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RESEARCH ARTICLE

Environmental Modifiers Reduced the Ammonia Levels, Improved the in-House Environment and Resulted in Improvement in the Production Parameters of Broilers

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A B S T R A C T

High ammonia concentration leads to many serious problems inside the poultry houses. Different parameters are associated with the increased ammonia concentration that can accelerate the production of toxic gases inside the shed. Factors associated with high ammonia production are moisture contents of litter, ambient temperature, poor ventilation, humidity and related problems. For first 15 days of brooding, birds were kept under same conditions of management. These birds were divided into 5 distinct groups (A-E) and litter material of group C, D and E was treated with different modifiers (aluminum sulphate, aluminum silicate and *Yucca* extract), respectively. Temperature, moisture and microbial count of air and litter were observed. The duration of the trial was 4 weeks. Dropping composition, fecal appearance, clinical signs, breast and foot pad lesions were also monitored throughout the trial. High ammonia concentration was associated with the decreased weight gain, and increased FCR. It was noted that ammonia concentration was significantly ($P<0.005$) lower in treated groups as compared to the positive control group. The study observed the effect of increased ammonia concentration and provided the more convincing and affordable treatment to lower the ammonia production in poultry houses.

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INTRODUCTION

Poultry industry is the 2nd largest industry of Pakistan. Poultry population in Pakistan is estimated nearly 319 million, out of which 160 million is broiler's stock (Liaqat, 2018). There are different housing systems to raise poultry birds. Deep litter system is extensively practiced in Pakistan. Although litter is essential for poultry house bedding, but high volatilized ammonia is produced from it. Other sources of ammonia include high moisture, feed waste and excreted nitrogen, which accumulates in the litter (Zhao *et al.,* 2016). Litter management is associated with the decreased emission of ammonia in the poultry house. Litter condition has an important role in the management of the farm because it is directly related to the performance of the birds. High moisture content is the major cause of the lesions: pod dermatitis, scabby hocks, breast lesions and exposes the birds to the respiratory infections. Increased ammonia concentration leads to the formation of caked litter that negatively regulate the growth, welfare and carcass quality of birds. Acidifying agents are used in the litter to reduce the stress and pathological lesions. These agents reduce the high level of moisture contents in the litter. If the level of ammonia is 20 ppm for persistent period, it can lead to several disorders (Beker *et al.,* 2004). The incidence of ascites and respiratory lesions caused by ammonia was reduced by the use of different acidifying agents. Dry granular acidic compound, sodium bisulfate has been used to control the high ammonia concentration and the pathogen count in the litter, especially the *Escherichia coli* (Pope and Cherry, 2000).

Acidic nature of the litter does not allow different bacteria and enzymes to produce the excess ammonia. Some acidifiers (Ferrous sulphate and phosphoric acid) are

not recommended in the poultry houses because of their toxic effects. *Yucca schidigera* extract treated litter has been found effective in decreasing the ammonia concentration without affecting the performance of the birds. The weight gain and FCR were improved, when *Yucca* extract in combination with coccidiosis vaccine was used (Alfaro *et al.*, 2007). Aluminum sulphate and bismuth sulphate are extensively used for the treatment of litter to reduce the emission of ammonia. Their mode of action is to lower the water-soluble contents of ammonia and phosphorus (Moore *et al*., 1995). Aluminum sulphate is the more effective treatment to reduce the ammonia formation from poultry litter material (Gilmour *et al.*, 2004).

Based on the valuable results of acidifiers to reduce the ammonia level in poultry sheds, present study was designed to test the powdered alum, aluminum silicate nano particles and *Yucca* extract to manage the ammonia emissions, litter pH, microbial load and in the improvement of the broiler weight and FCR. Association of ammonia emissions with different parameters has also been evaluated.

MATERIALS AND METHODS

Plan of study: Birds were purchased from a local hatchery of Faisalabad. They were reared in the brooding section for first 15 days. After that they were randomly and equally divided into five separated sections, sealed with the help of a polythene sheet. Each group had 20 birds as given in Table 1. The experiment was conducted in the experimental shed, Department of Pathology. Litter material used in the separated sections was the saw dust. The litter used during brooding was equally mixed with litter material of each group. The treatments were applied by using a special gun to sprinkle the powder in the sealed compartments. To produce the ammonia gas, moisture contents of the litter material were raised inside these compartments from the start of the trial.

Parameters studied: Concentration of ammonia, ambient temperature and humidity level were monitored by using digital ammonia meter and hygrometer regularly. Microbial count was determined using sterilized nutrient rich petri dish. The body weight gain and feed intake were also recorded. The pH of litter materials was determined using pH meter. Other parameters included foot pad and breast lesions, fecal contents, fecal consistency and clinical signs due to high ammonia level were scored on daily basis (Table 2). Samples of litter were collected and were processed for microbiological analysis (Jennifer *et al.*, 2004). Air samples for number of culturable microorganisms (NCM) were collected and analyzed for microbiological count (Petkov *et al.*, 2006). Briefly, at a height of 0.50 m from the floor/ soil, the air sampling was performed in sterile cardboard cylinders of 1-3m. Then in situ an opening of a cardboard cylinder was placed on sterile petri dishes with nutrient agar. The other side of the cylinder was covered with a lid of the petri dish. Cylinders remained in vertical position for 15 min. After that, cardboard cylinders were removed, and petri dishes were covered with their lids. The plated petri dishes were transported to the laboratory in a bag with ice. Samples were analyzed for microbiological count on the urgent basis. Moisture level was also determined in the litter, by performing proximate analysis.

FCR was calculated by using the formula:

$$\text{FCR} = \frac{\text{Weight gain}}{\text{Feed consumed}}$$

Statistical analysis: The obtained data were statistically analyzed by analysis of variance (ANOVA) and means were compared by Tukey's test in SAS statistical version 9.2 (SAS, 2007).

RESULTS

Live body weight: The statistical analysis showed significantly ($P<0.05$) lowered weight gain in the positive control group as compared to the negative control group except at 4th week (Table 3). The weight gain in the groups treated with potassium aluminum sulphate, aluminum silicate and *Yucca* extract was significantly ($P<0.05$) lowered as compared to the negative control group in 1st week. But during 2nd week, all the treatment groups were non-significantly ($P>0.05$) different in comparison with the negative control group. During 4th week, all the treatment groups showed significantly ($P<0.05$) higher weight than the negative control group. In 1st to 4th week, potassium aluminum sulphate and aluminum silicate treated groups showed significantly ($P<0.05$) higher weight gain from positive control group. *Yucca* extract treated group showed significantly ($P<0.05$) higher weight gain than that of the positive control group during 3rd and 4th week of trial.

Air microbial count: The air microbial count (AMC) of litter in the positive control group was significantly ($P<0.05$) higher from the negative control group (Table 3). During the 1st week of trial, potassium aluminum sulphate and aluminum silicate treated groups had significantly ($P<0.05$) lower AMC than that of the negative control group but only potassium aluminum sulphate treated group showed similar results in 2nd week. Similarly, all the treated groups had significantly ($P<0.05$) lower AMC than that of the positive control group except in *Yucca* extract at 4th week.

Litter microbial count: The litter microbial count (LMC) of positive control group was significantly ($P<0.05$) higher than that of the negative control group (Table 3). The LMC in potassium aluminum sulphate treated group was significantly ($P<0.05$) lower from that of the negative control group except during 1st and 3rd week of trial. LMC in aluminum silicate treated group was significantly ($P<0.05$) lower from the negative control group during 2nd and 3rd week of trial. All treated groups showed significantly ($P<0.05$) lower LMC than that of the positive control group.

Air ammonia level: The level of ammonia in the positive control group was significantly ($P<0.05$) higher from the negative control group (Table 3). The air ammonia level in the potassium aluminum sulphate treated group was significantly ($P<0.05$) higher from the negative control group except during 4th week. Ammonia level in aluminum silicate and *Yucca* extract treated groups were significantly ($P<0.05$) higher from the negative control group during the trial period. All the treatment groups showed significantly ($P<0.05$) lower air ammonia level than that of the positive control group throughout the experimental period.

Table 1: Treatment scheme followed during whole of trial is given below

Groups	No. of birds	Treatment	Method of application
A	20	Negative Control (Treatment was provided)	
B	20	Positive Control (No treatment was provided)	Moisture was applied to the litter.
C	20	Potassium aluminium sulphate (Grind form)	10% (w/w) in the grind form applied to the litter.
D	20	Aluminium silicate (Powdered form)	15g/m2 was applied to the litter after a week
E	20	*Yucca* plant extract (Liquid form)	1ml/10L yucca extract in the drinking water.
Total	100		

Table 2: Scoring of different parameters on the basis of their signs and lesions

Groups	Score 1	Score 2	Score 3	Score 4
Fecal score	Normal	Loose	Watery and mucoid	Bloody and watery
Clinical signs	Normal	Swollen eyelids	Swollen eyelids and lacrimal discharge	Swollen eyelids, lacrimal discharge, gasping and ascities
Breast lesions	Normal	Mild lesion	Moderate lesions	Severe lesions
Foot pad lesions	Normal	Mild lesion	Moderate lesions	Severe lesions

Table 3: Mean +SD values of Live body weight, air microbial count, litter microbial count, air ammonia level, relative humidity and temperature

Groups	Week 1		Week 2		Week 3		Week 4	
	Mean± SD	% change	Mean± SD	% change	Mean± SD	% change	Mean± SD	% change
				Live Body Weight				
Negative control	900±20.32	-	1449.05±17.26	-	1961±37.93	-	2070±127	-
Positive control	819±21.27*	9.00	1364±49.68*	5.86	1813±137*	7.54	2043±154	1.30
Potassium aluminium sulphate	879±32.94*¥	7.32	1477±28.32¥	8.28	2079±86.60*¥	14.67	2403±158*¥	17.62
Aluminium silicate	842±18.48*¥	2.80	1475±164.90¥	8.13	1943±24.85¥	7.17	2365±156.45*¥	15.76
Yucca schidigera extract	833±23.56*	1.70	1408±45.97	3.22	1929±24.95¥	6.39	2305.25±217.02*¥	12.82
				Air microbial count				
Negative control	323333±9291		350000±10000		369333±7505		445333±22501	
Positive control	348666±8082*	7.84	400000±10000*	14.28	434000±15394*	17.50	490000±24979.99*	10.03
Potassium aluminium sulphate	297666±2516*¥	14.62	326000±6557.44*¥	18.50	344400±9406.38¥	20.64	390000±32695.57¥	20.40
Aluminium silicate	309000±5567*¥	11.37	337333±8504.90¥	15.66	360766.67±18457.07¥	16.87	405333±27300¥	17.27
Yucca schidigera extract	322333±8326¥	7.55	361333±5131¥	9.66	359000±19974¥	17.28	425000±35028	16.67
				Litter microbial count				
Negative control	241±20.07		280±10.00		293±15.27		303±5.77	
Positive control	293±26.16*	21.57	320±10.00*	14.28	353±11.54*	20.47	376±15.27*	24.09
Potassium aluminium sulphate	188±20.13*¥	35.83	220±10.00¥	31.25	246±5.77*¥	30.31	290±26.45¥	22.87
Aluminium silicate	207±15.30¥	29.35	250±10.00*¥	21.87	266±5.77*¥	24.64	310±30.00¥	17.55
Yucca schidigera extract	225±21.79¥	23.20	266±5.77¥	16.87	283±11.54¥	19.83	323±25.16¥	14.09
				Air ammonia level				
Negative control	7±0.97		9±0.78		12±1.00		13±1.36	
Positive control	15±1.27*	114.28	17±2.69*	88.88	25±2.67*	108.33	42±8.02*	223.07
Potassium aluminium sulphate	9±1.97*¥	40	11±0.95*¥	35.29	14±2.29*¥	44	17±3.06¥	59.52
Aluminium silicate	12±1.67*¥	20	14±1.57*¥	17.64	20±3.20*¥	20	22±1.64*¥	47.61
Yucca schidigera extract	12±0.755*¥	20	15±2.03*¥	11.76	21±1.46*¥	16	24±1.26*¥	42.85
				Litter Relative Humidity				
Negative control	64±2.63		63±0.75		69±2.85		69±4.75	
Positive control	74±4.42*	15.62	79±0.79*	25.39	84±4.19*	21.73	84±3.92*	21.73
Potassium aluminium sulphate	68±1.91*¥	8.10	68±0.53*¥	13.92	69±2.79¥	17.85	75±4.84¥	10.71
Aluminium silicate	73±2.67*	1.35	75±2.19*¥	5.06	78±1.53*¥	7.14	83±4.56*	1.19
Yucca schidigera extract	77±1.51*	4.05	78±0.90*	1.26	78±2.41*¥	7.14	83±5.32*	1.19
				Litter Temperature				
Negative control	28.14±1.21		28.57±0.79		27.28±1.11		26.33±0.51	
Positive control	29.42± 0.53	4.54	28.28±0.49	1.01	27.42±1.13	0.51	25.83±1.47	1.89
Potassium aluminium sulphate	27.85±1.06¥	5.33	27.71±0.48*	2.01	27.42±0.97	0	27.16±0.75	5.14
Aluminium silicate	28.42±1.39	3.39	28.81±0.37	1.87	26.72±0.75	2.55	25.66±1.03	0.65
Yucca schidigera Extract	27.71±1.25¥	5.81	29.71±0.75*¥	5.05	27.57±1.27	0.54	26.50±0.54	2.59

The values with asterisk (*) and yen sign (¥) are showing the significant (P<0.05) difference from the negative and positive control group respectively. The percent difference of positive control group is from negative control group and the percent difference of all treatment groups is from positive control group.

Table 4: Mean +SD values of FCR, litter pH and moisture contents of litter

Groups	FCR		Litter pH		Moisture contents of litter	
	Mean± SD	% change	Mean± SD	% change	Mean± SD	% change
Negative control	1.57±0.12		8.34±0.82		8.98±0.82	
Positive control	1.69±0.15	7.64	9.52±0.95*	14.14	17.5±1.29*	94.87
Potassium aluminium sulphate	1.52±0.11	10.05	5.12±0.82*¥	46.21	11.34±0.82*¥	35.2
Aluminium silicate	1.58±0.11	6.50	6.64±0.82*¥	30.25	13.20±0.96*¥	24.57
Yucca schidigera extract	1.54±0.11	8.87	7.66±0.50¥	19.53	14.19±0.96*¥	18.91

The values with asterisk (*) and yen sign (¥) are showing the significant (P<0.05) difference from the negative and positive control group respectively. The percent difference of positive control group is from negative control group and the percent difference of all treatment groups is from positive control group.

Table 5: Mean +SD values of fecal appearance, clinical signs, breast lesions and footpad lesions scores

Groups	Fecal appearance		Clinical signs		Breast lesions		Footpad lesions	
	Mean± SD	% change	Mean± SD	% change	Mean± SD	% change	Mean± SD	% change
Negative control	1.70±0.72¥	-	1.52±0.64¥	-	1.92±0.87	-	1.55±0.64¥	-
Positive control	2.48±1.01*	+31.45	2.67±1.04*	+43.07	2.52±1.12	+23.81	2.48±1.09*	+37.5
Potassium aluminium sulphate	1.67±0.68*	-32.66	1.52±0.64*	-43.07	1.92±0.83	-23.81	1.70±0.67*	-31.45
Aluminium silicate	1.78±0.70*	-28.22	1.81±0.62*	-32.21	2.15±0.95	-14.68	2.04±0.98	-17.74
Yucca schidigera extract	1.96±0.76	-20.97	2.07±0.83*	-22.47	2.11±0.93	-16.27	2.11±0.97	-14.92

The values with asterisk (*) and yen sign (¥) are showing the significant (P<0.05) difference from the negative and positive control group respectively. The percent difference of positive control group is from negative control group and the percent difference of all treatment groups is from positive control group.

Relative humidity of litter: The humidity level of the litter in the positive control group was significantly (P<0.05) higher from the negative control group (Table 3). The humidity level in potassium aluminum sulphate treated group was significantly (P<0.05) higher from that of the negative control group in 1st and 2nd week. The humidity level of aluminum silicate and *Yucca* extract treated group was significantly (P<0.05) higher from the negative control group during the whole trial (Table 3). The group treated with potassium aluminum sulphate had significantly (P<0.05) lower humidity level from that of the positive control group throughout the trial. While the group treated with aluminum silicate had significantly (P<0.05) lower relative humidity as compared to the positive control group during the 2nd and 3rd week of trial. The group treated with *Yucca* extract only showed significantly (P<0.05) lower value in 3rd week of trial than the positive control group.

Litter temperature: The litter temperature did not show significant (P<0.05) difference in birds of positive control from the negative control group during the whole trial (Table 3). During 2nd week, the potassium aluminum sulphate treated group had significantly (P<0.05) lower temperature as compared with the negative control group. The temperature of *Yucca* extract treated group was significantly (P<0.05) higher from that of the negative control group during 2nd week of the trial. During rest of trial, temperature of all treated groups showed non-significant (P>0.05) results as compared with the negative control group. In 1st week, potassium aluminum sulphate and *Yucca* extract treated groups showed significantly (P<0.05) lower temperature as compared with the positive control group. *Yucca* extract also showed significantly (P<0.05) lower temperature from that of the positive control group in 2nd week of trial.

Litter pH: The Litter pH of the Potassium aluminum sulphate and aluminum silicate treated groups was significantly (P<0.05) lower from the negative control group (Table 4). The litter pH of *Yucca* extract treated group was non-significantly (P>0.05) lower from that of the negative control group. All treated groups showed significantly (P<0.05) lower pH than that of the positive control groups.

Moisture content of litter: The moisture contents of the litter showed that positive control group had significantly (P<0.05) higher moisture contents from that of the negative control group (Table 4). The treatment groups showed significantly (P<0.05) higher moisture contents from that of the negative control group but had significantly (P<0.05) lower moisture contents as compared with the positive control group.

Feed Conversion Ratio: The statistical analysis did not show any difference in the treatment groups as compared to both negative and positive control groups (Table 4).

Fecal appearance: The fecal appearance showed significant (P<0.05) difference in the positive control group from that of the negative control group (Table 5). The group treated with potassium aluminium sulphate and aluminium silicate showed significantly (P<0.05) lowered fecal appearance score, while *Yucca schidigera* extract showed non-significantly (P>0.05) lowered fecal score than that of the positive control group.

Clinical signs: The statistical analysis showed significantly (P<0.05) higher clinical signs in the positive control group than that of the negative control group (Table 5). All the treatment groups showed significantly (P<0.05) lowered clinical signs scores than that of the positive control group (Table 4).

Breast lesions: The statistical analyses of breast lesions scores (BLS) showed non-significantly (P>0.05) higher lesions in the positive control group from that of the negative control group (Table 5). All the treatment groups showed non-significantly (P>0.05) lowered breast lesions than that of the positive control group.

Foot pad lesions: The footpad lesions scores (FLS) showed significantly (P<0.05) higher lesions in the positive control group from that of negative control group (Table 5). The group treated with potassium aluminium sulphate showed significantly (P<0.05) lowered footpad lesions, while aluminium silicate and *Yucca schidigera* extract treated groups showed non-significantly (P>0.05) lowered footpad lesions than that of the positive control group.

DISCUSSION

High ammonia concentration is associated with the impairment of the immune system that can lead to the diseased condition as reported by Wang *et al.* (2010). High ammonia concentration effect carcass quality, feed consumption, FCR and leads to the development of various clinical signs including oedema, gasping, ocular discharge, bloody feces and abnormal feed intake. High ammonia is the reason of several disorders as it affects the growth rate, damages the respiratory tract epithelium and produces conjunctival lesions at level below 20 ppm for persistent period (Beker *et al.*, 2004). Moore *et al.* (2007) reported that, higher ammonia level compromises the immune system and make the bird susceptible to diseases and damage the respiratory system. Aziz and Barnes (2009) also found respiratory lesions, ascites, conjunctivitis and

damaged cornea of the eyes due to high ammonia concentration. There was a high incidence of breast blisters and foot pad dermatitis due to high ammonia concentration as found by Sahoo *et al.* (2016).

The weight gain in the groups treated with potassium aluminum sulphate, aluminum silicate and *Yucca extract* against experimentally raised ammonia was significantly (P<0.05) increased by 17.62, 15.62 and 12.82%, respectively as compared to the positive control group. Celen and Alkis (2009) also found improved bird performance in terms of body weight gain, when litter was treated with aluminum chloride in combination with aluminum sulphate. Due to the increased ammonia level, air and litter microbial counts observed were significantly (P<0.05) lower in potassium aluminum sulphate, aluminum silicate and *Yucca extract* treated groups by 20.40, 17.27, 16.67% and 22.87, 17.55 and 14.09%, respectively as compared with the positive control group. Similar results were reported by Smith *et al.* (2004), when aluminum chloride was used in combination with the dietary phytase to lower the air and litter microbial count. The potassium aluminum sulphate, aluminum silicate and *Yucca extract* treated groups showed significantly (P<0.05) reduced levels of air ammonia and humidity by 59.52, 47.61 and 42.85% and 10.71, 1.19 and 1.19%, respectively from that of the positive control group. Previously, researchers used different acidifying agents to lower the moisture level and soluble phosphorus contents of litter to reduce the emission of ammonia (Gilmour *et al.*, 2004). At the start of trial, litter temperature was observed lower in the potassium aluminum sulphate, aluminum silicate and *Yucca extract* treated groups by 5.33, 3.39 and 5.81%, respectively but was high in potassium aluminum sulphate and *Yucca extract* treated groups by 5.14 and 2.59%, respectively at the end of the trial.

The pH of the litter was significantly (P<0.05) reduced in the potassium aluminum sulphate, aluminum silicate and *Yucca extract* treated groups by 46.21, 30.25 and 19.53%, respectively as compared with the positive control group. Sahoo *et al.* (2016) found reduced litter pH in the acidifier treated groups, while treating with alum sulphate and sodium bisulphate. The moisture contents of litter were also reduced in the potassium aluminum sulphate, aluminum silicate and *Yucca extract* treated groups by 35.2, 24.57 and 18.91%, respectively from that of the positive control group. High moisture contents affect the live body weight and other parameters, but showed good recovery, when they were shifted to the dry litter (Mayne *et al.*, 2007). Lazarevic *et al.* (2014) also found reduced moisture contents, litter pH and microbial count by using the *Yucca schidigera* in the feed.

Increased moisture and ammonia levels were directly associated with the physiological changes of the feces and clinical signs as observed during the present study. The groups treated with potassium aluminium sulphate and aluminium silicate showed significantly (P<0.05) lowered fecal appearance score by 32.66 and 28.22%, respectively than that of the positive control group. The change in fecal appearance is attributed to the level of nitrogen (N) in the feces (Valentine, 1964), which is possibly decreased by the use of these modifiers. The potassium aluminum sulphate, aluminum silicate and *Yucca extract* treated groups showed significant (P<0.05) improvement in the clinical signs by 43.07, 32.21 and 22.47%, respectively than that of the control positive group. It has been reported that chemical modifiers decrease the chances of respiratory signs (Ritz *et al.*, 2004). Treated litter groups had the reduced mortality as compared to the control positive group. Kristensen (2000) reported that high ammonia level can cause serious respiratory diseases and irritation to mucous membrane of eyes and respiratory system. Alloui *et al.* (2013) found mortality related issue due to high concentration of ammonia. Breast lesion score in the potassium aluminum sulphate, aluminum silicate and *Yucca extract* treated groups was significantly (P<0.05) lower from that of the positive control group by 23.81, 14.68 and 16.27%, respectively. Only the potassium aluminum sulphate treated group showed significantly (P<0.05) lowered footpad lesion score by 31.45% than that of the positive control group. McWard and Taylor (2000) reported that the use of modifiers causes no gross changes in the lungs, air sacs and in the respiratory tract, while Nadir *et al.* (2013) only found conjunctivitis like lesions and mortality. The reduction in lesions scores and mortality is probably associated with the improvement in the microbial score and reduction in the ammonia levels, thus decreasing the chances of stress and infection in the treatment groups.

Mean FCR was lowered in the potassium aluminum sulphate, aluminum silicate and *Yucca extract* treated group by 10.05, 6.50 and 8.87%, respectively than that of the positive control group. FCR was not significantly (P<0.05) affected when the birds were exposed to elevated ammonia level. Line *et al.* (2002) also found improved performance of birds, when potassium aluminum sulphate was used in combination with sodium bisulphate. The group treated with potassium aluminum sulphate performed better than that of the aluminum silicate and *Yucca extract* treated groups as reported by Zarnab *et al.* (2019).

Conclusions: It was concluded that high ammonia level adversely affects the performance of birds. Based on this study, use of different acidifying agents including potassium aluminum sulphate, aluminum silicate and *Yucca schidigera* extract are effective in reducing the atmospheric ammonia level and improve the bird health.

Authors contribution: The MTJ and MAJ planned and conducted the research trial. The MHA, NT, SUKB and SMF drafted the detail of manuscript, helped in data editing and entering, data analysis and construction of tables. The IJ and RH reviewed the manuscript and added references according to journal style. All authors approved and read the final manuscript.

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