

**RESEARCH ARTICLE**

Effects of Induced High Ammonia Concentration in Air on Gross and Histopathology of Different Body Organs in Experimental Broiler Birds and its Amelioration by Different Modifiers

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ARTICLE HISTORY (18-477)

Received: December 13, 2018

Revised: February 25, 2019

Accepted: March 08, 2019

Published online: May 01, 2019

Key words:

Ammonia

Broilers

Immune system

Modifiers

Toxicity

ABSTRACT

The study was carried out on 100 broiler birds to ascertain the effect of ammonia and different ammonia modifiers on various parameters. For this purpose, the birds were divided into five groups and different treatments were allotted including potassium aluminium sulphate, aluminium silicate and *Yucca schidigera* plant extract. A completely sealed environment was created to produce ammonia. Results showed that ammonia at 25 ppm did not affect the carcass weight, relative organs weight and antibody titer. Gross and histopathological changes like pale liver, congestion in lungs and necrosis in kidneys were observed in positive control group, while minor changes were seen in all treatment groups. Grossly, bursa of Fabricius appear smaller in size, while on histological examination it showed depletion of lymphocytes in positive control. The level of urea (18.53 mg/100 ml), creatinine (0.30 mg/100 ml), AST (53.0 IU/L) and ALT (7.50 IU/L) were increased. The antibody titer (5.65 log²) was not affected by 25 ppm of ammonia however, it was 35% higher than both the control groups in aluminium silicate group (7.67 log²). A lower spleen weight (0.10 g) and kidney (0.73g) weight, while higher levels of urea (15.83 mg/100 ml) and creatinine (0.31 mg/100 ml) were observed in *Yucca* treated groups. The AST (53 IU/L) and ALT (8.26 IU/L) were found significantly (P<0.05) higher in alum treated groups than both control groups. All the treatments reduced the effects of ammonia in broilers, however, in overall aluminium silicate results were better than other treatments. The study concludes that, all the three amendments are useful in reducing the ammonia and improves the production and function of the broiler chicken.

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To Cite This Article: Zarnab S, Chaudhary MS, Javed MT, Khatoun A, Saleemi MK, Ahmed T, Tariq N, Manzoor F, Javed I, Zhang H, Zhenhua X and Peng Y, 2019. Effects of induced high ammonia concentration in air on gross and histopathology of different body organs in experimental broiler birds and its amelioration by different modifiers. Pak Vet J, 39(3): 371-376. <http://dx.doi.org/10.29261/pakvetj/2019.068>

INTRODUCTION

The generation of ammonia in poultry houses is a common problem effecting the both layers and broiler chicken (Kocaman *et al.*, 2006). The production of poultry results in the release of several pollutants to the atmosphere, like ammonia which has dangerous effects on poultry health and environment (Roumeliotis and Heyst, 2008). NH₃ gas is generated by the decomposition of uric acid which is volatilized (Rothrock *et al.*, 2008). During

urination nitrogen is excreted in three forms uric acid, urea and NH₃. Two enzymes uricase and urease causes the conversion of uric acid into the NH₃ in the presence of high temperature, pH and moisture (Maliselo and Nkonde, 2015). Long time exposure or higher levels of ammonia like 75 and 125 ppm leads to the failure of respiratory system and also effecting the health status of the birds (Naseem and King, 2018). The ammonia causes damaging effect, with symptoms including sneezing, irritation of trachea, inflammation of air sac, conjunctivitis and also

cause difficulty in breathing (Ritz *et al.*, 2004). Broilers of twenty days of age develop different signs like acute poisoning when revealed to NH₃ at (770 mg/kg) in poultry house. Pericardial effusion, hepatic inflammation, fibroblasts proliferation of heart, necrosis of hepatocytes and increased weight of different organs have been reported (Zhang *et al.*, 2006). Immense level of ammonia has also been noticed to decrease the LDH level in blood of chicken, also causes damaging effect to cardiac muscles, and increased organ weight (Liu *et al.*, 2010; Luo and Yang, 2012). The disease chances increase like Newcastle, and blindness and also cause decrease levels of plasma T3 and T4 and plasma cholesterol (Fidanci *et al.*, 2010). Kidneys have more chances of damage at high levels of ammonia because of lesser bird's movement results in kidney dropsy in broiler birds (Zhang *et al.*, 2014). The major reason for litter amendment usage is ammonia control and the performance benefits and fuel savings (Kim *et al.*, 2012).

The use of different chemical amendments has lowered the ammonia production in the poultry houses providing better conditions including health, production, function and better utilization of feed (Moore *et al.*, 2000). Potassium aluminium sulphate is white crystalline solid leads in the reduction of ammonia and eliminates harmful effects of litter on the environment (Moore *et al.*, 2000).

Yucca schidigera is a plant extract having polyphenolic compounds which are urease inhibitors and thus causes the reduction of ammonia. It improves feed utilization and is beneficial for immunity and has antioxidant effects. It can be added as a feed supplement as its property of raising the efficiency of feed, immune and antioxidative function in poultry birds. Enhances bird's growth and production, improves the metabolic efficacy, decreases the ammonia level, and increases the feed utilization in production of broilers (Sahoo *et al.*, 2015). Significant increase in weight was recorded in the *Yucca* treated groups when compared to yeast cell walls and *Yucca schidigera* extraction in the diet of chickens (Gurbuz *et al.*, 2011). The 38% reduction in ammonia was observed by using commercial preparation of *Yucca schidigera* (Karamanlis *et al.*, 2008).

Aluminium silicate a fine dry yellowish powder having bactericidal effect reduces the ammonia production and thus provides healthier condition for the bird. aluminium silicate is a yellow powder with Nano-structure and nanometer TiO₂ with bactericidal effect. It eliminates the pungent odour of NH₃, decreases humidity, good disinfectant and kill germs. It keeps a good environment and decreases the dose of chemical disinfectant (Commercial literature).

There are different ways to manage the issue of ammonia like acidifiers, alkaline materials, absorber, inhibitors, and enzymatic litter treatment. Keeping in view of the above facts, a study was planned to ascertain the effects of ammonia on broilers performance and the amelioration of ammonia effect by different modifiers.

MATERIALS AND METHODS

Ethical approval: The proposed design of study was approved by IBC, UAF ensuring comfort and welfare of the birds.

Experimental animal: A total of hundred, day old broiler birds were kept and purchased from local commercial hatchery. Birds were kept under suitable managemental environment for first fifteen days.

Experimental design: After first 15 days, birds were randomly allocated into 5 different groups (A-E) and each group had twenty birds. Then these chicks were placed in five different compartments environmentally sealed by the use of polythene sheet. Sawdust was placed as bedding material. The ammonia was produced by increasing the humidity level and by sprinkling of NH₃ solution (20 ml) equally in all groups except negative control. Treatments which were given to birds in groups A-E are presented in Table 1.

Total duration of the trial was for 27 days onwards. Treatments were given to birds after division. Temperature and humidity were recorded with hygrometer and ammonia was measured twice a day with ammonia meter.

Sample collection: Birds were weighed and after forty-two days of age. Birds were observed for pathological changes in different organs/tissues. The abdominal and thoracic cavities were opened and required tissues including kidneys, lungs, intestine, liver, heart, gizzard, proventriculus, spleen, thymus and bursa were weighed. Gross abnormalities on these organs were recorded. All the collected tissues were then proceeded for histopathological studies (Bancroft and Gamble, 2007). Serum was separated from blood for estimation of serum enzymes by using commercially available kits including AST (Merck; Cat #5.17521.0001) and ALT (Merck; Cat # 5.17531.0001) and LDH (Merck; Cat # 5.17652.0001). Serum creatinine (Merck; Cat # 5.17551.0001) and blood urea (Merck; Cat # 5.17611.0001) were also determined by using commercially available kits. The final calculation of each enzyme/creatinine/urea were made following the method as described for each kit. The serum antibody titers were also determined by HA and HI against NDV (Wang *et al.*, 2010).

Different parameters were performed during the experiment

- 1) Carcass weight of all the birds after slaughtering.
- 2) Weight gross and histopathology of different body organs (liver, lungs, heart, kidney, intestine, gizzard, proventriculus, spleen, bursa and thymus)
- 3) Antibody titer, serum enzymes (ALT, LDH, AST), urea and creatinine.

Data analysis: The data obtained were statistically analyzed by using analysis of variance technique and the means of treatment were compared by DMR by using SAS statistical software version 9.2 (SAS, 2007) on personal computer. The significance level was considered at (P<0.05). The antibody titer and geometric mean titer against ND were also worked out.

RESULTS

Carcass weight and Organs weight: The results of carcass weight revealed 14.4% decrease due to presence of 25ppm ammonia in the sealed environment. Different modifiers improved the carcass weight ranging from 8.6-12.7%. The relative weight of different organs showed a

non-significant increase or decrease from the negative control group. The kidney weight was significantly ($P<0.05$) lower in Yucca treated group than positive control group (Table 2).

Among the lymphoid organs, the relative weight of thymus and bursa did not show statistical difference in the treatment groups than control groups. However, the relative weight of spleen was significantly ($P<0.05$) decrease in positive control group than negative control group. The relative weight of spleen significantly ($P<0.05$) improved in aluminium sulphate and aluminium silicate groups than positive control group with 25% improvement in aluminium sulphate, while 36% improvement in aluminium silicate groups (Table 3). However, it did not improve in Yucca treated group.

Antibody titer: The log antibody titer against NDV did not show the effect of 25pp ammonia as the titer was almost similar in positive control ($5.65 \log^2$) to that of negative control group ($5.67 \log^2$). The effect of modifiers including aluminium sulphate ($6.00 \log^2$) and aluminium silicate ($7.67 \log^2$) showed an improvement in antibody titers than both control groups. However, it decreased $5.00 \log^2$ in Yucca treated group than the control groups (Table 4).

Serum biochemistry: The effect of 25ppm of ammonia resulted in significant ($P<0.05$) increase in serum AST (53.0 IU/L), urea (18.53 mg/dl) and creatinine (0.30 mg/dl) levels. The aluminium silicate caused significant reduction in serum AST (25.33 IU/L) which was beyond the values of negative control group (40.0 IU/L), while it

caused 29.21 and 11.11% improvement in blood urea and serum creatinine levels than positive control group. Aluminium sulphate caused improvement of 11.96, 1.24, 8.5 and 11.11% in serum LDH, AST, urea and creatinine. Yucca treatment resulted in 17.05% improvement in blood urea (12.47 mg/dl), while the values of LDH (1.43 IU/L) and creatinine (0.31mg/dl) increased beyond those in negative control group (15.83 mg/dl; >1.24 IU/L; >0.25 mg/dl, respectively). The values of AST (25.83 IU/L) and ALT (5.93 IU/L) further decreased from negative control group (<40.0 IU/L; <6.57 IU/L, respectively) (Table 5).

Gross lesions: In positive control group, pale liver was observed in 75% birds, while bursa appeared smaller and mild congestion in lungs and kidney was observed in 33% birds. Congestion in lungs were observed in 33% birds of aluminium sulphate and aluminium silicate treated groups, while wrinkled kidneys were seen in 33% birds of Yucca treated group.

Histopathology: Histopathological changes were observed in lungs, kidneys, liver and bursa of Fabricious. Lungs tissues showed moderate degree of congestion (Fig. 1) in 75% of positive control group, while in 33% of birds treated with aluminium sulphate. In kidneys, coagulative necrosis along with detachment of tubular epithelial cells (Fig. 2) was observed in 33% birds of positive control group. In liver, hepatocytes showed necrosis and inflammation (Fig. 3) in 33% birds of positive control group, while Bursa of Fabricious showed mild depletion of lymphocytes in follicles (Fig. 4).

Table 1: Experimental design

Groups	No. of Birds	Treatment 15 days of age	Dose Rate
A	20	Positive control	Ammonia was produced by creating wet litter conditions (sprinkled water @ 20 ml)
B	20	Negative control	
C	20	Potassium aluminium sulphate (Powdered aluminium sulphate)	Litter treatment (30 g/m ²) Once every week (Ammonia was produced by creating wet litter conditions)
D	20	Aluminium silicate nanoparticles	Litter treatment (Spray powder at 15g/m ² once every week) (Ammonia was produced by creating wet litter conditions)
E	20	<i>Yucca schidigera</i> extract	Drinking Water (1ml/10liter), Daily (Ammonia was produced by creating wet litter conditions)

Table 2: The Relative weight (Mean± SD) of various body organs are presented with percent (%) change in the weight from negative control group

Group	Carcass weight (grams)	Liver weight (grams)	Heart weight (grams)	Kidney weight (grams)	Intestine weight (grams)	Lung's weight (grams)	Proventricul us weight (grams)	Gizzard weight (grams)
A (Positive control)	1583.33±104.08 -14.41%	3.64±0.55 4.6%	0.64±0.19 -1.5%	0.98±0.42 11.4%	7.67±1.85 -16.7%	0.68±0.15 -5.6%	0.53±0.10 -13.1%	3.27±0.17 -7.4%
B (Negative control)	1850±260.58	3.48±0.49	0.65±0.11	0.88±0.80	9.21±0.72	0.72±0.18	0.61±0.09	3.53±0.88
C (Pot. Aluminium sulphate)	1733.33±251.66 8.6%	3.58±0.63 -1.6%	0.75±0.03 17.2%	0.86±0.79 -13.95%	8.98±2.29 14.6%	0.66±0.43 -3.03%	0.51±0.07 -3.92%	4.02±1.55 18.65%
D (Aluminium silicate)	1750.00±50.00 9.52%	3.61±0.57 -0.8%	0.54±0.12 -18.51%	0.80±0.51 -22.5%	8.59±1.54 10.71%	0.54±0.09 -25.92%	0.59±0.81 10.16%	2.94±0.05 -11.22%
E (<i>Yucca schidigera</i>)	1800±264.58 12.07%	3.31±0.27 -9.96%	0.56±0.08 -14.28%	0.73±0.08 -34.24	7.28±1.11 -5.35%	0.69±0.17 1.44%	0.54±0.06 1.85%	3.56±0.32 8.14%

Table 3: The values of atmospheric ammonia gas (ppm), relative weight of immune organs

Groups	Ammonia (ppm)	Thymus (gm)	% Change in thymus weight from positive cont. roll	Spleen (gm)	% Change in spleen weight from positive control	Bursa of Fabricious (gm)	% Change in bursa of Fabricious weight from positive control
A (+ve control)	25.1	0.19±0.15	26.7	0.09±0.00**	40	0.11±0.03	35.3
B (-ve control)	11.1	0.15±0.01	-	0.15±0.01	-	0.17±0.03	-
C (Aluminium sulfate)	13.3	0.24±0.05	20.83	0.12±0.02¥	25	0.16±0.03	31.25
D (Aluminium silicate)	16.3	0.18±0.05	-5.55	0.14±0.01¥	35.71	0.17±0.04	35.3
E (<i>Yucca schidigera</i>)	14.8	0.18±0.06	-5.55	0.10±0.01**	10	0.17±0.01	35.3

** indicate significant difference from negative control group; ¥ indicate significant difference from positive control group; (Mean ± SD) and % change in weight in birds of various groups from negative control group.

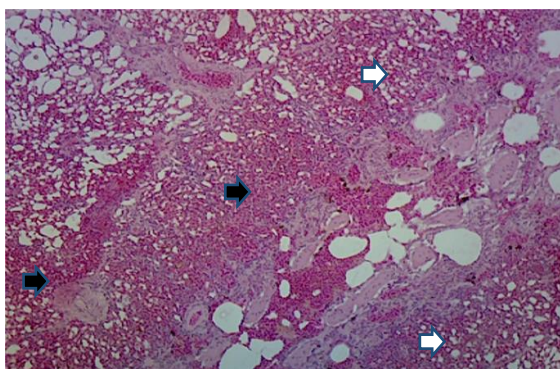


Fig 1: Lungs showing severe congestion (black arrow), Alveoli are collapsed (white arrow). The changes seen in some slaughtered birds of positive control group (H&E staining 200X).

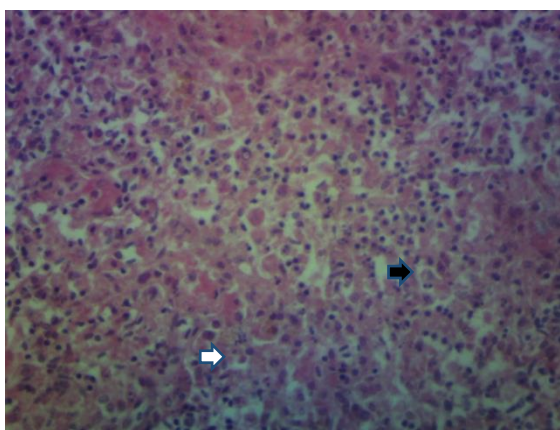


Fig. 2: The liver showing necrosis (white arrow) and inflammatory changes (black arrow) in positive control group. (H&E staining 200X).

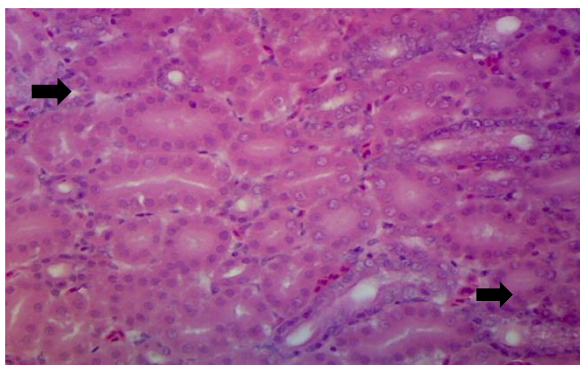


Fig. 3: Coagulative necrosis with pyknotic nuclei are seen in the kidneys. The changes seen in some slaughtered birds of positive control group (H&E staining 200X).

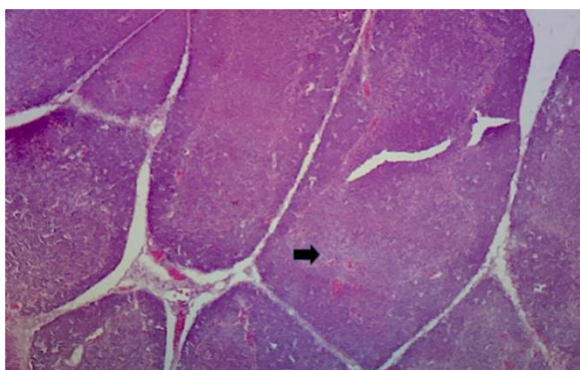


Fig. 4: Bursa of Fabricius showing mild depletion of lymphocytes in follicles in some slaughtered birds of positive control group (H&E staining 200X).

Table 4: The values of atmospheric ammonia concentration (ppm) and antibody titer (Log₂, % Values and GMI) by HA and HI against Newcastle disease virus in birds

Groups	Ammonia (ppm)	Log ₂ Titer	% Change in antibody titer from positive Control	% Change in antibody titer from negative cont. roll	Geometric mean titer
A (+ve control)	25.1	5.65	-	-	51
B (-ve control)	11.1	5.67	0.35	-	51
C (Aluminium sulfate)	13.3	6.00	5.8	5.5	64
D (Aluminium silicate)	16.3	7.67	26.33	35.27	203
E (<i>Yucca schidigera</i>)	14.8	5.00	-13	-11.81	32

DISCUSSION

Immense level of NH₃ in poultry house has damaging effect on the broiler birds, especially at earlier days. increased ammonia level makes immune system of the birds more exposed to diseases and causes disturbance to different respiratory organs Wang *et al.* (2010).

Ammonia emission also leads to environmental or health issues. Ammonia is lethal not only to poultry birds but also to poultry farmers (Choi and Moore, 2008). Inhaled air carrying harmful volatile compounds causes respiratory problems in chicken (Terzich *et al.*, 2007). Increased ammonia in air causes inflammation of conjunctiva, harmful for the cornea of eyes and produces damage in the upper respiratory tract in chicken (Aziz and Barnes, 2009). Different measures are being employed to decrease the ammonia in poultry houses, including gas absorbers, enzyme inhibitors, litter chemical treatments, etc. (Choi and Moore, 2008). Current study was carried out to determine the effects of ammonia and different modifiers on different body organs, immune system and some blood biochemical parameters. The results of the study revealed that the mean ammonia level in positive control group was 25 ppm which resulted in 14.4% decrease in the carcass weight of the chicken after 27 days of the study trial. There was non-significant reduction in the weights of different body organs, while increase in kidney and liver weight. However, a decrease of 26.7, 35.3 and 40% was observed in the weight of thymus, bursa of Fabricius and spleen. Previously, Miles *et al.* (2004) reported a non-significant effect of 50 ppm ammonia level on the immune organs. Wang *et al.* (2010) also discussed non-significant ($P>0.05$) difference in weight of immune organs than the control group at 52 ppm of ammonia. Both Wang *et al.* (2010) and Miles *et al.* (2004), however, found lower weight of immune organs, though the decrease in weight was statistically non-significant. During present study also, the weight of the immune organs was lower, as in previous study the magnitude of decrease was not reported so we cannot compare the effect of 25 ppm vs 50 or 5 ppm. However, all the studies confirmed a negative effect of ammonia on immune organs. Antibody titer against Newcastle disease virus was not affected by the ammonia at 25 ppm level. However, Wang *et al.* (2010) reported a significant decrease in the antibody titer at 21 days of treatment due to the ammonia of 26ppm and higher. The results of the current study do not corroborate well with those of Wang *et al.* (2010), which might be due to the difference of experimental conditions as present study was carried out

Table 5: The values of atmospheric ammonia gas (ppm), serum enzymes and relative weight of immune organs (Mean \pm SD) in birds of various groups

Groups	Ammonia (ppm)	LDH(IU/L)	AST(IU/L)	ALT(IU/L)	Urea(mg/dl)	Creatinine (mg/dl)
A (+ve control)	25.1	1.03 \pm 0.06 -16.9	53.0 \pm 1.00** 32.5	7.50 \pm 0.50 14.15	18.53 \pm 0.47** 37.23	0.30 \pm 0.01** 20
B (-ve control)	11.1	1.24 \pm 0.14	40.0 \pm 2.00	6.57 \pm 0.51	11.63 \pm 0.55	0.25 \pm 0.01
C (Aluminium sulfate)	13.3	1.17 \pm 0.21 11.96%	53.00 \pm 1.53** 1.24%	8.26 \pm 0.55**¥ 9.2%	12.47 \pm 0.45¥ -8.5%	0.27 \pm 0.15 14.81%
D (Aluminium silicate)	16.3	1.33 \pm 0.29 22.55%	25.33 \pm 1.53**¥ -109.23%	6.43 \pm 0.40¥ -16.64%	14.34 \pm 0.35**¥ -29.21%	0.27 \pm 0.20 -11.11%
E (<i>Yucca schidigera</i>)	14.8	1.43 \pm 0.15 27.97%	25.83 \pm 1.04**¥ -105.18%	5.93 \pm 0.20¥ -26.47%	15.83 \pm 1.89**¥ -17.05%	0.31 \pm 0.20** 3.22%

** indicate significant difference from negative control group; ¥ indicate significant difference from positive control group.

during the peak winter season in Pakistan when the temperature outside was on an average was 9/22°C (minimum/maximum). However, the inside temperature was maintained around 35°C. All the serum biochemical parameters including ALT, Creatinine, AST and urea increased by 14.15, 20.0, 32.5 and 37.23%, while the level of LDH decreased by 16.9%. The increase in AST, urea and creatinine was significant ($P < 0.05$). These results indicate damage occurring in the liver and kidneys, which was also reflected by the change in weight on these organs. It also corresponded with gross and histopathological observations, as the liver appeared pale, smaller bursa, and congested kidneys and lungs. The histopathological examination revealed moderate congestion in lungs of 75% birds of positive control group, while 33% in birds of aluminium sulphate treated group. Liver showed coagulative necrosis in about 33% of birds. In kidneys, the changes including coagulative necrosis with detached tubular epithelial cells were seen in 33% birds of positive control group. The changes in the kidneys were also observed during present study. Luo and Yang (2012) also reported similar changes in ammonia affected birds. Zhang *et al.* (2006) also reported microscopic changes in liver and kidneys in ammonia affected birds with swelling of hepatocytes along with necrotic changes in both the organs. Naseem and King (2018) also reported severe damage to myocardial tissue and kidney due to ammonia. However, both these studies did not indicate the level of ammonia affecting the body organs. The higher levels of ALT and AST in *common carp Fingerlings (Cyprinus carpio)* have also been reported after exposure to high ammonia (Abbas, 2006). The levels of AST and ALT may have been increased due to necrosis of tissues (Niles *et al.*, 1998), or because of the leakage from the degenerating tissues into the blood (Abbas, 2006). Therefore, the results of the present study indicate that the ammonia at 25 ppm level is even toxic to the birds, though the toxicity appears to be mild to moderate in nature.

The results of the current study indicated that the modifiers helped to increase the weight of immune organs. In present findings the relative weight of spleen was also significantly ($P < 0.05$) higher in aluminium sulphate and aluminium silicate treated group than positive control group but was non-significantly different from negative control group. The antibody titer was increased by the use of aluminium silicate and aluminium sulphate, while it was decreased by the use of *Yucca* treatment. Different treatments proved beneficial in eliminating the negative effects of ammonia on thymus,

spleen and bursa of Fabricius. Results indicated the treatment on litter with alum and aluminium silicate was more effective in improving the bird's immune status. Moore and Edwards (2007) also showed that litter treated with aluminium sulphate (alum) has positive effect on field application because it lessens the production of NH₃ and water soluble phosphorous. Alkis and Çelen (2009) reported that litter applied with aluminium sulphate resulted in body weight gain, and also improved other parameters. In the present study, kidney weight was lowered significantly in *Yucca* treated group than positive control group. The aluminium silicate and aluminium sulphate cause significant improvement in the serum biochemical parameters, similarly, *yucca* also results in improvement in most of the biochemical parameters, except for LDH and creatinine, which corresponded with the weight of kidney and pathological change in kidney. Lowe *et al.* (1997) found increased level of urea in the rat given *Yucca* extract and assumed that this increased level of urea was due to the contents of saponin in *Yucca* plant. Ritz *et al.* (2004) reported that chemical modifiers like aluminium sulphate eliminate the chances to cause respiratory lesions. However, Mcward and Taylor (2000) also reported that the use of aluminium sulphate caused no gross changes in the lungs and air sacs were observed cleared with respect to control group. They also revealed that high concentration of NH₃ causes damage to the respiratory system and also a damaging factor for the health of birds. These findings were similar as observed during present study.

Conclusions: Based on this study it can be concluded that atmospheric ammonia adversely affects the performance of broiler birds. It is concluded that the use of modifiers, including alum, aluminium silicate and *Yucca schidigera* are effective in reducing the atmospheric ammonia level and there is difference in the effect of these compounds. However, excessive use of *Yucca* causes adverse effects on kidneys.

Authors contribution: SZ, MSC and MTJ contributed in planning the research, data analysis and manuscript writing. AK and MKS helped in proof reading. TA helped in data manipulation. NT helped in data analysis. FM and IJ contributed in proof reading. HZ, XZ and YP executed research plan.

Acknowledgements: The help extended by Bai Yunhua (China) by providing the ammonia meter and supply of Aluminium silicate nanoparticles for the study purpose is highly acknowledged.

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