Journal of Animal and Veterinary Advances 9 (22): 2802-2806, 2010
ISSN: 1680-5593

Effect of Atmospheric Ammonia on Growth Performance and Immunological Response of Broiler Chickens

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Abstract: The experiment was conducted to investigate the effect of atmospheric ammonia (0, 13, 26, 52 ppm) on growth performance and immunological response of broiler chickens during the 0-3 week period. *Arbor Acres* (AA) strain broiler chicks (n = 480, half males and half females, 1 day old broilers) were randomly allocated to four treatments. Each treatment had 12 replicates of 10 birds. The experiment lasted 3 weeks. At the end of the experiment, 12 birds from each treatment were randomly selected for determining the effect of ammonia on broilers. Results showed that Body Weight (BW) and Feed to Gain ratio (F/G) were significantly depressed ($p<0.05$) by 5.0 and 3.0%, respectively for the 52 ppm ammonia concentration as compared with the 0 ppm treatment. Relative lymphoid organ weights, both Concanavalin (ConA) and Lipopolysaccharide (LPS) induced peripheral lymphocyte proliferation were not significantly affected ($p>0.05$) by ammonia treatment but the relative organ weight decreased with increasing ammonia concentration. Newcastle Disease Virus (NDV) hemagglutination inhibition antibody titer were lower ($p<0.05$) exposed to the 26 and 52 ppm ammonia than that of the 0 ppm treatment group. This study indicated that high atmospheric ammonia levels lowered growth performance and immunological response of broiler chickens.

Key words: Ammonia, growth performance, immunological response, broiler, lymphoid, China

INTRODUCTION

Pollutants of poultry houses include organic and inorganic dust, pathogens and other microorganisms as well as gases such as ammonia, hydrogen sulfide, carbon dioxide, carbon monoxide, nitrous oxide and methane in poultry houses. Among them, atmospheric ammonia is a major aerial pollutant (Carlile, 1984).

Ammonia in poultry houses of course has been recognized as a problem for many years. Several studies reported that ammonia can reduce bird growth performance (Reece *et al*., 1981; Miles *et al*., 2004; Beker *et al*., 2004) and increase susceptibility to disease and increase subsequent mortality (Reece *et al*., 1980; Kristensen and Wathes, 2000) (the latter at high concentrations of atmospheric ammonia). Additional studies showed that the bursa of Fabricius of birds subjected to 25 and 50 ppm ammonia from 4-8 weeks of age weighed less after infectious bronchitis vaccination than those not subjected to ammonia (Kling and Quarles, 1974). Caveny *et al*. (1981) also noticed this effect of ammonia on vaccine stressed broilers. They pointed out that ammonia at 25-50 ppm and vaccination with Newcastle disease virus at 21 days adversely affected broiler cockerel feed efficiency. Valentine (1964) suggested that ammonia concentrations of 60-70 ppm predisposed affected birds to respiratory disease and increased the risk of secondary infections.

Atmospheric NH_3 in poultry facilities, although rarely studied directly has long been recognized as a significant environmental problem in both laying hen and broiler growout facilities (Reece *et al*., 1981). In practice, ammonia release is caused by related actions of fecal content of litter, moister and temperature. Poultry are often exposed to the atmosphere with ammonia levels exceeding 50 ppm (Quarles and Caveny, 1979). However, despite of the harmful effects of ammonia in poultry houses has been reported by several workers, more information is needed to ascertain consequences under practical low exposure levels (Beker *et al*., 2004). In addition, Kristensen and

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Wathes (2000) also suggests ammonia levels <25 ppm are aversive to chickens and should be investigated for other effects on bird welfare and performance in the future.

Therefore, the objectives of the study reported herein were to determine the effect of low level and long term ammonia on growth performance and immunological response in broiler chickens.

MATERIALS AND METHODS

All procedures were approved by the Beijing Administration Office of Laboratory Animals.

Animals, housing and experimental design: About 480, one day old Arbor Acres male broiler chickens (Beijing Arbor Acre Broiler Co., Beijing, China) were randomly allotted into four treatment groups. Each treatment had 12 replicates (10 broilers in each replicate). The Control group (CTRL) was given 0 ppm gaseous ammonia. The experimental group (CORT) was given different levels of gaseous ammonia (13, 26, 52 ppm). Birds were housed in four environmentally controlled chambers and given *ad libitum* access to water and a standard corn-soybean basal diet containing ME 3.01 Mcal kg^{-1}; crude protein 21.5%. The diet was formulated in order to meet the National Research Council (NRC, 1994) recommend requirements for all nutrients. The chickens were initially started at 35°C then the chamber temperature was gradually decreased by 3°C weekly to 25°C by the end of 3 weeks. About 24 h of artificial light was supplied (Hu *et al*., 2010). Experimental period was 3 weeks from 1-21 days of age.

All birds were inoculated with the Infectious Bronchitis and Newcastle disease IV strain vaccine (Lohmann Animal Health GmbH and Co. KG) on day 7 by intranasal and intraocular administration and with infectious bursal disease vaccine (Schering-Plough Animal Health) on day 14 by intranasal and intraocular administration.

Ammonia addition: Anhydrous ammonia was metered continuously into three of the chambers to maintain levels of 13, 26 and 52 ppm ammonia for 24 h. Ammonia pressure-relief valve (Shanghai Pressure-relief Valve Co., Shanghai, China), rubber tube and glass-float flowmeters (No. LZB-3WB) were used to control the speed of ammonia flow into the chambers. Thrice a day, aerial ammonia concentration was measured in different sites of the chamber with a MiniWarn Multi-Gas Monitor (Draeger Co., Germany). The concentration of ammonia in the control chamber was near 0 ppm.

Performance: Average Body Weight (BW) was weighed as a group and feed consumption per replicate was measured at 3 weeks. Feed efficiency was calculated as the feed to gain ratio for each chamber accordingly. Average Daily Feed Intake (ADFI) was calculated for the continuous 21 days. Mortality was monitored and recorded daily.

Sampling: At 7 and 14 days of age, 12 birds from each treatment (1 bird per replicate) were randomly selected and blood samples were taken from the wing vein collected in 5 mL eppendorf tubes. Serum samples were analyzed for anti-NDV titers. At 21 days of age, 12 birds from each treatment were randomly selected and weighed then blood samples were obtained from the wing vein into heparinized blood collection tubes for the lymphocyte proliferation assay and into serum tubes for anti-NDV titers and serum IgG, IgM, IgA assay. These birds were slaughtered after blood sampling. Thymus, spleen and bursa were removed and weighed. Relative lymphoid organ weight was expressed as (lymphoid organ weight (g)/BW kg).

Analysis: The Peripheral Blood Mononuclear Cells (PBMC) were isolated from peripheral blood using Lymphocyte Density-gradient Centrifugation Medium (Shanghai Chemical Company, China) and centrifuged at 3,000 rpm for 30 min (Dong *et al*., 2007). Briefly, 3 mL heparinized blood sample was mixed with an equal volume of Hank's solution and carefully layered on the surface of lymphocyte separation medium. The lymphocyte band was collected and washed 3 times (3,000 rpm for 10 min) with RPMI-1640 culture medium (Sigma-Aldrich, Inc., St. Louis, MO) without foetal bovine serum. Peripheral blood mononuclear cells were counted and cell viability was determined by the trypan blue exclusion method. PBMC were suspended and adjusted to 1×10^7 mL^{-1} in RPMI 1640 medium containing 100U mL^{-1} of penicillin and streptomycin, 24 mmol L^{-1} Hepes solution, 2 mM of Gln and 10% heat-inactivated foetal bovine serum (Qian Yuan Hao Bio-logical Co., Ltd.).

A 190 μL quantity of PBMC suspension incubated in 96 well tissue culture plates adding either 10 μL Concanavalin A (ConA, 1000 μg mL^{-1}, Sigma, USA) or Lipopolysaccharide (LPS, 500 μg mL^{-1}, Sigma, USA). No cell suspension was added to control wells. After 72 h incubation at 39°C in 5% CO_2 humid incubator, 10 μL of 3-(4,5-dimethylthiazolyl)-2,5-diphenyl tetrazolium bromide (5 mg mL^{-1}, Amresco, USA) was added into each well incubating for another 4 h and then 100 μL quantity of sodium dodecanesulfonate was added into each well and shaken for 30 min to dissolve the precipitation completely.

The light absorbance was measured at 570 nm with Enzyme-linked Immunosorbent Assay Reader (BIO-RAD

Model 550, USA) (Dong *et al*., 2007). The Stimulation Index (SI) was calculated as the absorbance of mitogen-stimulated cells divided by the absorbance of unstimulated, control (media only) cells.

Newcastle disease virus HI antibody quantification was done by using the hemagglutination and HI procedures. The nonheparinized blood samples (1.5 mL chicken^{-1}) were placed at 37°C for 2 h and then centrifuged at 3,000 rpm for 10 min. Serum samples were collected and frozen at -20°C for assays. Briefly, HI tests were carried out by using serial 2-fold dilutions of serum and 4 hemagglutination units of the NDV antigen (China veterinary drugs censorship Institute).

Serum dilutions ranged from 1:2-1:2, 048. The geometric mean titer was expressed as reciprocal $\log_2$ values for the highest dilution that displayed HI (Dong *et al*., 2007).

Serum IgG, IgA, IgM concentrations were measured by using immunoturbidimetric assay (Shan *et al*., 2007; Madhavan *et al*., 2002).

Statistical analysis: Data were analyzed by SPSS 13.0 software for windows (SPSS Inc., Chicago, IL, USA). The effect of treatment was determined by one-way ANOVA with post hoc Duncan's multiple-range tests to identify between treatment differences. Means were considered significantly different at the $p<0.05$ level and data were expressed as means±SD.

RESULTS AND DISCUSSION

Effects of ammonia on growth performance in broiler chickens: The effects of ammonia on the performance of 3 weeks broiler chickens are shown in Table 1. Birds in CTRL (0 ppm NH_3 group) have 5.3% ($p<0.05$) improvement on Body Weight (BW) and 2.6% ($p<0.05$) deficit on feed to gain ratio (Feed/Gain) compared with the 52 ppm NH_3 group. There was no significant effect on BW or feed to gain ratio for birds reared at 13 or 26 ppm ammonia. ADFI and feed to gain ratio (Table 1) were also numerically poorer with the increasing levels of ammonia concentration but there were no noted statistically significant effects on ADFI however, a significant difference ($p<0.05$) on feed to gain ratio was observed between 52 ppm aerial ammonia treatment and CTRL (0 ppm NH_3 group). The mortality which noted in 52 ppm treatment was higher than the rest treatment but the differences were not significant.

Table 1: Effect of ammonia on the growth performance in 3 weeks broiler chickens

NH_3 (ppm)	ADFI (g)	BW (g)	Feed/gain (g:g)	Mortality (%)
0 (control)	44.97±1.57	678.17±25.46^{a}	1.49±0.02^{a}	0.83
13	44.37±1.64	667.34±29.69^{ab}	1.50±0.06^{ab}	0.83
26	44.14±2.03	662.44±32.76^{ab}	1.51±0.11^{ab}	0.83
52	43.54±2.44	644.17±29.75^{b}	1.53±0.03^{b}	1.67

^{a,b}Means in a column with no common superscript differ significantly ($p<0.05$). Each value represents the mean of twelve replicates (using ten birds per replicate)

Miles *et al*. (2004) noticed that male broilers exposed to 25, 50 and 75 ppm aerial ammonia concentrations had 2, 17 and 21% reductions in BW, respectively when compared with controls. The magnitude of the results of Miles *et al*. (2004) is so much bigger than that of this study, perhaps related to different breed of broilers and different age. In previous research, Reece *et al*. (1980, 1981) did a lot of research in this area. They exposed broiler chickens to high levels of ammonia ranging from 50-200 ppm during the brooding period and found weight gain and feed conversion during brooding were adversely affected for all exposure levels. Also, they exposed broiler chickens to low levels of ammonia covering 0-50 ppm that are more prevalent in practice than the high levels during 0-28 days to determine the effect of ammonia on broilers. Body weight was reduced by 4% at 25 ppm group but there was no discernible effect on feed conversion or mortality. The results of body weight and mortality in this study are partly in agreement with results reported by Reece *et al*. (1980, 1981). In other research, Charles and Payne (1966a, b) studied and found that broilers exposed to atmospheres of 78-106 ppm NH_3 had slower growth rates and poorer appetites than those grown in NH_3-free conditions and also illustrated high ammonia decreased egg production, feed intake and body weight in hens exposed to 106 ppm of NH_3 for 7 weeks. The current research agree with previous research mentioned above.

In this study, there was no effect of treatment on Average Feed Intake per day (ADFI) or mortality. However, treatment means for mortality (Table 1) at 3 week generally increased as aerial ammonia concentration upgraded. We have observed that mortality in the 52 ppm group was twice that of the other groups, this may be result from respiratory disease induced by high ammonia. It can be supported by that symptoms of cough and rale on many chicks in 52 ppm group during the course of experiment.

In conclusion, broilers exposed to concentrations >26 ppm of atmospheric ammonia may had a trend of reducing in BW and feed efficiency and generally perhaps had greater mortality. Body weights depression and lack of effect on feed efficiency correspond with earlier research (Miles *et al*., 2004; Reece *et al*., 1981; Kling and Quarles, 1974). The results of this study demonstrated that broiler performance at 3 weeks was reduced at the highest level of ammonia.

Table 2: Effect of ammonia on lymphoid organ weights in 3 weeks broiler chickens

	Relative lymphoid organ weights		
NH_3 (ppm)	Thymus (g kg^{-1} of BW)	Spleen (g kg^{-1} of BW)	Bursa (g kg^{-1} of BW)
0 (control)	3.29±0.13	0.94±0.31	2.33±0.60
13	3.06±0.33	0.85±0.11	2.23±0.69
26	3.04±0.51	0.78±0.17	2.21±0.82
52	3.00±0.26	0.74±0.15	2.17±0.56

In this experiment, n = 48

Table 3: Effect of ammonia on peripheral blood lymphocyte proliferation in 3 weeks broiler chickens

	Peripheral blood lymphocyte proliferation	
NH_3 (ppm)	ConA	LPS
0 (control)	1.04±0.11	1.02±0.11
13	1.02±0.06	0.98±0.06
26	0.98±0.14	0.95±0.09
52	0.93±0.12	0.93±0.18

In this experiment, n = 24

Effects of ammonia on relative lymphoid organ weights in broiler chickens: The effects of ammonia on relative lymphoid organ weights (3 weeks) are shown in Table 2. Relative weights of lymphoid organs were reduced with increasing ammonia levels but the results were not significant and were similar to those reported by Caveny *et al.* (1981). Caveny *et al.* (1981) exposed broiler cockerel to 0, 25 and 50 ppm ammonia found the bursa of fabricius weights as a percentage of live body weight was not significantly affected by treatment. Both the current research and Caveny's results were similar to those reported by Kling (1973).

Effects of ammonia on peripheral blood lymphocyte proliferation and serum anti-NDV titers in broiler chickens: The effects of ammonia on lymphocyte proliferation in broiler chicks are shown in Table 3. The Lymphocyte Proliferation of 52 ppm group induced by ConA and LPS are reduced by 10.58 and 8.82%, respectively compare with control group. There was no significant effect of ammonia level on the proliferation of T and B lymphocytes stimulated by ConA and LPS, despite the higher proliferation in the control group. The Peripheral Blood Lymphocyte Proliferation is representative of the ability of immune cells and the test results revealed that high ammonia will reduce the level of cellular immunity in chickens.

The effects of ammonia on the Serum anti-NDV titers in Broiler Chickens are shown in Table 4. There was no effect of ammonia level at 7 days. At 14 days of age, serum antibody titer of control group was increased by 38.5% ($p<0.05$) compare with 52 ppm group. At 21 days of age, serum antibody titer was greater in the control group than both the 26 and the 52 ppm group. This result

Table 4: Effect of ammonia on anti-Newcastle disease virus antibody titers (log_2) in 1-3 weeks broiler chickens

	Age (days)		
NH_3 (ppm)	7	14	21
0 (control)	6.75±1.04	4.50±0.53^{a}	3.88±1.36^{a}
13	6.75±1.04	4.00±0.76^{ab}	3.50±1.20^{ab}
26	6.63±1.06	3.88±0.99^{ab}	2.63±1.06^{bc}
52	6.50±1.07	3.25±0.89^{b}	1.88±0.64^{c}

$^{a-c}$Means in a column with no common superscript differ significantly ($p<0.05$) and data expressed as log_2. In this experiment, n = 48

Table 5: Effect of ammonia on serum IgG, IgA, and IgM in 3 weeks broiler chickens

	Item		
NH_3 (ppm)	Ig G (g L^{-1})	Ig A (g L^{-1})	Ig M (g L^{-1})
0 (control)	0.215±0.014^{a}	0.168±0.015^{a}	0.099±0.016^{a}
13	0.210±0.012^{ab}	0.162±0.011^{ab}	0.093±0.010^{ab}
26	0.201±0.014^{ab}	0.155±0.015^{ab}	0.086±0.017^{ab}
52	0.197±0.016^{b}	0.147±0.017^{b}	0.078±0.018^{b}

^{a,b}Means in a column with no common superscript differ significantly ($p<0.05$). In this experiment, n = 24

indicated that high ammonia would lower the level of antibodies in broilers with the trend of damaging humoral immunity.

Effects of ammonia on serum IgG, IgA, IgM: The effects of ammonia on the serum IgG, IgA, IgM concentration are shown in Table 5. Rearing birds in 52 ppm ammonia reduced serum IgG, IgA and IgM by 8, 12 and 21%, respectively compared to birds reared with 0 ppm ammonia. There was no significant effect of 13 and 26 ppm ammonia despite Ig levels falling with increasing ammonia concentration. However, in general the tendency of serum immunoglobulins concentration declined with the increase of ammonia concentration. In addition, immunoglobulin secretion is a complex metabolic process playing an important role in the process of the immune system to fend off antigen. The increased concentrations of serum immunoglobulins, cytokines and C4 could regulate and enhance the immune functions that provide health benefits for combating disease challenges (Turner *et al.*, 2002). These data show that chronic ammonia stress may affect immunoglobulin stability and decreased Ig levels in high ammonia conditions would result in decreased responses to infection.

CONCLUSION

On the basis of this study, it is concluded that atmospheric ammonia can adversely affect broiler growth performance and immunological response. These results demonstrate that broiler in the presence of ammonia may cause even a greater economical loss due to the poor performance. This would support a management recommendation to increase ventilation rates in the broiler

house during the successive weeks of the growth cycle of the birds to remove ammonia stress as quickly as possible without chilling the birds.

ACKNOWLEDGEMENTS

This research was financially supported by a grant (2006BAD14B06) from National Science and Technology Pillar Program in the 11th 5 years Plan Period, People's Republic of China. We gratefully acknowledge all the teachers and students for helping in this study.

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