live export

Decreasing shipboard ammonia levels by optimising the nutritional performance of cattle and the environment on ship during live export

Project LIVE.202
Final Report prepared for MLA and LiveCorp by:

Geoff Tudor[1], Jeisane Accioly[2], David Pethick[2], Nick Costa[2], Eric Taylor[2] and Colin White[3]
[1]Western Australian Department of Agriculture
[2]Division of Veterinary and Biomedical Sciences, Murdoch University
[3]CSIRO, Livestock Industries

Published by Meat & Livestock Australia Ltd
ABN 39 081 678 364
ISBN 1 74036 3442

December 2003

MEAT & LIVESTOCK AUSTRALIA

LIVECORP

The livestock export program is jointly funded by the livestock exporters and producers of Australia

TABLE OF CONTENTS

Acknowledgements

The guidance, expert advice and openness of the live export R&D advisory committee in helping to shape this research is greatly acknowledged. Wesfeeds, Glenforest Feeds and Gilmac are thanked for the patience and expert advice in making the experimental diets. The Vasse Research Station under the expert leadership of Mr Frank Coupar is thanked for managing the feeding experiments. The Veterinary Farm at Murdoch University under the management of Mr David Brockway is thanked in the management of cattle at Murdoch University. The skilled technical assistance of Mrs Barbara Waldoch, Mr Ken Chong and Mr Malcolm Boyce is gratefully acknowledged.

Part funding for this project was from the Cooperative Research Centre for Cattle and Beef Quality which is greatly acknowledged.

Funding from CSIRO Livestock Industries for Jeisane Accioly to undertake a PhD program as part of this project is gratefully acknowledged.

1. General Background

For cattle undergoing live export to the Middle East from southern Australia there is little or no dietary adaptation period and animals come from various backgrounds. Therefore it is paramount that the animals are offered a safe and highly palatable diet. The trade has so far relied on diets highs in rumen degradable nitrogen (N) in order to meet these requirements but this in turn increases the risk of ammonia gas build up. As the majority of vessels used in the trade were not purpose built, inefficient ventilation can sometimes exacerbate the air quality problem.

Health concerns are warranted for both animals and stockpersons exposed to high levels of atmospheric ammonia for long periods of time. According to the standards set by the American Occupational And Health Safety Administration (NOHSC 1995) ammonia levels for humans should not exceed 25ppm while some European standards are even lower (10ppm-The National Swedish Board of Occupational Safety and Health 1990). If ammonia is breathed continuously in sufficient concentration, it can result in serious health effects. Studies performed in pigs have demonstrated that excess ammonia exposure can cause reduced performance, lung damage (Gustin et al.1994, Urbain et al 1994) and eventually pneumonia (Drummond et al.1978).

2. Abstract

This project had the objective of investigating alternative methods for decreasing shipboard ammonia levels through nutritional manipulation and the use of chemical additives to the bedding. In addition, effects of dietary formulation on cattle live weight gain were investigated. A series of feeding experiments were performed with cattle offered pelleted rations in an effort to understand the effects of diet on animal performance, urinary nitrogen excretion and urinary pH. Dietary scenarios were discovered that could reduce urinary nitrogen excretion while maintaining animal performance. These included increasing the quality of the roughage (replacing straw with hay), replacing some of the dietary lupin grain with canola meal or by adding dietary acidifiers to reduce urine pH. Dietary acidifiers were able to reduce the urinary pH to below 7 and so markedly reduce the volatilisation of ammonium to ammonia. In addition some preliminary studies investigating the lung inflammatory response of cattle suggested no deleterious impact of atmospheric ammonia if the level is kept below 20ppm.

3. Executive summary

3.1 Diet formulation - the effects of varying protein source and roughage quality.

The aim of this section of the work was to develop a simple method for assessing nitrogen excretion when different diets were fed to cattle In addition the effects of roughage quality, cereal grain level and protein level/type were tested for effects on live weight gain, feed intake and urinary nitrogen excretion. The key components and results were:

1. A spot sampling procedure (one sample of urine or blood post feeding) has been developed which can be readily used to test the impact of diet on urinary nitrogen output.
2. A series of rations based on 50% roughage inclusion were tested for effects on weight gain and urinary nitrogen excretion (ME 8.5-10.4 MJ/kg DM, CP 9.7-18.8% DM) – the following conclusions were made:

    a. Roughage quality (cereal hay instead of cereal straw) had a significant positive impact on live weight gain – where possible high quality roughage should be used in pellets if live weight gain is desirable.

b. A high level of dietary true protein (from either canola meal or lupin grain) in combination with good quality roughage gave the best live weight performance. This was driven primarily by the availability of ME
c. High lupin grain inclusion (30%) allowed good live weight gain on straw based diets but at the cost of a high urinary nitrogen excretion.
d. Canola meal promoted lower levels of urinary nitrogen excretion.
e. High versus low *in vitro* digestible (IVD) cereal hay (ME = 9.1 vs 8.1 MJ/kg DM) was perhaps too subtle a change to detect differences in performance over the short feeding period – both gave satisfactory performance. However an extreme reduction in roughage quality, such as poor quality straw (i.e. ME = 4.9 MJ/kg DM, CP = 1.8% DM) did have a detrimental effect on performance.
f. High cereal roughage diets (70%) and lower cereal grain inclusion (10%) are feasible alternatives for the live export trade and can result in good performance as long as good quality roughage is used. One of the clear advantages is the suggestion that high roughage allows for a rapid achievement of full intake compared to diets having lower roughage (55%) a higher cereal grain (18%) inclusion.
g. An all roughage diet fed as lucerne cubes allowed for moderate live weight gain (= to the straw, high lupin inclusion diet) but at the expense of very high urinary nitrogen excretion.

3. The results show that the traditional diets used by the live export trade can be replaced by alternative diets that provide the same intake, similar or better performance and most importantly lower urinary N. Roughage quality seems to be the key for designing such diets. If roughage quality cannot be changed an alternative is to capitalise on the special quality of canola meal which has a lower rate of rumen degradable protein translating into lower rates of nitrogen excretion. To allow palatability, and delivery of more 'safe' ME on diets formulated around straw there is little option but to increase true protein inclusion (lupin grain or canola meal) and under these situations some substitution of lupin grain by canola meal is recommended if reducing nitrogen excretion is a primary goal. Both of these conclusions (roughage quality, protein inclusion/source) involve an increase in the cost of the final ration. In conclusion the cost of diet should not be the only thing to be considered when choosing a diet for long haul voyages; live weight gain and N excretion should also be considered.

3.2 Using dietary additives to acidify urinary pH and so reduce ammonia volatilisation.

This phase of the work addressed dietary manipulation of urinary pH since it is well known that ammonium volatilisation to ammonia gas is minimal when the effluent pH is below 7. Key findings were:

1. The addition of lime (1 or 2%) as a pellet binder increased urinary pH to about 7.5-8. That is, most live export diets will induce a high urinary pH.
2. Gypsum, when added to the diet decreased urinary pH but was not a satisfactory binder.
3. Gypsum when added at safe levels to protect against sulphur toxicity (i.e. <0.5%) was not able to reduce urinary pH in the presence of 1-2% lime
4. Either NH_4Cl (1.4%) or $CaCl_2$ (1%) when added in the presence of lime reduced urinary pH to below 7 (= significant reduction in ammonia volatilisation) and allowed good intake and live weight gain.
5. NH_4Cl addition alone (1.4%) reduced intake and performance, indicating care is needed when adding acidic salts.
6. Urinary acidifiers are a viable option for reducing ammonia volatilisation and should allow more flexible feed formulation such as increased inclusion of lupins. Further work

is required to see if dietary acidifiers negatively impact on adaptations to heat stress. The acidifiers produced a small reduction in blood bicarbonate levels and this might negatively impact on adaptations to heat stress but this result is by no means certain.

3.3 Bedding and other dietary additives.

In this part of the work several commercially available products were tested for their efficacy to reduce ammonia emission when added to the bedding of feed. Key findings were:

1. Gypsum added to sawdust bedding at an inclusion rate of 50% was effective in reducing ammonia emission. The effect is reduced at high temperatures (30°C, dry bulb). The main effect of gypsum is to reduce bedding pH which means that the industry can use either a dietary acidifier or gypsum in the bedding. The dietary addition of urinary acidifiers is likely to be more effective.
2. De-Odorase (Alltech-Kentucky, USA) in the bedding reduced ammonia emission in a variable manner, but was ineffective when added to the diet
3. Several other bedding compounds were tested for their ability to reduce ammonia volatilisation (Agrotain, Zeolite, Spongolite, Stable Plus). Most products resulted in some depression of ammonia emission but none were better than either gypsum except for Agrotain. Unfortunately Agrotain was considered to have potential toxic effects of its own.

3.4 Room simulations of final recommendations

Cattle were kept in rooms designed to simulate live export ship conditions and dietary scenarios were tested to see if ammonia emission could be reduced. The results show that a reduction in urinary N translates into reduced ammonia emission. In addition, dietary and/or bedding acidifiers will work on board to reduce ammonia volatilisation.

3.5 Lung health

Most of the studies investigating the effect of high atmospheric ammonia on respiratory tract have been performed in either pigs or poultry and the possible effect of atmospheric ammonia on ruminant lung is poorly understood. This section of the work had the aim to investigate the effect of exposure to high atmospheric ammonia on bovine lung.

1. Chronic exposure to high ammonia levels can cause an inflammatory response in the lung consistent with irritation.
2. This type of response does not occur if ammonia levels are kept under 20 ppm.

4. Nutritional Studies

4.1 Effect of Diet on Nitrogen Excretion

Urinary nitrogen excretion accounts for 55-65% of total nitrogen excretion and it is typically measured using a 24-hour urine collection. This method makes analysis of various diets using large number of animals impractical.

The aim of this experiment was to determine if a single sample technique (spot sample) could predict daily nitrogen output and if so at what time in relation to feeding this sample should be collected in order to achieve the highest degree of predicability. This would facilitate the use of more animals and diets for subsequent testing of potential commercial rations.

4.1.1 Method

- Studies were undertaken at Murdoch University using Angus steers each with a rumen fistula. A 24-hour urinary/faecal collection was performed using urinary harnesses while the cattle were kept in crushes.
- Three (3) levels of dietary crude protein with lupin inclusion as the primary variate. Crude protein levels were 9, 12.5, 15% (table 1).
- Six (6) rumen fistulated steers were fed diets for 21 days and then a 24-hour urinary and faecal collection was undertaken. Cattle were cycled through diets in a Latin square design.
- Live weight and feed intake were monitored. Animals were fed once a day around 8 am and residues were collected the following morning before fresh food was offered.
- Subsequent to each 24h collection period five spot samples of blood and urine were obtained over 3 days

Table 1: Composition of experimental diets

Diets	Lupins%	Barley %	Oats %	Mill mix %	Cereal Straw %	Marylime %	Crude Protein %	ME MJ / kg DM
1	10	20	10	11	46	2	9	9
2	21	20	0	16	41	2	9.6	12.5
3	32	13	6	7	40	2	10	15

The following measurements were taken:

- Faeces: total weight, dry matter and total N.
- Blood: ammonia, urea and creatinine.
- Rumen: pH, ammonia, and volatile fatty acid (VFA) concentrations.
- Urine: volume, pH, ammonia, urea, creatinine, uric acid and allantoin.

4.1.2 Results and Conclusions

Although there was a trend for daily weight gain to increase with an increase in crude protein level of diet this was not significantly different (P>0.05).

Table 2: Correlations (r^2) between: 1) blood urea nitrogen (BUN) and total urinary nitrogen (TUN); and 2) urinary urea: creatinine ratio (UR_CR) and TUN for five spot samples

Correlation/Time of sample	3h post feeding	6h post feeding	11h post feeding	15h post feeding	23h post feeding
BUN x TUN	0.73 (11)†	--	0.80 (11)	0.73 (7)	0.51 (15)
UR_CR x TUN	0.83 (16)	0.87 (16)	0.88 (17)	0.79 (14)	0.61 (17)

† No. samples from which r^2 determined

There were insufficient numbers of blood samples taken at the 6h post-feeding spot sample for statistical analysis. All r^2 values were significantly different ($P<0.05$). The results (table 2) show that urinary urea:creatinine ratio can be used to provide a reliable estimation of TUN at various times of the day. Blood urea can also been used to estimate TUN. The options for both blood and urine were considered important since a spot urine sample is easy to collect from heifers but difficult from steers.

4.2 Effect of Roughage Quality and Fermentable Energy/Protein on Intake, Performance and Nitrogen Excretion in Cattle

The aim of this experiment was to investigate the best combination of roughage source (straw/hay), barley, protein inclusion (18%/30%) and protein source (lupin grain/canola meal) to provide an alternative low-N residue diet to those currently used in the trade, without adverse effect on intake and performance.

4.2.1 Method

Ninety-eight Angus cross heifers (approximately 18 months of age) were randomly allocated to individual pens (5m x 20m). The pens were grouped in 14 blocks of seven pens each. The heifers were adapted to the pens for a period of one week when they were fed chopped pasture hay (ryegrass and sub clover) *ad lib*. After adaptation the animals were offered one of the 7 experimental pelleted diets listed in Table 3. Diet allocation was also randomly allocated and the heifers had not been exposed to a pelleted diet previously.

Table 3: Composition of experimental diets.

Treat.	Cereal straw† %	Oaten Hay† %	Lupins %	Barley %	Canola Meal %	Lime %	Bentonite %	Lucerne%
1	50		18	30		2		
2	50		30	18		2		
3	50			18	30	2		
4		50	18	30		2		
5		50	30	18		2		
6		50		18	30	2		
7							2	98

† Cereal straw ME = 4.9MJ/kg DM, CP = 1.8% DM; Oaten hay ME = 9.1MJ/kg DM, CP=6.1% DM.

Experimental diets were fed at 2.5% (as fed) of body weight and feed intake was measured daily. Animals were fed approximately at 8 am every morning and residues were collected before fresh food was offered. Body weight was measured weekly and used to readjust feeding rate. After at least 22 days of the dietary treatments samples of rumen fluid, urine and faeces were obtained approximately four to six hours post feeding. Blood was collected by venipuncture into vacutainer tubes (Sodium EDTA). Rumen samples were collected via nasogastric tube of 20 mm internal diameter with the sample drawn by a manually operated pump attached to the tube. Urination was stimulated by massaging perineum, bellow the vulva. Faeces were collected directly from the rectum.

4.2.2 Results

One week after the introduction of the diets 5 animals (one each from diets: 1, 4, 5, 6 and 7) were removed from the experiment, as they would not eat their pelleted (= 5% shy feeders). One extra animal was also removed on the second week as it stopped eating its pelleted diet (diet 6). Statistical analysis presented here contains data obtained from the 91 animals remaining in the study.

When only initial and final live weight were taken into consideration there was no effect of diet on live weight gain (Figure 1), but when daily liveweight gain over the 21 days was considered (Figure 2) the effect of diet on gain was highly significant ($P<0.001$). There was no significant effect of diet on intake but when intake was considered over time there was a significant ($P<0.05$) drop in feed intake on the high barley diets (diets 1 & 4) on the second day of experiment. This is thought to be due a subclinical acidosis. Diet significantly ($P<0.05$) affected feed conversion efficiency (FCE, Figure 3). Roughage type (Figure 4) also had a highly significant ($P<0.001$) effect on daily gain with animals fed diets containing hay putting on more weight then those fed diets containing straw or lucerne. Additionally there was a significant roughage x protein source interaction such that high rates of true protein inclusion (as either canola meal or lupin grain) in the presence of higher quality roughage (hay) gave the best live weight gain. The major factor driving live weight gain was the ME density of the diet (Figure 2).

Figure 1: Live weights over 21 days of animals kept on seven experimental diets.

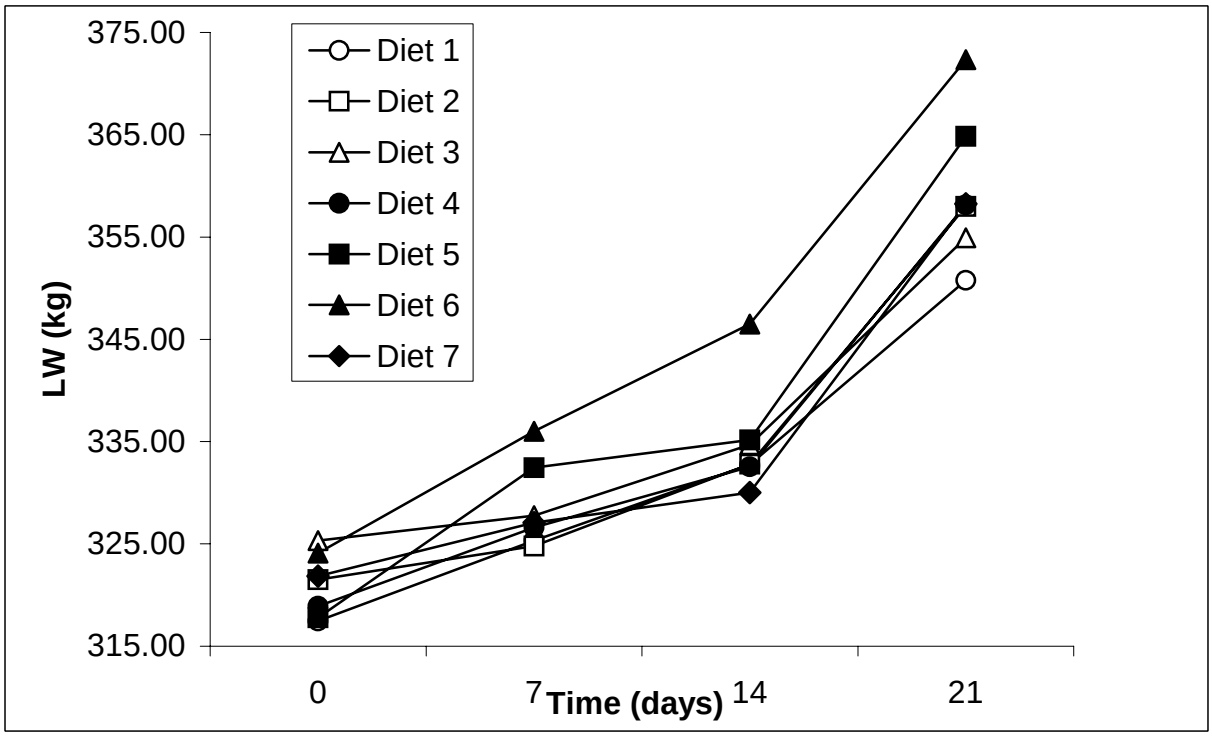


Figure 2: Mean daily gain for animals kept on seven experimental diets over 21 days and ME for each diet.

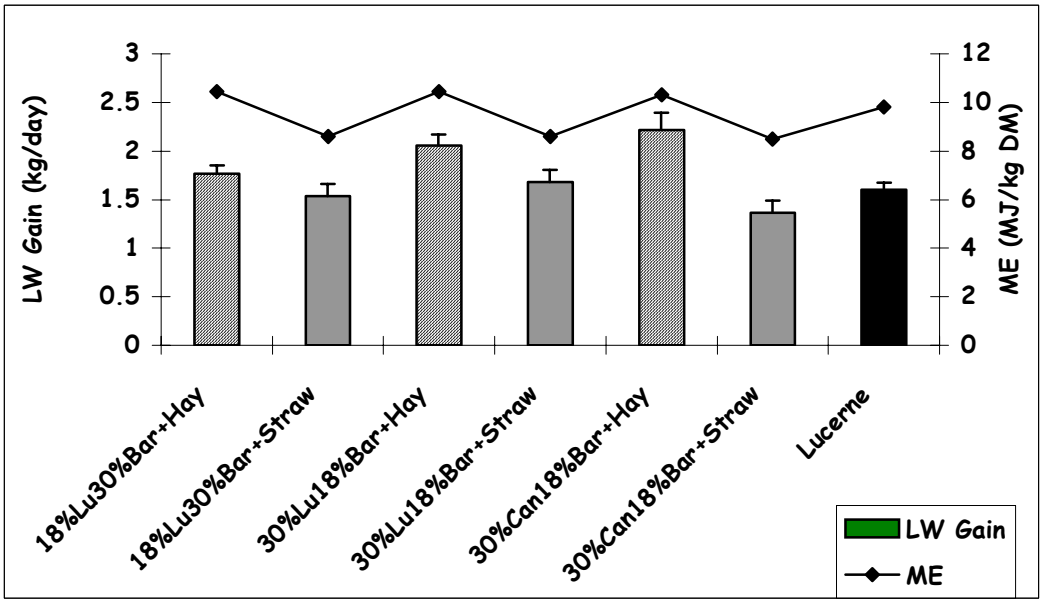


Figure 3: Feed conversion ratio based on mean daily gain and average daily intake of cattle fed 7 experimental diets over 21 days.

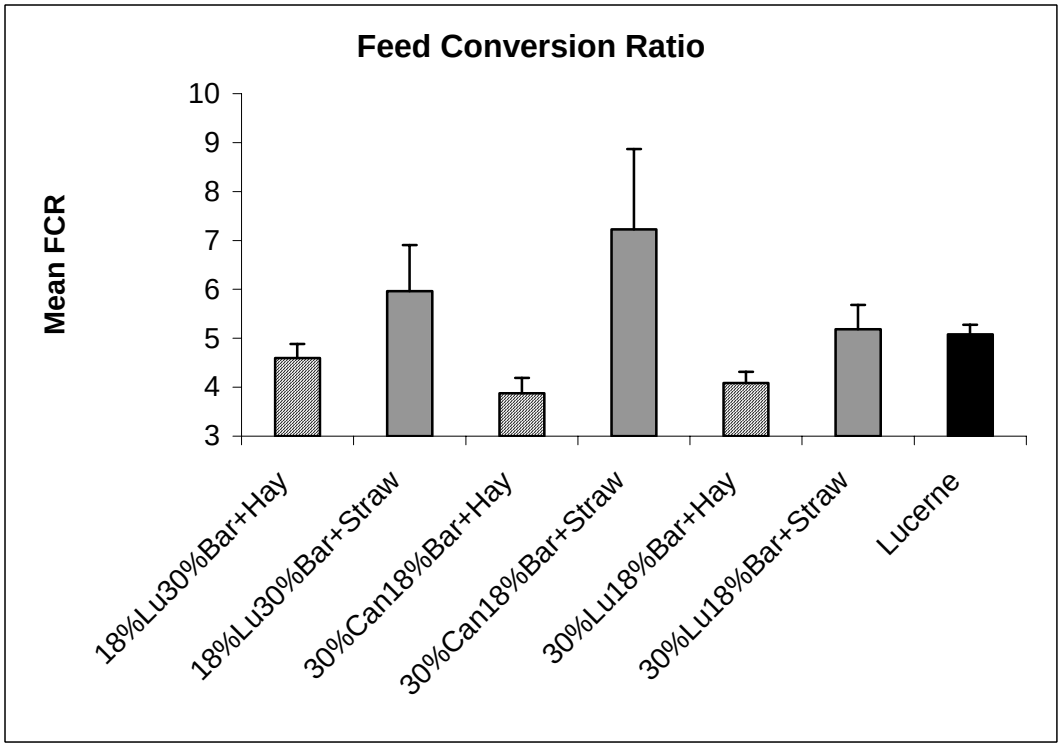
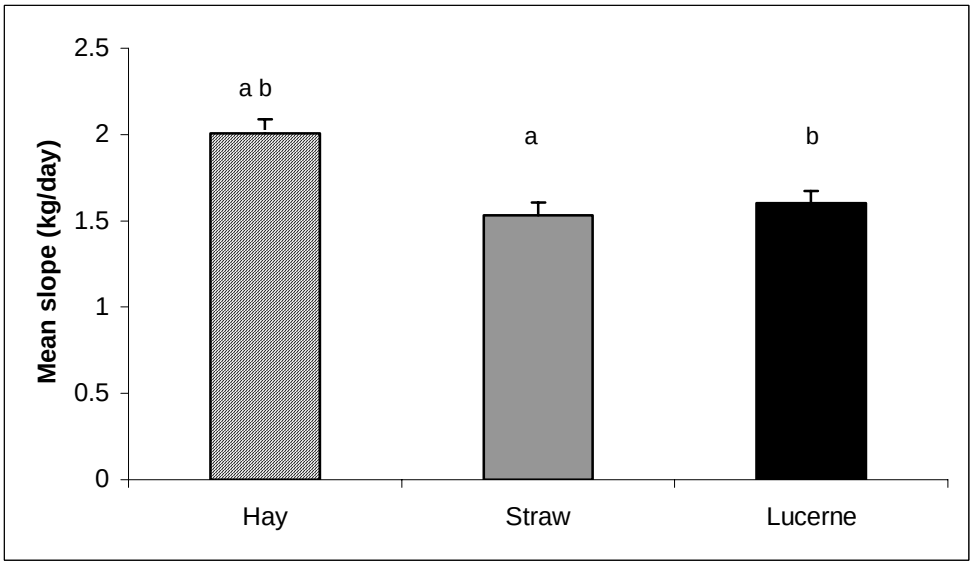


Figure 4: Effect of roughage quality on mean daily gain for animals kept on seven experimental diets over 21 days. Bars with same letters are significantly different (P<0.05)



There was a significant (P<0.001) effect of diet on estimated TUN (Table 4). The inclusion of lupin grain at 30% (diets 2 and 5) resulted in the higher nitrogen excretion and lucerne cubes gave an even higher response. Canola meal inclusion into the diet (diets 3 and 6) resulted in a marked reduction of nitrogen excretion at the same crude protein content. Diet 5 performed the best overall in terms of cost-effective gain as assessed by $/kg of gain, allied to a significant reduction in estimated total urinary nitrogen excretion compared with the equivalent straw diet (diet 2).

Table 4: Energy and protein concentrations in diets, estimated total urinary nitrogen excretion, costs of the diets and cost of live weight gain in cattle fed the seven diets. Values present are means of n number of animals in the group.

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	56.9	164	0.74
2 (14)	8.61	12.54	1.68	81.3	170	0.71
3 (14)	8.48	12.44	1.37	61.4	215	1.11
4 (13)	10.44	12.85	1.77	55.7	194	0.77
5 (13)	10.44	15.67	2.06	75.5	200	0.70
6 (12)	10.30	15.53	2.22	58.9	245	0.80
7 (13)	9.80	18.80	1.60	99.9	390	1.77

† Prices used to calculate the cost of diets $/tonne (as at Oct., 2001): Barley $215, Lupin $270, Canola Meal $420, Hay $150, Straw $90 and Lucerne Cubes $390.

4.2.3 Conclusions

Animals on diets combining good quality roughage (hay instead of straw) and a high level of dietary true protein (from either canola meal or lupin grain) had the best performance. This is driven primarily by the availability of ME. Diet did not affect feed intake when averaged across the 22 days. However we speculate that the drop in intake on the second day of experiment on the high barley diets was due to a mild transitory acidosis. The results show that the traditional diets used by the live export trade (diets 2 and 7) can be replaced by alternative diets that provide the same intake, similar or better performance and most importantly lower urinary N.

Roughage quality seems to be the key for designing such diets. If roughage quality cannot be changed, an alternative is to capitalise on the special quality of canola meal which has a lower rate of rumen degradable protein translating into lower rates of nitrogen excretion. To allow palatability, and delivery of more 'safe' ME on diets formulated around straw there is little option but to increase true protein inclusion (lupin grain or canola meal) and under these situations canola meal should be considered as an option if reducing nitrogen excretion is a primary goal. Both of these conclusions (roughage quality, protein inclusion/source) involve an increase in the cost of the final ration. In conclusion the cost of diet should not be the only thing to be considered when choosing a diet for long haul voyages, live weight gain and N excretion should also be considered.

4.3 Effect of pellet binders on urinary pH

Volatilisation of ammonium (liquid) into ammonia (gas) is greatly affected by effluent pH and decreasing effluent pH can bring volatilisation to a halt. This experiment tested the hypotheses that lime (calcium oxide hydrate), which is often used as a binder in export pellets, will promote alkaline urine, and that urinary pH could be decreased by substituting gypsum for lime. In addition gypsum will act as a pellet binder.

4.3.1 Method

Forty eighteen-month old angus-crossed heifers were randomly placed in individual pens and fed hay *ad libitum* for five days and then allocated based on live weight to one of the four experimental diets (Table 5). Animals were fed the diets at a rate of 2.25% of body weight on a dry matter basis for 19 days, when final samples were collected. The animals were fed daily at approximately 8 am and residues were collected and measured every morning before fresh food was offered. Urinary pH was measured at the last day of hay feeding period (day 0) and days 5, 14 and 19 of the pellet feeding period. Daily feed intake and weekly live weight were also recorded. At day 19 a blood sample was taken for blood gases analysis and faecal pH was also measured. All samples were collected approximately four to six hours post feeding.

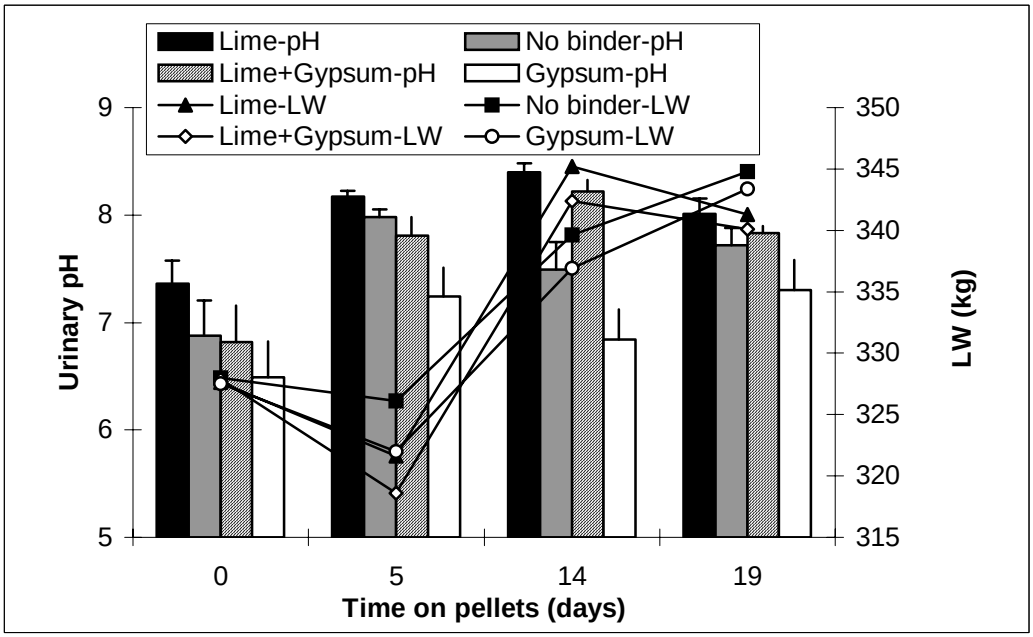
Table 5: Composition of experimental diets.

Ingredient	Diet 1	Diet 2	Diet 3	Diet 4
Oat hay (%)	52	50	49	51
Barley (%)	30	30	30	30
Lupins (%)	18	18	18	18
Gypsum (%)	-	-	1	1
Lime (%)	-	2	2	-

4.3.2 Results

Lime produced an alkaline urinary pH that was significantly ($P<0.05$) reduced compared with using gypsum as a binder (Figure 5). However, gypsum in combination with lime was not powerful enough to acidify urine (Figure 5). Analysis of variance demonstrated that diet did not significantly ($P>0.05$) affect intake, weight gain, faecal pH, blood pH, pO_2 or pCO_2. The blood HCO_3 in animals fed the diet containing gypsum was significantly lower than those receiving the diet with a gypsum-lime combination ($P<0.05$).

Figure 5: Urinary pH (bars) and live weight (lines) of cattle fed one of four experimental diets for 19 days.



4.3.3 Conclusions

Addition of lime to the basal diets resulted in an increase urinary pH while gypsum reduced the urinary pH. Gypsum did work as a good binder (solid pellets resistant to breaking down), but more time was required in the manufacturing of pellets since initially the ground diet flowed more slowly through the pellet press. However after adjusting the temperature and water inclusion the manufacturing problems were overcome to some extent. Further investigations of alternatives for urinary acidification of live export diets were therefore undertaken.

4.4 Effect of pellet binders and acid salts on urinary pH

Recent publications have demonstrated that addition of acid salts such as calcium chloride or ammonium chloride to diets can be used successfully to decrease ammonia emissions from pig facilities. In addition it is well known in the dairy industry that acidic salts can be used to reduce urine pH. This experiment evaluated the best combination of binders such as lime and gypsum with acid salts such as calcium chloride or ammonium chloride to decrease urinary pH in cattle.

4.4.1 Method

Ninety heifers were randomly placed in pens (5m x 20m) and adapted during one week during which time they were fed chopped pasture hay (ryegrass and sub clover based) *ad lib*. Once adapted to the pens the animals were randomly allocated to one of the six experimental diets (Table 6). Animals were fed the diets at a rate of 2.25% of body weight on the dry matter basis for 21 days The animals were fed daily at approximately 8 am and residues were collected and measured every morning before fresh food was offered. Urinary pH was measured at the last day of hay feeding period (day 0) and on day 14 of the pellet feeding period in all animals while on days 7 and 21 urinary pH was measured in a sample of 42 animals (7 animals from each treatment). All samples were collected approximately four to six hours post feeding.

Table 6: Composition of experimental diets.

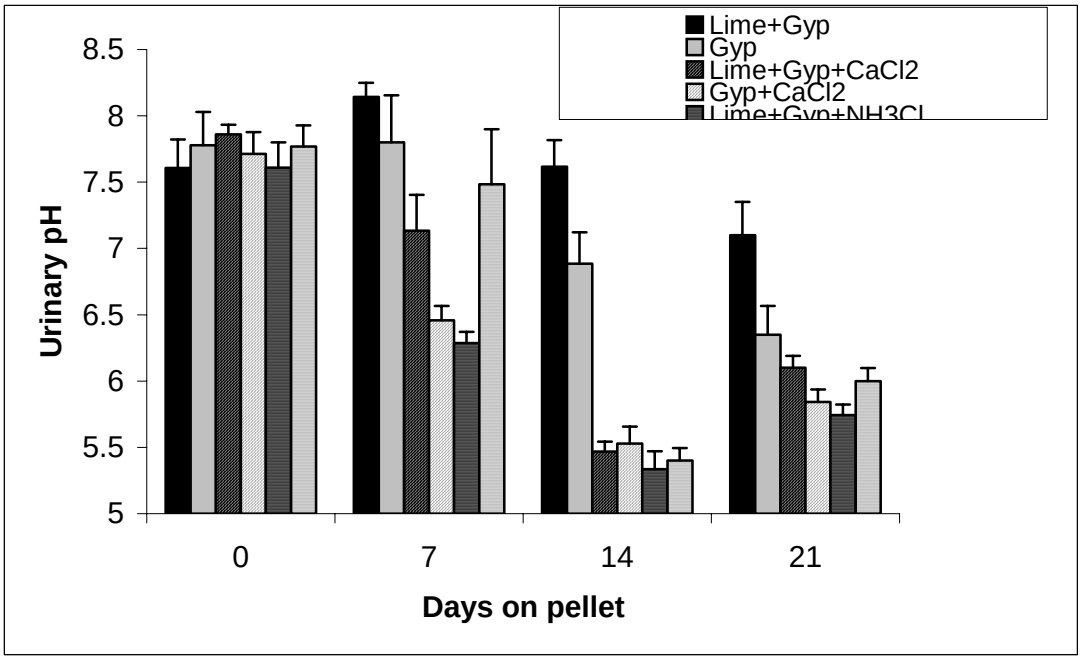
Diet (n)	Hay (%)	Barley (%)	Lupins (%)	Lime (%)	Gypsum (%)	$CaCl_2$ (%)	NH_4Cl (%)
1 (13)	49.5	30	18	2	0.5	0	0
2 (15)	51.5	30	18	0	0.5	0	0
3 (12)	48.1	30	18	2	0.5	1.4	0
4 (14)	50.1	30	18	0	0.5	1.4	0
5 (12)	48.5	30	18	2	0.5	0	1
6 (13)	50.5	30	18	0	0.5	0	1

n= number of animals per diet.

4.4.2 Results:

Eleven animals were removed from the experiment (with no bias towards any of the diets) for either refusing to eat the pellets or because they escaped from their individual pens. Overall calcium chloride and ammonium chloride significantly ($P<0.05$) reduced urine pH compared to gypsum and lime or even gypsum alone (Figure 6).

Figure 6: Effect of dietary treatments on urinary pH. The results shown are means ± SEM for animals per group indicated in Table 5.



4.4.3 Conclusions

Ammonium chloride and calcium chloride were equally effective in lowering urinary pH compared with diets containing 2% lime. This effect on lowering urinary pH was significant even in the presence of 2% lime.

4.5 Effect of urinary acidifiers and hay quality and quantity on animal performance, urinary N excretion (potential ammonia emission) and urinary pH in cattle fed shipper diets

This experiment had the following objectives:

- Demonstrate that both NH_4Cl and $CaCl_2$ can successfully be used as urinary acidifiers in the presence of lime as a binder without affecting intake, animal performance and urinary N excretion.
- Demonstrate that a lower (1% instead of 2%) lime inclusion will not affect pellet quality and will reduce urinary pH.
- Evaluate the effect of different quality hays (low versus high IVD) on intake, animal performance, urinary pH and N excretion.
- Evaluate the effect of rate of inclusion of hay on intake, animal performance, urinary pH and N excretion.

4.5.1 Method

Ninety-six Angus-cross heifers (approximately 18 months of age) never before fed pellets were randomly placed in individual pens and fed oaten-hay *ad libitum* for 7 days. In order to mimic what usually occurs under live export conditions a 18 hour feed curfew was applied before animals were introduced to one of the 6 experimental diets (Table 7). The group was divided in two blocks (A and B) of 48 animals and in each block 8 animals were allocated accordingly to live weight to each of the experimental diets. Pellets were fed at 3% live weight on dry matter basis and daily intake and weekly live weight were recorded. The animals were fed daily at approximately 8 am and residues were collected and measured every morning before fresh food was offered. At day 0 (last day on hay) a urine sample was collected for pH measurements and electrolyte analysis from all animals. All samples were collected approximately four to six hours post feeding. Urine pH was also measured from animals in block A on days 7 and 21. At day 14 the main collection was performed using all animals and the following samples were taken

- Urine: pH, electrolytes, urea and creatinine.
- Blood: electrolytes, urea and blood gases.
- Faeces: pH.

Table 7: Composition of experimental diets.

Diet	Hay High IVD (%)†	Hay Low IVD (%)†	Barley (%)	Lupins (%)	Lime (%)	CaCl2 (%)	NH4Cl (%)
1	54.6		25	18	1	0	1.4
2	55.6		25	18	0	0	1.4
3	56		25	18	1	0	0
4	55		25	18	1	1	0
5	0	54.6	25	18	1	0	1.4
6	69.6		10	18	1	0	1.4

† High IVD hay = 59% IVD or 9.2MJ ME/kg DM, Low IVD hay = 52% IVD or 8.1MJ ME/kg DM.

4.5.2 Results

Four animals were removed from the trial due to behavioural problems. The diet containing NH_4Cl alone without binder (lime) negatively affected feed intake (Figure 9) and consequently decreased live weight gain (Figure 8). The diet containing lime alone (diet 3) resulted in the best intake and live weight gain, but despite the reduction in lime inclusion relative to previous experiments, urinary pH remained high (Figure 7). Both $CaCl_2$ and NH_4Cl (should use $CaCl_2$ or calcium chloride etc consistently throughout the report) addition reduced urinary pH in the presence of 1% lime. There was no difference in live weight gain or feed intake between the high and low IVD hay. The high roughage diet (diet 6) promoted live weight gain equal to all other diets. In addition the intake on the high roughage inclusion diet was higher during the first 4-5 days after the introduction period (Figure 9).

Figure 7: Effect of the six experimental diets on urinary pH over 21 days.

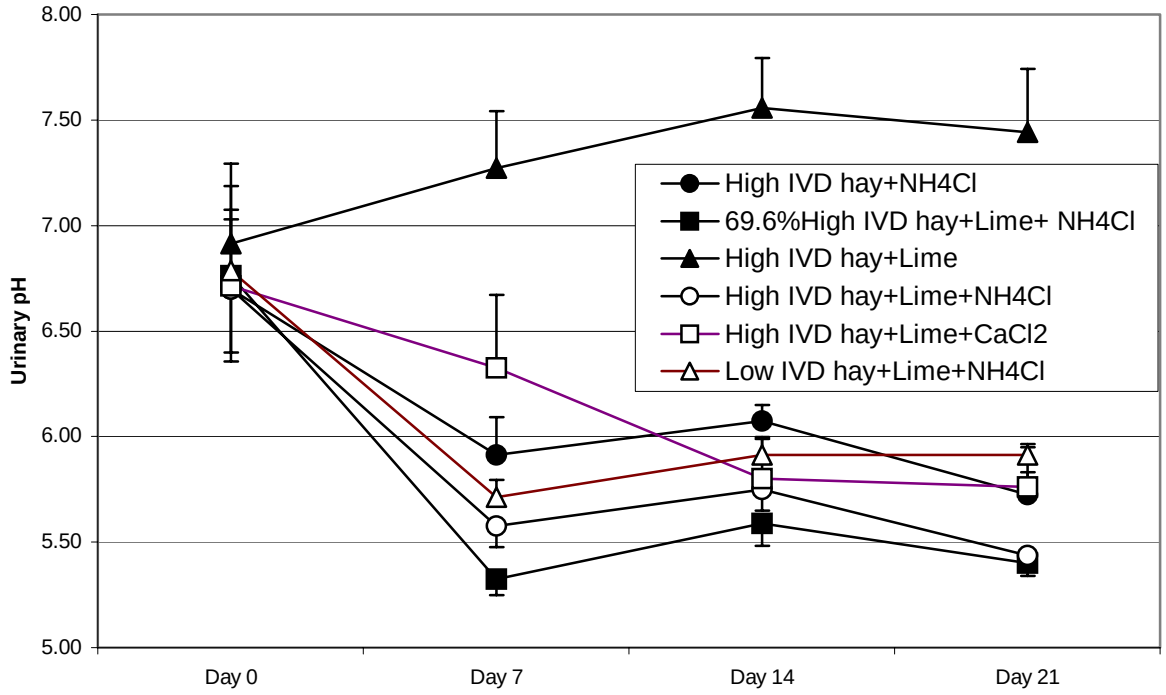


Figure 8: Effect of six experimental diets on live weight over 21 days.

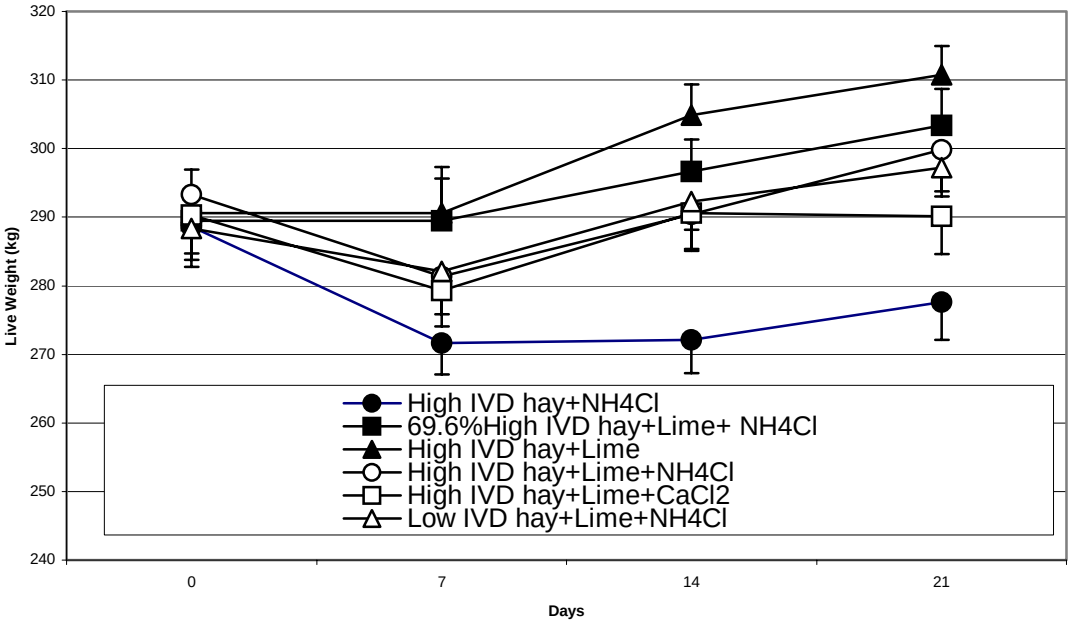
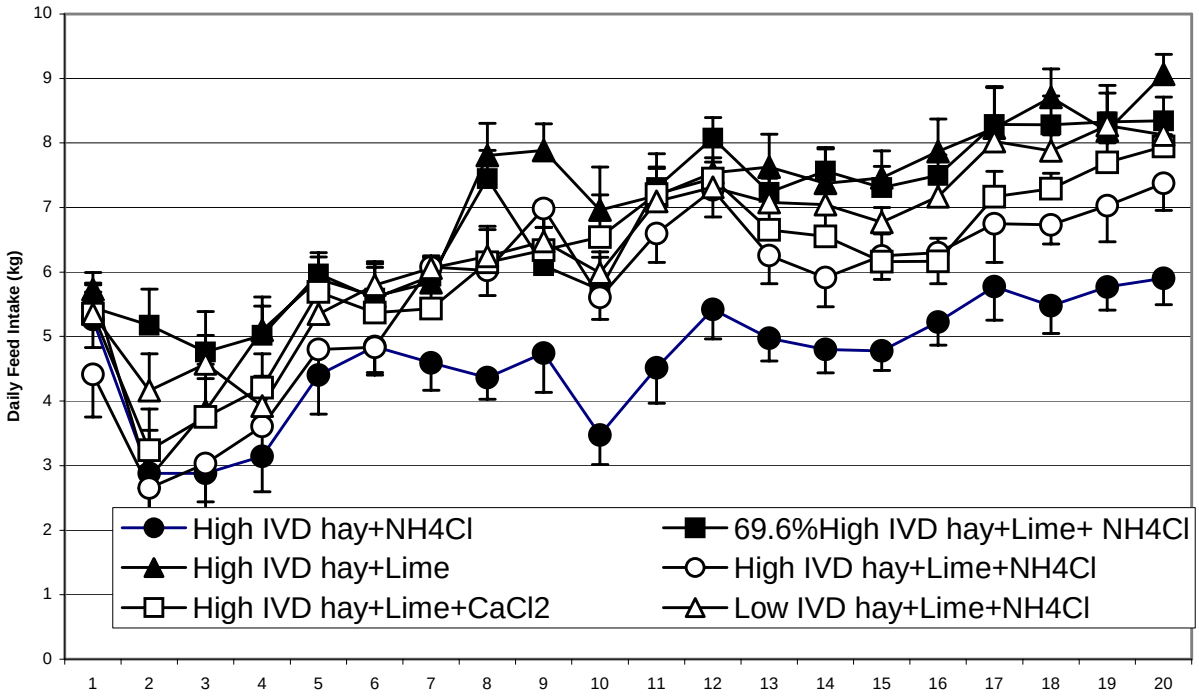


Figure 9: Effect of six experimental diets feed intake over 21 days.



4.5.3 Conclusions

- Acid salts decrease urinary pH but can negatively affect intake if used without a binder. Special attention should be given to diet formulation when using acid salts.
- High roughage diets (70%) and lower cereal grain inclusion (10%) are a feasible alternative for live export and can result in good performance as long as good quality roughage is used. One of the clear advantages is the suggestion that high roughage allows for a rapid

achievement of full intake compared to diets having lower roughage (55%) and a higher cereal grain (18%) inclusion.

- High versus low IVD hay was perhaps too subtle a difference to detect differenced in performance over the short feeding period. It would appear that an extreme reduction in roughage quality (i.e. straw) is needed before a detrimental effect on performance is detected.
- Even 1% lime inclusion will cause an elevation of urinary pH.

5. Additives Screening Studies

Little or no information is available as to the effectiveness of various additives for reducing the emission of ammonia from beef cattle effluent. Given this several experiments have been performed to evaluate either 'in feed' or 'in bedding' additives. In the preliminary work described here ship conditions were simulated by placing cattle effluent into plastic drums and then monitoring subsequent ammonia production in the gas space above the effluent. This was thought to be a practical and low cost method to screen additives before applying them in full-scale simulation experiments.

5.1 General Method

For these experiments cattle were fed pelleted diets containing around 12.5% crude protein at 2.5% BW. Animals were on the diet for at least 5 days prior to collection of manure. One of two methods was used to collect manure. With the first method animals were placed in a cattle crush and fitted with urinary harnesses to collect urine. Faeces were collected directly from the rectum and then mixed with urine in the proportion of 2.5kg of faeces for each litre of urine (based on information from actual expected proportions from the 24 hour collection performed in the first nutritional experiment). With the second method animals were placed in a pen for about 24 hours where drains were occluded with plastic film, this was done under cool temperatures (<20°C dry bulb) to minimise ammonia volatilisation. In both cases material collected was stored in sealed buckets and refrigerated for around 24 hours before experimentation.

Eighty litre sealed plastic drums were used for the simulation. The ammonia content of the atmosphere above the effluent was measured using Dräger tubes with access via a 7mm hole drilled through the top which was sealed after measurement. For each experiment 1kg of pine shavings and 3.5kg of manure was placed in each drum (estimated from live export recommendation for space allocation and an estimate for the rate of sawdust/ animal body weight/ manure output). The drums were then placed in a controlled temperature room (26°-32°C) and ammonia emission monitored over 4 to 5 days.

5.2 Agrotain

Agrotain is a urease inhibitor used to increase the efficiency of urea fertiliser when used in a cropping program. Urinary urea breakdown by urease (an enzyme present in microorganisms) results in ammonium (liquid) and at high pH ammonium volatilises into ammonia.

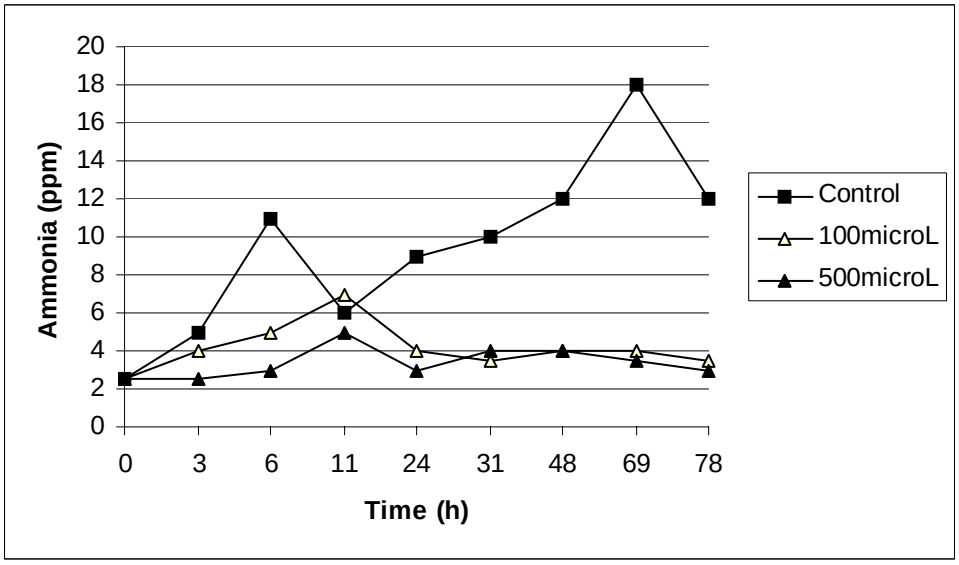
5.2.1 Method

In this trial 8 drums were used: 2 controls (no additives), 3 containing 100μL of Agrotain and 3 containing 500μL of Agrotain. In order to obtain better homogenisation of the contents of the bucket the 100μL Agrotain was added to 0.9 ml of distilled water and the 500μL to 9.5 ml of distilled water.

5.2.2 Results and Conclusions

The urease inhibitor Agrotain is highly effective at reducing the volatilisation from cattle effluent (Figure 10). Unfortunately Agrotain has a very strong and offensive odour and there is no information on its potential toxicity in cattle.

Figure 10: Effect of Agrotain on ammonia emission from drums containing simulated live export manure-bedding mixture.



5.3 De-Odorase

De-Odorase is a product containing the extract of the *Yucca* plant that is known to bind ammonia. This product has been used in the pig industry in both feed and bedding.

5.3.2 De-Odorase in Bedding

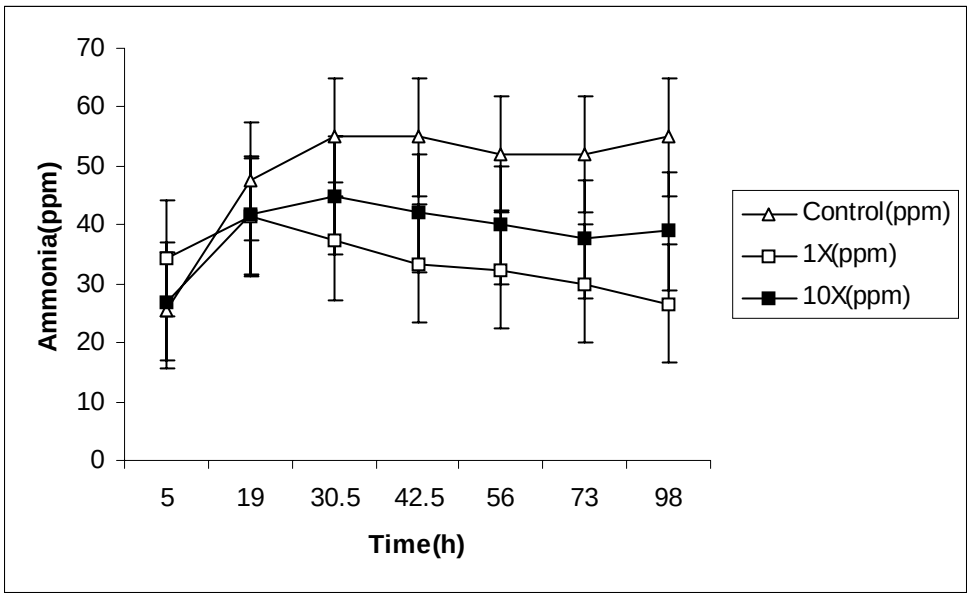
5.3.2.1 Method

In this experiment 8 drums were used: 2 controls (no additives), 3 containing 0.0455g of De-odorase (recommended inclusion rate) and 3 containing 0.455g of De-odorase (10 times recommended inclusion rate).

5.3.2.2 Results and Conclusions:

De-ordorase, when added to the bedding, was effective at reducing the volatilisation of ammonia from cattle effluent (Figure 11) although the response was variable and not dose responsive.

Figure 11: Effect of De-odorase on ammonia emission from drums containing simulated live export manure-bedding mixture.



5.3.3 De-Odorase in feed

5.3.3.1 Method

Four fistulated animals were used in this experiment. Animals were fed pellets only (control) and 2 animals were fed pellets +3 g of De-Odorase daily in a crossover design with one-week adaptation between periods. Animals were fed the diet for 5 days and then a faeces/urine collection was performed. The experiment was done in two runs.

Run 1
In this experiment 5 drums were used: 2 with manure from control animals and 3 with manure from animals receiving De-Odorase

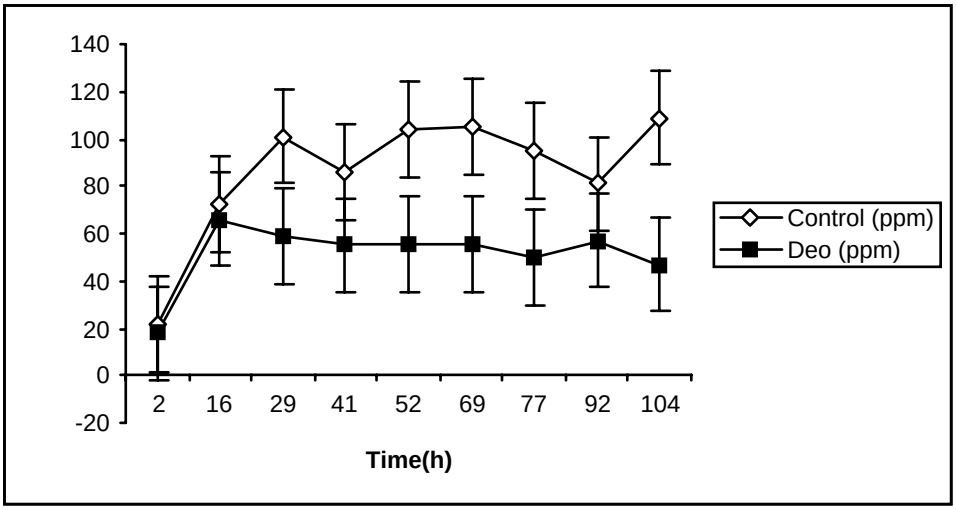
Run 2
In this experiment 4 drums were used: 2 with manure from control animals and 2 with manure from animals receiving De-Odorase

5.3.3.2 Results and Conclusions:

Run 1

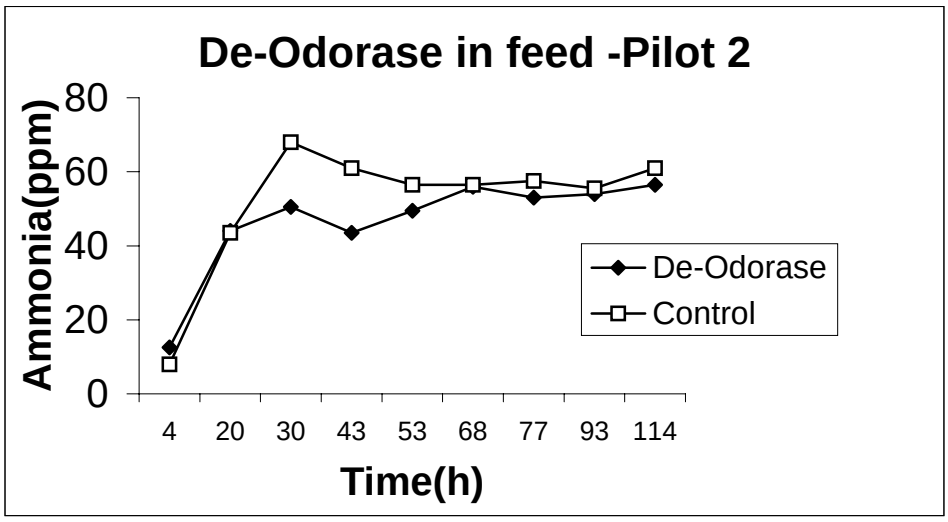
There was a significant ($P<0.05$) effect of De-Odorase (Figure 12).

Figure 12: Effect of feeding De-Odorase on ammonia emission from drums containing simulated live export manure-bedding mixture.



Run 2
There was no significant (P<0.05) effect of De-Odorase (Figure 13).

Figure 13: Effect of feeding De-Odorase on ammonia emission from drums containing simulated live export manure-bedding mixture.



De-Odorase when added to the feed of cattle tended to reduce the subsequent volatilisation of ammonia from cattle effluent but the response was variable and more experimental replicates are needed to understand the source of this variation.

5.4 Gypsum

Ammonia is highly volatile at neutral pH. If the pH of cattle effluent can be lowered then ammonia volatilisation should be decreased. Calcium sulphate (gypsum) is a low cost and widely used acidifying agent that should directly decrease the pH of the bedding and so reduce NH_4 volatilisation.

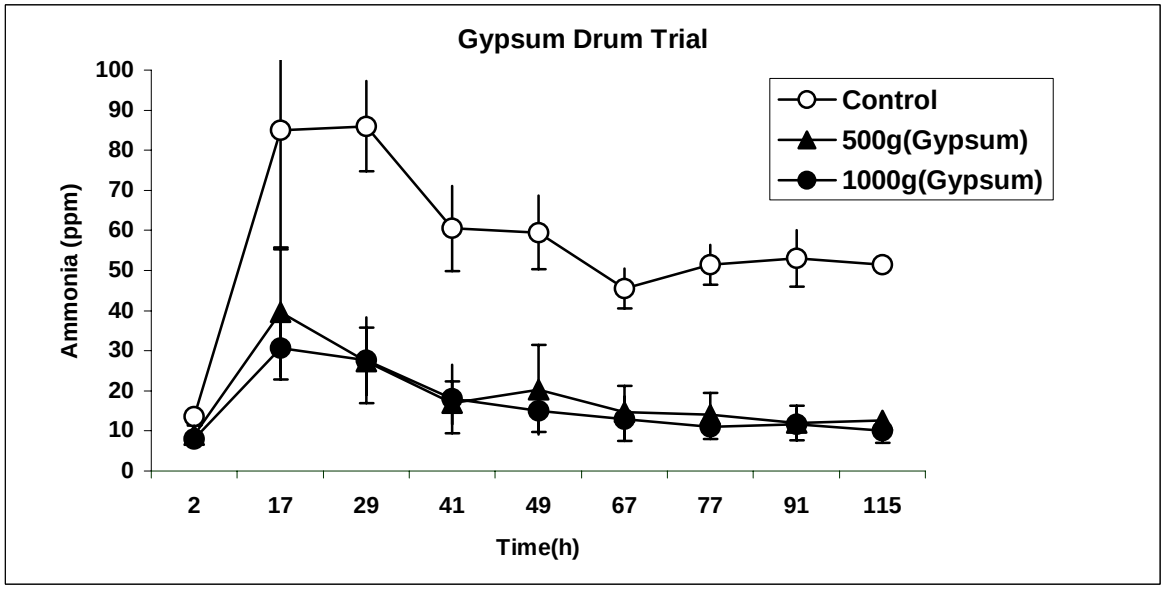
5.4.1 Method

In this experiment 8 drums were used: 2 controls (no additive), 3 containing 500 g of Gypsum and 3 containing 1000 g of Gypsum.

5.4.2 Results and Conclusions

Gypsum when added to cattle effluent was highly effective in reducing ammonia volatilisation (Figure 14).

Figure 14: Effect of Gypsum on ammonia emission from drums containing simulated live export manure-bedding mixture.



5.4 Effect of charged clays added to bedding material on ammonia emission - *in vitro* study

5.4.1 Spongolite and Zeolite

5.4.1.1 Method

Effluent from cattle kept in the environmental simulation rooms was collected over 24 h and stored under refrigeration over night. One kg of sawdust alone (control) or mixed with either 200 g or 600 g of one of the clays (Zeolite or Spongolite) was placed in 80 l drums and 3.5 kg of effluent was added to each drum. The drums were then sealed and placed in a controlled environmental room with the temperature around 32ºC dry bulb and ammonia emission monitored over 3-5 days. In the experiment where Spongolite was tested, extra drums were used with 500 g of gypsum mixed to the sawdust as a positive control.

5.4.1.2 Results and Conclusions

Graphs 15 and 16 show the results of these trials. Although both Zeolite and Spongolite at a higher dose (60%) significantly ($P<0.05$) decreased ammonia emission from cattle effluent they did not perform as well as gypsum (Figure 15 and 16) which is considerably cheaper. Therefore further investigations of these products were not carried out.

Figure 15: Effect of Zeolite on ammonia emission from drums containing cattle effluent mixed to treated or untreated sawdust.

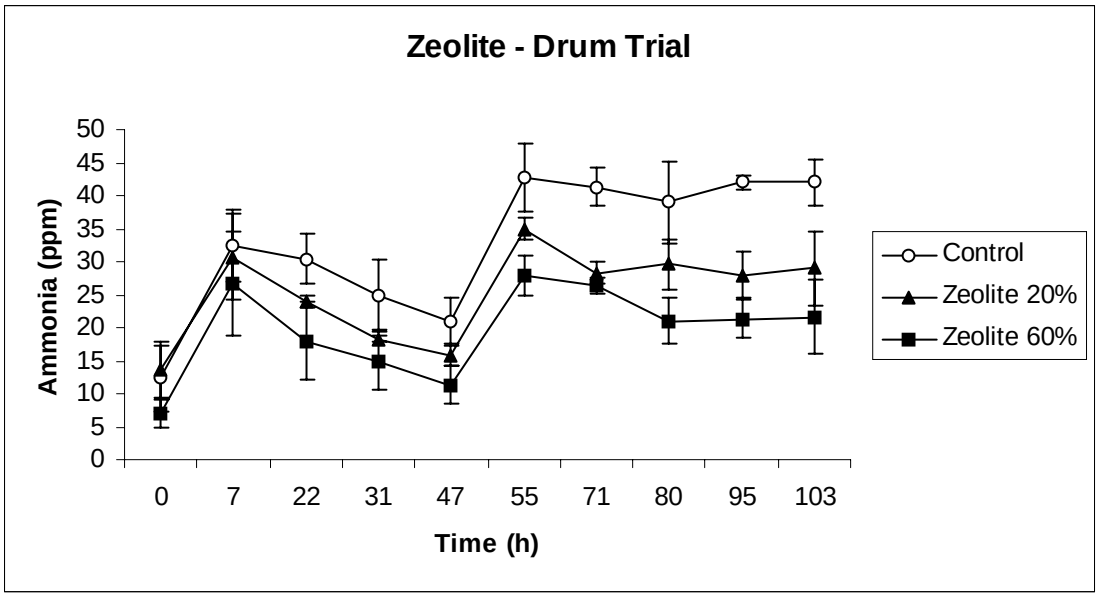
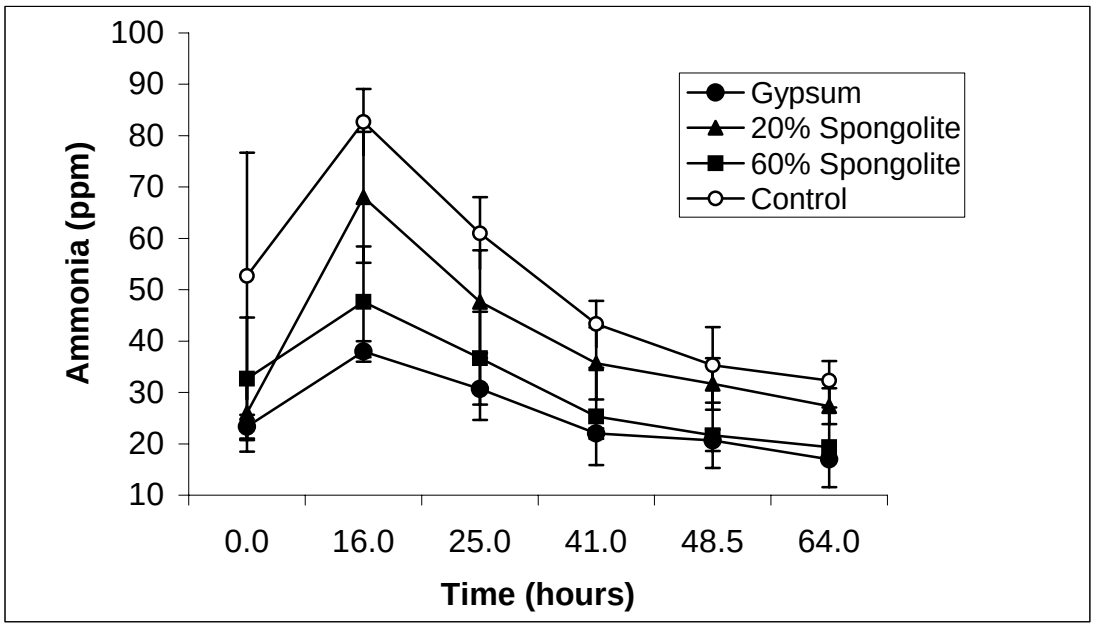


Figure 16: Effect of Spongolite on ammonia emission from drums containing cattle effluent mixed to treated or untreated sawdust



5.4.2 Stable-Plus

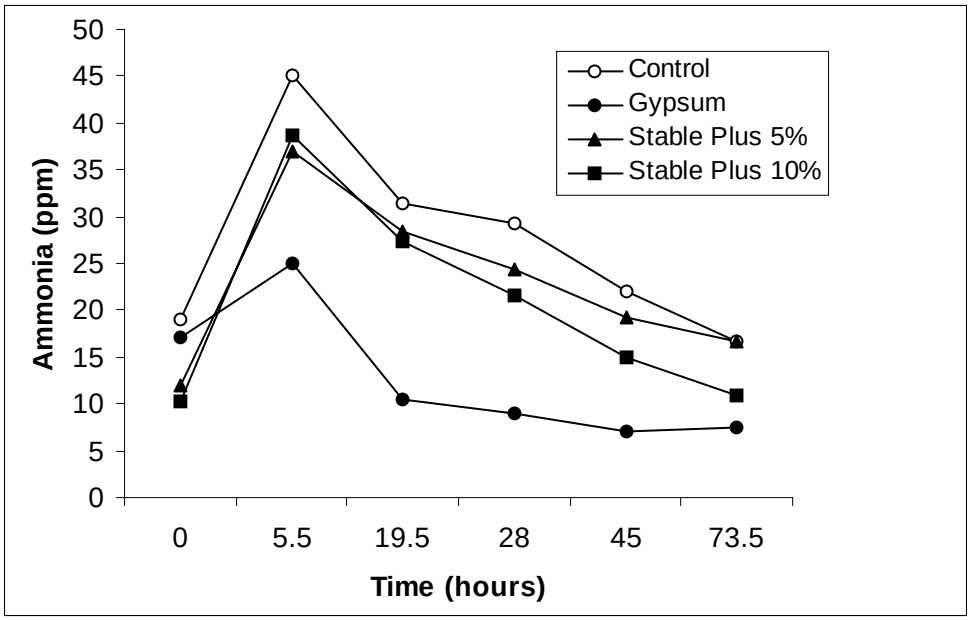
5.4.2.1 Method

One kg of wood shavings alone (control, n=3) or mixed with either 5 g (n=3) or 10 g (n=3) of Stable-Plus (ammonia binder product) was placed in 80 l drums and 3.5 kg of effluent was added to each drum. The drums were then sealed and placed in a controlled environmental room with temperature around 32ºC dry bulb and ammonia emission monitored over 3 days. As a positive control, extra drums (n=2) containing 500 g of gypsum mixed with wood shavings were also monitored.

5.4.2.2 Results and Conclusions

Figure 17 shows the results for ammonia emissions. Stable-Plus did not significantly ($P<0.05$) decreased ammonia emissions, while gypsum showed a significant reduction in ammonia emission. Stable-Plus is not a useful alternative for reducing ammonia emissions during live cattle export.

Figure 17: Effect of Stable-Plus on ammonia emission from drums containing cattle effluent mixed to treated or untreated sawdust.



5.4.3 Overall conclusions for bedding and dietary additives which reduce ammonia production.

- Gypsum was the best bet for addition to the bedding to reduce ammonia emissions.
- Dietary addition of De-odorase requires more work to confirm or refute its efficacy as an ammonia reducing agent.
- Dietary inclusion of gypsum has been discussed above.

6. Final testing of Ammonia reducing strategies using 'on board' simulation experiments

Following the *in vitro* environmental investigations as described above, two rooms were designed for testing ammonia reducing options in cattle under simulated 'on board' conditions. The rooms were located at Murdoch University and reproduced the conditions found onboard live export vessels (i.e. stocking density and pen air turnover). Both rooms were under negative pressure ventilation with air leaving through one outlet (35 cm X 35 cm) and entering through one inlet (58 cm X 28 cm). The air supply to each room was approximately 1,000 cubic metres per hour. The rooms' 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 one pen (3.64 m X 2.05 m) where animals were placed. Each pen had 2 feeders (40 cm X 77 cm each) and a water trough (30 cm X 33 cm). The pen air turnover (PAT) was 134 m/h. 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 to not interfere with room ventilation.

6.1 Use of gypsum 'in bedding' on the control of ammonia emission from cattle effluent – simulation rooms reproducing live export conditions

Ammonia is highly volatile at alkaline pH. The urine of cattle is typically about 8.0. If the pH of cattle effluent can be lowered, then ammonia volatilisation should be decreased. Gypsum (calcium sulphate) is a low cost and widely used acidifying agent.

6.1.1 Method

Ten Angus cross steers around 12 months of age and with an average weight of 263.6 kg were assigned randomly to one of the two simulation rooms. Animals were fed lucerne cubes (Hycube Industries Pty Ltd, Victoria, Australia) to induce a high rate of nitrogen excretion by the cattle and so a high potential ammonia load. The diet was fed once a day at 2.25% (DMI) of body weight and animals were on the diet for one week before the experiment was initiated.

The bedding used in both rooms consisted of 75 kg of sawdust, but in the treatment room the sawdust was mixed with 32.5 kg of Gypsum. The trial was carried out in 4 replicates with the bedding treatment being swapped in between rooms in each replicate. There was a break of approximately two and a half days in between replicates when the rooms were aired and steam cleaned. The animals were kept outdoors in a sand yard and on in the same diet regimen during these periods.

The animals were placed in the rooms at 10 am on day one when the first set of measurements was taken. The animals were kept in the rooms for 5 days and 2 measurements were taken everyday at 8:30 am and 2 pm in order to sample values from both cool and warm times of the day. The following parameters were measured: ammonia concentration (Neotox single gas monitor Mk5), CO_2 concentration (Testo 445 measuring instrument with CO_2 probe), temperature and relative humidity (Testo 445 measuring instrument with combined temperature and humidity probe). At each measurement set, 5 measurements were taken: four measurements were taken from the platform above the pens. The instruments were lowered to 130 cm above the ground to sample air at a human breathing level and to 100 cm to sample air at an animal breathing level. These measurements were done in the middle of the pen and approximately 40 cm from the north south wall. A fifth measurement was also taken from outside the pen but inside the room at approximately at 120 cm above the ground. At the time of each measurement the animals were observed for any symptoms of respiratory irritation such as coughing and presence of abnormal nasal discharge. After the first measurement was taken at day 3 the bedding was mixed, as there was a tendency for effluent to accumulate in

the middle of the room with clean sawdust displaced to the sides and corners of the pens. This procedure was carried out for animal welfare reasons.

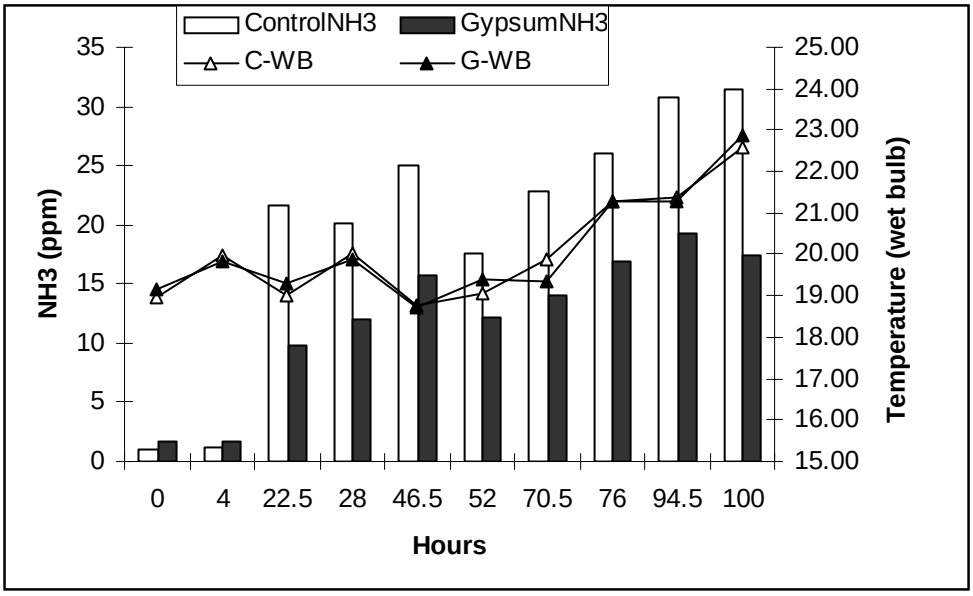
At day five after the animals were removed from the rooms they were submitted to a physical examination. This included measurement of body temperature, heart and respiratory rate and recording the presence of abnormal nasal discharge and abnormal respiratory sounds.

At the beginning and at the end of each replicate the airflow (PSI – 500 Air Velocity Meter) was measured in each room with vents both clean and dirty and an average used as the flow for each replicate. Also at the end of each replicate the effluent's pH (pHBoy-P2-Shindengen) was measured from a homogenised sample taken from 5 spots in each room.

6.1.2 Results

Gypsum significantly (P<0.001) decreased ammonia emission (Figure 18). There was a trend for animals to put on more weight while in gypsum treated replicas. Coughing increased with time but there was no significant difference (P>0.05) between treatments. There was no significant difference (P>0.05) in temperature and humidity between the rooms.

Figure 18: Room's wet bulb temperature (WB) and effect of bedding treatment with gypsum on ammonia emissions (ppm) in simulation rooms.



6.1.3 Conclusions

Gypsum when added to pine straw bedding at a ratio ~2:1 was effective in reducing ammonia emission for 5 days from the effluent of cattle kept under simulated live export conditions. This reduction is thought to be due to bedding acidification. Treating bedding with Gypsum may be an alternative used in a multilateral approach to controlling ammonia emission during live export.

6.2 Use of De-Odorase 'in feed' for decreasing ammonia emission from beef cattle effluent – simulation rooms reproducing live export conditions

De-Odorase is an extract from *Yucca* plant that is known to bind ammonia. This product has been used successfully to reduce ammonia emissions from livestock operations (Weaver 1993). .

6.2.1 Method

Ten Angus cross steers approximately 12 months of age and average weight of 263 kg were assigned randomly to one of the two simulation rooms. The bedding used in both rooms was sawdust at 75 kg/pen.

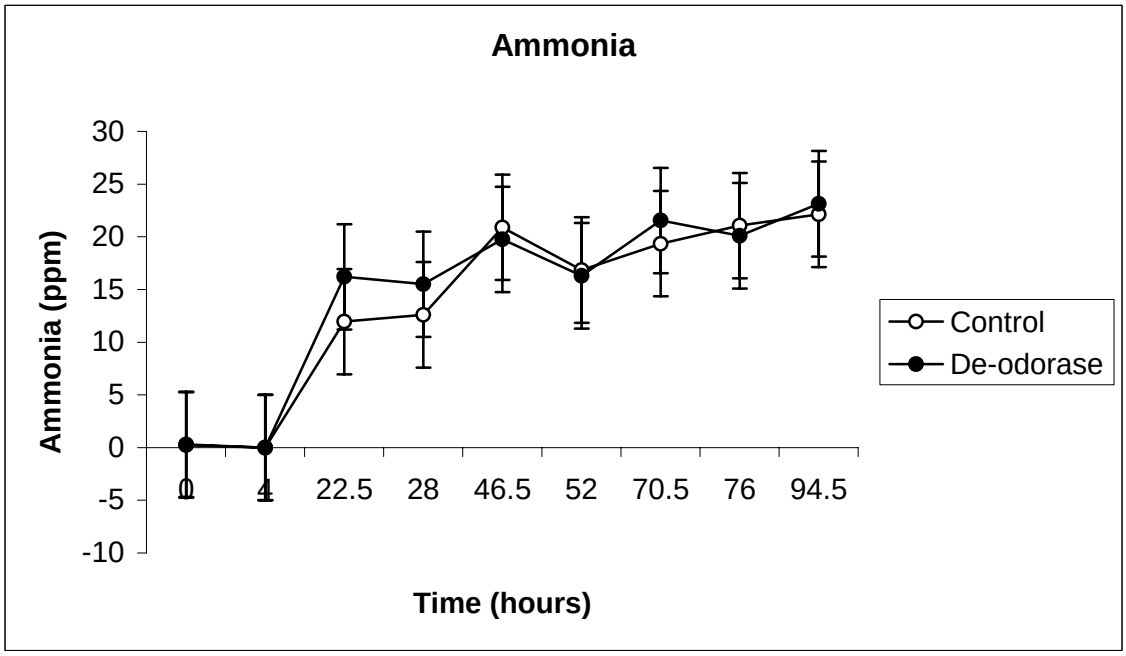
Animals were fed lucerne cubes (Hycube Industries Pty Ltd, Victoria, Australia). The diet was fed once a day at a rate of 2.25% of body weight based on dry matter intake and animals were on the diet for five days before the experiment was initiated. While one group would receive the Lucerne cubes only the other group received a 25 g daily dose of De-Odorase – diluted in 150 ml of distilled water which was mixed throughout the cubes just before feeding. Each group was introduced to their treatment diet 3 days before being placed in the rooms.

The trial was carried out in 4 replicates with feeding treatment being swapped between rooms for each replica. There was a break of approximately 3 days in between replicas when the rooms were aired and steam cleaned and animals kept outdoors on a sand yard. (during this period they were introduced to the new diet treatment).The animals were placed in the rooms at 10 am on day one when the first set of measurements was taken. The following parameters were measured: ammonia and CO_2 concentration in the air, air temperature and relative humidity. Other procedures were as described in section 6.1.1.The animals were kept in the rooms for 5 days and 2 measurements were taken everyday at 8:30 am and 2 pm in order to sample values from a cool and warm time of the day.

6.2.2 Results

De-Odorase did not significantly ($P>0.05$) affect ammonia emission in this simulation room model (Figure 19). Humidity, temperature and ventilation were not significantly ($P>0.05$) different between rooms. Coughing significantly ($P<0.05$) increased with time but there was no treatment effect on its occurrence.

Figure 19: Ammonia emission (ppm) from cattle effluent fed diets containing or not the additive De-Odorase while kept in simulation rooms.



6.2.3 Conclusion

In feed De-Odorase is not effective in decreasing ammonia emission from cattle effluent in simulation rooms.

6.3 Effect of acidifying salt ($CaCl_2$) on ammonia emission under simulated conditions

In this experiment the effect of dietary $CaCl_2$ on air ammonia concentration was tested in cattle under live export simulated conditions.

6.3.1 Method

Subsequent to experiments 6.1 and 6.2, the simulation rooms were fitted with climate control devices. The rooms were climate controlled using supply air fans, humidifiers and electric duct heaters. The 2 supply air fans were set to deliver 300 l/s at 150 Pa. The air flow rates in the rooms were 15 air changes per hour exhaust rate or 210 l/s. The 2 steam humidifiers had a steam capacity modulation range of 10 to 33 kg/hr. The system was controlled by independent electronic temperature and absolute humidity controls, with one sensor in each room providing feed back control. The temperature and absolute humidity could be adjusted manually via panel mounted controllers on the front of the control panel.

Eight Angus cross heifers were divided in 2 groups and fed one of the two experimental diets (table 8). The initial average live weight for the group fed the basic diet was 398 kg and for the group fed the basic diet plus $CaICl_2$ 386 kg. Animals were fed diets at 2.25% of body weight on a dry matter basis. The diet was fed for four days before the animals were placed in one of the simulation rooms for approximately four days Need to be consistent throughout – either 4 or four – a good rule is to spell one to nine and use the numerals for 10 and above. Atmospheric ammonia, CO_2, temperature and humidity were measured throughout the period the animals were in the rooms with measurements being performed twice a day in five locations in each of

the rooms. The animals had a rest of two and a half days before the experiment was repeated. The dietary treatment remained the same for each room with the animals moving from one room to another. Three replicates were performed. Animals were weighed immediately before and after each repeat.

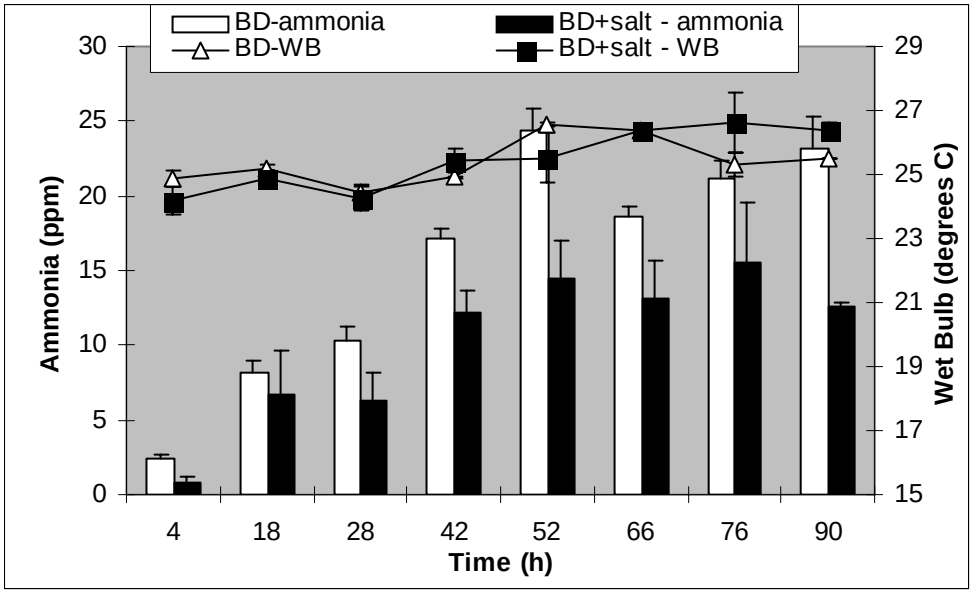
Table 8 Percent composition of diets for assessment of atmospheric ammonia under simulated conditions of live export.

Diet	Straw	Barley	Lupins	Lime	$CaCl_2$
Basic diet	55.5	25	18	1.5	
Basic diet + $CalCl_2$	55	25	18	1	1

6.3.2 Results and conclusions

There was no significant difference ($P>0.05$) on live weight changes, CO_2, temperature or humidity between treatments. Dietary treatment affected ammonia emissions with the addition of $CalCl_2$ significantly ($P < 0.05$) decreasing ammonia emission (Figure 20).

Figure 20: Effect of dietary treatment on ammonia emissions (bars) from manure of cattle kept under live export simulated conditions (wet bulb temperature shown as a line).



BD = basal diet; BD+salt = basal diet plus $CaCl_2$.

The results show that dietary manipulation can decrease ammonia emission. The diets used in this experiment and a crude protein content of 9.5% (basic diet) and 9% (basic diet + $CalCl_2$). If compared with the emissions recorded from cattle fed lucerne cubes (section 6.1) where dietary crude protein was 18.8% the results demonstrated that ammonia emissions could be decreased by decreasing protein content of the diets. The results also demonstrate that addition of $CalCl_2$ to the diet decreases ammonia emissions even further.

6.4 Effect of modified diet in combination with gypsum addition to the bedding on ammonia emission under simulated condition

In this experiment the effect of dietary $CaCl_2$ and gypsum in the bedding on air ammonia concentration was tested in cattle under live export simulated conditions.

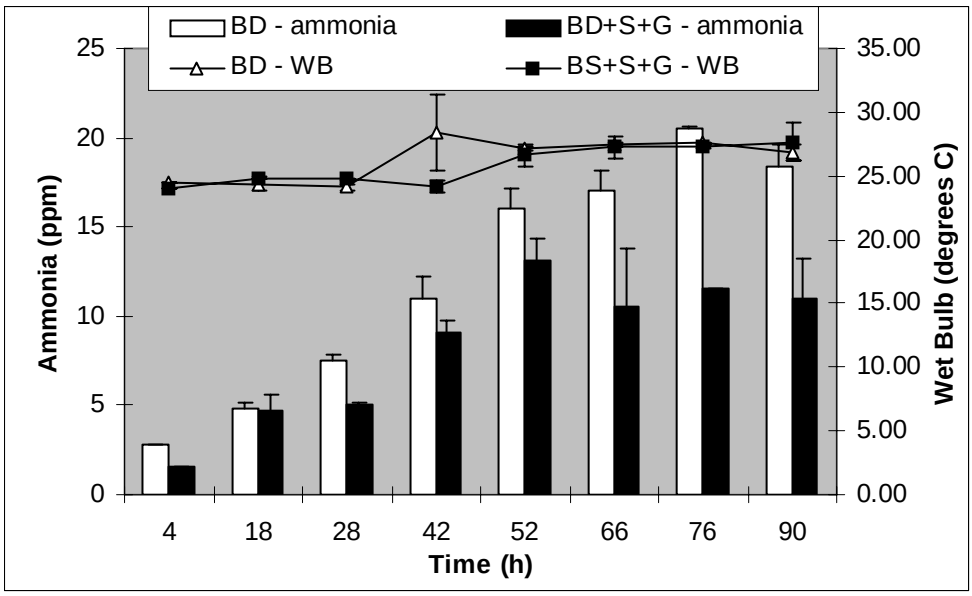
6.4.1 Method

This experiment was also performed after the experimental rooms were fitted with climate control devices. The same design as experiment 6.3 was applied with the addition that the 70kg of wood shaving used as bedding in both rooms were mixed with 32.5kg of gypsum in the room where the animals were receiving the Basic diet + $CalCl_2$.
The initial average live weight for the animal on the basic diet was 434 kg while for the animals on the Basic diet + $CalCl_2$ was 412 kg. The experiment was repeated twice.

6.4.2 Results and conclusions

The dietary $CaCl_2$ plus gypsum in the bedding treatment reduced gaseous ammonia levels indicating a very positive effect ($P<0.05$) (Figure 21). However when the results are compared with the previous section using dietary $CaCl_2$ alone it would appear that addition of gypsum to the bedding alone gave little additional benefit.

Figure 21: Effect of dietary treatment and gypsum addition to bedding on ammonia emissions (bars) from manure of cattle kept under live export simulated conditions (wet bulb temperature shown as a line).



BD = basal diet; BD+S+G = basal diet plus $CaCl_2$ and gypsum added to the bedding.

7. Lung Health Studies

There is very little scientific information on the effects of continuous exposure to high ammonia levels on the bovine lung. Physical examination of cattle used in the simulation room experiments did not produce objective information on the effect ammonia on their lungs. An experiment was therefore designed where bronchial alveolar lavages (BAL) were performed a few days before and immediately after the animals were placed in the simulation rooms.

7.1 High protein diet (Lucerne cubes – 18.8% CP)

7.1.1 Method

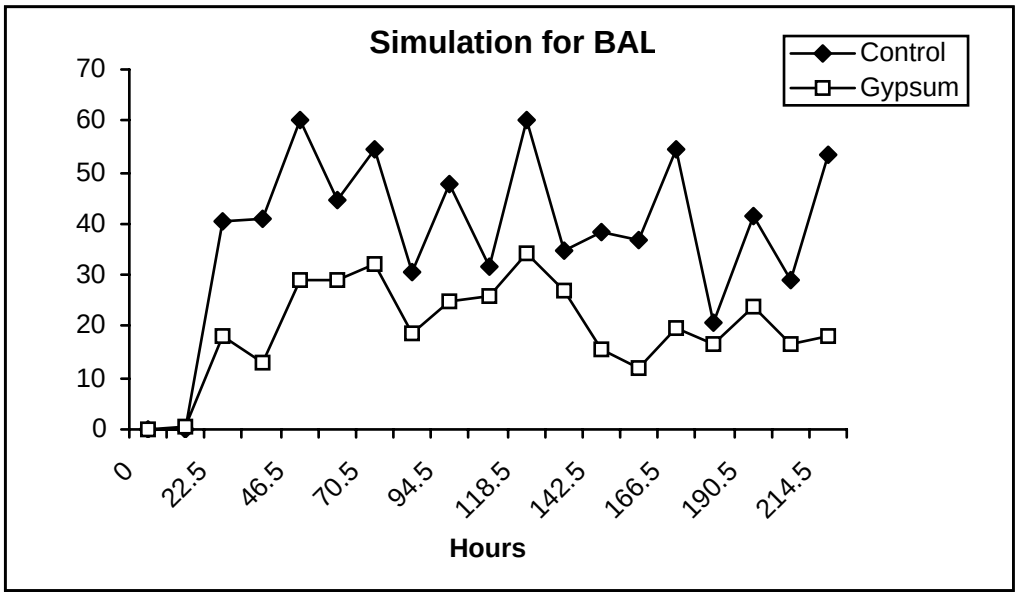
Ten Angus cross heifers were kept in the simulation rooms for 10 days (two groups of five with a avarage total live weight of 1688kg in each room). Animals were fed a lucerne cube diet at 2.25% of live weight on a dry matter basis. They were introduced to the diet 5 days before the experiment was started. An attempt was made to keep ammonia levels in one of the rooms low (below the 25ppm occupational health and safety recommended levels – NOHSC 1995) by treating the bedding with gypsum while the other room was untreated and left to achieve high ammonia levels. This experiment was performed at the start of autumn 2001 (before the rooms were fitted with controlled environment equipment) and as the environmental temperatures were getting lower a portable heater was used in each of the room to keep temperature closer to the live export conditions. Controlling the environment of the rooms proved very difficult.

A complete blood cell count was also performed at the same time as the BALs.

7.1.2 Results and conclusions

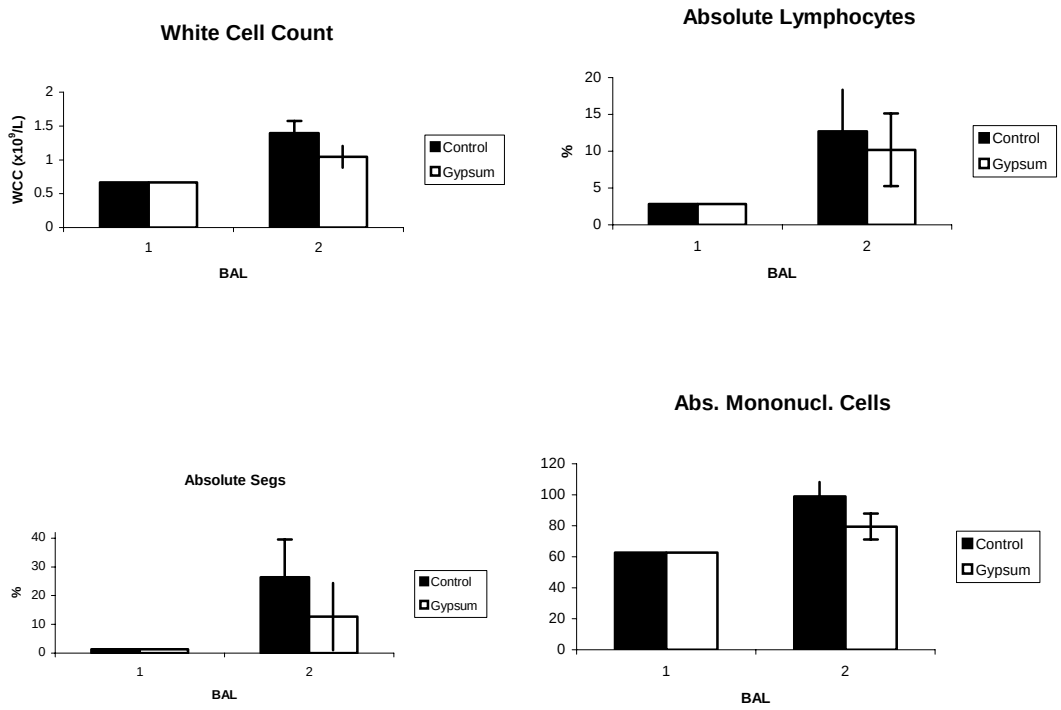
Once temperature got higher then around 30-32ºC (dry bulb), gypsum was not very efficacious in maintaining low ammonia levels and so both sawdust and gypsum had to be added frequently (at least once a day) to the room in order to decrease ammonia levels. Despite this, in some instances ammonia levels achieved higher levels than those limits recommended by Australian occupational health and safety authorities (Figure 22). The maximum reliable reading for the ammonia measuring device (Neotox single gas monitor Mk5) used for measurements was 60 ppm and so it is likely that on some occasions ammonia levels were higher than 60ppm.

Figure 22: Ammonia levels in rooms during experimental trial to investigate the effect of ammonia levels on bovine lung.



There were no significant differences (P>0.05) in live weight gain and blood cell results between the two treatments or between initial and final measurements (Figure 23). There was a significant increase in white cell count and mononucleated cell counts on the BALs performed after the animals left the rooms. There was a trend for the responses to be more marked in the animals that were placed in the untreated room (Figure 23; higher ammonia levels).

Figure 23: Cell counts from brochio- alveolar lavages performed in cattle kept in rooms with high ammonia levels



The BAL results showed that the animals developed a non-specific inflammatory response and this is consistent with irritation. As the dust levels in both rooms was very low, ammonia is the most logic-attributed cause for this irritation. Considering that the animals in the experiment were not under the same sort of stress encountered by animals during live export, this finding is very significant in terms of the potential role that high ammonia levels can play in predisposing live export animals to pneumonia. Further investigations in this area will be carried out once the environment in the simulation rooms is under better control.

7.2 Lower protein diets (9-9.5% CP)

This experiment was performed after the simulation rooms were fitted with climate control devices.

7.2.1 Method

This experiment was carried out in two sections using a total of 6 groups of 4 Angus cross heifers. Animals were fed the diets described in table 7 at 2.25% live weight on a dry matter basis for at least 5 days before first BAL was performed. The first half of the experiment consisted of one group of animals kept outdoors at all times and fed the basic diet, one group fed the basic diet plus 9 days in the simulation rooms, and a group of animals fed the basic diet +$CaCl_2$ that also spent 9 days in the simulation rooms. The total live weight for each group was respectively: 1480 kg, 1508 kg and 1520 kg.

The second half of the experiment consisted of one group of animals kept outdoors at all times and fed the basic diet +$CaCl_2$, one group fed the basic diet and that spent 9 days in the simulation rooms, and a group of animals fed the basic diet +$CaCl_2$ that also spent 9 days in the simulation rooms. The average live weight for each group was respectively: 1646 kg, 11652kg and 1650kg.as above

To assure that ammonia levels would be kept low at all times, the room with animals receiving the basic diet +$CaCl_2$ had the bedding changed every 2 to 3 days. Bronchio-alveolar lavage, total blood cell count, arterial blood gases and plasma Haptoglobin (an acute phase protein that can indicate inflammation) were measured 4 days before the animals were placed in simulation rooms and immediately after they left the rooms.

7.2.2 Results and conclusions

There were no significant differences ($P>0.05$) in either live weight gain or clinical parameters between treatments. There was no significant effect of time on clinical parameters, i.e. parameters did not change from initial values after animals spent time in the simulation rooms. Figures 24 and 25 show the atmospheric ammonia levels for the two sections of the experiment. The outdoor level of ammonia was 0 ppm at all times.

Figure 24: Ammonia emissions in rooms with cattle fed either the basal diet +$CaCl_2$ (mean±sem:10.63 ±0.3 ppm) or basic diet (18.62 ±0.6 ppm)

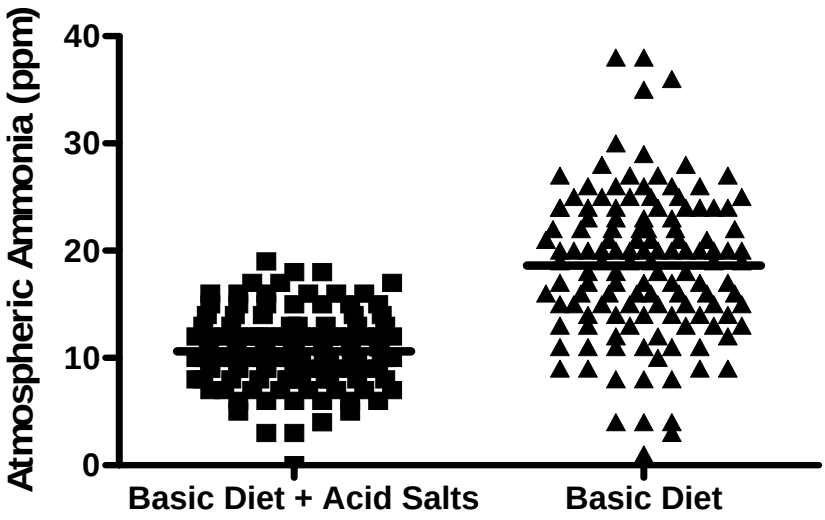
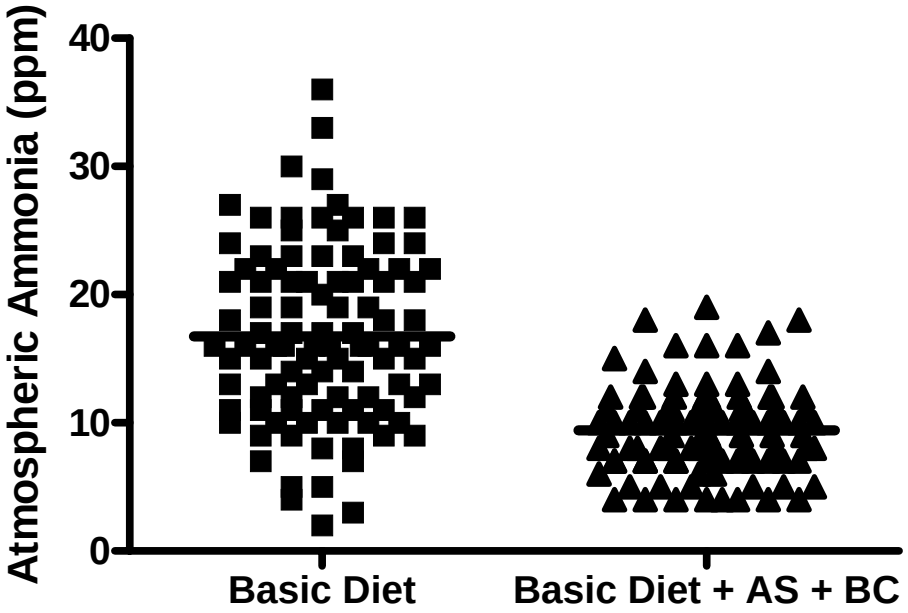


Figure 25: Ammonia emissions in rooms with cattle fed either basic diet (mean±sem: 16.76 ±0.68 ppm) or basic diet +$CaCl_2$ (9.42 ±0.34 ppm)



These results indicate that cattle kept under conditions of less than 20 ppm atmospheric ammonia for 9 days did not develop a lung inflammatory response when kept under live export simulated conditions.

8. Overall conclusions and industry significance

8.1 Diet formulation - the effects of varying protein source and roughage quality.

The aim of this section of the work was to develop a simple method for assessing nitrogen excretion when different diets were fed to cattle. In addition the effects of roughage quality, cereal grain level and protein level/type were tested for effects on live weight gain, feed intake and urinary nitrogen excretion.

1. A spot sampling procedure (one sample of urine or blood post feeding) has been developed which can be readily be used to test the impact of diet on urinary nitrogen output.

2. A series of rations based on 50% roughage inclusion were tested for effects on weight gain and urinary nitrogen excretion (ME 8.5-10.4 MJ/kg DM, CP 9.7-18.8% DM) – the following conclusions were made:

    a. Roughage quality (cereal hay instead of cereal straw) had a significant positive impact on estimated live weight gain – where possible high quality roughage should be used in pellets if live weight gain is desirable.
    b. A high level of dietary true protein (from either canola meal or lupin grain) in combination with good quality roughage gave the best performance. This was driven primarily by the availability of ME.
    c. High lupin inclusion (30%) allowed good live weight gain on straw based diets but at the cost of a high urinary nitrogen excretion.
    d. Canola meal promoted lower levels of urinary nitrogen excretion.
    e. High versus low IVD cereal hay (9.1 vs 8.1 MJ/kg DM) was perhaps too subtle a change to detect differences in performance over the short feeding period – both gave satisfactory performance. However an extreme reduction in roughage quality, such as poor quality straw (i.e. 4.9 MJ/kg DM, CP 1.8% DM) would have a detrimental effect on performance.
    f. High cereal roughage diets (70%) and lower cereal grain inclusion (10%) are feasible alternatives for the live export trade and can result in good performance as long as good quality roughage is used. One of the clear advantages is the suggestion that high roughage allows for a rapid achievement of full intake compared to diets having lower roughage (55%) and a higher cereal grain (18%) inclusion.
    g. An all roughage diet fed as lucerne cubes allowed for moderate live weight gain (equivalent to the straw, high lupin inclusion diet) but at the expense of very high urinary excretion of ammonia.

3. The results show that the traditional diets used by the live export trade can be replaced by alternative diets that provide the same intake, similar or better performance and most importantly lower urinary N. Roughage quality seems to be the key for designing such diets. If roughage quality cannot be changed an alternative is to capitalise on the special quality of canola meal which has a lower rate of rumen degradable protein translating into lower rates of nitrogen excretion. To allow palatability, and delivery of more ‘safe’ ME on diets formulated around straw there is little option but to increase true protein inclusion (lupin grain or canola meal) and under these situations some substitution of lupin grain by canola meal is

recommended if reducing nitrogen excretion is a primary goal. Both of these conclusions (roughage quality, protein inclusion/source) involve an increase in the cost of the final ration. In conclusion the cost of diet should not be the only thing to be considered when choosing a diet for long haul voyages, live weight gain and N excretion should also be considered.

8.2 Using dietary additives to acidify urinary pH and so reduce ammonia volatilisation.

This phase of the work addressed dietary manipulation of urinary pH since it is well known that ammonium volatilisation to ammonia gas is minimal when the effluent pH is below 7.

1. The addition of lime (1 or 2%) as a pellet binder increased urinary pH to about 7.5-8. That is most live export diets will induce a high urinary pH.
2. Gypsum, when added to the diet decreased urinary pH but was not a satisfactory binder.
3. Gypsum when added at safe levels to protect against sulphur toxicity (i.e. <0.5%) was not able to reduce urinary pH in the presence of 1-2% lime.
4. Either NH_4Cl (1.4%) or $CaCl_2$ (1%) when added in the presence of lime reduced urinary pH to below 7 (= significant reduction in ammonia volatilisation) and allowed good intake and live weight gain.
5. NH_4Cl addition alone (1.4%) reduced intake and performance, indicating care is needed when adding acidic salts.
6. Urinary acidifiers are a viable option for reducing ammonia volatilisation and should allow more flexible feed formulation such as increased inclusion of lupins. Further work is required to see if dietary acidifiers negatively impact on adaptations to heat stress. The acidifiers did result in a small reduction in blood bicarbonate levels and this might negatively impact on adaptations to heat stress but this result is by no means certain.

8.3 Bedding and other dietary additives.

In this part of the work several commercially available products were tested for their efficacy to reduce ammonia emission when added to the bedding of feed.

1. Gypsum added to sawdust bedding at an inclusion rate of 50% was effective in reducing ammonia emission. The effect is reduced at high temperatures (30°C dry bulb). The main effect of gypsum is to reduce bedding pH which means that the industry can use either a dietary acidifier or gypsum in the bedding. The dietary addition of urinary acidifiers is likely to be more effective.
2. De-Odorase in the bedding reduced ammonia emission in a variable manner, but was ineffective when added to the diet.
3. Several other compounds were tested for their ability to reduce ammonia volatilisation (Agrotain, Zeolite, Spongolite, Stable Plus). Most products resulted in some depression of ammonia emission but none were better than either gypsum except for Agrotain. Unfortunately Agrotain was considered to have potential toxic effects of its own.

8.4 Room simulations of final recommendations

Cattle were kept in rooms designed to simulate live export ship conditions and dietary scenarios were tested to see if ammonia emission could be reduced. The results show that a reduction in urinary N translated into reduced ammonia emission. In addition dietary and/or bedding acidifiers will work on board to reduce ammonia volatilisation.

8.5 Lung health

1. Chronic exposure to high ammonia levels can cause an inflammatory response in the lung consistent with irritation.
2. This type of response does not occur if ammonia levels are kept under 20 ppm.

9. Recommendations

1. Decrease ration protein and balance with energy by using good quality roughage.
2. Target protein level is 10-11% with a ME of 10 MJ/kg DM, which means a 1.0% to1.5% drop in protein from existing levels. However higher protein diets will cause little problem with ammonia emission if acidic salts are used.
3. Cost of diet should not be the only consideration.
4. The inclusion of lime as a binder at 1% is effective in terms of pellet quality
5. Acid salts are useful for decreasing ammonia emissions and can deliver acidic urine even in the presence of 2% lime. Further work is needed to see if they have a negative interaction with heat stress.
6. Ammonia levels should be monitored onboard in order to identify problem pens and readjust stock density.
7. According to the preliminary findings of this project on the effect of high atmospheric ammonia on cattle lung, ammonia levels below 20 ppm should be the target during live export.

10. Publications

Publications resulting from this project were:

Accioly, J.M., Tudor, G.D., Taylor, E.G., White, C.L., Costa, N.D., Pluske, J.R. and Pethick, D.W. (2002). Effect of roughage quality and fermentable energy/protein on intake, performance and nitrogen excretion in cattle fed export diets. *Asia Pacific Journal of Clin Nutrition* **11** (S): S250.

Accioly, J. M., Pearce, K., Taylor, E., Costa, N. D., White, C. L., Tudor, G. D. Pluske, J.R., Williams, I.H. , and Pethick, D.W. (2002) A spot sample method for predicting nitrogen excretion in cattle fed export diets. *XXII World Buiatrics Congress*, Hannover, Germany. Abstract 30-323.

Accioly, J.M., Beatty,D.T., Barnes,A.L., Pethick,D.W., Taylor,E.G., Tudor,G.D., White,C.L., Maloney,S.K., McCarthy,M.R., Pluske,J.R. and Costa,N.D.(2003). Nutrition During Live Export of Cattle. *Recent Advances in Animal Nutrition in Australia*, **14**, 49-56.

11. References

Drummond, J.G., Curtis, S.E., and Simon, J. (1978) "Effects of atmospheric ammonia on pulmonary bacterial clearance in the young pig." *Am.J. Vet.Res.* 39: 211-212.

Gustin, P., Urbain, B., Prouvost, J-F. and Ansay, M.. (1994) 'Effect of atmospheric ammonia on pulmonary hemodynamics and vascular reactivity in pigs: interaction with endotoxins." *Toxicl. Appl. Pharmacol.* 125: 17-26.

National Occupational Health and Safety Commission (1995) "Exposure Standards for Atmospheric Contaminants in the Occupational Environment." pp 75, Australian Government Publishing Service, Canberra.

The National Swedish Board of Occupational Safety and Health. (1990) "Occupational exposure limit values." p13, AFS, Solna, Sweden.

Urbain, B.,Gustin,P., Prouvost, J-F. and Ansey, M.(1994) Quantitative assessment of aerial ammonia toxicity to the nasal mucosa by use of nasal lavage method in pigs. *American Journal of Veterinary Research*,55: 1335-1340.

Weaver, D. (1993) Effect of de-odorase (*Yucca schidigera* extract) on ammonia levels in a commercial dairy operation. *Journal of Dairy Science* 76 (Suppl. 1), 258.