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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INTRODUCTION

The generation of ammonia in poultry houses is a common problem affecting 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 cause 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 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 antioxidative 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 Nanostructure 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 25ppm ammonia as the titer was almost similar in positive control (5.65 log2) to that of negative control group (5.67 log2). The effect of modifiers including aluminium sulphate (6.00 log2) and aluminium silicate (7.67 log2) showed an improvement in antibody titer in Yucca treated groups than both control groups. However, it decreased 5.00 log2 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 (25.33 IU/L) 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 (0.31 mg/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         | Litter treatment (30 g/m2) Once every week (Ammonia was produced by creating wet litter conditions) |
| C      | 20           | Potassium aluminium sulphate (Powdered aluminium sulphate) | Litter treatment (Spray powder at 15g/m2 once every week) (Ammonia was produced by creating wet litter conditions) |
| D      | 20           | Aluminium silicate nanoparticles | Drinking Water (1ml/10liter), Daily (Ammonia was produced by creating wet litter conditions) |
| E      | 20           | Yucca schidigera extract | |

**Table 2:** The relative weights (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)| 1583.3±104.08          | 3.64±0.55            | 0.64±0.19            | 0.98±0.42             | 7.6±1.85                | 0.68±0.15              | 0.53±0.10                      | 3.27±0.17               |
| B (Negative control)| 1414.1±104.08          | 3.49±0.49            | 0.65±0.11            | 0.88±0.80             | 9.2±0.72                | 0.72±0.18              | 0.61±0.09                      | 3.53±0.88               |
| C (Pot.            | 1733.3±251.66          | 3.58±0.63            | 0.75±0.03            | 0.86±0.79             | 8.98±2.29               | 0.66±0.43              | 0.51±0.07                      | 4.02±1.55               |
| D (Aluminium sulphate) | 1750.0±50.00          | 3.61±0.57            | 0.54±0.12            | 0.80±0.51             | 8.59±1.54               | 0.54±0.09              | 0.59±0.81                      | 2.94±0.05               |
| E (Yucca schidigera)| 1800.2±264.58          | 3.31±0.27            | 0.56±0.08            | 0.73±0.08             | 7.28±1.11               | 0.69±0.17              | 0.54±0.06                      | 3.56±0.32               |

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

| Groups | Ammonia (ppm) | Thymus weight (gm) | % Change in thymus weight from positive control | Spleen weight (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          | 1.2                                          | 0.12±0.02%        | 25                                            | 0.16±0.03               | 31.25                                                    |
| C (Aluminium sulfate) | 13.3        | 0.24±0.05          | 20.8                                          | 0.14±0.02%        | 25                                            | 0.16±0.03               | 31.25                                                    |
| D (Aluminium silicate) | 16.3       | 0.18±0.05          | -5.5                                          | 0.14±0.01%        | 35.7                                          | 0.17±0.04               | 35.3                                                     |
| E (Yucca schidigera) | 14.8         | 0.18±0.06          | -5.5                                          | 0.10±0.01%**      | 10                                            | 0.17±0.01               | 35.3                                                     |

**indicate significant difference from negative control group; Y indicate significant difference from positive control group; (Mean ± SD) and % change in weight in birds of various groups from negative control group.**
**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 Fabricious 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 26 ppm 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.
The results of the current study indicated that the use of Yucca causes adverse effects on the health of birds. These findings were similar as observed by 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, Mceward 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.

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