THE EFFECTS OF AERIAL AMMONIA AND STREPTOCOCCAL ORGANISMS ON THE FEED INTAKE, IMMUNE FUNCTION AND PHYSIOLOGY OF THE PIG

Tim W Murphy, Colin F Cargill
Pig and Poultry Production Institute, South Australian Research and Development Institute, University of Adelaide, Roseworthy Campus, Australia

Introduction

Growth rates and feed :gain efficiency of pigs raised under commercial conditions in Australia are well below their genetic potential, and the values that could be achieved if the animals were housed under 'ideal' conditions (1). This difference has a significant impact on the potential profitability of a pig enterprise. There are many factors within a commercial piggery environment that can contribute to the depression in feed intake, growth rate and efficiency of feed use and act to increase the stress level of the pigs. The stressors can be divided broadly into three categories; social, climatic and hygiene. In most commercial environments several stressors are acting simultaneously. Hygiene and air quality in intensive animal housing is a major concern to producers, employees, housing and farming specialists, and veterinarians involved in the intensive livestock farming industries. In recent years a number of reports have highlighted the negative effects of sub-optimal air quality and hygiene on the health and production of animals, as well as the health of workers (3,7).

Material and Methods

A series of experiments were conducted to quanitify the effects of varying concentrations of aerial ammonia and viable *Streptococcal* organisms on the feed intake, immune function and physiology of 16 week old gilts.

The experiments were conducted in the Research Facility of the PPPI Roseworthy Piggery, University of Adelaide. The experimental room (24^{o}C, 55% RH), was cleaned and disinfected between each trial, and hosed three times daily during the trial. Pigs were removed from the room during cleaning and the pit was flushed every second day. Pigs with faecal matter on their skin were washed and dried and returned to their stall. Other stressors, such as stocking rate, background ammonia and bacterial levels were minimised.

Large White x Landrace respiratory disease-free gilts, housed individually on a partially slatted floor, were used in each experiment. Pigs were weighed daily and given access to water at all times. They were fed 2.5 kg of a commercial diet daily, divided into equal portions (morning and afternoon). Twenty pigs were used in each experiment with 5 pigs per treatment, replicated 4 times.

Ammonia gas in nitrogen was pumped into the feed bin at a rate of 12L/min for 20 minutes while pigs were eating, and each pig was observed to ensure maximum exposure. Concentrations of ammonia used were 0, 10, 25 and 50ppm. After feeding, pigs were dosed intranasally with a solution of *Viridans streptococcus* suspended in buffered saline at a concentration of $2x10^5$ cfu/ml. The organisms used were collected from the airspace of a shed housing growing pigs, using a 6-stage Andersen Sampler loaded with Columbia Horse Blood Agar plates.

Pigs were weighed daily and any uneaten food was collected and weighed.

Pigs were bled prior to pollutant exposure and again 14 days later, and lymphocyte proliferation, phagocytosis (as a measure of neutrophil function), and surface markers CD4, CD8, CD21 measured. Blood analysis included T cell proliferation assays - expressed as CCPM measuring the incorporation of 3HT into actively dividing cells in response to a mitogen and Phagocytosis assay - expressed as % granulocytes (eosinophils and neutrophils) with phagocytic potential. Pigs were slaughtered at the end of each experiment and lungs examined grossly for lesions.

Data were analysed by ANOVA (Statistix 7.1)

Results

No lesions were observed in lungs examined macroscopically after slaughter.

There was a significant reduction in the growth rate of pigs exposed to ammonia compared with untreated controls (0 ppm) and the reduction increased as levels increased from 10 to 50ppm (Table 1). Growth rate suppression was further increased when pigs were exposed to bacteria as well. Similar reductions were also recorded in feed efficiency (Table 1).

Table 1. Mean growth rate (ADG) and feed efficiency (FCR) pre- and post- exposure with NH_3 or NH_3 and bacteria ($2x10^5$ CFU/ml) (NH_3 + B).

NH_3	ADG	FCR	NH_3 + B	ADG	FCR
0	796 ± 25.8^{a}	3.12 ± 0.11^{f}	0	691 ± 24.5^{b}	3.61 ± 0.14^{f}
10	754 ± 27.4^{a,b}	3.41 ± 0.13^{f}	10	555 ± 20.8^{c}	4.24 ± 0.17^{f}
25	713 ± 27.2^{b}	4.01 ± 0.14^{f}	25	464 ± 23.6^{d}	5.76 ± 0.16^{g}
50	590 ± 40.3^{c}	5.85 ± 0.45^{g}	50	264 ± 32.8^{e}	10.97 ± 1.27^{h}

a→h.– ADG/FCR different superscripts significantly different (P<0.05)

Although lymphocyte proliferation (as measured by the stimulation index) was not consistently increased in pigs exposed to ammonia, a significant increase was recorded in pigs exposed to both ammonia and bacteria (Table 2). In the latter groups, the stimulation index increased as concentrations of ammonia increased.

Table 2. Mean lymphocyte proliferation (SI) pre- and post- exposure with ammonia (NH3) or ammonia and bacteria ($2x10^5$ CFU/ml) (NH3 + B).

NH3	Before (SI)	After (SI)	NH3 + B	Before (SI)	After (SI)
0	38.90 ± 4.66^{a}	46.40 ± 5.19^{a}	0	45.20 ± 2.71^{a}	65.50 ± 4.09^{b}
10	47.40 ± 5.31^{a}	56.3 ± 6.96^{a}	10	47.00 ± 3.48^{a}	92.1 ± 7.15^{c}
25	38.80 ± 5.34^{a}	46.60 ±6.23^{a}	25	50.60 ±3.03^{a}	152.10 ± 8.23^{d}
50	35.50 ± 4.97^{a}	47.4 ± 5.89^{a}	50	42.80 ± 2.99^{a}	178 ± 13.37^{e}

a→e – Rows with different superscripts significantly different (P<0.05)

While phagocytic activity (expressed as % granulocytes - eosinophils and neutrophils with phagocytic potential) in pigs exposed to ammonia tended to increase, a significant increase was recorded in pigs exposed to both ammonia and bacteria (Table 3). In the latter groups, the neutrophil phagocytic activity increased as concentrations of ammonia increased.

Table 3. Mean phagocytosis activity pre- and post-exposure to NH3 and NH3 and bacteria (NH3 + B).

NH3	Before	After	NH3 + B	Before	After
0	9.30 ± 1.03 [a]	11.25 ± 1.22 [a]	0	15.25 ± 1.30 [a]	28.25 ± 2.28 [c]
10	10.85 ± 0.81 [a]	13.10 ± 0.99 [a]	10	13.30 ± 0.80 [a]	33.20 ± 2.34 [d]
25	10.10 ± 1.07 [a]	14.65 ± 1.62 [a]	25	14.55 ± 0.94 [a]	51.00 ± 3.24 [e]
50	11.75 ± 1.04 [a]	17.63 ± 1.55 [b]	50	14.65 ± 0.96 [a]	66.50 ± 4.47 [f]

a→e – Rows with different superscripts significantly different (P<0.05)

Discussion

While neither overt clinical signs, nor macroscopic lesions suggestive of respiratory disease, were observed in pigs throughout the experiments, significant production effects and immune changes were recorded. This finding is consistent with previous reports where immune responses and growth rate suppression was demonstrated in disease free pigs exposed to poor air quality and hygiene (5).

While exposure to bacteria appeared to have a greater effect than ammonia on growth rate and feed efficiency, as well as aspects of immune function, the most significant effects were observed in pigs exposed to high levels of ammonia followed by bacteria. This agrees with previous studies (8) where pleurisy prevalence was higher in sheds with both high levels of ammonia and bacteria, compared with sheds with concentrations of ammonia below 5ppm and levels of bacteria above 1.5 $x10^5$. Hence, we hypothesise that ammonia damages the integrity of the mucosa allowing bacteria, or their toxins, better access to the animal's immune tissues.

The bacteria used in the study are part of the normal flora of the airspace in pig sheds and commonly referred to as faecal Streps. They are non-pathogenic but do contain chemicals such as alpha-glucans and peptydoglycans in their cell walls, both of which are known to be immunogenic. While the proportion of live and dead or decaying bacteria was not assessed, dead bacteria, which have released the cell wall toxins, could be expected to be more harmful than living organisms (2). In previous studies (8) the concentration of Streptococcal organisms in the airpsace of a pig shed was identified as a major risk factor for high pleurisy prevalence in growing pigs. Murphy (7) also reported a significant negative correlation between the concentration of bacteria in the airspace and growth rate and a similar correlation between stocking density (m^3/pig) and bacterial concentration. The immune response and production effects recorded after 14 days expsoure, without overt clinical signs, suggests an acute response. Whether the reaction would become muted over time was not studied. However pigs need not show clinical signs of disease to have reduction in performance (9). Pigs exposed to pathogenic organisms at a dose lower than that which causes overt clinical disease may develop immunity to the organisms but with a resulting depression in performance. In this study, pigs with high stimulation of the immune system ate 5.5% less feed and grew 17% more slowly than pigs with low immune stimulation.

Conclusion

There is strong evidence that many of the factors (social, climatic and hygiene) which reduce the performance of pigs raised in commercial environments act to increase the stress level of the pigs. This suggests that the removal of one stressor should have a positive effect on performance (4).

The results indicate a close relationship between growth rate and air quality, in particular aerial ammonia and *Streptococcus* sp. bacteria. Building hygiene has also been shown to be one of the most important factors associated with post-weaning enteric diseases in pigs (6).

Acknowledgements

The study was supported in part by Australian Pork Limited. Fresh whole blood was analysed at the CSIRO AAHL facility in Geelong, Victoria, and the LSA Biochemistry laboratory at the Roseworthy Campus of the University of Adelaide.

References

1. Black, J.L., Giles, L.R., Wynn, P.C., Knowles, A.G., Kerr, C.A., Jones, M.R., Strom, A.D., Gallagher, N.L., Eamens, G.J. (1997). A review – Factors limiting the performance of growing pigs in commercial environments. *In "Manipulating Pig Production VIII"*, pp. 9-36, ed. P.D. Cranwell. (Australian Pig Science Association: Adelaide).
2. Fogelmark, B., Sjostrand, M., Rylander, R. (1994). Pulmonary inflammation induced by repeated inhalations of beta(1,3)-D-glucan and endotoxin. *International Journal of Experimental Pathology*. **75**: 1-7.
3. Hartung, J. 1994. Environment and Animal Health. *In Livestock Housing* eds, Wathes, C.M. and Charles, D.R.
4. Hyun, Y., Ellis, M., Riskowski, G., Johnson, R.W. (1998). Growth performance of pig subjected to multiple concurrent stressors. *Journal of Animal Science*. **76**: 721-727.
5. Knowles, A.G., Seccombe, A.M., Giles, L.R., Lee, C., Bryden, W.L., Husband, A.J. (1997). Effect on environment and group size on immunological parameters in weaner pigs. *In "Manipulating Pig Production VI"*, pp. 310, ed. P.D. Cranwell. (Australian Pig Science Association:Canberra).
6. Madec, F., Leon, E. (1999). The role of management and husbandry in enteric disorders. *In "Manipulating Pig Production VII "*, pp. 200-209, ed. P.D. Cranwell. (Australasian Pig Science Association:Adelaide).
7. Murphy, T., Cargill, C., Carr, J. (2000). The effects of stocking density on air quality. *Proceedings of the 16th International Pig Veterinary Society Congress*, Melbourne, Australia, pp 326.
8. Skirrow, S., Cargill, C., Mercy, A., Nicholls, R., Banhazi, T., Masterman, N. (1995). Risk factors for pleurisy in pigs. *(DAW 28P) Report to Pig Research and Development Corporation*, Canberra. pp 3-45.
9. Williams, N.H. (1998). Impact of immune system activation on pig growth and amino acid needs. *In "Progress in Pig Science"*, pp. 583-592, eds. J. Wiseman, M.A. Varley, J.P. Chadwick. (Nottingham University Press: Nottingham, UK).