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The Effect of Ammonia and Humidity in Poultry Houses on Intestinal Morphology and Function of Broilers

[1, 2]Wei Feng-Xian, [2]Xu Bin, [3]Hu Xiao-Fei, [2]Li Shao-Yu, [1]Liu Fu-Zhu,
[2]Sun Quan-You, [2]Jiao Yu-Ping and [2]Wang Lin-Yi
[1]College of Animal Science and Technology,
Northwest A&F University, 712100 Yangling, P.R. China
[2]Institute of Husbandry and Veterinary, [3]Henan Key Lab for Animal Immunology,
Henan Academy of Agricultural Science,
450002 Zhengzhou, P.R. China

Abstract: A single study has been conducted to determine the effects of atmospheric ammonia (NH_3) and humidity on the small intestinal morphology and function of broiler chickens. A total of 288, 21 days old broilers were exposed to either 30 parts per million (ppm) (CTRL) or 70 ppm of atmospheric NH_3 (EXP) for a total of 21 days. In each group one third were kept at 35% Relative Humidity (RH) (L), one third at 60% RH (M) and one third at 85% RH (H) for 21 days. The average initial Body Weight (BW), average final BW, ADFI, ADG and the ratio of feed intake to weight gain (F:G) were recorded >21 days for the 6 treatment groups of 48 broilers, namely CTRL + L, CTRL + M, CTRL + H, EXP + L, CTRL + M, CTRL + H. At the end of the experiment, the Villus Height (VH) and Crypt Depth (CD) of duodenum and jejunum and ileum as well as the absorptive function (xylose uptake) were determined. The Secretory Immunoglobulin A (SIgA) concentration in the small intestine was also measured as a parameter of intestinal immunological function. The results showed that exposure to 70 ppm of ammonia significantly decreased all of the measured parameters except F:G which was increased significantly ($p<0.05$). Exposure to 85% RH resulted in significantly decreased average final BW, ADFI, ADG, VH and CD of duodenum, jejunum and ileum, D-xylose level in plasma, SIgA concentration in duodenal mucosa and increased the F:G of broiler chickens compared with 60% RH exposure, respectively ($p<0.05$). The chickens exposure to 30% RH had lower ADG, ileal VH, duodenal and ileal CD, D-xylose concentration and higher F:G than those to 60% RH, respectively ($p<0.05$). But there was no significant interactive effect between ammonia and humidity ($p>0.05$). In conclusion, high level of ammonia in poultry house depressed the development of small intestinal morphology and subsequently the absorptive and defensive function of the small bowel, resulting in poorer growth performance of broiler chickens. Neither a high nor low level of relative humidity in the poultry house benefited the growth performance and intestinal development of broiler chickens.

Key words: Broiler chicken, atmospheric ammonia, relative humidity, intestinal morphology, intestinal function, China

INTRODUCTION

There are many kinds of harmful gases in livestock houses such as NH_3, H_2S, CO_2 and CH_4 with NH_3 being the most detrimental gas to animal health (Carlile, 1984). Ammonia is a highly irritating, colorless gas and its concentration in poultry houses is associated with a number of factors including diet, litter, temperature, humidity (Alhomidan *et al*., 2003; Ritz *et al*., 2004). Atmospheric NH_3 concentration in poultry facilities has been recognized as the most important factor when considering the bird's performance and worker's health (Reece *et al*., 1981; Fairchild *et al*., 2009). Broiler feed intake, feed efficiency and final body weight have been shown to decrease with exposure to levels of NH_3 ranging from 25-125 ppm (Charles and Payne, 1966; Johnson *et al*., 1991; Miles *et al*., 2004). With chronic exposure to NH_3 birds may also suffer from damage to the respiratory tract lining, dyspnea and lowered resistance to respiratory diseases, conjunctivitis, increased ascites and subsequent high mortality (Elliott and Collins, 1982; Carlile, 1984; Miles *et al*., 2004). Decreased vaccination efficiency has also been related to the NH_3 concentration in broiler house (Kling and Quarles, 1974).

Corresponding Author: Liu Fu-Zhu, College of Animal Science and Technology, Northwest A&F University, 712100 Yangling, P.R. China

The small bowel is a metabolically active tissue and its growth is critical for the overall development and growth of a young animal (Spratt *et al*., 1990; Ziegler *et al*., 2003). The intestinal epithelium of birds is responsible for the growth potential after hatching (Uni *et al*., 1998) and the body weight increase is dependent on the development of intestinal morphology and function (Yamauchi and Tarachai, 2000). The SIgA in small intestinal mucosa serves as the first line of defense against microorganism through a mechanism called immune exclusion, protecting the animal against invasion by the luminal microflora and toxins (Corthesy, 2007). However, small bowel development and function is affected by many stressors (Thompson and Applegate, 2006; Hu and Guo, 2008; Hu *et al*., 2010).

Researchers had supposed that the reduced growth performance of broilers from ammonia exposure (Kling and Quarles, 1974; McFarlane *et al*., 1989a, b; Johnson *et al*., 1991; Beker *et al*., 2004) was probably due to intestinal lesions (Quarles and Fagerberg, 1979). Furthermore, it is reported that the effect of ammonia with other simultaneous stressors is generally additive on chicken growth (McFarlane *et al*., 1989a, b). High levels of humidity are also detrimental in poultry houses (Weaver and Meijerhof, 1991; Yahav *et al*., 1995). However, there is scant literature about the effect of NH_3 and/or humidity on the intestinal development and function of broilers. Based on the literature we hypothesize that ammonia and/or moisture in poultry house are detrimental to the development of intestinal morphology and function of broiler chickens and to test the consequences of varying NH_3 and humidity levels on the small intestinal morphology and function of broilers was determined.

MATERIALS AND METHODS

Animals, housing and experimental design: Before initiating the experiment the research group investigated many broiler farms in North China to determine the range in poultry house ammonia concentrations and relative humidity and in different seasons. Researchers found that the ammonia concentration in poultry houses was variable, especially in Winter, ranging from 30-70 ppm. The relative humidity varied from 35-85%. The experimental design is as a 2×3 factorial with 2 between-subject factors. The poultry house contained two levels of ammonia concentration, 30 ppm as Control (CTRL) and 70 ppm as Experimental (EXP). There were three levels of relative humidity at each ammonia concentration, namely 35% (L) and 60% (M), 85% (H). Thus, there were 6 groups: CTRL + L, CTRL + M, CTRL + H, EXP + L, EXP + M and EXP + H. Five hundred, 1 day old male commercial broiler chickens (Arbor Acres) were fed with diet containing 21.5% crude protein, 1.00% calcium, 0.45% available P and 2.90 Mcal kg^{-1} ME by a calculated basis from 1-21 days of age.

At day 21, the birds were weighed individually and 288 chickens with bodyweights closest to the mean were selected and placed in 36 equal pens (8 birds/pen). The birds were allotted to 6 treatments 6 pens per treatment. The basal diet contained 19.5% crude protein, 0.90% calcium, 0.40% available P and 3.00 Mcal kg^{-1} ME by calculation. Birds were kept in 3-tiered metal batteries in programmable artificial climate chambers, temperature + 1.0°C, RH + 2.5% and ammonia + 1.0 ppm. About six programmable artificial climate chambers were employed with 6 pens in each chamber. Anhydrous ammonia was metered continuously into the chambers to maintain 24 h levels of 30 or 70 ppm ammonia which was determined daily as well as the humidity level. During the study, ambient temperature was maintained at 26°C. All chickens had free access to feed. About 24 h of artificial light was supplied. The experimental period was 21 days, from 22-42 days of age. All the birds in the experiment were taken care as the guideline of the Institutional Animal Ethics Committee of Northwest A&F University. The experiment was undertaken in state Key Laboratory of Animal Nutrition in Beijing.

Sampling and analysis: The production parameters, i.e., initial BW, final BW, Average Daily Gain (ADG), Average Daily Feed Intake (ADFI) and the ratio of feed intake to Weight Gain (F:G) were calculated for each pen continuously from 21-42 days of age. At 42 days of age, one bird per pen was removed for sampling (six birds/treatment, n = 6). The birds were slaughtered and samples of about 1 cm of medial duodenum (apex of the duodenum) and medial jejunum (midway between the point of entry of the bile ducts and Meckel's diverticulum) and medial ileum (midway between Meckel's diverticulum to the ileocecal junction) taken and fixed in buffered 4% formal-saline solution before processing for embedding in paraffin. Histological examination was carried out according to the method described by Uni *et al*. (2001). Briefly, villus height was determined from the tip of the villus to the villus crypt junction and crypt depth was defined as the depth of the invaginations between adjacent villi. About one gram of mucosa was taken from the remaining duodenum and after flushing with ice-cold normal saline and wiping with filter stydy, homogenized with 9 mL of distilled water and then centrifuged at 12000×g for 10 min at 4°C. The supernatant was taken for the determination of SIgA concentration, employing a kit

(Bethyl, texas, USA) and the SigA concentration given as nanograms per gram (ng g^{-1}) of mucosa. Xylose absorption was measured using six birds (one bird per pen) for each treatment. A dose of 0.5 g kg^{-1} D-xylose at concentration of 5% (wt./vol. distilled water) was given to each bird by oral gavage.About 1 h later blood was taken from the wing vein and the plasma xylose concentration determined by the method described by Doerfler *et al.* (2000).

Statistical analysis: The data were analyzed by ANOVA with ammonia level and humidity level in poultry house as main effects and for interactions using the GLM procedure (SPSS13.0 Software for Windows, SPSS Inc., Chicago, IL). One way ANOVA was also employed for analyzing the differences between 6 treatments. Differences between mean values were considered to be statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

Performance of broiler chickens: The data about average initial BW, average final BW, ADG and ADFI of broilers are shown in Table 1. There was no marked difference of average initial BW of birds between the six treatments. Exposure to high ammonia produced a lower ADFI, ADG and final BW than those in CTRL ($p<0.05$). However, the F:G did not differ significantly between CTRL and EXP. Chickens exposed to a high Humidity (H) had decreased ADFI, ADG, final BW and increased F:G than those exposed to Low (L) and Moderate humidity (M) ($p<0.05$). Moreover, the chickens exposed to moderate humidity had higher ADG and lower F:G than those to low humidity ($p<0.05$). The interactive effect on performance of broilers between ammonia and humidity was not significant ($p>0.05$).

Table 1: Effect of ammonia and humidity on performance of broilers[1, 2]

Treatments	Initial BW (g)	Final BW (g)	ADFI (g)	ADG (g)	F:G
CTRL+L	722.630	2906.000^{bc}	177.870^{bc}	103.470^{b}	1.720
CTRL+M	721.560	2939.580^{c}	182.580^{c}	107.590^{c}	1.700
CTRL+H	720.940	2730.500^{a}	169.250^{a}	95.480^{a}	1.780
EXP+L	723.650	2836.770^{b}	171.680^{ab}	100.630^{b}	1.710
EXP+M	726.790	2880.830^{bc}	174.690^{b}	102.570^{b}	1.700
EXP+H	723.540	2713.330^{a}	168.860^{a}	94.750^{a}	1.780
SEM	4.650	11.160	1.050	0.550	0.010
Ammonia					
CTRL	721.710	2863.750	176.570	102.520	1.750
EXP	724.660	2810.310	171.740	99.320	1.730
Humidity					
L	723.140	2868.240^{b}	174.780^{b}	101.920^{c}	1.730^{c}
M	724.180	2910.210^{b}	178.630^{b}	105.080^{b}	1.700^{b}
H	722.240	2721.140^{a}	169.050^{a}	95.080^{a}	1.780^{a}
p-value					
Ammonia	0.753	0.039	0.028	0.014	0.203
Humidity	0.986	0.000	0.003	0.000	0.000
Ammonia x Humidity	0.983	0.614	0.323	0.287	0.109

[1]n = 48 per treatment (8 birds/replicate, 6 replicates/treatment). For within column comparisons, values with differing superscripts have significant differences with $p<0.05$; [2]CTRL = 30 ppm ammonia level; EXP = 70 ppm ammonia level; L = 30% relative humidity; M = 60% relative humidity; H = 85% relative humidity

Morphological structure of small bowel of broiler chickens: As shown in Table 2, the VH and CD in all small intestinal segments of the chickens in CTRL groups were significantly higher than those in EXP ($p<0.05$). Overall birds exposed to moderate humidity had higher VH and CD in all three segments of small bowel than those exposed to high humidity ($p<0.05$) and to those exposed to a low humidity but the differences were not significant, apart from duodenal and ileal CD and ileal VH which did show significant change ($p<0.05$). There was no significant interactive effect on morphological structure of small bowel of broilers between ammonia and humidity ($p>0.05$).

Intestinal function of broiler chickens: Both ammonia and humidity levels influenced D-xylose absorbtion and duodenal SIgA concentration (Table 3). A high level ammonia decreased the D-xylose absorbtion and the SIgA concentration in duodenal mucosa ($p<0.05$). Birds treated with a moderate humidity gave a higher D-xylose concentration in their plasma and SIgA concentration in duodenal mucosa than those with exposed to a high humidity ($p<0.05$). Moreover, birds exposed to a moderate humidity also had higher D-xylose concentration than those at a low humidity ($p<0.05$). The interactive effect on intestinal function of broilers between ammonia and humidity was not significant ($p>0.05$).

In the present study, all of the broiler chickens have the same initial body weight but after 3 weeks exposure to 70 ppm ammonia (EXP) had a lower final BW, ADFI and ADG than those exposed to 30 ppm ammonia (CTRL) but there was no significant difference of feed ratio between EXP and CTRL which is in accordance with that of earlier studies (Reece *et al.*, 1981; Miles *et al.*, 2004). The exposure to high levels of atmospheric ammonia results in eye damage and consequently birds may experience difficulty in finding feed and water sources. Overall growth performance can therefore be affected by increased NH_3 concentration (Ritz *et al.*, 2004). Beker *et al.* (2004) found that there were no significant differences in blood uric acid and blood urea nitrogen of birds exposed to 30 or 60 ppm atmospheric ammonia, suggesting that the protein metabolism of the birds is not affected by different levels of atmospheric ammonia. Thus, efficiency of feed conversion might be expected to be unchanged by the ammonia level. Charles and Payne (1966) also found less live-weight gain in birds exposed to high levels of

Table 2: Effect of ammonia and humidity on small intestinal villus height and crypt depth of broilers[1, 2]

	Duodennum (μm)		Jejunum (μm)		Ileum (μm)	
Treatments	VH	CD	VH	CD	VH	CD
CTRL+L	1419.450^{ab}	403.810^{bc}	958.100^{0ab}	363.360^{ab}	909.310^{a}	333.900^{ab}
CTRL+M	1473.400^{a}	439.160^{a}	980.280^{0a}	386.130^{a}	956.590^{b}	363.600^{b}
CTRL+H	1291.000^{bc}	408.530^{bc}	953.800^{ab}	329.500^{cd}	904.630^{a}	328.390^{a}
EXP+L	1270.670^{c}	378.730^{c}	906.360^{bc}	341.390^{bc}	888.430^{a}	303.010^{a}
EXP+M	1319.990^{bc}	411.660^{ab}	944.920^{ab}	350.560^{bc}	917.650^{ab}	321.120^{a}
EXP+H	1242.470^{c}	384.380^{bc}	879.840^{c}	308.970^{d}	888.060^{a}	298.420^{a}
SEM	17.760	3.810	6.660	4.060	5.250	4.360
Ammonia						
CTRL	1394.620	417.170	964.060	359.660	923.510	341.960
EXP	1277.710	391.590	910.370	333.640	898.050	307.520
Humidity						
L	1345.060^{ab}	391.270^{a}	932.230^{ab}	352.370^{b}	898.870^{a}	318.450^{a}
M	1396.700^{b}	425.410^{b}	962.600^{b}	368.340^{b}	937.120^{b}	342.360^{b}
H	1266.730^{a}	396.450^{a}	916.820^{a}	319.230^{a}	896.340^{a}	313.410^{a}
p-value						
Ammonia	0.006	0.006	0.002	0.008	0.032	0.002
Humidity	0.034	0.007	0.044	0.001	0.013	0.041
Ammonia x Humidity	0.422	0.983	0.513	0.713	0.663	0.811

[1]n = 6 per treatment (1 birds/replicate, 6 replicates/treatment). For within column comparisons, values with differing superscripts have significant differences with $p<0.05$; [2]CTRL = 30 ppm ammonia level; EXP = 70 ppm ammonia level; L = 30% relative humidity; M = 60% relative humidity; H = 85% relative humidity

Table 3: Effect of ammonia and humidity on D-xylose level in plasma and the SIgA concentration in duodenal mucosa of broilers[1, 2]

Treatments	D-xylose level in plasma (mmol L^{-1})	SIgA concentration in duodenal mucosa (ng g^{-1})
CTRL+L	2.660^{b}	64.380^{bc}
CTRL+M	3.990^{c}	71.450^{c}
CTRL+H	1.550^{a}	60.460^{bc}
EXP+L	2.010^{ab}	54.820^{ab}
EXP+M	2.670^{b}	62.880^{bc}
EXP+H	1.510^{a}	47.380^{a}
SEM	0.120	1.750
Ammonia		
CTRL	2.730	65.430
EXP	2.050	55.030
Humidity		
L	2.340^{a}	59.600^{ab}
M	3.310^{b}	67.160^{b}
H	1.530^{c}	53.920^{a}
p-value		
Ammonia	0.006	0.006
Humidity	0.000	0.016
Ammonia x Humidity	0.083	0.859

[1]n = 6 per treatment (1 birds/replicate, 6 replicates/treatment). For within column comparisons, values with differing superscripts have significant differences with $p<0.05$; [2]CTRL = 30 ppm ammonia level; EXP = 70 ppm ammonia level; L = 30% relative humidity; M = 60% relative humidity; H = 85% relative humidity

ammonia but associated this with a lowered food intake partly due to a reduced energy requirement caused by a lowering of body heat loss. Atmospheric humidity in poultry houses is another major factor that may influence the growth of the birds (Weaver and Meijerhof, 1991; Yahav *et al*., 1995). In the present study, birds kept at a moderate Relative Humidity (RH = 60%) had higher final BW, ADFI and ADG than those at high Relative Humidity (RH = 85%) or at low Relative Humidity (RH = 35%) which is in agreement with results previously published (Yahav *et al*., 1995; Yahav, 2000). Yahav (2000) found that feed conversion efficiency of birds was not affected by RH whereas researchers found that birds kept under conditions of moderate humidity were more efficient at feed conversion. The different results for feed conversion could be due to differences in the strain of birds used. Yahav (2000) used Cobb chickens and levels of relative humidity used in the experimental design (RH was 40-45% (low level), 60-65% (moderate level) and 70-75% (high level). Yahav (2000) had also found that RH had no effect on growth performance of turkeys. However, it was reported that birds at low or high RH showed a decline in plasma T_3 concentration (Yahav *et al*., 1995; Yahav, 2000) and that feed intake of broiler was positively correlated with the plasma T_3 level (Yahav *et al*., 1995, 1996; Yahav, 2000). T_3 is one of the growth-controlling hormones and is important as a growth promoter in chickens (Harvey *et al*., 1981). These facts would support the present observation that birds at a moderate RH had a better growth performance than those at low or high RH.

A decline or restriction of the feed intake of chickens results in a decreased VH and CD of the small intestinal epithelium (Mitchell and Carlisle, 1992; Yamauchi *et al*., 1996; Hu and Guo, 2008; Hu *et al*., 2010). Hu and Guo (2008) found that stress lowered the feed intake of birds which subsequently delayed the intestinal epithelial cell proliferation and in turn decreased small intestinal VH and CD of birds. In the present study a moderate RH even with a high level of ammonia increased the VH and CD of small intestinal three segments of birds. The D-xylose, a poorly metabolized pentose is absorbed from the small intestine primarily by passive diffusion and to a lesser

extent by the same active transport system responsible for absorbing glucose and galactose. The xylose absorption assay has proven to be a reliable indicator of intestinal absorptive function (Doerfler *et al*., 2000). Histologically, the malabsorptive condition is reflection of villus atrophy hence a reduction of villus surface area with a concomitant loss of absorptive area. Villus surface area is positively correlated to villus height (Mitchell and Carlisle, 1992) and a decreased VH lowered the absorptive capacity of the small bowel (Yamauchi *et al*., 1996). In the present study exposure to a high level of ammonia or a high RH decreased small intestinal VH so decreasing the absorptive capacity reflected in lower plasma D-xylose level.

The SIgA in intestine is biosynthesized in B cells in the corona (mantle zone) and released as a complex of polymeric IgA (pIgA, mainly dimers) and following cleavage at the luminal surface the cleaved extracellular portion is defined as bound secretory component (Corthesy, 2007). Intestinal luminal SIgA has a powerful capacity to prevent adhesion and entry of antigen into and through the epithelium (Nagler-Anderson, 2001). SIgA modified the IgG-mediated reactions and serves to seclude bacteria from contact with mucosal membranes and depresses inflammatory reactions at the site of infection (Magnusson and Stjernstrom, 1982). It has also been found that SIgA may contribute to biofilm formation in the gut (Bollinger *et al*., 2003). So, the SIgA concentration in intestinal mucosal membranes is a reliable indicator of the status of immune function in the intestinal mucosa (Zhang *et al*., 2007). The decreased feed intake leads to a local deficiency of nutrient in the small intestine depresses intestinal protein synthesis with a concomitant lowering of intestinal weight (Mitchell and Carlile, 1992; Hu *et al*., 2010). Thus, a decrease in feed intake is likely to depress the local biosynthesis of SIgA so, the decreased small intestinal SIgA concentration found in broilers exposed to high level ammonia and/or humidity is compatible with this.

CONCLUSION

Researchers suggest that high levels of atmospheric ammonia and humidity in poultry houses produces a delay in proliferation of intestinal epithelial with a subsequent reduction of intestinal villus height and crypt depth which in turn impairs absorptive function (xylose absorption) and the immunological function (SIgA concentration) of the small bowel of broiler chickens may partially explain the observed decrease in the broiler's growth performance. Maintaining a moderate humidity can in some measure mitigate the consequences of a high ammonia level.

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