainfall, shade in the feedlot and wind speed.

The influence of ventilation on ammonia has been reviewed in the report SBMR.002 (2001). Managing ventilation rate reduced heat load and should have the added benefit of keeping atmospheric ammonia at lower levels. Nevertheless the authors noted that the ammonia levels measured, ie 15 ppm typically below deck with some readings reaching 20 – 30 ppm, were higher than expected from calculations based on available literature (SBMR.002 2001). These higher than expected values must be of some concern for the industry as in many

instances the atmospheric ammonia concentration was higher than allowable limits for animal housing in Europe.

The diets provided for cattle are formulated for palatability and sustained productivity during sea transport. Most formulations are based on straw because it is chemically consistent and available in large quantities. As a result of using straw, the grain component of the rations must have high energy and protein density and therefore comprise mainly barley and lupins, both of which contain a high proportion of rumen degradable protein especially when they have been ground for production of pellets. High levels of rumen degradable protein can lead to high rumen ammonia concentrations that cross the rumen wall and are detoxified as urea in the liver. This leads to a high output of urinary nitrogen. The urea component of urinary nitrogen completes the cycle and forms the basis of high atmospheric ammonia during sea transport. Reformulating these rations using better quality roughage such as cereal hays that are also lower in nitrogen than lucerne hay decreases the urinary nitrogen output in a cost-effective manner. In addition using ingredients with lower rumen degradable protein such as canola meal also reduces the potential nitrogen substrate for atmospheric ammonia. This type of comparison was tested by Accioly et al. (2002) who fed the following diets shown in Table 3.

Table 3. Energy and protein concentrations in diets, estimated total urinary nitrogen excretion, costs of the diets and cost of liveweight gain in cattle fed the seven different diets listed above. Values presented are means of n number of animals in the group shown in parenthesis.

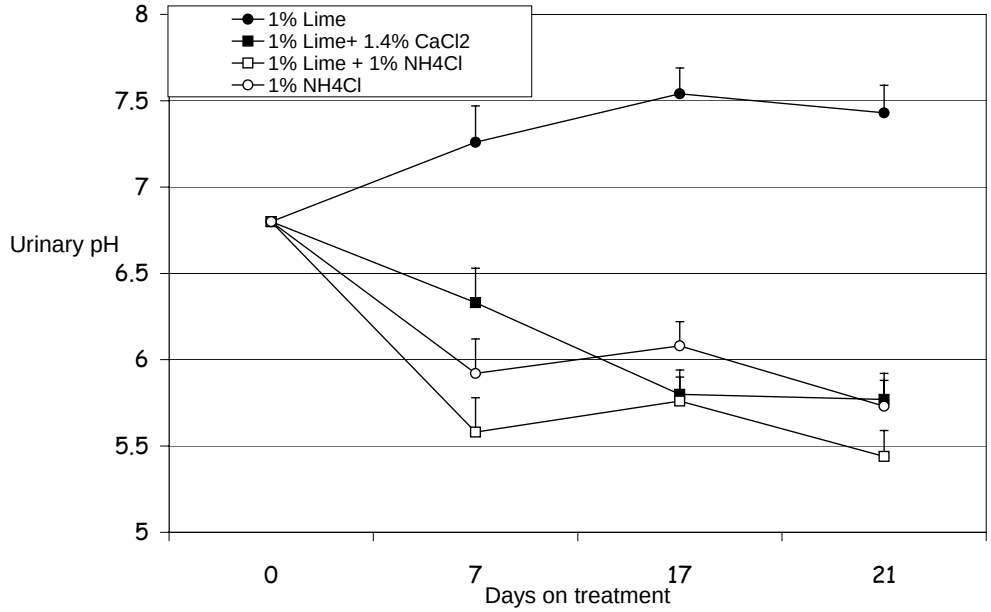
Diet (n)	ME MJ / kg DM	Crude Protein %	Gain kg/day	eTUN g/d	Costs $/tonne	Costs of LW gain ($/kg)
1 (13)	8.61	9.72	1.53	56.94	164	0.74
2 (14)	8.61	12.54	1.68	81.27	170	0.71
3 (14)	8.48	12.44	1.37	61.39	215	1.11
4 (13)	8.61	12.85	1.77	55.72	194	0.77
5 (13)	10.44	15.67	2.06	75.51	200	0.70
6 (12)	10.30	15.53	2.22	58.85	245	0.80
7 (13)	9.80	18.80	1.60	99.92	390	1.77

Diet 1 consisted of 50% straw, 30% barley and 18% lupins. Diet 2 consisted of 50% straw, 18% barley and 30% lupins. Diet 3 consisted of 50% straw, 18% barley and 30% canola meal. Diet 4 consisted of 50% hay, 30% barley, 18% lupins. Diet 5 consisted of 50% hay. 18% barley, and 30% lupins. Diet 6 consisted of 50% hay, 18% barley and 30% canola meal. All of the diets from 1- 6 contained 2% lime as a binder. Diet 7 consisted of 98% lucerne and 2% bentonite. The nutrient composition, liveweight gain, estimated total urinary nitrogen (eTUN), costs of the diets and of the liveweight gain are presented in Table 3.

Urinary nitrogen excretion was significantly lower in cattle fed diets based on cereal hay rather than straw even at higher dietary protein percentages (diet 2 vs 5, Table 3). Moreover, canola meal significantly reduced urinary nitrogen excretion at the same dietary protein content as lupins (Diet 5 vs 6 Table 3). Thus there is significant scope for reformulating rations for cattle with the aim of reducing atmospheric ammonia. In addition, the higher cost of moving in this direction of reducing ammonia levels can be offset by cost-effective liveweight gain during sea transport (Table 3).

Current live export diets can result in a urinary pH around 8.0 that is a contributing factor to atmospheric ammonia through the chemistry of ammonia/ ammonium equilibrium that favours ammonia volatilisation.

Figure 3. Effect of the acid salts, ammonium chloride and calcium chloride, on the pH of urine in cattle fed export rations.

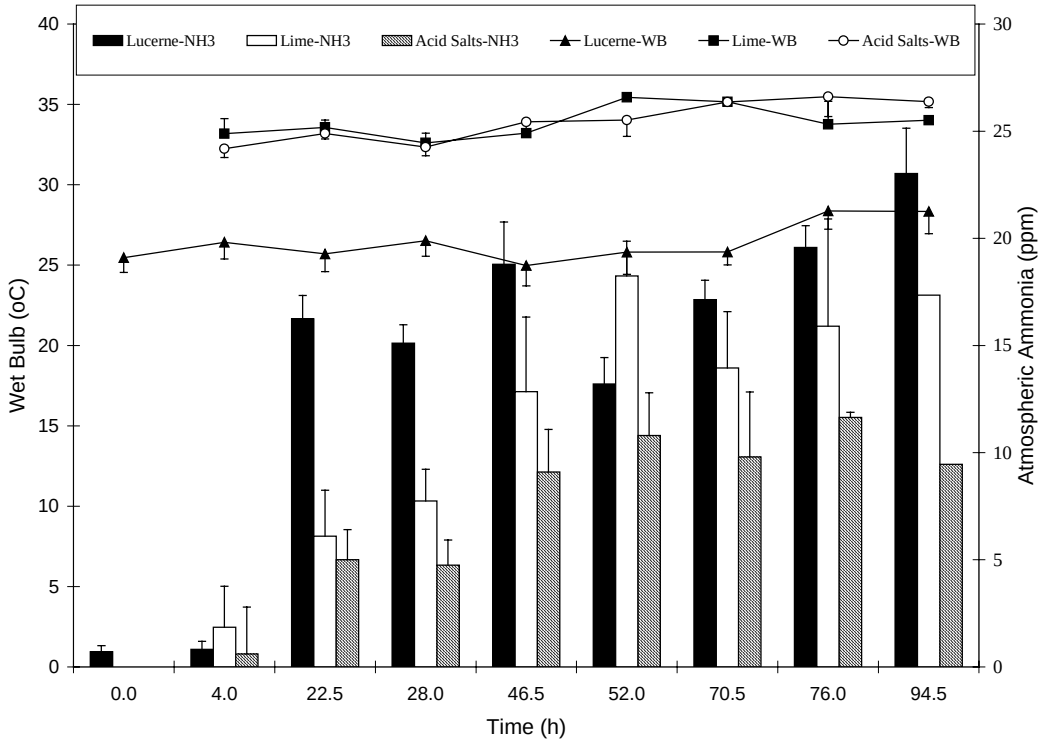


Lime is the most commonly used binder in export diets and is the major contributor to this alkaline urinary pH, even at 1% inclusion rather than the previous 2% standard. Addition of gypsum to export diets decreased urinary pH substantially but gypsum was not as effective a binder as lime.

Acid salts, such as ammonium chloride or calcium chloride, decrease urinary pH more efficiently than gypsum even in the presence of 2% lime. However, these acid salts can negatively affect intake if used without a binder such as lime. Therefore, care must be taken when using acid salts in formulations to reduce ammonia to ensure that lime is present to overcome any check on intake. Accioly et al. (2003) demonstrated this effect using rations based on these acid salts (Figure 3). It is important to note that ammonium chloride had a

greater effect on lowering urinary pH than any potential direct contribution to atmospheric ammonia. The powerful effect of acid salts on not only urinary pH but also atmospheric ammonia is demonstrated in Figure 4. Lucerne diets and export diets using lime as a binder both resulted in atmospheric ammonia concentrations of 25 ppm or more in rooms maintained under simulated conditions of temperature and humidity for live export. In contrast the addition of ammonium chloride reduced the volatilisation of the ammonium to ammonia. The ammonia concentration increased in these rooms under a set of conditions that did not exceed 30^{o}C wet bulb (Figure 4).

Figure 4. Effect of lucerne, lime and acid salts containing diets on atmospheric ammonia under simulated conditions of heat and humidity for live export.



Thus the combination of ventilation rate, appropriate selection of ingredients based on cereal hay to balance energy and protein and addition of acid salts should be additively effective in reducing atmospheric ammonia. Obviously the next step is to validate this combination on a series of actual voyages where all these factors are used to decrease atmospheric ammonia.

13 Threshold levels and critical exposure levels of atmospheric ammonia for humans

The National Occupational Health and Safety Commission (NOHSC) is the Australian body established by the Commonwealth Government to develop, facilitate and implement a national occupational health and safety strategy. The exposure standards detailed by the

NOHSC represent airborne concentrations of ammonia that, according to current knowledge, should neither impair the health of nor cause undue discomfort to 'nearly all workers' (NOHSC:1003 1995). The exposure standards apply to long-term exposure to a substance over an eight-hour day, for a five-day week, over an entire working life. It is important to note that exposure standards do not represent 'no-effect' levels that guarantee protection to every worker. Following on from this, exposure standards are not fine dividing lines between satisfactory and unsatisfactory working conditions or safe levels. However, exposure standards are indicative of where and when appropriate control measures are required.

The Australian (NOHSC:1003 (1995)), American (OHSA, 1989), Canadian (Canadian Centre for Occupational Health and Safety 2001) and UK (Groot Hoerkamp et al. 1998) occupational health and safety authorities list the following limits for exposure to atmospheric ammonia. Humans can smell ammonia when the concentration exceeds 0.6 ppm (Canadian Centre for Occupational Health and Safety 2001). The threshold limit value time-weighted average (TLV-TWA) is 25 ppm over an eight-hour working shift. This limit on exposure recorded as protective against irritation to the eyes and the respiratory tract and minimizes discomfort. On the other hand, volunteers noticed nose and throat irritation at 24 ppm (Luttrell 2002). The threshold limit value for short-term exposure limit (TLV-STEL) and permissible exposure short-term exposure limit (PEL-STEL) are both 35 ppm. The permissible exposure limit time-weighted average (PEL-TWA) is 50 ppm. Groups of 5 and 6 human subjects were exposed to respective ammonia concentrations of 30 and 50 ppm (Luttrell 2000). The volunteers subjectively rated irritation for the 10-minute exposures. No moderate or higher irritation was reported from the group at the lower exposure; however four out of 6 people reported moderate nose and throat irritation at 50 ppm (Luttrell 2002). The threshold for bronchoconstriction, determined as a 20% increase in airway resistance, was 85 ppm following 10 breaths of ammonia via a mouthpiece (Douglas and Coe, 1987). The immediate dangerous to life and health (IDLH) concentration for ammonia is 300 ppm (Luttrell 2002). Death of humans has occurred following a 5-minute exposure to 5000 ppm (Canadian Centre for Occupational Health and Safety 2001).

Notwithstanding this consensus on ammonia standards in many countries including Australia, the TLV-TWA limit is lower [ie 10 ppm for stockmen] in Sweden (The National Swedish Board of Occupational Safety and Health 1990). This lower limit for Sweden is not justified either from survey results or on longer working hours than the standard 8-hour, five-day week accepted for other countries.

The Australian Government's Department of the Environment and Heritage maintains a National Pollutant Inventory that includes atmospheric ammonia. On a relative health hazard spectrum of 0 – 3 ammonia registers as 1.0. A score of 3 represents a very high hazard to

health, 2 represents a medium hazard and 1 is harmful to health. A substance that scores a relatively high health hazard is arsenic at 2.3, and ammonia is one of the lowest at 1.0. While the relative health hazard takes inot account ammonia's toxicity it does not take into account exposure to ammonia. Human exposure is reflected in National Pollutant Inventory (NPI) rank given to a substance. Approximately 400 substances were considered for inclusion on the NPI reporting list. A risk rating is given based on health and environment hazard identification, and human and atmospheric exposure to the substance. Some substances are grouped together at the same rank with 208 ranks in total. Ammonia is ranked as 45 out of the 208 ranks which places it in approximately the bottom 25% of the NPI ranks.

It is important to reiterate the ammonia concentrations reported in the investigation of the ventilation efficiency on livestock vessel (SBMR.002, 2001): "Typical levels below decks were 15 ppm with readings commonly reaching 20 – 30 ppm". Therefore, typical values of atmospheric ammonia on livestock vessels are below the TWA for humans. However, concentrations above the TWA ie 30 ppm, are commonly reached which is a cause for concern. Of more concern, and need for action, are the circumstances where the STEL concentration of 35 ppm is exceeded. In feedlots, the values for atmospheric ammonia of 25 – 30 ppm are also a concern for workers in feedlots who might be working near feed or water troughs.

14 Symptoms of ammonia toxicity and mode of action of ammonia in humans

The symptoms in humans exposed to atmospheric ammonia are summarised in Table 4. The route of entry of ammonia is most likely to be inhalation, or through the eyes and skin. Ammonia is severely irritating to the nose, throat and lungs in humans. Symptoms of ammonia inhalation include burning sensations, coughing, wheezing, shortness of breath, headache and nausea. Overexposure to atmospheric ammonia is toxic to the central nervous system with signs including unconsciousness and convulsions. Upper airway damage is more likely and can result in bronchospasm (closing of the airway). Vocal chords are particularly vulnerable to corrosive effects at high concentrations. Lower airway damage may result in fluid build up and haemorrhage. Underlying conditions aggravated by exposure to ammonia include asthma, emphysema, dermatitis and eye disease. Nevertheless, repeated exposure to ammonia may develop a tolerance (or acclimatisation) to irritating effects after a few weeks (Canadian Centre for Occupational Health and Safety 2001). Even after long-term exposure, ammonia is not a carcinogen or likely carcinogen.

Table 4 Human Health Effects from Breathing Ammonia

Short-term Exposure (less than or equal to 14 days)		
Levels in Air (ppm)	Length of Exposure	Description of Effects*
0.5		Minimal risk level.
50	Less than 1 day	Slight, temporary eye and throat irritation and urge to cough.
500	30 minutes	Increased air intake into lungs; sore nose and throat.
5000	Less than 30 minutes	Kills quickly.
Long-term Exposure (greater than 14 days)		
Levels in Air (ppm)	Length of Exposure	Description of Effects*
0.3		Minimal risk level
100	6 weeks	Eyes, nose and throat irritation.

*These effects are listed at the lowest level at which they were first observed. They may also be seen at the higher levels.

Source: Agency for Toxic Substances and Disease Registry, Division of Toxicology, 1600 Clifton Road, E-29, Atlanta, Georgia 30333

15 Critical levels of atmospheric ammonia at which animal symptoms appear and affect performance.

The critical value of atmospheric ammonia where cattle welfare and production is adversely affected should be set at 25 ppm, ie the same as the general TWA for humans and higher than the 20 ppm European standard for pig housing systems (CIGR 1984). This is achievable under current production systems as noted in previous reports (SBMR.002, 2001). It is important to remember that the TWA of 25 ppm for humans is set for regular exposures of 8 to 10 hours maximum, repeated each day for five days. For the majority of the day, such persons reside in low or no-ammonia environments. However, the level of 25 ppm for humans is already under review. Sweden has declared a lower TWA of 10 ppm for agricultural workers notwithstanding the lack of evidence in support of this level. During sea transport to some overseas markets, cattle are continuously exposed to environments where ammonia can be 25 ppm or more for at least five, and frequently over 10 days. This means that cattle during sea transport are exposed to far higher concentrations of atmospheric ammonia than those measured in most production systems for ruminants in Europe (0 to 8

ppm), and these levels of exposure occur at higher temperatures and humidity than any measured in Europe or north America. In feedlots in Australia, the TWA for humans can be exceeded for parts of the day. Given that the TWA is set for at least 8-hours per day, then atmospheric ammonia values in excess of 25 ppm for short periods should not be an occupational health concern for humans. However, cattle present a different problem since they are in this type of atmosphere for longer.

Pigs should have a greater resistance to disease at higher levels of ammonia than cattle because of the better anatomical structure of their respiratory system. An explanation for this difference is the poor capacity and response to infection of the bovine (and ruminant) respiratory system. As part of one of the most effective dose-response studies in pigs, Urbain et al. (1994) proposed 15 ppm as the maximal ammonia concentration to be tolerated in the air of pig buildings. During this study, the temperature was 23.7 ± 0.5 ^{o}C and the relative humidity was around 75% which equates to a wet bulb temperature of between 19.8 to 20.8 ^{o}C, ie much lower than the wet bulb temperatures during sea transport to the Middle East. The mean dust concentration was approximately 7 x 10^{6} ± 2 x 10^{6} particles/m^{3}. However, the authors added a cautionary note that this recommendation of 15 ppm did not take into account the fact that the effects of ammonia can be enhanced by dust. It is important to note that not only did Urbain et al. (1994) adopt a more appropriate dose-response range than most studies, but they also developed a new, more sensitive method for assessment of ammonia-induced inflammatory response in defining 15ppm as a new no-effect level for atmospheric ammonia. Nevertheless, they did not have a value below 25 ppm in their range, so they cannot state with any certainty that 15 ppm is more appropriate than 25 ppm, only that at 25 ppm they did observe inflammatory responses in pigs.

The rates of ammonia generated by sheep during sea transport assessed on either a m^{3}/s or mg/h basis are the same as cattle when measured on the same units of live weight (SBMR.002, 2001). It is unlikely that sheep or goats are going to be qualitatively or quantitatively different to cattle in their respiratory responses to ammonia. Therefore, the same critical value for atmospheric ammonia of 25 ppm should be applied to sheep and goats.

Adopting 25 ppm as a critical level for atmospheric ammonia for ruminants has the advantage of being in line with most of the North American, British and Australian standards for humans. Moreover, this level is more conservative than the European standards for humans and livestock since these are set at lower levels of atmospheric ammonia concentration. Moreover, this is an achievable level for atmospheric ammonia for sea transport systems and feedlots. Setting critical atmospheric ammonia at 25 ppm will maintain the welfare and well-

being of livestock during sea transport and in feedlots as well as meeting the occupational health standards for the stockmen and other crew who manage them.

16 Significant gaps in our information

Assessment of respiratory function in cattle or sheep is fraught with difficulty. The usual physical measures and the equipment designed to assess them are designed for humans who respire vertically, ie at 90^{o} to ruminants who respire horizontally. The most common method used for assessment of respiratory function and distress in cattle is respiratory rate. Bronchioalveolar lavage (BAL) can be used as a method for assessment of lung dysfunction. Costa et al (2003) collected BAL samples from cattle prior to their entry for nine days into rooms that were heated and humidified. All of the cattle were fed diets consisting of lucerne (2%) and bentonite (2%) with either standard wood shavings bedding or bedding that included gypsum and which was changed regularly.

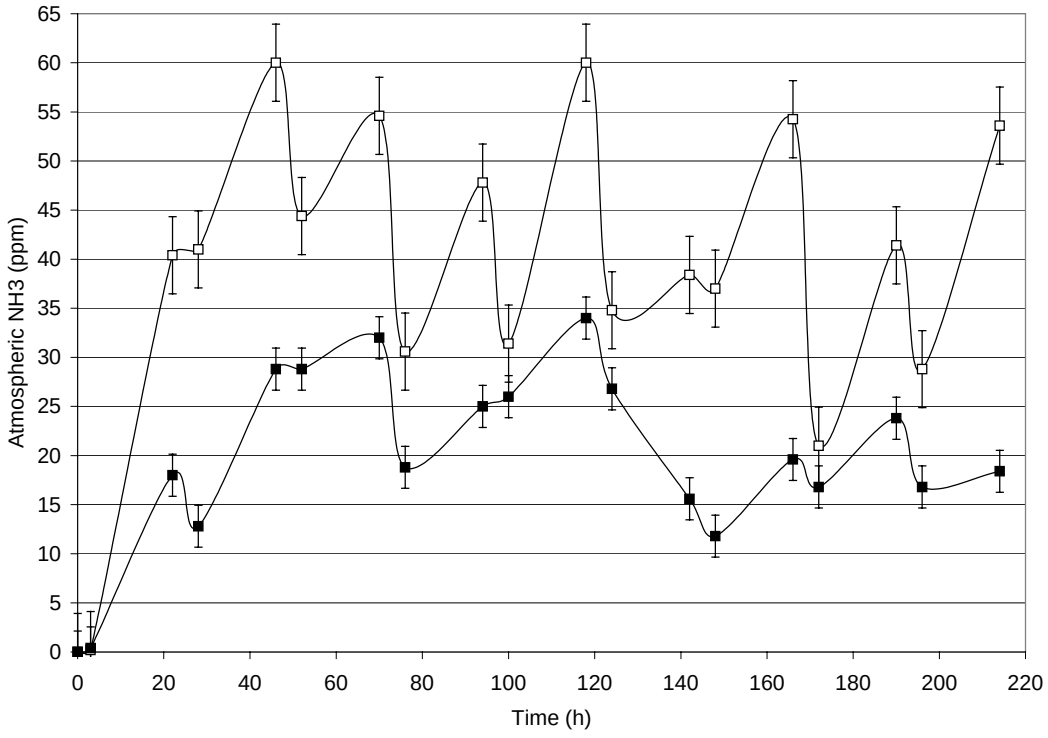


Figure 5 Atmospheric ammonia in simulation rooms at Murdoch University where cattle were housed for nine days and fed lucerne/bentonite diets. Bedding was either pine shavings maintained throughout the nine days for lucerne/bentonite diet (❑) or pine shavings plus gypsum as an acidifying agent that was changed on day 5 of the nine days (■).

These conditions resulted in mean atmospheric ammonia concentration of 42.3 ±2.8 ppm in the room where cattle were fed lucerne with no changes of bedding for over nine days. In

comparison, adding gypsum and changing the bedding reduced the mean concentration to 22.0 ± 1.6 ppm.

These values for atmospheric ammonia lie on either side of the TWA of 25 ppm, which in many countries is the operational and actionable limit. It is quite clear that the simple acts of changing the bedding and maintaining an acid environment halved the atmospheric ammonia to below 25 ppm in this instance (Figure 5). Nevertheless both groups of cattle showed increases in the indicators of inflammation such as lymphocyte, white cell count, segmented cell fragments and mononucleated cells in cattle compared with values for the same indicators before entry to the simulation rooms (Figure 6).

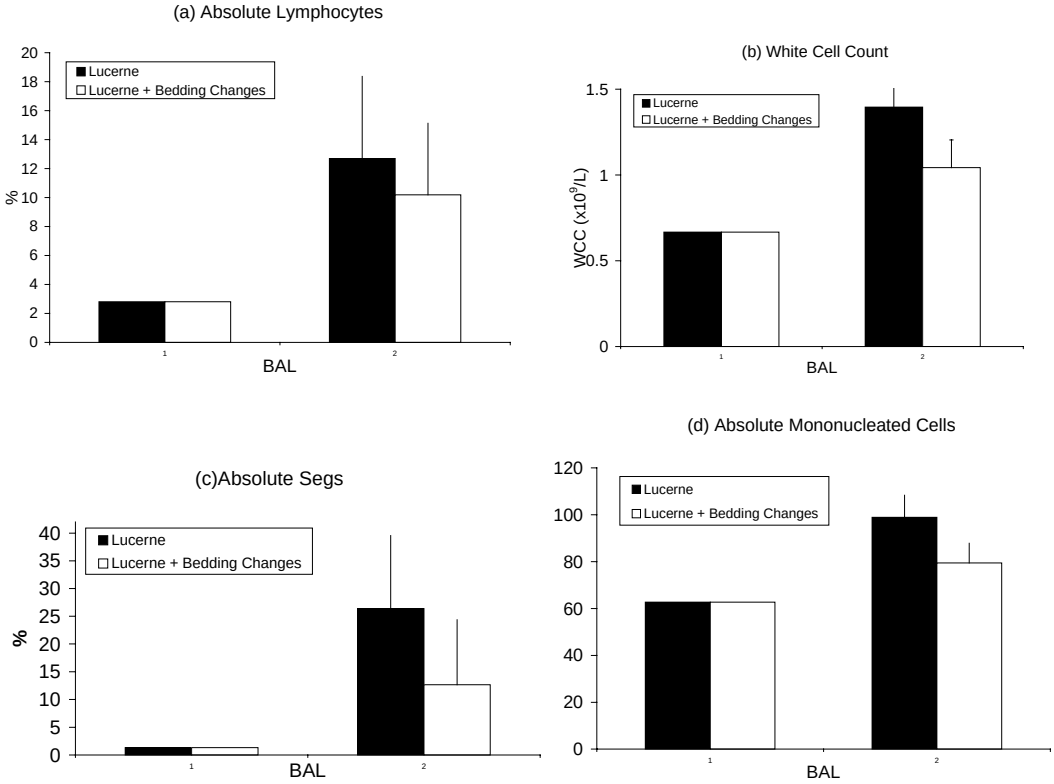


Figure 6 Bronchioalveolar lavage (BAL) values for (a) lymphocytes, (b) white cell count, (c) segmented cells, and (d) mononucleated cells in cattle fed lucerne/bentonite diets and maintained for nine days in simulation rooms at Murdoch University. Bedding was either pine shavings maintained in the rooms throughout the nine days for lucerne/bentonite diet or pine shavings plus gypsum (as an acidifying agent) that were changed on day 5 of the nine days.

This is significant since the bedding changes and the addition of gypsum did lower the ammonia below the TWA for humans, yet there was still the initial signs of inflammation in the lungs of these cattle. The mean value of 22.3 ppm is slightly lower than the 25 ppm used by Urbain et al. (1994) in their studies with pigs. Atmospheric ammonia is frequently higher than 22ppm at 0.25 metres above the ground in Australian feedlots.

Moreover, all four parameters of inflammatory response were higher in cattle housed in the room where the atmospheric ammonia was higher at 42.3 ppm. Nevertheless cattle still showed inflammatory responses in room with the lower ammonia of 22 ppm, ie below the TWA of 25 ppm. In fact, both rooms were uncomfortable for humans to work in for long periods even though the lower ammonia was at a level considered reasonable for 8-hours of exposure.

Few objective measures of inflammation resulting from exposure to elevated atmospheric ammonia have been undertaken to date and more work needs to be done. Firstly we need to confirm the validity of bronchioalveolar lavage as a sensitive method for assessing respiratory function, and secondly we must develop regression relationships with other factors that would be more easily assessed onboard vessels. The encouraging feature of bronchioaveolar lavage in cattle is that it supports the findings using nasal lavage in pigs (Urbain et al. 1994) that inflammation commenced at or below the human TWA limit of 25 ppm. However in neither instance for either pigs or cattle were there significant effects on liveweight gain at 25 ppm.

17 Conclusions

The totality of the evidence justifies defining a critical level for atmospheric ammonia in cattle, sheep and goats at 25 ppm. Adoption of this level balances welfare requirements against the levels that are reasonably achievable by best practice in the industry. Atmospheric ammonia should not normally exceed these levels in outdoor feedlots. As for cattle undergoing sea transport, atmospheric ammonia has already exceeded 25 ppm during some voyages and in some decks. However, actions such as: adequate ventilation, change of bedding and feeding lower protein diets containing acid salts, should be taken by ship's masters, stockmen and feed manufacturing and export industry to comply with the recommendation of 25 ppm for atmospheric ammonia. As part of this process, more data should be compiled on atmospheric ammonia levels onboard vessels during sea transport of animals. This would be simplified if continuous, real-time measurements could be logged. Troublesome areas onboard vessels could then be confirmed and an appropriate plan of action developed.

In feedlots, shading and keeping moisture levels to minimum should be part of the best practice to keep atmospheric ammonia levels as low as practicable. Keeping moisture levels to a minimum, especially under shade, should be part of the best practice. Mean values of 15 ppm should still be assessed for any long-term respiratory effects in cattle given the conclusions by Urbain et al. (1994) from their studies in pigs. Of greater concern are the values of 25 ppm or more near the ground, and the length of time that cattle are in that

atmosphere. More accurate specification of protein requirements, and improved formulation of feedlot diets to optimise the utilisation of feed nitrogen should be a priority for the industry, from both a profit and atmospheric aspect. Putting dietary acidifiers in feedlot rations should also decrease atmospheric ammonia. Finally, methods to assess lung function should be validated, new types of bedding developed and the frequency of replacement established.

There are a number of areas of concern about the effects of atmospheric ammonia where there is little to no information. These areas warrant further investigation. Firstly, an abattoir study could be undertaken to quantify any difference in the respiratory capacity and efficiency for *Bos taurus* versus *Bos indicus*. While the differences in respiratory efficiency between wild ruminants and domesticated cattle have been shown allometrically, no studies are available to confirm any differences between B. *taurus* and B. *indicus*. Practically, this could be performed on respiratory collected from abattoirs. Live weight, carcase weight, lung volume and ratios of upper respiratory system to total respiratory size could be measured. This information will provide a basis for comparison of these cattle to handle ammonia load during periods of high heat load, especially at high wet-bulb temperatures. Even at atmospheric ammonia of 22ppm and 28^{o}C wet bulb, cattle showed the initial signs of respiratory inflammatory response. The simulations rooms at Murdoch University could be used to study the combined effects of heat load and atmospheric ammonia. The studies led by Dr Anne Barnes deliberately kept the ammonia levels low (<8 ppm) to remove ammonia as a confounding factor during heat stress in *taurus* vs *indicus* cattle. It would be worthwhile to set ammonia as close to 25 ppm as possible for an extended period of perhaps 11 days during which wet-bulb temperatures increased to 32^{o} (for 4-5 days). The standard measures, including blood electrolyte and gas analysis and respiratory rates used by the Barnes group could be augmented with BAL assessment. Experiments like this could quantify the direct (alveolar function) and indirect (acid-base balance) effects of ammonia on heat tolerance. Any and all of these parameters are affected by feed intake and composition of the feed. Most of the ammonia studies in the simulation rooms would provide information of acute effects on animals under heat load.

On the other hand, the study of chronic effects of ammonia at 15 – 25 ppm on the respiratory system of cattle in feedlots requires a different approach. There are a number of options to lower atmospheric ammonia in feedlots. Each of the measures should be aimed at reducing atmospheric ammonia near ground level. Acidifying feedlot rations with ammonium chloride or calcium chloride will decrease the volatilisation of ammonia. This is a relatively straightforward means of reducing ammonia and the levels and effects have been documented in LIVE.202. Increasing the area of feedlots and decreasing cattle density is another factor that will decrease ammonia concentration. Moreover, appropriate shading that still keeps moisture levels to minimum should be part of the best practice to keep atmospheric

ammonia levels as low as practicable. Feeding strategies that limit intake in feedlot cattle during early summer have been used a successful tool for enhancing animal comfort by alleviating the combined effects of high climatic and metabolic heat load (Mader et al. 2002). Optimising the nitrogen content of the feed is an important factor that needs to be taken into account and more attention could be paid to this nutritional parameter. In all of the above, dust is also a compounding factor on ammonia effects whether in feedlots or during live export. Little information is available on the chronic interaction of ammonia and dust on respiratory capacity.

All of the projects suggested above need to be prioritised and funded if critical ammonia levels are to put on a sounder basis for feedlot and live export conditions. Nevertheless, setting critical atmospheric ammonia levels at 25 ppm is a practical level that will allow the feedlot beef industry and the live export industry to monitor and develop strategies to comply with this recommendation.

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19 Appendix 1: Anatomy and Physiology of the Ruminant Respiratory System

The ruminant respiratory tract is entered via wide nostrils set laterally within the naked nasolabial plate of cattle, or the smaller, haired nasal plate of sheep and goats (Nickel et al. 1979). The nasal cavity is relatively long, but narrower than might be predicted from its external dimensions, due to the impingement of large conchae (Dyce et al. 1987). Its epithelium is typical histologically, with a ciliated respiratory epithelium and scattered T-cell populations (Schmidt et al. 2000). As in most species, the ventral nasal meatus provides the principal respiratory passage to the nasopharynx. The middle nasal meatus provides access to an extensive system of paranasal sinuses, which excavate much of the bone surrounding the caudal nasal passage and orbit. This sinus complex is particularly well developed in cattle, where it also surrounds much of the cranial cavity and even enters the horns. The pharynx is anatomically unremarkable, except for the partial division of the nasopharynx by a membranous septum, which terminates in a distinct pharyngeal tonsil. Though the oropharynx is narrow, the palatine arrangement is such that ruminants are capable of mouth breathing when required (Dyce et al. 1987). Functionally, the pharynx of ruminants is notable for the continuous flow of saliva, and frequent passage of both regurgitated cud and large volumes of eructated rumen gases. This results in a comparatively higher incidence of microbial contamination of the trachea, albeit at a low level (Mullenax et al. 1964).

The ruminant larynx is comparatively short and compact, with the laryngeal entrance directed relatively dorsally. Vestibular folds and laryngeal ventricles are absent, though a small median ventricle is present in sheep and goats (Dyce et al. 1987). The transverse distance between the vocal folds (rima glottidis) represents the limiting dimension for endotracheal intubation (Lodge 1969). The laryngeal area is well endowed with lymphoid tissue.

It has been observed that the diameter of the bovine trachea (approximately 33 mm in a 450-500 kg animal) is proportionately small when compared with, for instance, horses of comparable size (46 mm). The trachea is smaller again in beef breeds compared to dairy animals (Lodge 1969). At a length of 650 mm (Getty 1975), the bovine trachea has a comparatively high ratio of length:diameter (~20), as does the goat (15 x 300 mm) (Phalen et al. 1983). From these dimensions, a comparatively high degree of tracheal airflow resistance could be predicted according to Poiseuille's Law. The ovine trachea (17 x 220 mm) is better proportioned, but is similarly narrow when compared with, for instance, a 10kg beagle (16 mm etc) (Shafie et al. 1978; Phalen et al. 1983). The ruminant trachea is U-shaped in cross-section, and is slightly deeper than it is wide. The trachealis muscle is internal to the

cartilage rings, and covers a concentration of dorsal retromucosal lymphoid tissue (Dyce et al. 1987).

The bovine thorax is notably short and wide, and the lungs correspondingly small (Getty 1975). Perhaps due to poor compliance of the chest wall (due to their wide stiff ribs), cattle are relatively more reliant on the diaphragm for breathing than other species (Dyce et al. 1987). The right and left sides of the thorax are completely divided by particularly strong mediastinal pleura. The right thoracic cavity is considerably larger, reflecting the marked asymmetry of the lungs. Thick visceral pleura cover the lungs, and are continuous with pronounced seams of interstitial connective tissue dividing adjacent lobes and lobules. This very high degree of lobulation prevents collateral ventilation between secondary lobules, which in some species (for example dogs) can provide up to 96% of normal tidal volume to obstructed airspaces (Robinson 1982). Thus in cattle (as in pigs) airway obstruction is much more functionally damaging than in those species with effective collateral ventilatory pathways. Lobulation also predisposes cattle to interstitial emphysema, which readily develops during prolonged periods of dyspnoea (Mariassy et al. 1975). However the connective tissue septa also confer a low degree of mechanical interdependence, thus it is possible to observe healthy parenchyma directly adjacent to consolidated lobules in diseases such as chronic bronchopneumonia (Robinson 1982). A similar degree of lobulation is not obvious in the sheep, and is only observed in the cranial and middle lung lobes of goats. As might be expected, these species have a greater capacity for collateral ventilation, due to the presence of accessory bronchiole-alveolar communications (Robinson 1982).

The ruminant tracheobronchial tree is distinguished by a strongly monopodial system of branching (*i.e.* diameters of daughter airways at branching are markedly dissimilar) (Phalen et al. 1983). A tracheal bronchus is present, arising proximal to the main tracheal bifurcation to supply the right cranial lung lobe (Lodge 1969). Respiratory bronchioles are either short or absent in cattle, creating a sudden transition from terminal bronchioles to alveolar ducts (Iovannitti et al. 1985). However, the goat does have 5-6 orders of respiratory bronchioles (Phalen et al. 1983). The cell population of the tracheobronchial tree is largely similar to that of other species, though the sheep is better studied than for cattle. Ovine tracheal epithelium contains 31% ciliated cells, 41% mucous cells, and 28% basal cells. This comparative abundance of the stem cell-like basal cells suggests an inherent potential for recovery from cytotoxic injury (Mariassy et al. 1983). Brush cells are present in low numbers and are not found distal to the small bronchi (Allan 1978). At the level of the bronchiolar transition, the abundance of mucous and ciliated cells declines rapidly. Below this level, Clara cells are the only secretory cell present (~65% of cell population) and submucosal glands are absent (Mariassy et al. 1983). Clara cells are similarly abundant in bovine bronchioles (Iovannitti et

al. 1985). Ovine Clara cells display ample smooth endoplasmic reticulum (SER), and numerous ovoid secretory granules. By contrast those of cattle lack SER, but contain large amounts of cytoplasmic glycogen. These characteristics suggest a less important secretory role for the Clara cells of this species (Plopper 1983).

The bovine mucociliary system is well-developed in the upper airways, but has been shown to be patchy and incomplete at the level of the smaller bronchioles (Iovannitti et al. 1985). This system consists of a viscous gel layer overlying a low-viscosity sol, in which the cilia of the underlying epithelial cells beat. Tracheal ciliary activity is similar to that of humans (Wills et al. 1998), and the normal rate of mucociliary flow (15 mm/min) is close to the mean for domestic animals (Veit et al. 1978). This rate is known to be reduced by ammonia, and by factors which reduce lowering of the head to graze, such as transport (Veit et al. 1978). Mucus gland openings are asymmetrically distributed, with the number in the ventral portion much greater than the dorsal half of the trachea, in both sheep (1.01 *vs* 0.56 per mm^2) and cattle (1.54 *vs* 0.56 per mm^2) (Choi et al. 2000). While the summed volume of bovine tracheal glands is comparatively large (Choi et al. 2000), the depth of airway surface liquid (ASL) as measured by freeze-fracture is unremarkable (23±3 µm) (Widdicombe et al. 1997). Mucociliary clearance has been measured radiometrically in calves, and is comparable with that of other species (Jones 1983).

The lack of respiratory bronchioles in cattle is accompanied by a relatively simple acinar arrangement of the alveoli. Mariassy et al. (1975) reported an almost total lack of interalveolar pores (pores of Kohn), though a subsequent study found them to be more frequent (Iovannitti et al. 1985). The mean alveolar diameter is 97µm in cattle and 74µm in sheep (Altman et al. 1971; Shafie et al. 1978). The ruminant blood-air barrier consists of a thin layer of surfactant fluid, the flattened alveolar type I cell, an epithelial basal lamina, a continuous and well-developed capillary basal lamina (though this may fuse with that of the epithelium), and a thin capillary endothelium. The pulmonary capillaries are not fenestrated (Epling 1964). The thickness of this barrier has been measured in sheep (arithmetic mean 1.87±0.18µm, harmonic mean 0.68±0.03µm) but not in cattle (Meban 1980). Bovine surfactant (produced by alveolar type II cells) has a similar composition to that of other species, with the exception of low levels of triacylglycerol and cholesterol ester (Yu et al. 1983). Several authors have noted a low abundance of alveolar macrophages compared to similar mammals (Rybicka et al. 1974; Mariassy et al. 1975). As these cells are thought to be important in the maintenance of lung sterility, this would appear to compromise bovine resistance to bacterial infection (Veit et al. 1978). However alveolar macrophages were ‘readily’ found in another study (Iovannitti et al. 1985), and are recovered in large numbers by bronchoalveolar lavage (Corstvet et al. 1982). In addition, intravascular macrophages are

common within pulmonary capillaries, and this may represent a substantial local phagocytic pool (Rybicka et al. 1974).

Alveolar type I cells of the ruminant lung possess several unique properties, such as the presence on the luminal surface of a substantial glycocalyx, numerous highly anionic microdomains, and an elaborate complex of tubulocisternal endoplasmic reticulum (a type of ER thought to be involved in active transepithelial movement of ions). These modifications are thought to be an adaptation to the unique gaseous environment of the ruminant lung, in which a cocktail of ruminal gases is delivered deeply into the lung during eructation (Dougherty et al. 1962; Dougherty et al. 1962; Atwal 1999). These gases include methane, hydrogen sulphide, formaldehyde, ammonia, and CO_2 (Kuhlmann et al. 1985), and are inhaled into the lungs in surprisingly large volumes (*eg.* 33.9L per hour in a Guernsey cow) (Dougherty et al. 1962).