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EFFECT OF ATMOSPHERIC AMMONIA ON BOVINE LUNG

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SUMMARY

The effect of atmospheric ammonia on bovine lung was investigated in 3 experiments by means of bronchio-alveolar lavage (BAL). Cattle were placed in rooms simulating live export conditions and atmospheric ammonia was monitored throughout. Bronchio-alveolar lavage was performed 4 days before the cattle were placed in rooms and immediately after they left the rooms. The cattle were introduced to experimental diets 5 days before the first BAL was performed. In all experiments, wood shavings were used as bedding and, in room 2, these were mixed with gypsum (2:1) and partially changed every 2-3 days. In experiment 1, 10 Angus cross heifers were fed lucerne cubes diets (2.25% of liveweight (LW)) with 5 placed in each simulation room for 9 days. Atmospheric ammonia was significantly ($P<0.05$) higher in room 1 (42.3 ± 2.8 ppm) than in room 2 (22.0 ± 1.6 ppm). In experiments 2 and 3, 3 groups of 4 Angus cross heifers were used. They were fed (all at 3% of LW) either a basic export diet (BD) or a basic diet containing $CaCl_2$ (BD + $CaCl_2$). Two groups were kept in the simulation rooms for 12 days, and a third group was kept outdoors in a sand yard. In experiment 2, the animals in room 1 were fed BD while the animals in room 2 and outdoors were fed the BD + $CalCl_2$. Atmospheric ammonia was significantly different ($P<0.05$) among the 3 groups, and the values were 16.8 ± 0.68, 9.4 ± 0.34 and 0 ± 0 ppm, respectively, for rooms 1 and 2, and outdoors. In experiment 3, animals in room 1 and outdoors were fed the BD while the animals in room 2 were fed the BD + $CalCl_2$. Atmospheric ammonia values were18.6 ± 0.6, 10.6 ± 0.3 and 0 ± 0 ppm, respectively, for rooms 1 and 2, and outdoors. In experiments 1, there was a significant increase in total white cell and mononucleated cell counts ($P<0.05$) on the BAL samples performed after the animals left the rooms. There were no changes on BAL samples in either experiment 2 and 3. The recommended time-weighted average safety levels for atmospheric ammonia for humans (25 ppm) were exceeded on a number of occasions in both rooms in experiment 1. On the other hand, atmospheric ammonia did not exceed 25 ppm in either experiment 2 or 3. The dust levels in both rooms and sand yard were very low, therefore, ammonia was the most logically attributable cause for the irritation developed by the animals in experiment 1.

Keywords: ammonia, live export, bronchio-alveolar lavage, cattle

INTRODUCTION

Ammonia emissions in livestock buildings result mainly from the degradation of urea in urine and undigested proteins in faeces (Rom and Dahl 1997). Prolonged exposure to high atmospheric ammonia levels can have deleterious effect on livestock (Drummond *et al.* 1976, 1980; Gustin *et al.* 1994). While the Australian National Occupational Health and Safety Commission has set the threshold limit value time weighted average (TLV-TWA) for atmospheric ammonia on 25 ppm, currently there are no regulations on livestock exposure.

Respiratory disease is 1 of the main causes of death in feedlot cattle (Dunn *et al.* 1998; Kelly and Janzen 1986) and in cattle in long haul voyages (Norris *et al.* 2003). In both scenarios, most deaths can be attributed to pneumonia caused by bacteria such as *Mannheimia (Pasteurella) haemolytica, M. multocida or Haemophillus somnus* (Mosier 1997; Green 2000; Norris *et al.* 2003). All major bacterial respiratory pathogens are commonly found in clinically normal cattle, therefore, the presence of other factors such as viral infections, transportation, temperature extremes, dehydration, crowding and exposure to irritant gases are crucial for the development of disease (Mosier 1997; Green 2000).

Although it has been suggested that high atmospheric ammonia levels can be related to the development of respiratory illness in calves (Dewes and Goodall 1995), the effect of high atmospheric

ammonia in the bovine lung is unknown. Investigations on the effect of atmospheric ammonia on animals have been confined to other species (Drummond *et al.* 1976, 1980; Gustin *et al.* 1994; Van Wicklen *et al.* 1997). Publications on ammonia emissions from cattle facilities have focused on environmental issues (Voorburg and Kroodsma 1992; Bussink and Oenema 1998; Petersen *et al.* 1998; Nimenya *et al.* 2000). High levels of ammonia (>25 ppm) emissions have been recorded on board live export vessels carrying cattle between Australia and the Middle East and can also be encountered near ground level in feedlots. The objective of the study reported here was to investigate the effect of exposure to high atmospheric ammonia on the bovine lung. Bronchio-alveolar lavage (BAL) sampling was used as a means of objectively assessing respiratory responses by identifying any acute changes in the lung cell population.

MATERIALS AND METHODS

Three experiments were conducted in which live export conditions were reproduced in 2 simulation rooms. Bronchio-alveolar lavages were performed 4 days before the animals were placed in the simulation rooms and immediately after they were removed from the rooms. The animals were introduced to experimental diets (Table 1) 5 days before the first BAL was performed. In the last 2 experiments, 1 group of animals remained outdoors at all times on a sand pen adjacent to the simulation rooms. The animals were weighed and received a physical examination consisting of heart and respiratory rates, rectal temperature and pulmonary auscultation, immediately before and after being placed in the rooms to ensure they were in good health.

Table 1. Composition of experimental diets.

Diet	Straw %	Barley %	Lupins %	Lime %	$CaCl_2$ %	Lucerne hay %	Bentonite %	CP %	ME MJ/kg DM
Lucerne cubes						98	2	18.8	9.8
Basic diet	55.5	25	18	1.5				52.0	8.5
Basic diet+$CaCl_2$	55	25	18	1	1			58.6	9.0

Bronchio-alveolar lavage procedure

All animals were sedated with xylazine (0.04 mg/kg, intra venous). Butorphanol can help in suppressing the cough reflex when the BAL tube passes into the trachea so in the 2 last experiments, the xylazine was followed 10 minutes later by a dose of butorphanol (0.02 mg/kg intra venous). Approximately 10 minutes later, the animals were restrained with a head halter and a drink water gag was placed in the mouth to avoid possible damage to the BAL tube. A bronchio-alveolar tube (Cook® Veterinary Products – V/PBAL 240) was then inserted in 1 nostril to engage the pharyngeal area, and then advanced down the trachea (at this moment in the last 2 experiments, 10 mL of 2% lignocaine were injected via the BAL tube to minimise coughing). The lavage was performed using 50 mL of sterile 0.9% sodium chloride solution, and as much liquid as possible was sucked back into the collection syringe. The samples collected were delivered to the laboratory within 15 minutes. Samples were spun and a slide prepared for differential cell count. In the first experiment, differential counts were done on 100 cells, while in the other 2 experiments, differential counts were done on 500 cells.

Simulation rooms

The rooms reproduced the conditions found onboard live export vessels (i.e. stocking density and air turnover). The dimensions were: Room 1: 5.28 m x 2.34 m x 3.92 m and Room 2: 5.29 m x 2.31 m X 5.92 m. Each room had 1 pen (3.64 m x 2.05 m x 3.92 m) where the animals were placed. Each pen had 2 feeders (40 cm x 77 cm each) and 1 water trough (30 cm x 33 cm). A platform was built 2 metres above the pens to allow access for an operator to take measurements. The floor of this platform consisted of a combination of metal mesh and wooden pallets so as not to interfere with room ventilation. In the first experiment, a portable heater was used in each room to increase ambient temperature. In experiments 2 and 3, the rooms were fitted with a more sophisticated apparatus that permitted a better control of temperature and humidity. In all experiments, 70 kg of pine saw dust was placed in each simulation room, but in room 2, this was mixed with 35.5 kg of gypsum, and the bedding was also partially replaced every 2 to 3 days to decrease ammonia emissions.

Environmental measurements

Concentrations of ammonia (Neotox single gas monitor Mk5) and CO_2 (Testo 445 measuring instrument with CO_2 probe), together with temperature and relative humidity (Testo 445 measuring

instrument with combined temperature and humidity probe), were measured. Temperature and relative humidity were used to calculate wet bulb temperature, which is a good indicator of animal comfort. At each time, 4 measurements were taken from the platform above the pens. The instruments were lowered to 130 cm above the ground to sample air at human breathing level, and at 100 cm to sample air at animal breathing level. These measurements were done in the middle of the pen and approximately 40 cm from the door that gave access to the outdoor pen. In the outdoor pen, only 1 set of measurements was taken.

Animals and samples

Experiment 1. Ten 18-month old Angus cross heifers were randomly allocated to 2 groups of 5 and placed in the simulation rooms for 9 days. The total liveweight was 1694 kg and 1682 kg for rooms 1 and 2, respectively. They were fed the lucerne cube diet (Table 1) at 2.25% of liveweight on a DM basis. At the time of the BAL sampling, blood was collected from the tail vein for measurement of total cell count fibrinogen and total plasma protein. Total cell count included measures of erythrocyte, total leukocyte and platelet concentration, mean cell volume, haemoglobin, haematocrit, corpuscular haemoglobin and mean corpuscular haemoglogin concentration.

Experiment 2. Twelve 24-month old Angus cross heifers were randomly allocated to 3 groups of 4 and placed in the simulation rooms for 12 days while 1 group was kept in the sand yard adjacent to the simulation rooms. The total liveweight for each room was 1652, 1650 and 1646 kg for rooms 1 and 2, and outdoors, respectively. Animals in room 1 were fed the basic diet while the animals in room 2 and outdoors were fed the basic diet + $CaCl_2$. All diets were fed at 3% of liveweight on a DM basis. At the time the BAL sampling, blood was collected from the tail vein so that total cell count, fibrinogen, total plasma protein and blood gases measurements, and haptoglobin assays, could be performed. Blood was also collected from the tail artery so blood gases and arterial lactate could be measured. Haptoglobin was determined on a Cobas Mira analyser using a spectrophotometric assay (Tridelta Development Ltd, Ireland).

Experiment 3. Twelve 24-month old Angus cross heifers were randomly allocated to 3 groups of 4 and placed in the simulation rooms for 12 days while 1 group was kept in the sand yard adjacent to the simulation rooms. The total liveweight for each room was 1508, 1520 and 1480 kg for rooms 1 and 2, and outdoors, respectively. Animals in room 1 and outdoors were fed the basic diet while the animals in room 2 were fed the basic diet + $CaCl_2$. All diets were fed at 3% of liveweight on a DM basis. Same samples and measurements were performed as in experiment 2.

Statistical analysis

The results are presented as means ± standard errors of means. Laboratory variables and weight gain were analysed by analysis of covariance using the initial values as the covariate. The average of all 4 measurements in each room was used at any given time and analyse of variance was used to determine statistical differences ($P<0.05$).

RESULTS

Experiment 1. Atmospheric ammonia was significantly higher in room 1 (42.3 ± 2.8 ppm) than in room 2 (22.0 ± 1.6 ppm). The cut off point for the atmospheric ammonia measuring device was 60 ppm and, in room 1, this level was reached on more than 1 occasion. Despite bedding treatment and changes the ammonia level exceeded 25 ppm for almost 5 days in room 2. There was no significant difference in other environmental parameters (CO_2, wet bulb) between the 2 rooms. There was a significant increase in total white cell and mononucleated cell counts in the BAL samples collected after the animals left the rooms. There was no significant difference in BAL samples between treatments. There was no significant difference on blood parameters between either the first or second BALs or between treatments.

Experiment 2. Atmospheric ammonia was significantly different among the 3 groups, and the values were: 16.76 ± 0.68, 9.42 ± 0.34 and 0 ± 0 ppm, respectively, for rooms 1 and 2, and outdoors. There was no significant difference in other environmental parameters (CO_2, wet bulb) between the 2 rooms, but both CO_2 and wet bulb were significantly higher in the rooms than in the outdoor sand yard. There was no significant difference between treatments in BAL samples or in any of the blood parameters, between either the first or second BAL, or among treatments.

Experiment 3. **A**tmospheric ammonia values were 18.62 ± 0.6, 10.63 ± 0.3 and 0 ± 0 ppm, respectively, for rooms 1 and 2, and outdoors. The overall findings were similar to experiment 2.

DISCUSSION

In experiment 1, cattle in both treatments developed an inflammatory response after being exposed to high atmospheric ammonia levels of at least 22 ppm or more. This response, as shown by significant increases in total white cell and mononucleated cell counts, was consistent with irritation of the respiratory system. In both experiments 2 and 3, an inflammatory response was not observed in any of the 3 treatments. However, in experiment 1, the recommended time-weighted average safety levels for atmospheric ammonia for humans (ie 25 ppm) were exceeded on a number of occasions in both rooms. Atmospheric ammonia did not exceed 25 ppm in either experiment 2 and 3. Since there was no visible dust in either of the rooms or sand yard, then ammonia is the most logically cause for the irritation developed by the animals in experiment 1. Importantly, the animals in experiment 1 did not develop clinical respiratory disease. Thus, there is a clear sensitivity of the bovine respiratory system to an atmospheric ammonia challenge at or about the time-weighted limit of 25 ppm for humans. Some of the anatomical features of the bovine respiratory system, including relative narrowness of the upper respiratory tract, a high degree of anatomic compartmentalisation, small lung size and abundance of lymphoid tissue, and frequent exposure to rumen gases, might confer an inherent susceptibility to respiratory pathology and dysfunction. The measured volume of a cow lung was only 48% of that predicted by allometric regression (Gehr *et al.* 1981) and the alveolar surface area was also lower (77%) than that predicted allometrically. This lack of respiratory capacity in *B. taurus* cattle may be the result of domestication and selection for other traits (Gehr *et al.* 1981). The sensitivity of bovine lung to atmospheric ammonia may exacerbate heat stress, which is the main cause of death in cattle during long haul live export (Norris *et al.* 2003). Reducing risk factors to the respiratory system such as atmospheric ammonia by dietary manipulation (Accioly *et al.* 2003) is recommended. Further studies are necessary to determine the critical atmospheric ammonia levels for *B. taurus* cattle.

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