Broilers' head behavior as an early warning index of production and lung health under ammonia exposure

Qing Xiu Liu, Min Hong Zhang,[1] Ying Zhou, and Jing Hai Feng

Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, State Key Laboratory of Animal Nutrition, Beijing 100193, China

ABSTRACT This study investigated the effects of ammonia exposure (0, 15, 25, and 35 ppm) on head behavior, production performance and lung tissue morphology of broilers, and the relationship between head behavior, production performance, and lung tissue injury. In this experiment, a total of 264 AA commercial male broilers (21 d old) were assigned to 4 treatment groups with 6 replicates of 11 chickens for a 21-day trial period, the frequency of head-scratching and head-shaking behavior at the initial stage (2, 24, and 72 h) of ammonia exposure were recorded, and the production performance indices and the lung pathological changes after 21 d of ammonia exposure were observed. The correlation analysis was established between head behavior and production performance indices. Results showed that head-scratching behavior increased under 15 ppm ammonia for 72 h, head-shaking behavior increased when exposure to 15 ppm ammonia for 2, 24, and 72 h, and lung tissue was injured when exposure to 15 ppm ammonia for 21 d. However, exposure to 15 ppm ammonia did not influence growth performance. Compared with the control group, exposure to 25 ppm decreased the ADG and exposure to 35 ppm decreased the ADG, ADFI, and F/G. Furthermore, the increase in head-shaking frequency after 2 h and 24 h ammonia exposure was significantly associated with production performance and lung tissue injury after 21 d ammonia exposure. In conclusion, the head-shaking behavior at the initial stage of ammonia exposure can reflect the degree of harm of the later production performance and lung tissue health.

Key words: broilers, ammonia, head behavior, production performance, lung tissue morphology

2021 Poultry Science 100:100814
https://doi.org/10.1016/j.psj.2020.10.067

INTRODUCTION

Exposure to air pollution can cause various behavioral changes in humans, such as anxiety, depression, aggressive behavior, sleep rhythm, and activity changes (Lim et al., 2012; Bakian et al., 2015; Chen et al., 2018). A study reported evidence of positive association between the PM2.5 concentration and moderate to severe depressive and anxiety behaviors among older adults (Pun et al., 2017). On the other hand, air pollutants have been proved to be associated with chronic respiratory inflammation, systemic inflammation, neuroinflammation, behavior, and cognitive deficits. Healthy children and adult populations exposed to pollutants such as ozone and PM have shown extensive damage to the respiratory nasal epithelium and olfaction (Calderón-Garcidueñas et al., 2002). Impaired olfaction is important early changes in neurodegenerative diseases including Alzheimer's disease (**AD**) and Parkinson's disease (**PD**) (Kovacs et al., 1999). Moreover, air pollutant gases have a significant association with respiratory symptoms, including cough and wheeze (Zhang et al., 2015). In children, air pollution causes an increase in coughing (Gouveia et al., 2018) and multiple household air pollution indicators have been strongly associated with persistent cough (Coker et al., 2020). The above studies showed that air pollution can lead to changes in head behavior (behaviors associated with the head of the human or animal such as head-shaking, sneezing, etc.), and the degree of these changes are related to the degree of pollution. At present, it is generally accepted that ammonia is one of the most harmful air pollutants in poultry industry; however, whether ammonia exposure can affect broilers' head behavior have not been reported.

Continuous ammonia exposure will inevitably affect the physiological health and production of broilers. It was found that ammonia exposure reduced BW gain, feed intake, and feed conversion rates (Miles et al.,

Received July 10, 2020.

Accepted October 27, 2020.

[1]Corresponding author: zhangminhong@caas.cn

2004, 2006; Skinner-Noble et al., 2005; Li et al., 2017). Exposure to 25 ppm ammonia had negative effects on broiler production performance and immunity (Almuhanna et al., 2011). Atmospheric ammonia enters broilers' respiratory tracts through the mouth and nose, damages the respiratory system, and causes immune reactions (Olanrewaju et al., 2007). The main manifestation of inflammatory damage is the massive secretion of inflammatory cytokines. Articles from our laboratory found that exposure to 15 ppm low ammonia increased the level of IL-6 and IL-10 in the respiratory tract of broilers (Zhou et al., 2020). Effects of ammonia on production performance and respiratory systems of broilers have been reported. However, there is limited information available on the relationship between head behavior and growth performance, lung tissue injury under ammonia exposure.

tTherefore, in this study, the effect of different concentrations of ammonia on head behavior at 2-hour, 24-hour, and 72-hour after exposure begins, as well as on production performance and lung tissue morphology at 21 d were investigated. And we also explored the relationship between the head behavior at the initial stage of ammonia exposure and production performance and lung tissue injury after 21 d ammonia exposure. The study aimed to find a practical and reliable early indicator that reflects ammonia discomfort, and provide scientific basis for the timely and accurate adoption of ammonia control measures.

MATERIALS AND METHODS

All protocols related to animal use in this study were approved by the Institutional Animal Care and Use Committee of the Chinese Academy of Agricultural Sciences (permit number: IAS 2019-41).

Experimental Design

A total of 280 one-day-old male broiler chickens (Huadu Co. Ltd., Hebei, China) were housed in cages and in a temperature- and humidity-controlled room, with free access to feed and water, then reared to 21-day old. After the end of the 21 d brooding period, 264 broilers with similar weight were randomly divided into 4 treatment groups, each comprising 6 replicate cages (11 broilers per cage) for each treatment. The 4 treatment groups were the control group (0 ppm), the 15 ppm, the 25 ppm, and the 35 ppm ammonia group. The experimental period was from 21-day old to 42-day old. In the climate chamber, the temperature, humidity, ventilation, illumination, and ammonia concentrations were controlled by computer. All broilers received normal food and were fed ad libitum daily (Table 1), and immunized in accordance with routine vaccinations.

Table 1. Ingredients and nutrient compositions of the basal diet (g/kg diet as-fed basis).

Items	Content (%)	Items	Content (%)
Ingredients		Nutrient levels[2]	
Corn	56.51	ME/(MJ/kg)	12.73
Soybean meal	35.52	CP	20.07
Soybean oil	4.50	Ca	0.90
NaCl	0.30	AP	0.40
Limestone	1.00	Lys	1.00
$CaHPO_4$	1.78	Met	0.42
d L-Met	0.11	Met + Cys	0.78
Premix[1]	0.28		
Total	100.00		

[1]Premix provided the following per kg of the diet : VA 10,000 IU, VD3 3,400 IU, VE 16 IU, VK3 2.0 mg, VB1 2.0 mg, VB2 6.4 mg, VB6 2.0 mg, VB12 0.012 mg, pantothenic acid calcium 10 mg, nicotinic acid 26 mg, folic acid 1 mg, biotin 0.1 mg, choline 500 mg, Zn($ZnSO_4 \cdot 7H_2O$) 40 mg, Fe ($FeSO_4 \cdot 7H_2O$) 80 mg, Cu($CuSO_4 \cdot 5H_2O$) 8 mg, Mn($MnSO_4 \cdot H_2O$) 80 mg, I(KI) 0.35 mg, Se(Na_2SeO_3) 0.15 mg.

[2]Calculated values.

Sample Collection

On the 2 h, 24 h, and 72 h of the experiment, all broilers in the repetition were videotaped for 10 min each time to observe the broilers' head-scratching and head-shaking behavior, and the frequency of head-scratching and head-shaking behavior of the whole group of broilers within 10 min was recorded by frequency method.

Head-scratching: When the broiler stands, one foot supports the body, the head hangs down, the other foot lifts, and swiftly scratches the head.

Head-shaking: The head of the broiler leaves the center line and swings rapidly to the left or right.

The total intake and body weight of broilers were recorded, and the average daily feed intake (**ADFI**), average daily gain (**ADG**), and feed/gain (**F/G**) were calculated.

On the 21st day of the experiment (42-day-old broiler), one broiler was selected in each replicate cage, and the left lung tissues were isolated, washed with PBS. Then 1 cm^3 lung tissue was dissected and fixed with 4% paraformaldehyde solution, dehydrated, paraffin embedded, sliced (3 micron thick), stained with hematoxylin and eosin, observed, and photographed under optical microscope.

Statistical Analysis

All statistical analyses for factor measurements of the difference between groups were conducted using ANOVA available with the SAS 9.1 software. Spearman correlation analysis was conducted to study the relationship between head behavior indicators at the initial stage of ammonia exposure and production performance. The data were presented as means ± SEM. The replicate cage served as the experimental unit, and the $P < 0.05$ was considered statistically significant.

RESULTS

Effects of Ammonia Exposure on Broilers' Head Behavior

Effects of different ammonia concentrations on broilers' head behavior are shown in Table 2. With the increase of ammonia concentration, the head-scratching and head-shaking behavior increased significantly. Compared with the control group, the head-scratching behavior increased when exposure to 35 ppm ammonia at 2 h, exposure to 25 ppm ammonia at 24 h, and exposure to 15 ppm ammonia at 72 h ($P < 0.05$). However, head-shaking behavior increased when exposure to 15 ppm ammonia at 2, 24, and 72 h ($P < 0.05$).

Effects of Ammonia Exposure on Production Performance of Broilers

Effects of different ammonia concentrations on broilers' production performance are shown in Table 3. Under 15 ppm ammonia exposure, ADG, ADFI, and F/G were not significantly different from the control group ($P > 0.05$). Under 25 ppm ammonia exposure, ADG was significantly lower than the control group ($P < 0.05$), but there were no significant effects on ADFI and F/G ($P > 0.05$). Compared with the control group, ADG and ADFI were significantly decreased, and F/G was significantly increased under 35 ppm ammonia exposure ($P < 0.05$).

Effects of Ammonia Exposure on Lung Tissue Morphology of Broilers

Effects of different ammonia concentrations on lung tissue morphology are shown in Figure 1. Compared with the control group, some accessory bronchi in the 15 ppm group showed inflammatory cell infiltration. Exposure to 25 ppm ammonia, local tissue hemorrhage occurred, and a large amount of red blood cells were seen in some accessory bronchi. Exposure to 35 ppm ammonia, some of the pulmonary lobules showed connective tissue hyperplasia and local hemorrhage.

Correlation Analysis Between Head Behavior Indicators at the Initial Stage of Ammonia Exposure and Productive Performance Indicators

Correlation analysis between head behavior and growth performance is shown in Table 4. The ADFI was negatively correlated with the head-shaking times at 2 h and 24 h ($P < 0.05$), and negatively correlated with the head-scratching times at 72 h ($P < 0.05$). The ADG was significantly negatively correlated with the head-shaking times at 2 h, 24 h, and 72 h ($P < 0.05$), and significantly negatively correlated with the head-scratching times at 72 h ($P < 0.05$). The F/G was significantly positively correlated with the head-shaking times at 2 h, 24 h, and 72 h ($P < 0.05$).

DISCUSSION

Previous studies revealed that the existence of ammonia is closely related to production performance and respiratory tract health in broilers. Exposure to 15 ppm ammonia can significantly increase inflammatory cytokines in the trachea of broilers, but has no effect on production performance (Zhou et al., 2020). Exposure to 25 ppm ammonia environment can result in a decrease in the proportion of pectoral muscles and the slaughter late (Yi et al., 2016). Exposure to 30 ppm ammonia can cause anorexia in broilers, accompanied with weight loss, reduced production performance, and caused inflammation (Kristensen et al., 2000). Exposure to 50 ppm ammonia exposure can contribute to respiratory injury, such as damaged tracheal cilia, increased mucus secretion, degeneration and necrosis of tracheal epithelium and lung tissue (Xiong et al., 2016). Similarly, in our study, broilers exposed to 15 ppm ammonia concentration showed pulmonary fibrosis, accompanied by inflammatory cell infiltration, local bleeding, and other phenomena, but had no significant effect on production performance. Broilers exposed to 25 and 35 ppm ammonia showed reduced feed intake, BW gain, and feed utilization rates, and aggravated the degree of lung injury. The above results showed that with the increase of ammonia concentration, the production performance decreased and the lung injury gradually aggravated.

It is generally believed that when animals are in a bad condition, they will express their discomfort and maladjustment through behavior (David et al., 2015). It has been demonstrated that, when the concentrations of ammonia were set at approximately 0, 10, 20, and 40 ppm ammonia, laying hens significantly preferred fresh air to an ammoniated atmosphere (Wathes et al., 2002). Broiler chickens, when given a free choice between 4, 11, 20, and 37 ppm atmospheric ammonia, avoided the 2 higher concentrations and chose to spend equal amounts of their time in 4 and 11 ppm (Jones et al.,

Table 2. Effects of different ammonia concentrations on the frequency of head-scratching and head-shaking behaviors of broilers.

Items		0	15 ppm	25 ppm	35 ppm	P-value
2 h	Head-scratching	3.83 ± 2.93^{a}	$6.50 \pm 3.88^{a,b}$	$6.83 \pm 1.17^{a,b}$	7.33 ± 1.86^{b}	0.1372
	Head-shaking	7.33 ± 3.44^{a}	14.5 ± 3.94^{b}	29.2 ± 6.74^{c}	29.0 ± 7.48^{c}	<0.0001
24 h	Head-scratching	2.00 ± 1.79^{a}	$4.33 \pm 2.16^{a,b}$	4.83 ± 1.60^{b}	4.67 ± 2.50^{b}	0.0878
	Head-shaking	7.83 ± 3.06^{a}	26.0 ± 5.59^{b}	35.3 ± 9.67^{c}	36.2 ± 10.9^{c}	<0.0001
72 h	Head-scratching	2.17 ± 0.75^{a}	4.33 ± 1.51^{b}	4.17 ± 1.47^{b}	5.00 ± 1.67^{b}	0.0134
	Head-shaking	7.17 ± 2.14^{a}	18.0 ± 3.16^{b}	21.17 ± 4.4^{b}	18.5 ± 1.87^{b}	<0.0001

In the same row, the same letter superscripts mean no significant difference (P > 0.05), whereas with different small letter superscripts mean significant difference (P < 0.05).

Table 3. Effects of different ammonia concentrations on growth performance of broilers.

Items	Ammonia concentration/(ppm) 0	15	25	35	*P*-value
ADG/g	76.27 ± 2.28[a]	73.77 ± 2.31[a,b]	69.95 ± 3.16[b]	51.89 ± 3.47[c]	<0.0001
ADFI/g	129.43 ± 5.01[a]	127.21 ± 7.12[a]	126.26 ± 3.63[a]	106.89 ± 4.40[b]	<0.0001
F/G	1.70 ± 0.04[a]	1.72 ± 0.10[a]	1.81 ± 0.10[a]	2.06 ± 0.17[b]	<0.0001

[a-c]In the same row, the same letter superscripts mean no significant difference ($P > 0.05$), whereas with different small letter superscripts mean significant difference ($P < 0.05$).

2005). These results indicate that the birds experience ammonia as aversive, and birds, when in uncomfortable environment, display abnormal behaviors. An interesting phenomenon found in this study was that broilers exposed to 15 ppm ammonia for 2 h and 24 h showed abnormal head-shaking behavior, and this behavior significantly increased with the increase of ammonia concentration. Therefore, head-shaking behavior of broilers indicates that the ammonia is irritating or aversive, and we believe this specific behavior is a natural defense mechanism, such as coughing, that protects the respiratory tract from inhaling foreign bodies and by clearing excessive bronchial secretions (Andrani et al., 2019). Moreover, this specific behavior is significantly associated with the degree of lung tissue injury caused by the increase of ammonia concentration.

It is generally accepted that animal behavior is associated with animal production and welfare (Lawrence, 2008). When abnormal behavior occurs, it means that the animal welfare is damaged to a certain extent (Lu, 2014). In this study, the correlation analysis showed that there is a significant negative correlation between the frequency of head-shaking behavior after 2 h and 24 h ammonia exposure and the production performance after 21 d ammonia exposure. We believed that the reason for this result is that the frequency of head-shaking behavior reflects the degree of lung tissue injury, and different degrees of lung tissue injury lead to different degrees of decline in production performance.

In conclusion, this study shows that exposure to 15 ppm ammonia can increase head-shaking behavior and cause lung tissue injury, and has a tendency to reduce production performance. Ammonia concentrations above 25 ppm can significantly reduce growth performance. In the range of 15, 25, and 35 ppm ammonia concentration, the increased frequency of head-shaking behavior after 2 h and 24 h ammonia exposure could reflect the decline of production performance and the degree of lung tissue injury after 21 d of ammonia exposure. Thus, in actual production, head-shaking behavior can be used as an early indicator of the degree of lung injury and production performance changes caused by ammonia exposure in broilers. The above results will help us to use head behavior indicators to provide a warning of damaging ammonia concentrations, and take measures as early as possible.

ACKNOWLEDGMENTS

This study was supported by the National Key Research and Development Program of China (2016YFD0500509), and it were also supported by the Science and Technology Innovation Project of the Chinese Academy of Agricultural Sciences (ASTIP-IAS09) and the Special Fund for China Agriculture Research System (CARS-41).

DISCLOSURES

No conflict of interest exits in the submission of this article, and the manuscript is approved by all authors for publication. The work described was original research that has not been published previously, and not under consideration for publication elsewhere, in whole or in part. All the authors listed have approved the manuscript that is enclosed.

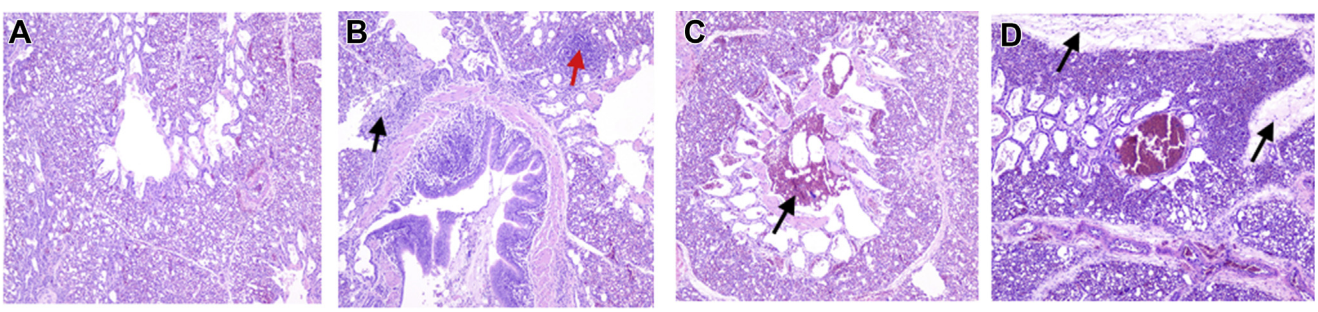

Figure 1. Lung tissue morphology of broiler under different concentrations of ammonia. (A) the control group; (B) the 15 ppm group (the black arrow: local necrosis of the tissue; the red arrow: infiltration of inflammatory cells); (C) the 25 ppm group (tissue hemorrhage); (D) the 35 ppm group (connective tissue hyperplasia). Lung tissue cells were stained with hematoxylin and eosin (HE). Graphs were observed at 20×, the size unit of the photograph is 50 μm.

Table 4. Spearman correlation analysis of head behaviors and production performance.

Items		ADFI/g		ADG/g		F/G	
		R^2	*P*-value	R^2	*P*-value	R^2	*P*-value
2 h	Head-scratching	−0.36504	0.0794	−0.34126	0.1027	0.19641	0.3577
	Head-shaking	−0.60165	0.0019	−0.70229	0.0001	0.48912	0.0153
24 h	Head-scratching	−0.24885	0.2410	−0.18903	0.3764	0.16858	0.4310
	Head-shaking	−0.50822	0.0112	−0.55452	0.0049	0.4312	0.0350
72 h	Head-scratching	−0.43057	0.0357	−0.48215	0.0170	0.35899	0.0849
	Head-shaking	−0.22825	0.2834	−0.47631	0.0186	0.48603	0.0160

REFERENCES

Almuhanna, E. A., A. S. Ahmed, and Y. M. Al Yousif. 2011. Effect of air contaminants on poultry immunological and production performance. Int. J. Poult. Sci. 10:461–470.

Andrani, F., M. Aiello, G. Bertorelli, E. Crisafulli, and A. Chetta. 2019. Cough, a Vital Reflex. Mechanisms, Determinants and measurements. Acta Biomed. 89:477–480.

Bakian, A. V., R. S. Huber, H. Coon, D. Gray, P. Wilson, W. M. McMahon, and P. F. Renshaw. 2015. Acute air pollution exposure and risk of suicide completion. Am. J. Epidemiol. 181:295–303.

Calderon-Garciduenas, L. C., B. Azzarelli, H. Acuña, R. Garcia, T. M. Gambling, N. Osnaya, S. Monroy, M. R. DEL Tizapantzi, J. L. Carson, A. Villarreal-Calderon, and B. Rewcastle. 2002. Air pollution and brain damage. Toxicol. Pathol. 30:373–389.

Chen, C., C. Liu, R. Chen, W. Wang, W. Li, H. Kan, and C. Fu. 2018. Ambient air pollution and daily Hospital Admissions for Mental Disorders in Shanghai, China. Sci. Total Environ. 613:324–330.

Coker, E., A. Katamba, S. Kizito, B. Eskenazi, and J. L. Davis. 2020. Household air pollution profiles associated with persistent childhood cough in urban Uganda. Environ. Int. 136:105471.

David, B., C. Mejdell, V. Michel, V. Lund, and R. O. Moe. 2015. Air quality in alternative housing systems may have an impact on laying hen welfare: Part II-ammonia. Animal 5:886–896.

Gouveia, N., and W. L. Junger, ESCALA investigators. 2018. Effects of air pollution on infant and children respiratory Mortality in Four large Latin-American Cities. Environ. Pollut. 232:385–391.

Jones, E. K. M., C. A. Wathes, and A. J. F. Webster. 2005. Avoidance of atmospheric ammonia by domestic fowl and the effect of early experience. Appl. Anim. Behav. Sci. 90:293–308.

Kristensen, H. H., L. R. Burgess, T. G. Demmers, and C. M. Wathes. 2000. The preferences of laying hens for different concentrations of atmospheric ammonia. Appl. Anim. Behav. Sci. 68:307–318.

Kovacs, T., N. J. Cairns, and P. L. Lantos. 1999. Beta-amyloid deposition and neurofibrillary tangle formation in the olfactory bulb in ageing and Alzheimer's disease. Neuropath Appl. Neurobiol. 25:481–491.

Lawrence, A. B. 2008. Applied animal behavior science: Past, present and future prospects. Appl. Anim. Behav. Sci. 2:191–197.

Li, J., T. Wang, W. Yao, L. Hu, Y. Gao, and F. Huang. 2017. Ammonia emission characteristic from livestock and poultry house and its harm to livestock and poultry health. Chin. J. Anim. Nutr. 29:3472–3481.

Lim, Y.-H., H. Kim, J. H. Kim, S. Bae, H. Y. Park, and Y.-C. Hong. 2012. Air pollution and symptoms of depression in elderly adults. Environ. Health Perspect. 120:1023–1028.

Lu, H. 2014. Relationship of livestock behavior with production and genetics. J. China Agric. Univ. 19:133–139.

Miles, D. M., S. L. Branton, and B. D. Lott. 2004. Atmospheric ammonia is detrimental to the performance of modern commercial broilers. Poul. Sci. 83:1650–1654.

Miles, D. M., W. W. Miller, S. L. Branton, W. R. Maslin, and B. D. Lott. 2006. Ocular responses to ammonia in broiler chickens. Avian Dis. 50:45–49.

Olanrewaju, H. A., W. W. Miller, W. R. Maslin, J. P. Thaxton, W. A. Dozier, J. Purswell, and S. L. Branton. 2007. Interactive effects of ammonia and light intensity on ocular, fear and leg health in broiler chickens. Int. J. Poult. Sci. 10:762–769.

Pun, V. C., J. Manjourides, and H. Suh. 2017. Association of ambient air pollution with depressive and anxiety symptoms in older adults: results from the NSHAP study. Environ. Health Perspect. 125:342–348.

Skinner-Noble, D. O., L. J. McKinney, and R. G. Teeter. 2005. Predicting effective caloric value of nonnutritive factors: III. Feed form affects broiler performance by modifying behavior patterns. Poult. Sci. 84:401–403.

Wathes, C. A., J. B. Jones, H. H. Kristensen, E. K. M. Jones, and A. J. Webster. 2002. Aversion of pigs and domestic fowl to atmospheric ammonia. Trans. ASAE. 45:1605–1610.

Xiong, Y., X. Tang, Q. Meng, and H. Zhang. 2016. Differential expression analysis of the broiler tracheal proteins responsible for the immune response and muscle contraction induced by high concentration of ammonia using iTRAQ-coupled 2D LC-MS/MS. Sci. China Life Sci. 59:1166–1176.

Yi, B., L. Chen, R. Sa, R. Zhong, H. Xing, and H. Zhang. 2016. High concentrations of atmospheric ammonia induce alterations of gene expression in the breast muscle of broilers (Gallus gallus) based on RNA-Seq. BMC Genomics 17:598.

Zhang, Q., M. Qiu, K. Lai, and N. Zhong. 2015. Cough and environmental air pollution in China. Pulm. Pharmacol. Ther. 35:132–136.

Zhou, Y., Q. Liu, X. Li, D. Ma, S. Xing, J. Feng, and M. Zhang. 2020. Effects of ammonia exposure on growth performance and cytokines in the serum, trachea, and ileum of broilers. Poult. Sci. 99:2485–2493.