RESEARCH REPORT
ANIMAL WELFARE

Title: Acute and chronic effects of ammonia on inflammation, immunology, endocrine function, performance, and behavior of nursery pigs - **NPB #03-159**

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Abstract: The objective was to determine acute or chronic effects of moderate (35 ppm) and high (50 ppm) concentrations of atmospheric ammonia (NH_3) on welfare of newly weaned pigs. Welfare measures included, inflammatory, immunological, hematological, metabolic, and stress parameters, as well as performance, and behavior. Two experiments were conducted using eight groups of 24 nursery pigs (per group) in environmental gas exposure chambers. Exp. 1 was an investigation in the chronic effects (20 d) and Exp. 2 in the acute effects (96 hrs post exposure) of atmospheric ammonia on pig welfare. Both, Exp. 1 and Exp. 2 were divided into two studies (48 pigs per study): the first study compared ammonia exposure of 50 vs. 0 ppm (control) and the second study 35 vs. 0 ppm. In Exp. 1 blood samples were obtained at d -1, 8, and 20 to be analyzed for hematological (blood cell differentials) and metabolic parameters (BUN, glucose, lactate, ammonia), cortisol, and haptoglobin. Pigs in Exp. 2 were bled to measure acute concentrations of cortisol, haptoglobin, as well as the pro-inflammatory cytokines TNF-α and IL-4. Blood samples were drawn before exposure at -72 h and again at 2, 4, 8, 12, 24 and 96 h after exposure to ammonia. Performance parameters (BW, DMI, ADG, and F:G) were measured and calculated in Exp. 1 for d –1, 8 and 20. Behaviors (body posture, feeding, and aggression) were video filmed and analyzed on d 3 and 19 of Exp. 1.

Exposure to ammonia did affect hematological and immunological parameters. Total white blood cells concentration, lymphocytes, and monocytes were approximately doubled ($P < 0.05$) in pigs exposed to 35 ppm vs. control animals. Other hematological (hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration) and metabolic (BUN blood ammonia, glucose, and lactate) parameters were similar between pigs that were exposed to ammonia vs. control animals. The acute phase protein haptoglobin was twice as high in 50 ppm ammonia exposed pigs compared to the control animals at d 8 and 20 (study 1) but not different between 35 ppm vs. control pigs (study 2). Pigs that were chronically exposed to ammonia (both 50 and 35 ppm, in study 1 and 2, respectively) showed increased ($P < 0.05$) cortisol concentrations on d 20 compared to control animals. Nursery pigs responded to acute ammonia exposure (with both 50 and 35 ppm) with an increase ($P < 0.05$) in serum cortisol. Ammonia exposed vs. control pigs were similar with respect to most of the performance variables. However, pigs exposed to 50 ppm of ammonia vs. the control tended to decrease DMI ($P = 0.096$) and feeding behavior ($P = 0.059$). In summary, exposure to 35 and 50 ppm atmospheric ammonia has affected systemic responses and increased cytological and biochemical markers of injury and inflammation like haptoglobin, total white blood cells, lymphocytes, and monocytes. Atmospheric ammonia also increased cortisol concentrations and tended to decrease feeding behavior resulting in a trend for lower dry matter intake.

Introduction: Despite the lack in understanding of acute and chronic effects of ammonia, it has been suggested that ammonia exposure increases inflammatory, immune, and neuroendorine stress responses in pigs (Asmar et al., 2001). The most common inflammatory pathway involves the induction of cytokines (e.g., IL-1, IL-4, IL-6, TNF-α) which mediate and regulate immunity, inflammation, and hematopoiesis in response to tissue damage (Kataranovski et al., 1999). Cytokines are produced *de novo* in response to an immune stimulus. Atmospheric ammonia is believed to cause the release of cytokines by alveolar macrophages and neutrophils, constituting a potent inflammatory response (Murata and Horino, 1999). The early phase of inflammation is characterized by acute phase protein (APP) responses. An increase of acute phase proteins like haptoglobin generally occurs during infection, injury, and tissue destruction, which make them a potent stress indicator (Weissman, 1990). Besides haptoglobin and cytokines, total white blood cell count, macrophages, neutrophils, and lymphocytes, are

generally viewed as indicators of inflammatory or immunological responses to stress (Asmar et al., 2001). Cortisol as a measure of the hypothalamic-pituitary-adrenal axis is widely used to describe the effect of a stressor on immune function (Tuchscherer et al., 1998). Spurlock (1997) suggested that inflammatory stress correlates with reduced feed intake and growth.

Current recommendations on upper ammonia limits in swine confinement buildings are mainly intended to provide occupational exposure limits (e.g., OSHA threshold of 50 ppm) for animal facilities because scientific evidence that ammonia exposure affects animal welfare and performance is scarce (Wathes et al., 2003).

Objective: To determine the effects of acute and chronic exposure of atmospheric ammonia (at concentrations of 50 and 35 ppm) on stress indicators like immunological and inflammatory agents, hematological, metabolic and endocrine parameters as well as growth performance and behavior of recently weaned nursery pigs.

Materials and Methods: ***Animals and housing***

Two experiments were conducted at the Swine Research Teaching and Outreach Facility at the University of California, Davis. Exp. 1 was conducted over the course of two studies of 20 d each (using 48 pigs/ study, 24 pigs/chamber) to evaluate the chronic effects of 50 vs. 0 ppm (study 1) and 35 vs. 0 ppm (study 2) atmospheric ammonia on welfare of weanling pigs. Exp. 2 was an investigation into acute effects of 50 vs. 0 ppm (study 3) and 35 vs. 0 ppm (study 4) of atmospheric ammonia for 96 h of exposure time.

The UC Davis air quality research facility is equipped with two new and identical environmental exposure chambers, each measuring H 10.7 m x W 4.8 m x H 3.1 m (159 m^3). One chamber (treatment chamber) had elevated ammonia (ammonia) concentrations (50 or 35 ppm, respectively) and the other (control chamber) was supplied with fresh air (0 ppm). The chamber ceiling has two inlet air ducts and one outlet air duct. Fresh air (396 m^3 per min) was supplied through the inlets air ducts to each room and the same amount of room air exited from the outlet air duct. Room temperatures were automatically kept at 22 °C.

For each of the four studies (two studies per experiment), male and female crossbred (Yorkshire/Hampshire) piglets (19.17 ± 1.08 d of age) were evenly distributed to six pens (1.2 m x 1.2 m, 4 pigs/pen) in each of two chambers (24 pigs per study per

chamber, 12 males and 12 females per chamber). Each pen was equipped with a six-hole feeder and a nipple waterer, both to which pigs had *ad libitum* access. Pigs were adapted to the housing conditions for 10 d before treatment was initiated. The University of California, Davis, Animal Care and Use Committee approved the study.

Ammonia gas exposure

The elevated ammonia concentration in the treatment chamber was achieved by mixing pure anhydrous ammonia gas (99.9%) with the fresh inlet air. The ammonia 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 flow-meter was used to adjust and monitor the ammonia flow rate. Swagelok fittings were used for all connections to prevent potential leaks of ammonia in the gas delivery system. The pure ammonia gas exited from the delivery tubing inside the inlet duct where the gas mixed with fresh air. To achieve 35 and 50 ppm ammonia concentrations inside the treatment chamber, pure ammonia gas flow rates were 0.7 and 1.0 L/min, respectively. The ammonia concentration was monitored using two instruments: (1) Draeger Pac III ammonia gas monitor (1 ppm accuracy; Draeger, Pittsburgh, PA), which was used three times/d; and (2) Dionnex (Dionex ICS90, Dionex Corp., Sunnyvale, CA) ion chromatograph (EPA reference method, EPA 40 CFR). For the EPA method, air from inside the chamber was sampled through a sampling train, which contained sulfuric acid. The atmospheric ammonia was trapped in the acid and analyzed in the laboratory using ion chromatography. The EPA reference method was conducted twice/wk to confirm the Draeger Pac III measurements. According to each measurement of ammonia concentration in the treatment chamber, the pure ammonia flow rate was fine adjusted to keep the changes in ammonia concentration at less than 5% of the required values. The gas delivery system as well as the environmental chambers performed flawlessly throughout the entire experiment.

Feeding

Pigs were fed *ad libitum* and the diet was a pelleted, corn-soy based ration (Table 1) with 19% crude protein.

Blood sampling

In Exp. 1, bleeding dates for the hematological, metabolic, immunological/ inflammatory, and endocrine assays were on d -1, 8, and 20. Six randomly chosen pigs (n = 6) were bled per chamber (one per pen) for hematological parameters and all 24

pigs for determination of cortisol and haptoglobin. In Exp. 2, six randomly chosen pigs were bled (one per pen; n = 6) before and after exposure to ammonia at –72, 2, 4, 8, 12, 24, 96 h. In both experiments, blood samples were obtained in the morning before feeding (between 0800 and 0900 h) via puncture of the anterior vena cava using BD vacutainers and 20-gauge 1 ½" disposable needles. Pigs were individually taken out of the chamber, fixated on a bleeding table, and bled in less than one min (per pig). NaFl whole blood samples (for determination of lactate and glucose) were mixed by inversion and centrifuged (2500xg for 5 min) within 15 min, frozen and transported on dry ice to the laboratory. EDTA whole blood samples were kept cold on ice at 4 °C and separated in three sub-samples. The first whole blood sub-sample was transferred to the laboratory on ice at 4 °C for the analysis of hematological parameters. The second whole blood sub-sample was centrifuged (2500xg 5 min) for 30 min, stored and transported on dry ice to be sent directly to the laboratory for metabolite (BUN, plasma ammonia, lactate, and glucose) analysis. The third whole blood sub-sample was kept cold and centrifuged (2000xg at 4 °C) for 20 min and plasma samples were stored at –70 °C for analysis of plasma TNF α. Whole blood samples for cortisol, IL-4 and haptoglobin determination were obtained and placed on ice for 2 h before centrifugation (2500 x g at 4 °C for 20 min) and stored at -70 °C until further analysis.

Blood Analysis

a. Blood metabolites and blood cell determination

Plasma BUN, glucose, lactate and ammonia concentrations were measured using an enzymatic method on an auto analyzer (Roche, Hitachi 717, Diamond Diagnostics, Holliston, MA). Blood cells were analyzed using an automated cell counter (Coulter Gen-S, Bayer, ADVIA 120 hematology analyzer, Diamond Diagnostics, Holliston, MA) for platelet count, white blood cell (WBC) count, red blood cell (RBC) count, hemoglobin, hematocrit, mean corpuscular volume (MCV, i.e. measurement of the average size of RBC), mean corpuscular hemoglobin (MCH, i.e. average weight of hemoglobin within RBC), mean corpuscular hemoglobin concentration (MCHC, i.e. concentration of hemoglobin in RBC), neutrophils, lymphocytes, monocytes, eosinophils, and basophils. All metabolite and cell determination measures were conducted at the Preclinical Research Service, IDEXX Laboratories (Sacramento, CA).

b. Cytokine determination

Total EDTA plasma TNF-alpha and serum IL-4 concentrations were measured with swine specific ELISA kits (Biosource International, Swine TNF-alpha, Swine IL-4, Camarillo, CA), using a biotinylated antibody sandwich system.

c. Haptoglobin determination

Serum haptoglobin concentration was measured using a commercial assay kit (Phase range haptoglobin assay kit; Tridelta Development, Greystones, Ireland).

d. Cortisol determination

Serum cortisol concentration was measured using radioimmunoassay technique as described by Daley et al. (1999).

Performance

Individual BW was measured by placing pigs into a bucket on a portable electronic scale that was placed next to their pens. In Exp.1, initial BW was 7.5 $\pm$ 0.32 kg (study 1), and 9.95 $\pm$ 0.57 kg (study 2). In Exp. 2, the initial BW was 6.83 $\pm$ 1.6kg (study 3) and 7.32 $\pm$1.10 kg (study 4) at –72 h. Feed intake was estimated by subtracting feed refusals from the feeders and the floor from feed provided to the pigs. Measures related to growth performance were ADG (kg/d/pen), and DMI (kg/d/pen).

Behavior

A video system was installed to allow for detailed analysis of behaviors during Exp. 1. One HTC-65C day/night color camera (CCD image sensor 1/3 “, 380 TV lines, 1 lux sensitivity; Inter-Pacific, Deerfield, IL) with wide angle lens (CE F1.4/1.6-3.4 mm; Inter-Pacific, Deerfield, IL) was bracket-mounted to the ceiling to cover the entire six pen area (2.44 x 3.66 m). Two time lapse video recorders (Samsung SLV-960A) were used to continuously record the behavior in 12-h time lapse mode. Video recorders were connected to video monitors (Sony SSM - 121) to allow surveillance of the pigs from the outside of the chambers. The video system was identical in both chambers. Four pigs in each pen were marked individually with an animal crayon marker (stripes, shoulder belts, spots, no marking) to allow for identification of individual pigs.

Behavioral data were obtained prior to the bleeding days to ensure undisturbed behaviors, namely on days 3 and 19.

Behavior data (as defined in Table 2) were analyzed using 10 min scan sampling intervals for body positions and aggression behavior and 5 min scan sampling intervals for feeding behavior (Martin and Bateson, 1993). Measured behaviors were directly entered from the video recordings into a computer spreadsheet at 10-min and 5-min

scan intervals (Mitloehner et al., 2001). Data were expressed as a percentage of time of total observations and square root-arcsine transformed (to achieve normal distribution) before further statistical analysis.

Experimental Design and Statistical Analyses

The design for the experiments was a Completely Randomized Design with pen (housing four piglets) as experimental unit and six replications (n = 6) per chamber. All blood analyses related data were analyzed as a split plot for repeated measures (either for day in Exp.1, or time in Exp. 2, respectively). The individual pig was treated as a random effect; therefore, PROC MIXED in SAS (SAS, Inst. Inc., Cary, NC.) was used for analysis. The model included treatment (tested with pen within chamber variance) and effects of day in Exp. 1 (or time in Exp. 2, respectively) as well as the interaction of treatment x day (or time) in the subplot. Performance and behavior data were analyzed using PROC GLM with treatment in the model and pen within chamber as the error term.

Results: *Immunological and hematological measurements*

Differential blood cell counts and blood metabolites for the chronic exposure experiment (Exp. 1) are summarized in Figure 1 and Tables 3 and 4. Chronic exposure to atmospheric ammonia did affect differential blood cell counts (Figure 1). Total white blood cells, lymphocytes, and monocytes were approximately doubled ($P < 0.05$) in pigs exposed to 35 ppm ammonia vs. control animals but no differences were found between 50 vs. 0 ppm pigs. In neither of the chronic exposure studies did hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), or mean corpuscular hemoglobin concentrations (MCHC) differ between ammonia exposed vs. control pigs (Tables 3 and 4). Pigs that were chronically exposed to ammonia (at both 50 and 35 ppm) vs. the control showed similar concentrations of blood metabolites, namely ammonia, BUN, glucose, and lactate (Tables 3 and 4).

Cortisol, acute phase protein, and cytokines

Pigs that were chronically exposed to either 50 or 35 ppm ammonia (in study 1 and 2 of Exp. 1, respectively) showed increased ($P < 0.05$) serum cortisol concentrations on d 20 compared to control animals (Figure 2). Additionally, chronic exposure of pigs to 50 ppm ammonia vs. control increased ($P < 0.05$) haptoglobin on d 8 and d 20.

In the acute exposure experiment (Exp. 2; Figure 3), pigs exposed to 50 ppm ammonia vs. the control increased serum cortisol at 12 h ($P < 0.05$) after gas exposure. Similarly, pigs exposed to 35 ppm ammonia vs. the control showed serum cortisol increases after 12 h ($P < 0.05$), 24 h ($P = 0.068$), and 48 h ($P = 0.077$). Acute exposure to ammonia did not affect haptoglobin or TNFα in either of the two studies. IL-4 concentrations were below the detection threshold concentration of the assay.

Performance

Performance results of the chronic ammonia exposure experiment (Exp. 1) are presented in Tables 5 and 6. BW between ammonia exposed vs. control pigs were similar between treatments in study 1 (50 vs. 0 ppm) and study 2 (35 vs. 0 ppm). Accordingly, the ADG did not differ between treatments in both studies of Exp. 1. However, Pigs exposed to 50 ppm ammonia (study 1) tended ($P = 0.096$) to show decreased DMI vs. control pigs.

Behavior

Body posture, feeding and aggressive behaviors on day 19 are presented in Tables 7 and 8. Feeding behavior tended to decrease ($P = 0.059$) in pigs exposed to 35 ppm ammonia (in both studies of Exp. 1; $P < 0.1$) vs. control animals. Aggression was observed in less than 1 % of the period between 0800 and 2000 h and was not different between the treatment groups.

Discussion: The individual role of ammonia in the development of respiratory disease remains unclear, though it acts synergistically with other pollutants and may influence the incidence and severity of biological agent-induced respiratory diseases. Ammonia is highly soluble in water and is supposed to be largely absorbed by the distal airway mucus. Ammonia can favor bacterial contamination of the lungs by reducing pulmonary clearance and by inducing airway mucosal inflammation (Drummond et al., 1980, 1981a, b). Ammonia can also affect cellular necrosis of alveolar tissues and lead to respiratory stress and edema. Stress has effects on and immune, endocrine, behavior, and performance measures (Hicks et al., 1998). Stress factors involve a series of natural defense reactions, which constitute homeostatic processes, namely inflammation. The early phase of inflammation elicits an acute phase response, which mobilizes the metabolic response of the whole organism. The most prominent acute phase response involves an increase in liver-synthesized serum proteins, called acute phase proteins (APP), which are believed to play a vital role in the physiological stress

response (Weissman, 1990). One of the most important acute phase proteins, haptoglobin, plays a vital role in the restoration of homeostasis after injury, tissue necrosis, or infection. Haptoglobin belongs to the major positive acute phase proteins in pigs, indicating inflammatory or infectious lesions by increasing serum concentrations (Lampreave et al., 1994). Haptoglobin is generally regarded as being a sensitive, though non-specific, indicator of inflammation and to assess health in pigs (Eckersall et al., 1996; Lipperheide et al. 2003). Grellner et al. (2002) suggested that serum acute phase protein concentrations in pigs are negatively correlated with body weight, suggesting that a prolonged activated cellular immune response is a detriment to growth. Our study showed that haptoglobin concentrations of pigs exposed to 50 ppm of ammonia were twice that of their peers in the control chamber. This high haptoglobin concentration on d 8 and 20 could indicate that the pigs exposed to 50 ppm of ammonia do not adapt or recover from the gas stimulus but invest significantly in the cleanup of cell debris. This continuing high haptoglobin concentration could be indicative for edema or for continuing alveolar necrosis to occur. However, pigs exposed to 35 ppm did not show increased haptoglobin concentrations, which might indicate that the pigs detoxified after the initial insult. Serum haptoglobulin may be a valuable indicator of stress in swine herds and a combination of serum haptaglobin and serum cortisol concentrations may be a more reliable indicator of disease status or stress in pigs than either parameter alone (Murato and Horino, 1999; Lipperheide et al., 2002). Interestingly, present experiments showed consistent increases of cortisol to ammonia exposed pigs not just in the acute but also in the chronic studies (d 20). This type of generalzed stress response is in contrast to the studies of Gustin et al. (1994) who did not find a generalized stress response (cortisol) at any concentration (25-100 ppm) of atmospheric ammonia exposure over 6 days. Our study is the first that found these relationships.

The most common inflammatory pathway involves the induction of cytokines (e.g. TNF-α, IL-1, IL-4, IL-6), which are regulatory proteins secreted by cells in response to tissue damage. They can mediate a variety of local and systemic biological functions involved in the control of acute phase protein expression (Kataranovski et al., 1999). Ammonia causes the release of cytokines by alveolar macrophages and neutrophils, constituting a potent inflammatory response (Murata and Horino, 1999). Harding et al. (1997) found correlations of haptoglobulin and plasma TNF and with prolonged stress. Our studies did not show a response of the cytokine TNF-α to ammonia exposure and

IL-4 was below the detectable concentration. Besides cytokines and acute phase proteins, total white blood cell count, macrophages, neutrophils, and lymphocytes, are seen as indicators of immunological responses to respiratory stress (Asmar et al., 2001). In the present studies, pigs exposed to ammonia at 35 ppm doubled total white blood cells, lymphocytes, and monocytes but not neutrophils indicating that a more long term chronic inflammatory response was performed (because lyphocytes and Monocytes have to be synthesized from the bone marrow first).

Spurlock (1997) suggested that pro-inflammatory cytokines correlate with reduced feed intake and growth. Additionally, Drummond et al. (1980) compared effects of 50, 100, and 150 ppm ammonia vs. the control (0 ppm) on performance and reported decreases in ADG of 12, 30 and 29%, respectively. Our studies comparing both 50 vs. 0 ppm and 35 vs. 0 ppm did not detect any effects on performance besides a trend of decreased dry matter intake at 50 ppm ammonia exposure. These results are in agreement with Wathes et al. (2004) who did not find any effect on productivity of chronic exposure of weaned pigs over 5 ½ weeks to ammonia concentrations up to 37 ppm.

Animal behavior is regarded as a sensitive indicator for what an animal prefers or dislikes. Earlier studies from Morrison et al. (1993) concluded that ammonia concentrations in commercial buildings are not sufficient to induce aversion to ammonia. More recent preference tests (Jones et al., 1996; Wathes et al., 2002) indicate that weaner pigs responded aversely to ammonia concentrations of 20 ppm and higher but this avoidance was delayed and explained by the possible development of a sense of malaise. If this is true, pigs should reduce their feed intake at levels higher than 20 ppm. Our results do not support that interpretation, although the trend for a decreased DMI at higher concentrations of 50 ppm might point toward that explanation. Operant responses of pigs to high levels (up to 100 ppm) of ammonia revealed a relative weak aversion to polluted air exposure while they were rooting for food (Jones et al., 1998).

Current recommendations on upper ammonia limits are mainly intended to provide occupational exposure limits (e.g., OSHA threshold for human exposure of 50 ppm) as the scientific evidence that ammonia exposure affects animal health and performance is scarce (Wathes et al., 2003). Recent studies failed to find an effect of chronic exposure to ammonia (up to 37 ppm) on resoiratory disease in weaned pigs (Done et al., 2004). Most of the existing guidelines and recommendations for animal houses are set at limits ranging from 20 to 50 ppm of ammonia. Our studies indicated

that pigs respond to ammonia with systemic inflammatory and stress responses. However, even 50 ppm does not dramatically seem to affect animal performance. Future studies should focus on the effects of ammonia on lung histopathology to determine what kind of damage occurs due to ammonia exposure that elicits the animal's immune and inflammatory response.

Implications: Exposure to atmospheric ammonia affects the welfare of newly weaned pigs. Systemic hematological, and immunological effects were observed as well as increases in acute phase response variables of pigs that were exposed to ammonia. These responses need to be further evaluated for their significance on the health and well-being of weaned pigs. Performance and behavior of pigs in response to ammonia concentrations also needs to be studied on a larger scale and under commercial field conditions.

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Table 1. Feed components

Feed Components	*% in diet*
Corn (ground)	58.3%
Soybean (47.5% protein)	26.5%
Akey Start 200 Base	8%
Fat	5%
Mono-Dical Phosphate	1.2%
Limestone Meal	0.9%
Salt	0.4%
Tylan 40	0.1%

Table 2. Behavioral ethogram.

Measure	**Definition**
Posture	
Upright	Pig assumes or maintains an upright position on extended legs while standing still or moving
Lying	Included lateral, semi lateral, ventral, or sternal recumbency and involved contact of the body with the ground
Behavior	
Aggression	Competitive or aggressive interactions between pen mates
Feeding	Pig head positioned in the feeder

Table 3. Least squares means, pooled standard errors, and probability values for metabolic and hematological measures in pigs exposed to 50 vs. 0 ppm of ammonia for 20 d.

	Treatment			
Item	50 ppm	0 ppm	SEM[a]	P-value
Number of pigs	6	6	-	-
Ammonia, ug/dL	112.28	94.52	19.33	0.52
BUN, mg/dL	9.34	10.09	5.22	0.92
Glucose, mg/dL	89.27	95.84	5.48	0.41
Lactate, mg/dL	61.58	47.68	7.97	0.23
WBC, thousand/uL	20.75	15.64	4.60	0.44
RBC, million/uL	6.65	7.07	0.27	0.31
Hemoglobin, g/dL	9.20	10.34	0.64	0.24
Hematocrit, %	28.78	32.57	2.83	0.36
MCV[b], fL	42.28	46.53	2.10	0.17
MCH[b], pg	13.63	14.70	0.49	0.14
MCHC[b], g/dL	32.61	31.61	1.02	0.50
Neutrophils, /uL	4459.92	4306.71	663.80	0.87
Lymphocytes, /uL	13,764.00	10,138.00	2702.23	0.36
Monocytes, /uL	1145.96	1132.27	438.57	0.98

[a] Pooled standard error.
[b] MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration

Table 4. Least squares means, pooled standard errors, and probability values for metabolic and hematological measures in pigs exposed to 35 vs. 0 ppm of ammonia for 20 d.

	Treatment			
Item	35 ppm	0 ppm	SEM[a]	P-value
Number of pigs	6	6	-	-
Ammomia, ug/dL	25.67	47.00	8.67	0.0948
BUN, mg/dL	6.17	7.00	1.22	0.63
Glucose, mg/dL	95.67	92.67	2.70	0.44
Lactate, mg/dL	63.50	69.08	13.63	0.78
WBC, thousand/uL	21.75	9.78	1.98	0.0003
RBC, million/uL	7.03	7.28	0.31	0.57
Hemoglobin, g/dL	10.90	11.55	0.43	0.30
Hematocrit, %	34.50	35.83	1.84	0.61
MCV[b], fL	49.50	49.33	1.92	0.95
MCH[b], pg	15.6	15.92	0.46	0.63
MCHC[b], g/dL	31.58	32.22	1.18	0.71
Neutrophils, /uL	3,408.17	3,029.37	963.98	0.78
Lymphocytes, /uL	11,014.00	6,228.25	956.85	0.0018
Monocytes, /uL	1116.26	515.37	155.11	0.0114

[a] Pooled standard error.

[b] MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration

Table 5. Least squares means, pooled standard errors, and probability values for performance by nursery pigs exposed to 50 vs. 0 ppm of atmospheric ammonia for 20 d.

	Treatment			
Item	50 ppm	0 ppm	SEM[a]	P-value
Number of pigs	24	24	-	-
Number of pens	6	6	-	-
Initial BW, kg	7.5	7.5	0.32	0.96
BW at d 20, kg	12.2	13	0.45	0.22
ADG, kg/d	0.26	0.29	0.022	0.25
DMI, kg/d	0.66	0.70	0.022	0.30

[a] Pooled standard error.

Table 6. Least squares means, pooled standard errors, and probability values for performance by nursery pigs exposed to 35 vs. 0 ppm of atmospheric ammonia for 20 d.

	Treatment			
Item	35 ppm	0 ppm	SEM[a]	P-value
Number of pigs	24	24	-	-
Number of pens	6	6	-	-
Initial BW, kg	10.2	9.7	0.57	0.57
BW at d 20, kg	19.4	19.1	0.84	0.82
ADG, kg/d	0.48	0.50	0.019	0.68
DMI, kg/d	0.39	0.44	0.019	0.096

[a]Pooled standard error.

Table 7. Least squares means (% of observations), standard errors, and probability values for behaviors (over 12 h on d 19) of pigs exposed to 50 vs. 0 ppm of atmospheric ammonia for 20 d.

	Treatment			
Measure	50 ppm	0 ppm	SEM[a]	P-value
Number of replicates (pens)	6	6	-	-
Number of animals	24	24	-	-
Upright posture	33.36	28.08	2.48	0.39
Lying posture	66.64	71.93	2.48	0.39
Feeding	7.64	8.92	0.82	0.64
Aggressive behaviors	1.81	0.78	0.47	0.70

[a]Pooled standard error.

Table 8. Least squares means (% of observations), standard errors, and probability values for behaviors (over 12 h on d 19) of pigs exposed to 35 vs. 0 ppm of atmospheric ammonia for 20 d.

	Treatment			
Measure	35 ppm	0 ppm	SEM[a]	P-value
Number of replicates (pens)	6	6	-	-
Number of animals	24	24	-	-
Upright	23.23	22.82	2.20	0.45
Lying	76.77	77.18	2.20	0.45
Feeding	7.76	8.03	0.94	0.059
Aggression	0.65	0.32	0.35	0.52

[a]Pooled standard error.

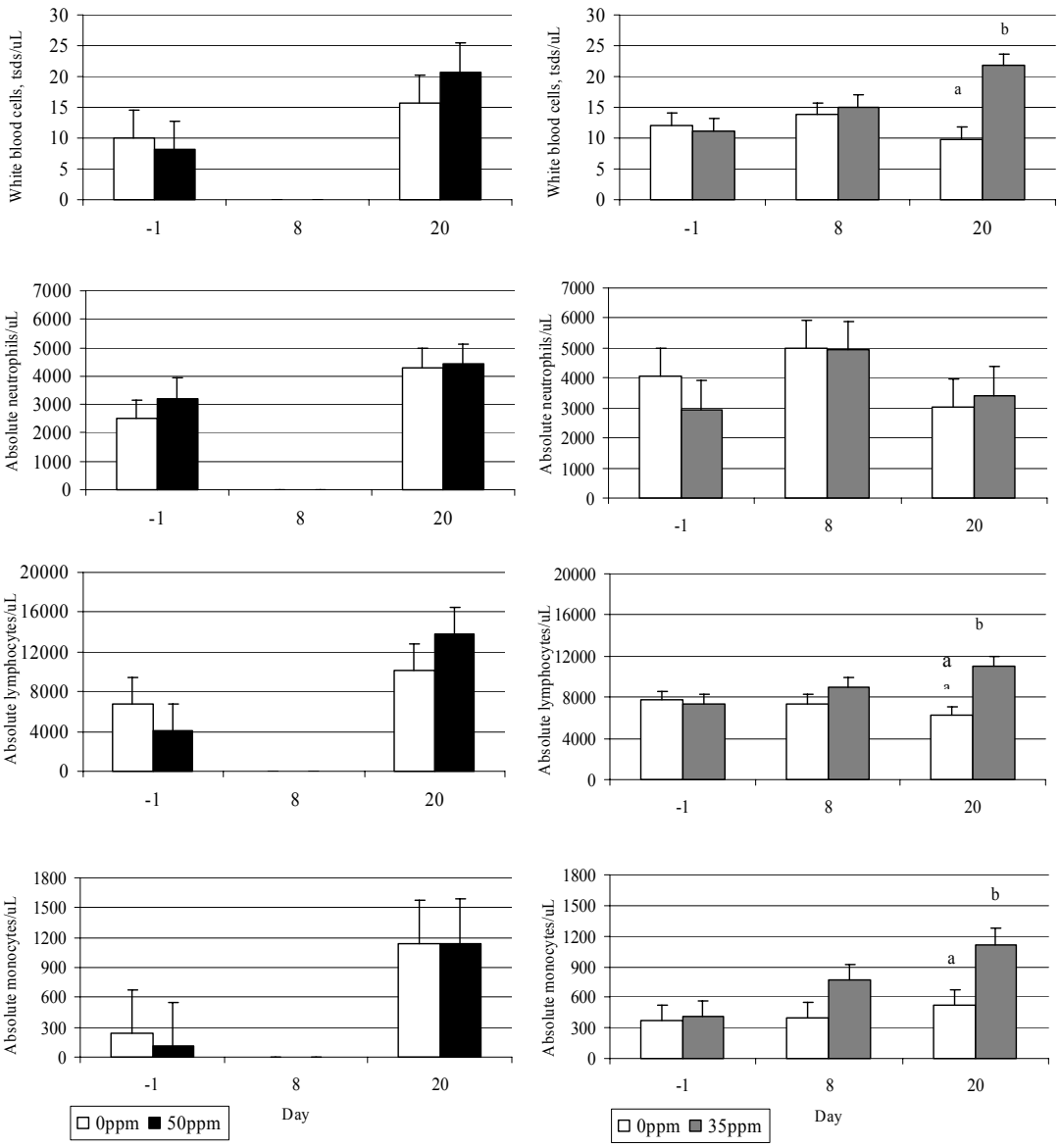

Figure 1. Hematological parameters of nursery pigs at d -1, 8, and 20 of chronic exposure to 50 vs. 0 ppm (Panel 1-3) and 35 vs. 0 ppm (Panel 4-6) of atmospheric ammonia, respectively. Least squares with different superscripts differ ($P < 0.05$). Note: Missing data in Panel 1-3 on d 8 were due to unintentional early thawing of samples.

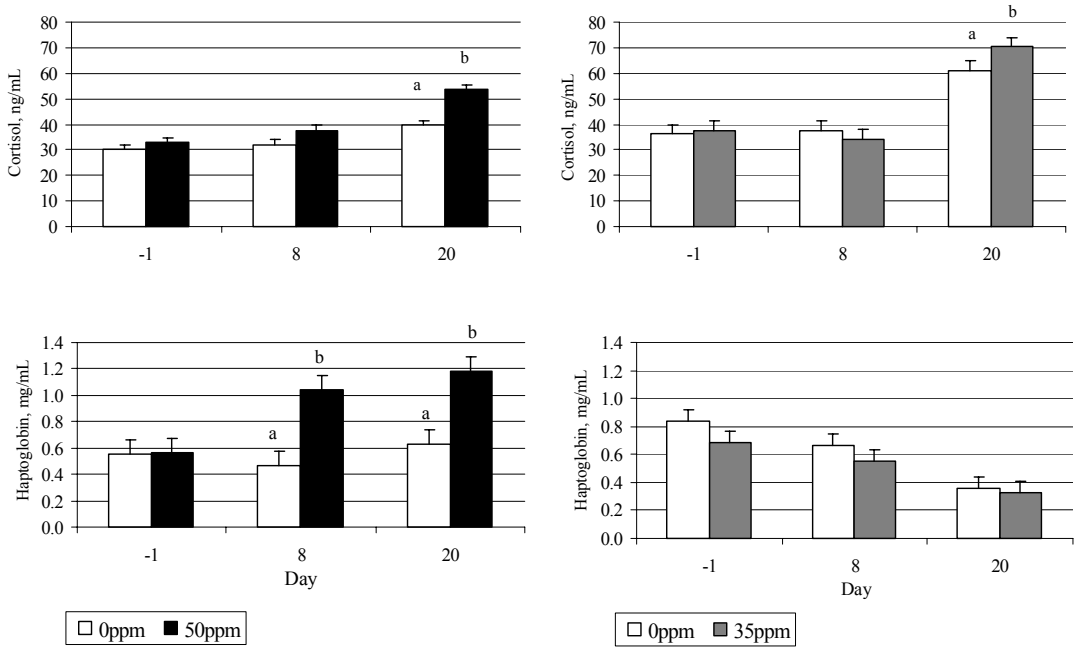

Figure 2. Serum cortisol and haptoglobin concentrations in nursery pigs at d -1, 8, and 20 of chronic exposure to 50 vs. 0 ppm (Panel 1-2) and 35 vs. 0 ppm (Panel 3-4) atmospheric ammonia, respectively. Least squares with different superscripts differ ($P < 0.05$).

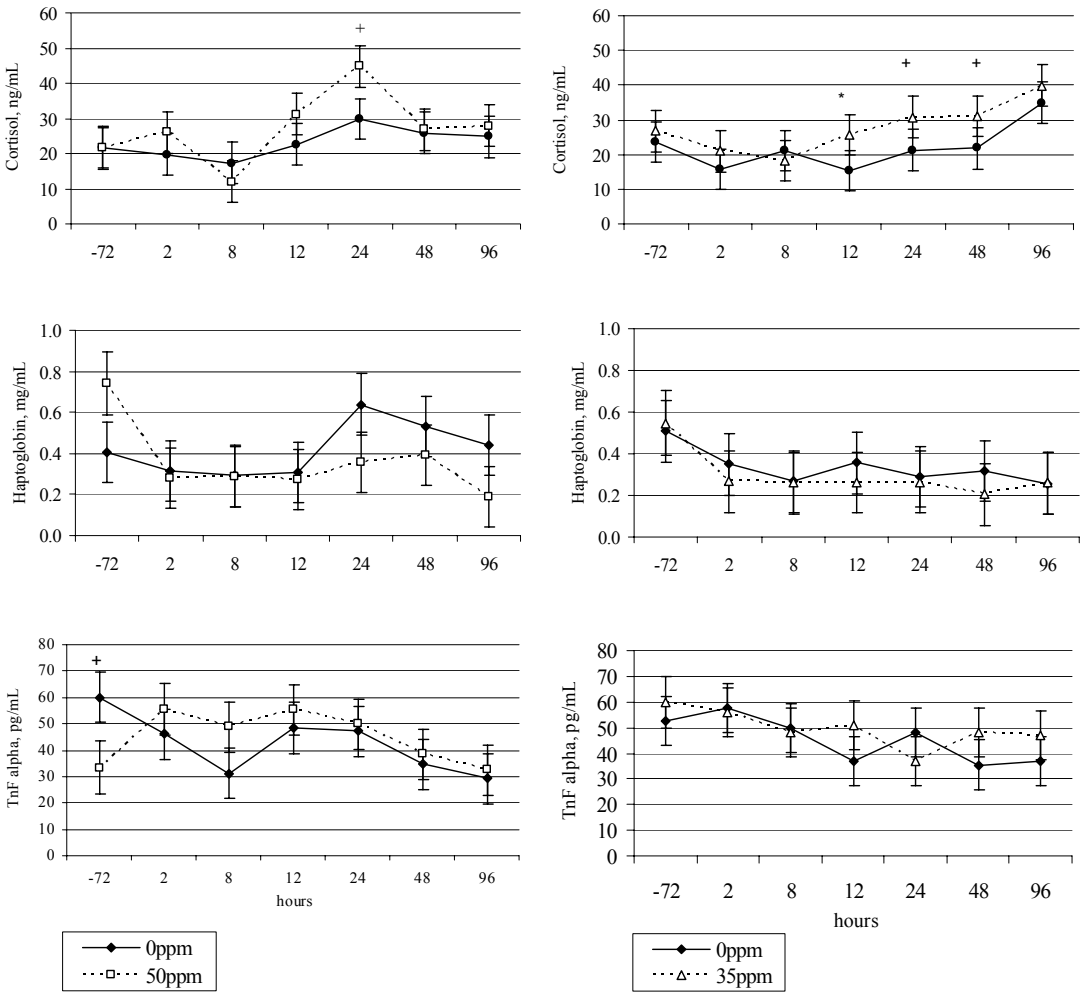

Figure 3. Serum cortisol, haptoglobin, and TNF-α concentrations during 96 h of acute exposure to 50 vs. 0 ppm (Panel 1-3) and 35 vs. 0 ppm (Panel 4-6) atmospheric ammonia in nursery pigs. Least squares means with * differ $P < 0.05$ and $^{+}$ tend to differ $P < 0.1$.