Acute and prolonged effects of ammonia on hematological variables, stress responses, performance, and behavior of nursery pigs

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Summary

Objectives: To determine acute and prolonged effects of 35 and 50 ppm concentrations of atmospheric ammonia (NH_3) on welfare of weaned pigs.

Materials and methods: Two experiments were conducted using gas exposure chambers to investigate prolonged effects (Experiment One; 19 days) and acute effects (Experiment Two; 96 hours) of NH_3. Each experiment included two studies: exposure to NH_3 at 0 and 35 ppm and at 0 and 50 ppm. In Experiment One, body weight, hematological and metabolic variables, and serum cortisol and haptoglobin were assessed, and behaviors were video-taped. In Experiment Two, serum cortisol and haptoglobin and plasma tumor necrosis factor-α were measured.

Results: Absolute counts of white blood cells, lymphocytes, and monocytes were greater in pigs exposed to 35 ppm NH_3 than in controls ($P < .05$). Serum haptoglobin was higher in pigs exposed to 50 ppm NH_3 for 7 and 19 days than in controls ($P < .05$). Serum cortisol concentrations were greater in pigs exposed to 35 or 50 ppm NH_3 for 19 days than in controls ($P < .05$). Less feeding behavior was observed in pigs exposed to 50 ppm NH_3 than in controls ($P < .05$). In acute studies, serum cortisol concentrations were greater in pigs exposed to NH_3 than in controls ($P < .05$).

Implications: Under the conditions of these studies, prolonged exposure to NH_3 is associated with increases in absolute monocyte, lymphocyte, and neutrophil counts and in serum cortisol and haptoglobin concentrations, but has no effect on pig growth performance.

Keywords: swine, ammonia, welfare, performance, stress response

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Resumen - Efectos prolongados y agudos del amonio en variables hematológicas, respuestas al estrés, desempeño, y conducta en cerdos en destete

Objetivos: Determinar los efectos prolongados y agudos de concentraciones de amonio atmosférico (NH_3) en concentraciones de 35 y 50 ppm en el bienestar de cerdos destetados.

Materiales y métodos: Se realizaron dos experimentos utilizando cámaras de exposición a gas para investigar los efectos prolongados (Experimento Uno; 19 días) y los efectos agudos (Experimento Dos; 96 horas) del NH_3. Cada experimento incluyó dos estudios: exposición al NH_3 a 0 y 35 ppm y a 0 y 50 ppm. En el Experimento Uno, se valoraron el peso corporal, las variables metabólicas y hematológicas, y el cortisol y la haptoglobina en el suero, y se videograbaron los comportamientos. En el Experimento Dos, se midieron el suero de cortisol, la haptoglobina, y el factor-α del plasma de la necrosis del tumor.

Resultados: Los conteos absolutos de las células blancas de la sangre, los linfocitos, y los monocitos fueron mayores en los cerdos expuestos a 35 ppm de NH_3 que en los controles ($P < .05$). La haptoglobina del suero fue mayor en cerdos expuestos a 50 ppm de NH_3 por 7 y 19 días que en los controles ($P < .05$). Las concentraciones de cortisol en suero fueron mayores en cerdos expuestos a 35 o 50 ppm de NH_3 por 19 días que en los controles ($P < .05$). Se observó un comportamiento de menor consumo de alimento en cerdos expuestos a 50 ppm de NH_3 que en los controles ($P < .05$). En estudios agudos, las concentraciones de cortisol en suero fueron mayores en cerdos expuestos de manera aguda al NH_3 que en los controles ($P < .05$).

Implicaciones: Bajo las condiciones de estos estudios, la exposición prolongada al NH_3 se relaciona con incrementos en los conteos absolutos de monocitos, linfocitos, y neutrófilos y en las concentraciones de cortisol y haptoglobina en suero, pero no tiene efecto en el desempeño de crecimiento el cerdo.

Résumé - Effets aigus et prolongés de l'ammoniaque sur des variables hématologiques, les réponses au stress, les performances, et le comportement des porcelets en pouponnières

Objectifs: Déterminer les effets aigus et prolongés de concentrations de 35 et 50 ppm d'ammoniaque atmosphérique (NH_3) sur le bien-être de porcs sevrés.

Matériels et méthodes: Deux expériences ont été effectuées en utilisant une chambre permettant l'exposition au gaz pour étudier les effets prolongés (Expérience 1; 19 jours) et les effets aigus (Expérience 2; 96 heures) du NH_3. Chaque expérience comportait deux études: exposition

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au NH_3 à des concentrations de 0 et 35 ppm, et exposition à des concentrations de 0 et 50 ppm. Au cours de l'Expérience 1, on a mesuré le poids corporel, des variables hématologiques et métaboliques, et les concentrations sériques de cortisol et d'haptoglobine, et enregistré les comportements sur bande vidéo. Lors de l'Expérience 2, on a mesuré le cortisol et l'haptoglobine sériques et le facteur-α nécrosant de tumeurs.

Résultats: Les nombres absolus de leucocytes, lymphocytes, et monocytes étaient plus élevés chez les porcs exposés à 35 ppm de NH_3 que chez les porcs témoins ($P < .05$). L'haptoglobine sérique était plus élevée chez les porcs exposés à 50 ppm de NH_3 pour 7 et 19 jours que chez les témoins ($P < .05$). Les concentrations de cortisol sérique étaient plus élevées chez les porcs exposés à 35 ou 50 ppm de NH_3 pendant 19 jours que chez les témoins ($P < .05$). Moins de comportements de prise de nourriture ont été observés chez les porcs exposés à 50 ppm de NH_3 que chez les témoins. ($P < .05$). Dans les études d'exposition aiguë, les concentrations de cortisol sérique étaient plus élevées chez les porcs exposés au NH_3 que chez les témoins ($P < .05$).

Implications: Dans les conditions expérimentales de ces études, une exposition prolongée au NH_3 est associée avec une augmentation absolue des comptes de monocyte, lymphocyte, et neutrophile et des concentrations sériques de cortisol et d'haptoglobine, mais aucun effet n'a été observé sur les perfor-

Despite the lack in understanding of acute and prolonged effects of ammonia (NH_3), it has been suggested that NH_3 exposure increases inflammatory, immune, and neuroendocrine stress responses in pigs.[1] The most common inflammatory pathway involves induction of cytokines (eg, IL-1, IL-4, IL-6, and tumor necrosis factor-α (TNF-α), which mediate and regulate immunity, inflammation, and hematopoiesis in response to tissue damage.[2] Cytokines are produced de novo in response to an immune stimulus. Atmospheric NH_3 is believed to cause release of cytokines by alveolar macrophages and neutrophils, constituting a potent inflammatory response.[3] The early phase of inflammation is characterized by acute phase protein responses. An increase in concentrations of acute phase proteins (eg, haptoglobin) generally occurs during infection, injury, and tissue destruction; thus, acute phase proteins are useful stress indicators.[4] High concentrations of haptoglobin and cytokines, and elevated counts of total white blood cells (WBC), macrophages, neutrophils, and lymphocytes, are generally viewed as indicators of inflammatory or immunological responses to stress.[1] Serum cortisol, as a measure of the hypothalamic-pituitary-adrenal axis, is widely used to describe the effect of a stressor on immune function.[5] Previous research has suggested that inflammatory stress correlates with suboptimal feed intake and growth.[6]

Current recommendations established by the US Occupational Safety and Health Administration (OSHA) on upper limits for NH_3 concentrations in swine confinement buildings are mainly intended to provide occupational exposure limits over an 8-hour period. The OSHA threshold for permissible worker 8-hour exposure is 50 ppm, and the short-term exposure limit (15 minutes) is 35 ppm.[7] While these two exposure standards relate to human health exposure, information on the effects of NH_3 on animal welfare is scarce, and no thresholds have been established in the United States to date.[8]

The objectives of this study were to determine the effects of acute and prolonged exposure to atmospheric NH_3 at concentrations of 35 and 50 ppm on welfare of recently weaned nursery pigs housed under controlled experimental conditions in environmental chambers. Welfare measurements included stress indices, hematological, metabolic, and endocrine variables, and growth performance and behavior.

Materials and methods

Animals, housing, and feeding

For each of four studies, male and female crossbred piglets (Yorkshire × Hampshire) weaned at 19.2 ± 1.1 days of age were distributed evenly by litter and gender into six pens (1.2 m × 1.2 m; four pigs per pen) in each of two chambers (24 pigs per study per chamber, 12 males and 12 females per chamber). Pigs were adapted to the housing conditions for 10 days after weaning, and exposure studies began when they were on average 29 days old.

The research was conducted at the Swine Research Teaching and Outreach Facility at the University of California, Davis, utilizing two identical environmental exposure chambers, each measuring 10.7 m long × 4.8 m wide × 3.1 m high (159 m^3). One chamber (treatment chamber) was supplied with NH_3 at concentrations of 35 and 50 ppm, and the other (control chamber) was supplied with fresh air (0 ppm NH_3). Each chamber ceiling had two inlet air ducts and one outlet air duct. Fresh outside air (37.4 m^3 per minute) was supplied through the inlet air ducts to each chamber and the same quantity of chamber air exited from the outlet air duct. Incoming air was unaltered except for heating or cooling. Room temperatures in each chamber were automatically maintained at 22°C ± 2°C. The slatted chamber floor was hosed clean with water once daily to remove excreta.

Each pen was equipped with a nipple waterer and a two-hole feeder that allowed access for two to three pigs to feed at any given time. In order to maintain ad libitum feed access, feeder reservoirs were re-filled once daily with a pelleted, corn-soy-based diet with 19% crude protein (as fed). Feed ingredients (on a dry matter basis) were corn (58.3%), soybean (26.5%), Akey Start 200 Base (Akey, Lewisburg, Ohio) (8%), fat (5%), mono-dical phosphate (1.2%), limestone meal (0.9%), salt (0.9%), and Tylan 40 (Elanco, Indianapolis, Indiana) (0.1%).

The University of California, Davis, Animal Care and Use Committee approved these studies.

Study design

Two experiments were conducted as completely randomized designs with pen as the experimental unit.[9,10] Each experiment included 48 pigs, with 24 pigs and six replications per chamber, and four pigs per pen. Experimental design followed common pathology and exposure studies in which the impact of two housing environments for swine differing in pathological loads were compared.[11,12]

In Experiment One, two 20-day studies were conducted, beginning 10 days post weaning (Day 0). Prolonged effects on welfare were evaluated in groups of pigs exposed to atmospheric NH_3 at 0 and 35 ppm in Study 1 and 0 and 50 ppm in Study 2. Blood samples and individual body weights (BW) were obtained Day -1 (pre ammonia exposure) and Days 7 and 19. Blood samples were obtained from all 24 pigs per chamber between 8:00 AM and 9:00 AM for cortisol, haptoglobin, and TNF-α assays. One pig per pen was randomly selected to be tested for hematology

measures (n = 6). Behavior was video-taped between 7 AM and 7 PM on Days 2 and 18, when blood samples were not collected, to ensure undisturbed behavior.

In Experiment Two, two 96-hour studies were conducted. Acute effects on welfare were evaluated in groups of pigs exposed to atmospheric NH_3 at 0 and 35 ppm in Study 3 and at 0 and 50 ppm in Study 4. Blood samples were collected from all 24 pigs per treatment group at 72 hours before and 2, 8, 12, 24, 48, and 96 hours after ammonia exposure began, and six samples (one pig per pen, as in Experiment One) were randomly selected for testing for cortisol, haptoglobin, and TNF-α assays (n = 6)

Ammonia gas exposure

Before the experiments started, capability of the chambers to produce uniform gas distribution was assessed. Therefore, gas mixing characteristics were determined and found satisfactory using sulfur hexafluoride (SF_6) tracer gas, which was released through the air inlet and measured in vertical and horizontal matrix planes. Chambers had forced ventilation at 3.8×10^4 L per minutes, resulting in a chamber residence time of approximately 6 minutes. The elevated NH_3 concentration in the treatment chamber was achieved by mixing pure anhydrous NH_3 gas (99.9% ammonia purity) with the fresh inlet air. The NH_3 gas cylinder was located outside the treatment chamber and connected to the incoming air duct using Teflon tubing. A regulator controlled the delivery pressure, and a mass flow controller was used to adjust and monitor the NH_3 flow rate. Swagelok fittings (Swagelok Company, Solon, Ohio) were used for all connections to prevent potential leaks of NH_3 in the gas delivery system. The pure NH_3 gas exited from the delivery tubing inside the inlet duct, where NH_3 mixed with fresh air.

To achieve 35 and 50 ppm NH_3 concentrations inside the treatment chamber, pure NH_3 gas flow rates were 0.7 and 1.0 L per minute, respectively. Concentration of NH_3 was monitored inside the animal pens at animal level using three instruments and methodologies. The first instrument, a Draeger Pac III NH_3 gas monitor (Draeger, Pittsburgh, Pennsylvania; 1 ppm accuracy), was used three times per day. The second instrument, a Pranalytica photoacoustic spectroscopy monitor (Pranalytica, Santa Monica, California), measured NH_3 concentrations continuously at animal level as described earlier by our laboratory.[13] The third instrument was a Dionex ICS90 ion chromatograph (Dionex, Sunnyvale, California) using an acid impinger sampling method.[13] For the latter method, air was sampled through Teflon tubing from the animal pens into sampling trains containing sulfuric acid. Atmospheric NH_3 was trapped in the acid and analyzed in the laboratory using ion chromatography. The acid impinger method was conducted to confirm the Draeger Pac III and Pranalytica sensor measurements (Experiment One, twice per week; Experiment Two, 10 hours after exposure began). The NH_3 flow rate was fine-tuned to keep the changes in NH_3 concentration within 5% of the required values.

Blood-sample collection and processing

Blood samples for both experiments were collected via puncture of the anterior vena cava using evacuated blood collection tubes and 20-gauge, 3.8-cm disposable needles. Pigs were individually removed from the chamber and restrained on a bleeding table, and blood was collected in < 1 minute per pig, minimizing the stress associated with the procedure.

Whole blood samples from tubes containing sodium fluoride (for determination of lactate and glucose) were mixed by inversion and centrifuged (2500*g* for 5 minutes) within 15 minutes of collection. The separated plasma samples were immediately transported (on dry ice) to the laboratory for same-day analysis.

Whole blood samples from tubes that contained EDTA were kept cold on ice at 4°C and separated into two subsamples per pig. The first subsample was transferred to the laboratory on ice at 4°C for hematological measurements. The second subsample was centrifuged (2500*g* for 5 minutes) within 30 minutes of collection, and plasma was subdivided into two portions. The first plasma portion was transported on dry ice directly to the laboratory for metabolite analysis (ie, blood urea nitrogen [BUN], plasma NH_3). The second plasma portion was stored at -70°C for analysis of TNF-α.

Whole blood samples for cortisol and haptoglobin determination were placed on ice for 2 hours before centrifugation (2500*g* at 4°C for 20 minutes) and the serum was stored at -70°C until analysis.

Hematology assays were conducted on the day the samples were collected. All frozen samples were thawed and assayed 2 days after the animal experiments were completed.

Hematology, clinical chemistry, and plasma NH_3 analyses

All metabolite and cell determination measures were conducted at the Preclinical Research Service, Idexx Laboratories (West Sacramento, California). Plasma BUN, glucose, lactate, and NH_3 concentrations were measured using an enzymatic method on an auto analyzer (Roche, Hitachi 717; Diamond Diagnostics, Holliston, Massachusetts). An automated cell counter (Coulter Gen-S Bayer, ADVIA 120 hematology analyzer; Diamond Diagnostics) was used for platelet count, red blood cell count, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), WBC count, and absolute counts of neutrophils, lymphocytes, monocytes, eosinophils, and basophils.

Assays for TNF-α, haptoglobin, and cortisol

Plasma levels of TNF-α were measured in duplicate using a single commercial kit (swine-specific biotinylated monoclonal antibody sandwich ELISA; Biosource International, Swine TNF-alpha; Camarillo, California) according to the supplier's instructions as described.[14] Serum haptoglobin concentration was measured using a single commercial kit (Phase range haptoglobin assay kit; Tridelta Development, Greystones, Ireland) as described by Petersen et al.[15] Serum cortisol concentration was measured in duplicate using a radioimmunoassay technique as previously described by Daley et al.[16] Interassay and intra-assay coefficients of variation for TNF-α, haptoglobin, and cortisol were < 8%.

Behavior (Experiment One)

Two identical video systems (one per chamber) were installed to allow for detailed analysis of behaviors. One HTC 65°C day-night color camera per chamber (CCD image sensor, 380 TV lines, 1 lux sensitivity; Inter-Pacific, Deerfield, Illinois) with wide angle lens (CE F1.4/1.6–3.4 mm; Inter-Pacific) was bracket-mounted to the ceiling to cover the entire six-pen area (2.44 m × 3.66 m). One time-lapse video

recorder (Samsung SLV-960A; Kyungki Do, Korea) per chamber was used to continuously record behavior in 24-hour time-lapse mode (2.78 mm tape per second, 12:1 compression). The four pigs in each pen were marked with an animal crayon marker (stripes, shoulder belts, spots, no marking) to allow for identification of individual pigs. Behavior data were analyzed on a per-pen basis using 10-minute scan sampling intervals for body positions and 5-minute scan sampling intervals for feeding behavior.[17] Measured behaviors were directly entered from the video recordings into a computer spreadsheet.[18] The list of measured behaviors (ethogram) included three categories: upright posture, defined as the pig assuming or maintaining an upright position on extended legs while standing still or moving; recumbency, the default behavior; and feeding behavior, measured and defined as the pig's head positioned in the feeder. Data were expressed for each behavior category as its percentage of total observation time. Such data are generally not normally distributed. Therefore, the arcsine transformation was applied to the square roots of percentage data to achieve normal distribution before further parametric statistical analysis.[18]

Performance (Experiment One)

Measures related to growth performance were BW (kg per pen) and ADG (kg per pen). Individual BW was measured using a portable electronic scale (accuracy ± 0.02 kg). Measurements of feed intake (as fed) were attempted by collecting feed refusals from the feeders and floor and subtracting them from feed provided to the pigs. As feed residuals partly fell through the slatted floor where they mixed with excreta, feed refusals were not measured and feed efficiency could not be calculated.

Statistical analyses

Behavior, BW, and blood analyses-related data were analyzed as a split-plot for repeated measures (for day in Experiment One or time in Experiment Two) using PROC MIXED in SAS (SAS Institute Inc, Cary, North Carolina). The model included treatment (tested with pen-within-chamber variance), effects of day in Experiment One or time in Experiment Two, and the interaction of treatment × day (or time) in the subplot. Average-daily-gain data were analyzed using PROC GLM. The model included treatment and pen-within-chamber as the error term.

Results

Experiment One: prolonged NH_3 exposure studies

Hematology, biochemistry, and NH_3 measurements. Absolute blood cell counts (Figure 1) and results for cortisol and haptoglobin assays (Figure 2) are expressed as least squares means. Prolonged exposure to atmospheric NH_3 affected absolute blood cell counts (Figure 1). On Day 19, WBC and absolute numbers of lymphocytes and monocytes in pigs exposed to 35 ppm NH_3 were approximately twice those in the control animals. Blood cell counts did not differ between groups exposed to 0 and 50 ppm NH_3. Hemoglobin, MCV, MCH, and MCHC were similar for control groups and those exposed to NH_3 (ranges 100 to 160 g per L, 50 to 68 fL, 17 to 23 pg, and 300 to 360 g per L, respectively). Concentrations of blood metabolites and plasma NH_3 were similar in controls and pigs exposed to NH_3 (BUN range, 3.3 to 3.6 mmol per L; glucose range, 4.9 to 5.3 mmol per L; lactate range, 5.29 to 6.84 mmol per L; NH_3 range 25.7 to 47.0 μg per dL).

Serum cortisol, haptoglobin, and TNF-α. Serum cortisol concentrations were greater on Day 19 ($P < .05$) in pigs exposed to either 35 or 50 ppm NH_3 than in control animals (Figure 2). Additionally, haptoglobin was higher on Days 7 and 19 ($P < .05$) in pigs exposed to 50 ppm NH_3 than in controls. Tumor necrosis factor-α was similar across treatments (range, 31 to 60 pg per mL).

Performance. Initial mean BW (Day 0) (± standard error of the mean [SEM]) were 9.95 ± 0.57 kg (Study 1) and 7.50 ± 0.32 kg (Study 2). On Day 19, BW of pigs exposed to 35 ppm NH_3 (19.4 ± 0.95 kg) and control pigs (19.1 ± 0.95 kg) did not differ ($P > .05$; Study 1), and BW of pigs exposed to 50 ppm NH_3 (12.4 ± 0.81 kg) and control pigs (12.9 ± 0.81 kg) did not differ ($P > .05$; Study 2). Accordingly, ADG did not differ between treatments in either study.

Behavior. Body posture, feeding, and aggressive behaviors on Day 2 were similar in pigs exposed to NH_3 and control pigs. However, on Day 18, time spent feeding was less (mean + SEM) in pigs exposed to 50 ppm NH_3 (11.27% ± 1.29% of time) than in control animals (12.27% ± 1.29% of time) ($P < .05$). Across both studies, the combined average percent feeding time for the control groups was 12.82% on Day 2 and 11.37% on Day 18. Finally, for all treatment groups, the range for upright posture (across both studies and on both observation days) was 30% to 42% of time, and the range for aggression was 0.4% to 2.2% of time.

Experiment Two: acute NH_3 exposure studies

Cortisol, haptoglobin, and TNF-α. Serum cortisol was greater in pigs exposed to 35 ppm NH_3 than in controls after 12 hours ($P < .05$), and tended to be greater after 24 hours ($P = .07$) and 48 hours ($P = .08$; Figure 3). Acute exposure to NH_3 did not affect serum haptoglobin or plasma TNF-α concentrations (Figure 3).

Discussion

The role of NH_3 in development of respiratory disease remains unclear, although it acts synergistically with other pollutants and may influence the incidence and severity of pathogen-induced respiratory diseases.[19] Ammonia is highly soluble in water and is presumably largely absorbed by the distal airway mucus. Ammonia can favor bacterial contamination of the lungs by decreasing pulmonary clearance and inducing airway mucosal inflammation.[20-22] Ammonia also can affect cellular necrosis of alveolar tissues and lead to respiratory stress and edema. Stress in general has effects on immune, endocrine, behavior, and performance measures.[23] Stress factors induce a series of natural defense reactions, which constitute homeostatic processes. The early phase of airway mucosal inflammation elicits an acute-phase response. Among the most prominent acute-phase responses is an increase in liver-synthesized serum proteins, ie, acute phase proteins, which are believed to play a vital role in the physiological stress response.[4] Haptoglobin, an acute phase protein, plays a vital role in the restoration of homeostasis after injury, tissue necrosis, and infection by scavenging heme released by damaged cells. Increased serum concentrations of haptoglobin are also indicative of inflammatory or infectious lesions.[24] Haptoglobin is generally regarded as being a sensitive, although non-specific, indicator of stress and is used to assess health in pigs.[25] Grellner et al[26] suggested that serum concentrations of acute phase protein in pigs are negatively correlated with BW, indicating that a prolonged activated cellular immune response is a detriment to growth. In our

Figure 1: White blood cell counts (least squares means) in nursery pigs exposed to atmospheric ammonia for 19 days at 0 versus 35 ppm (panels A through D) and at 0 versus 50 ppm atmospheric ammonia (panels E through H) (Experiment One; chronic exposure). Pigs weaned at 10 days of age were housed four per pen, with pen the experimental unit (six pens and 24 pigs per treatment). Exposure to ammonia began 10 days post weaning when the pigs were 29 days old (Day 0). Samples from one pig per pen were tested on Days -1, 7, and 19 (n = 6). Values within a panel with different letters differ ($P < .05$; ANOVA). Day 7 data for pigs on 50 ppm ammonia are missing because whole blood samples were unintentionally discarded.

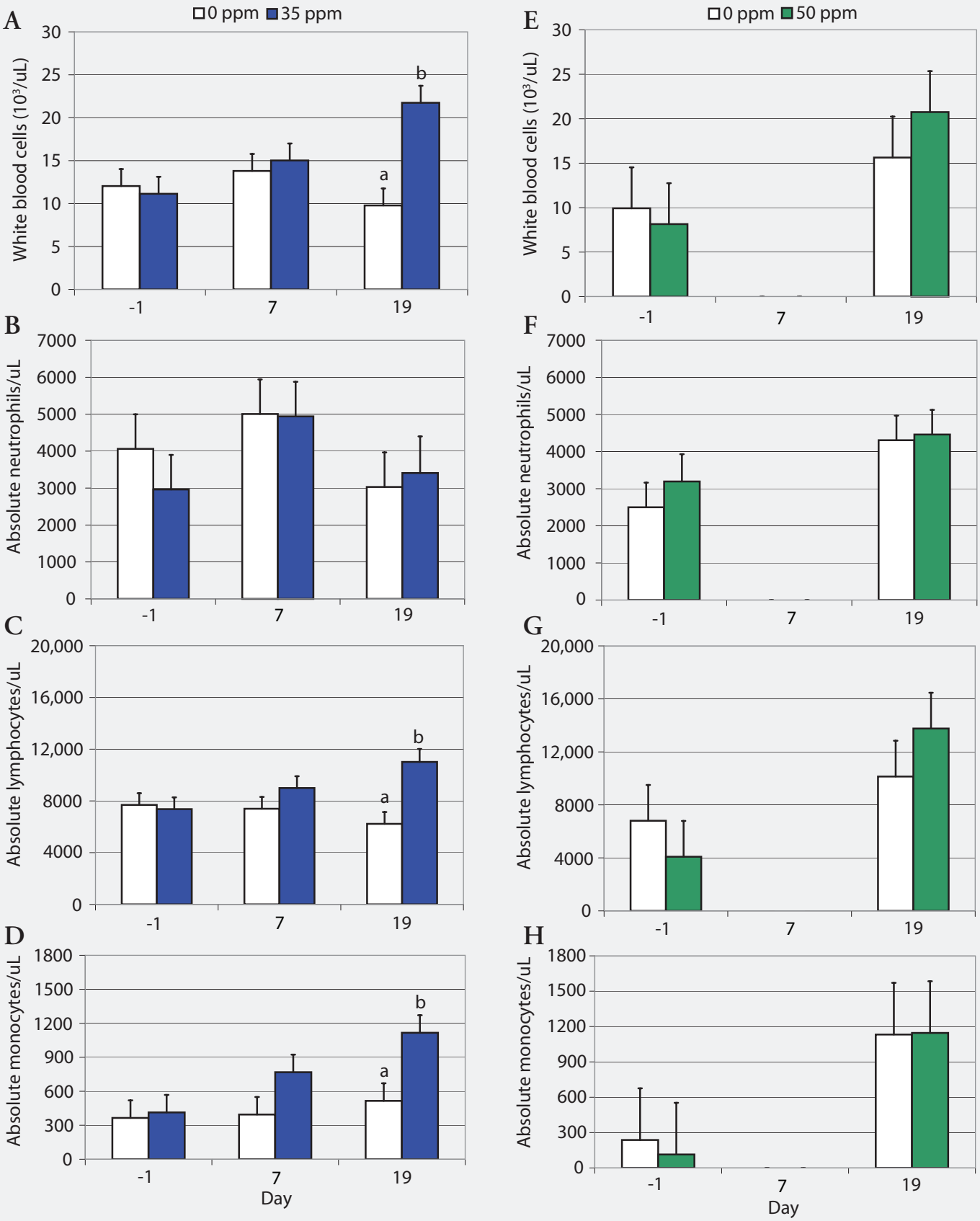
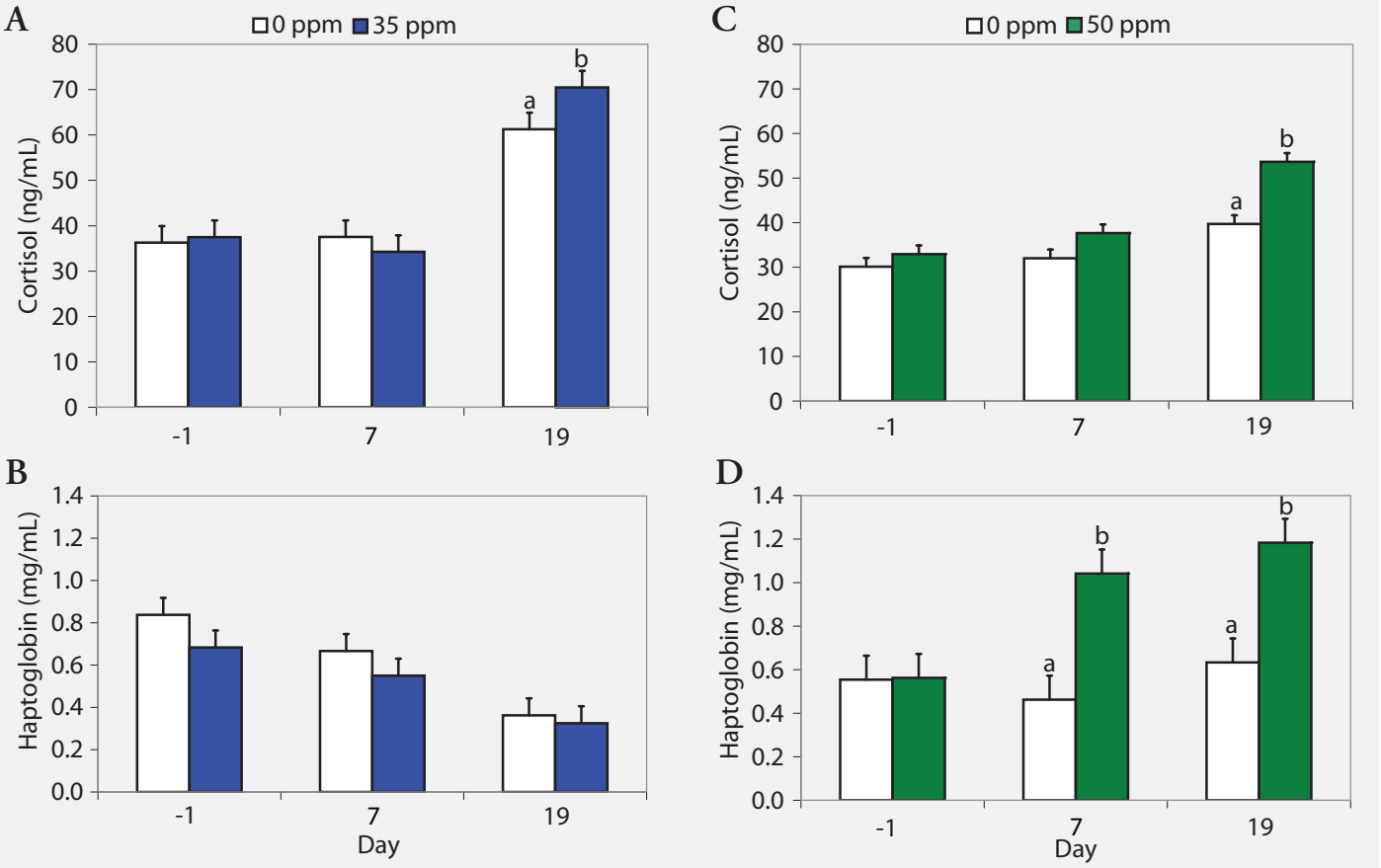


Figure 2: Serum cortisol and haptoglobin concentrations (least squares means) in nursery pigs (described in Figure 1) exposed to atmospheric ammonia at 0 versus 35 ppm (panels A and B) and 0 versus 50 ppm (panels C and D) at Days -1, 7, and 19 (Experiment One; n = 24). Values within a panel with different letters differ ($P < .05$; ANOVA).

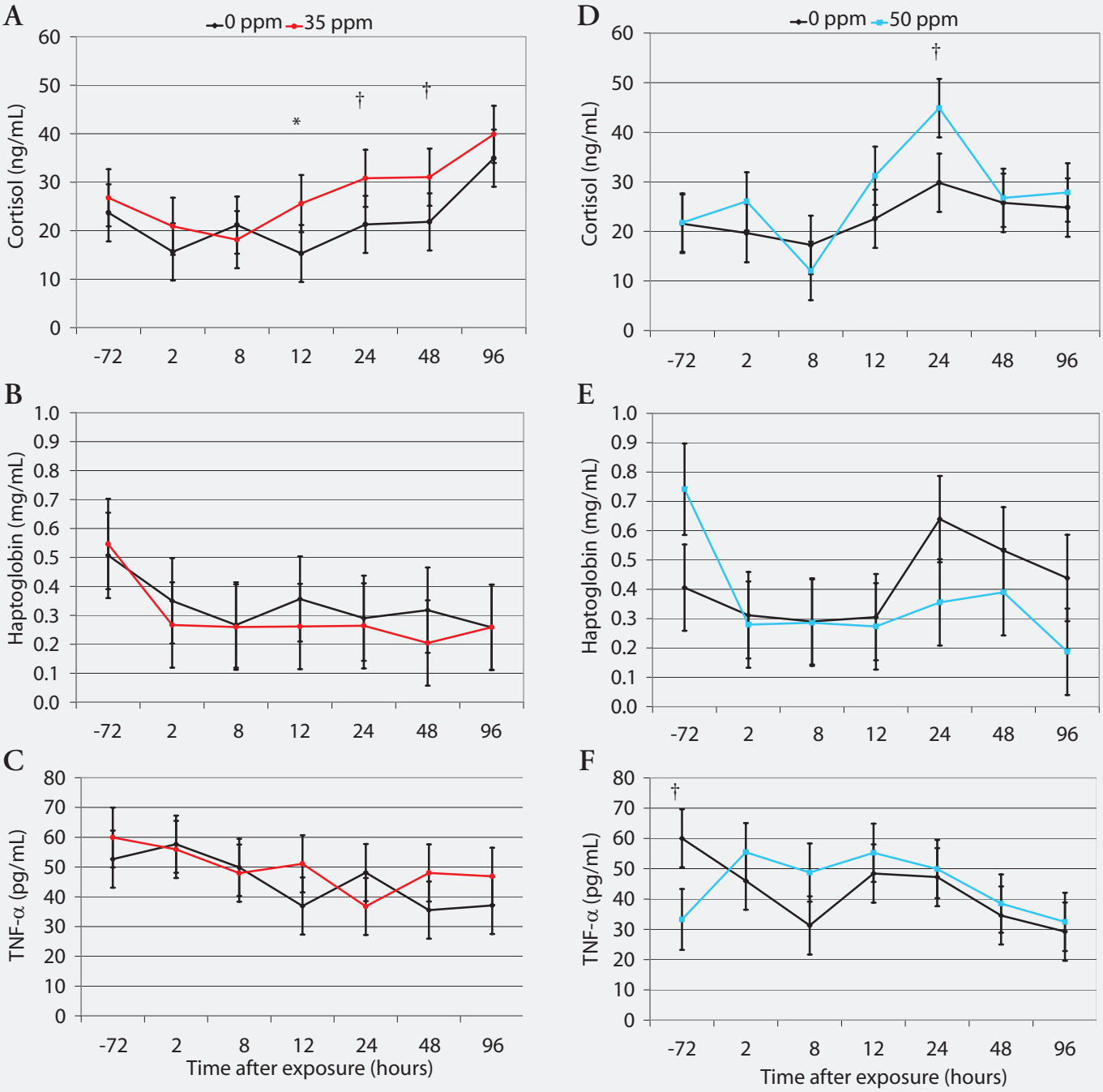


study, mean serum haptoglobin concentration of pigs exposed to 50 ppm of NH_3 was twice that of their peers in the control chamber. This high haptoglobin concentration on Days 7 and 19 might indicate that the pigs exposed to 50 ppm of NH_3 did not adapt to or recover from the gas stimulus, but invested significantly in the cleanup of cell debris. A continuing high haptoglobin concentration might indicate pulmonary edema or continuing alveolar necrosis; however, pigs exposed to 35 ppm did not show greater serum haptoglobin concentrations than the controls, which may indicate that these pigs detoxified after the initial insult. A combination of serum haptaglobin and serum cortisol concentrations may be a more reliable indicator of disease status or stress in pigs than either measurement alone.[3] Interestingly, our experiments showed a tendency for pigs exposed to NH_3 to have higher serum cortisol concentrations not only in the acute but also in the prolonged studies (Day 19). This is in contrast to the results of another study,[27] which showed no cortisol response to exposure to concentrations of 25 to 100 ppm of atmospheric NH_3 over a 6-day period.

Cytokines mediate a variety of local and systemic biological functions involved in the control of acute phase protein expression.[2] Ammonia causes the release of cytokines by alveolar macrophages and neutrophils, constituting an inflammatory response.[3] Correlations of haptoglobin and plasma TNF-α with prolonged stress were reported earlier.[28] Our studies did not show a response of the cytokine TNF-α to prolonged or acute exposure to NH_3, which may be explained by the large degree of variation in this parameter. In addition to cytokines and acute phase proteins, high total WBC count and absolute numbers of macrophages, neutrophils, and lymphocytes are considered indicators of immunological responses to respiratory stress.[1] In the present studies, pigs exposed to NH_3 at 35 ppm, compared to the controls, had much higher total WBC and absolute numbers of lymphocytes and monocytes, but numbers of neutrophils did not differ between treated and control groups. Absolute numbers of lymphocytes and monocytes were not consistently increased in groups exposed to 50 ppm in Study 2. We believe that the large degree of variance in these values masked what we expected to be significant differences (as exhibited in Study 1; exposure to 35 ppm).

The present experimental design followed those of common pathology and exposure studies[11,12] in which multiple subjects are tested per exposure room or building and the animal or pen, rather than the room, is considered the experimental unit. In these studies, pen was the experimental unit, and in Experiment One, we randomly selected one pig per pen for hematology testing, considering this randomly selected animal as representative of the pen of four animals. In addition, we considered the process of removing individual pigs from the chamber and collecting blood samples from each pig while separated from the group to be less stressful than collecting samples from the pigs among their peers.

Figure 3: Serum cortisol, haptoglobin, and tumor necrosis factor-α (TNF-α) in nursery pigs acutely exposed to ammonia gas at 0 ppm versus 35 ppm and at 0 ppm versus 50 ppm for 96 hours (least squares means; Experiment Two). Blood samples collected from six pigs per treatment (one per pen) at 72 hours before and 2, 8, 12, 24, 48, and 96 hours after ammonia exposure began were tested (n = 6). * Control and treatment values differ ($P < .05$; ANOVA); †Control and treatment values tend to differ ($P < .10$ ANOVA).



It should be noted that the treatment (ammonia concentration) was applied continuously in each ammonia treatment room in all four studies. In future studies, a larger sample size (ie, more pen replications) might be advantageous to increase statistical power, thereby addressing the issue of large variability, to determine whether similar effects occur in pigs exposed to NH_3 at 50 and 35 ppm.

Previous research in nursery pigs has suggested that pro-inflammatory cytokines correlate with low feed intake and growth.[6] Additionally, Drummond et al[20] compared effects of 50, 100, and 150 ppm NH_3 versus the control (0 ppm) on performance and reported ADG was lower by 12%, 30%, and 29%, respectively, compared to the controls. Our studies comparing pigs exposed to NH_3 at 0 and 35 ppm and at 0 and 50 ppm did not detect effects on performance, other than a trend toward low dry matter intake at 50 ppm NH_3 exposure. These results agree with those of others,[8] who found no effects of chronic NH_3 exposure (up to 37 ppm NH_3) on productivity of weaned pigs > 5.5 weeks of age.

Animal behavior is regarded as a sensitive indicator of what an animal prefers or

dislikes. Morrison et al[29] concluded that NH_3 concentrations in commercial buildings are not sufficient to induce aversion to NH_3. Although the experiment at hand does not address ammonia aversion and preferences, more recent preference tests[30,31] indicate that weanling pigs did prefer to avoid an area where NH_3 was ≥ 20 ppm, but this avoidance was delayed and explained by the possible development of a general sense of malaise. Even operant responses of pigs to high concentrations of NH_3 (up to 100 ppm) revealed a relatively weak aversion to polluted air exposure while they were rooting for food.[32] Although the concept of malaise in the context of motivational studies was not the question of concern in our work, one might expect that subclinically diseased pigs would decrease their feeding behavior (frequency and duration of feeding bouts) at NH_3 concentrations that were previously reported to affect the behavior of pigs. Our results, however, supported that hypothesis only at 50 ppm and not at 35 ppm NH_3 exposure. Pigs and other species typically reduce their overall activity during periods of inflammation, which is referred to as sickness behavior.[33] The lack of a difference in upright body postures between pigs exposed to 35 or 50 ppm and untreated pigs in Experiment One does not support the interpretation that pigs exposed to these NH_3 concentrations experienced a state of sickness.

Current recommendations on upper NH_3 limits are mainly intended to provide occupational exposure limits, as the scientific evidence that NH_3 exposure affects animal health and performance is scarce.[8,34] Synergistic effects of dust and NH_3 on swine health[35,36] and on occupational health of farm workers [35,37] have been reported and need to be considered accordingly. Recent studies failed to find an effect of a 5-week chronic exposure to NH_3 (≤ 37 ppm) on respiratory disease in weaned pigs.[36,37] Even after exposure to combinations of dust and NH_3, gross pathology was minimal and widespread minor pathological changes were of little significance.[36] Most existing guidelines and recommendations for animal houses set limits ranging from 20 to 50 ppm of NH_3. Our studies indicate that pigs respond to NH_3 with systemic stress responses; however, even 50 ppm does not affect animal growth performance over a 20-day period. Future studies should focus on the effects of NH_3 on lung histopathology to determine the kind of damage occuring due to NH_3 exposure that elicits the animal's physiological stress response.

Implications

- Under the conditions of this study, prolonged exposure of weaned pigs to atmospheric NH_3 elicits increases in WBC, absolute numbers of lymphocytes and monocytes, and serum cortisol and haptoglobin.
- Under the conditions of this study, exposure to NH_3 at concentrations of up to 50 ppm does not affect weight gain of pigs.
- Reduced feeding behavior at exposure to 50 ppm NH_3 implies that prolonged exposure (in combination with other factors) should be studied on a larger number of pigs.

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Conversion tables

Weights and measures converstions

Common (US)	Metric	To convert	Multiply by
1 oz	28.35 g	oz to g	28
1 lb (16 oz)	453.59 g	lb to kg	0.45
2.2 lb	1 kg	kg to lb	2.2
1 in	2.54 cm	in to cm	2.54
0.39 in	1 cm	cm to in	0.39
1 ft (12 in)	0.31 m	ft to m	0.3
3.28 ft	1 m	m to ft	3.28
1 mi	1.6 km	mi to km	1.6
0.62 mi	1 km	km to mi	0.6
1 sq in	6.5 cm^2	sq in to cm^2	6.5
0.15 sq in	1 cm^2	cm^2 to sq in	0.15
1 sq ft	0.09 m^2	sq ft to m^2	0.09
11.11 sq ft	1 m^2	m^2 to sq ft	11
1 cu ft	0.03 m^3	cu ft to m^3	0.03
35.32 cu ft	1 m^3	m^3 to cu ft	35
1 c (cup)	0.24 L	c to L	0.24
4.1667 c	1 L	L to c	4.2
1 gal (128 fl oz)	3.8 L	gal to L	3.8
0.264 gal	1 L	L to gal	0.26
1 qt (32 fl oz)	946.36 mL	qt to L	0.95
33.8138 oz	1 L	L to qt	1.1

Temperature equivalents

°F = (°C × 9/5) + 32

°C = (°F - 32) × 5/9

°C	°F
0	32
10	50
15.5	60
16	61
18.3	65
21.1	70
23.8	75
26.6	80
28	82
29.4	85
32.2	90
38.8	102
39.4	103
40.0	104
40.5	105
41.1	106
100	212

Conversion chart, kg to lb

Pig size	Kg	Lb
Birth	1.5 – 2.0	3.3 – 4.4
Weaning	3.5	7.7
	5	11
	10	22
Nursery	15	33
	20	44
	25	55
	30	66
Grower	45	99
	50	110
	60	132
Finisher	90	198
	100	220
	105	231
	110	242
	115	253
Sow	135	300
	300	661
Boar	360	800

1 tonne = 1000 kg

1 ppm = 0.0001% = 1 mg/kg = 1 g/tonne

1ppm = 1 mg/L