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RESEARCH ARTICLE

The Chicken In-House Environment Can be Improved by the Use of Nanotechnology

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A B S T R A C T

The broiler performance can be influenced by optimizing the in-house environment and the negative effects of litter on the environment of the poultry can be minimized by the utilization of nanoparticles to reduce the ammonia production. For this purpose, this study was performed for 42 days, including 540-day-old broiler birds to ascertain the best levels of nanoparticles. The birds were randomly divided at day 15 into A-I groups, where A was as positive control, B as negative control, and C as starch treatment group. The groups D, E and F were divided into three subgroups. Birds were treated with three types of nanoparticles, i.e., alum, zinc oxide and copper oxide. Nanoparticles were sprayed on litter material to kill the litter microbes and reduce the ammonia emission. Ammonia was produced by maintaining wet litter conditions by sprinkling water at the rate of 250 ml per day for first week of treatment trial. Ammonia, temperature, moisture, humidity, litter pH and feed consumption were recorded daily throughout the experiment. Air and litter microbial counts were recorded on weekly basis. Ammonia, humidity and pH levels depicted significantly ($P<0.05$) lower level in treatment groups (ZnO @ 500 mg/m^2), (ZnO @1000 mg/m^2) and (ZnO @ 1500 mg/m^2) from the control positive group in the 3rd week. Air microbial count was significantly ($P<0.05$) lower in ZnO (@1000 mg/m^2) group in 3rd week from the control positive group. In week 4, all the treatment groups showed significantly ($P<0.05$) lower litter microbial count from that of the control positive group. The study showed that copper oxide, zinc oxide and alum nanoparticles can be used as possible substitute to overcome the in-house environmental problems. However, there is need to investigate the real impact of these nanoparticles as environmental modifiers.

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INTRODUCTION

Poultry production faces many problems that include dust, bacterial and viral diseases as well as the production of many foul smelling odorous chemical compounds from litter material (Blake and Hess, 2001) The ammonia in poultry houses is the most important air pollutant, its irritating nature causes respiratory distress of the broiler birds that affect the immune system of the birds (Aziz and Barnes 2010; Maliselo and Nkonde, 2015). The ammonia content of the poultry house depends on ventilation, temperature and relative humidity (Ullman *et al*., 2004). Ammonia levels above 25 ppm have been associated with problems in the poultry houses (Zarnab *et al*., 2019) Temperature also plays an important role in increasing the bacterial activity and production of ammonia (Al-Homidan *et al*., 2003). The signs of ammonia toxicity are sneezing, tracheal irritation, air sac inflammation, conjunctivitis and dyspnea. The high ammonia levels cause reduction in weight gain, feed efficiency and causes damage to the respiratory system leading to mortality (Patterson and Adrizal, 2005).

However, to face this emerging challenge to treat bacterial infections in poultry, nanotechnology has been introduced to overcome the microbial resistance due to its wide range of antibacterial activity (Hallaj-Nezhadi and Hassan, 2013). Nanoparticles have antibacterial activity against both gram positive and negative bacteria, and fungi (Hallaj-Nezhadi and Hassan, 2013).

Different environmental modifiers like zinc, copper sulfate and alum can be used to improve the poultry house environment (Zarnab *et al*., 2019). The nanoparticles of copper have shown an important role in elimination of different microbial infections. Copper in the form of

$CuSO_4$ is known to have antibacterial activity (Raffi *et al*., 2010; Kon and Rai, 2013). Addition of zinc oxide in food materials is reported as a useful agent against different contaminants, that enhances the shelf life of the food, and keep it safe from spoilage (Stoimenov *et al*., 2002). Nanoparticles of zinc oxide have more definite property against microbes. It kills microbes by the production of reactive oxygen species (Reddy *et al*., 2007). The use of these nanoparticles is to maintain a healthy in-house environment, linked to low mortality rates, to have efficient poultry production, while preserving the consumer's health by avoiding antibiotic residues. There is considerable work being done in evaluating the use of alum, zinc and copper oxide nanoparticles in poultry houses to decrease the ammonia production. For this purpose, this study was planned to explore the improvement potential of nanoparticles of zinc, copper and alum in reducing the ammonia concentration in the chicken house environment.

MATERIALS AND METHODS

Ethical approval: The proposed design of study was approved by ethical committee of UAF ensuring comfort and welfare of the birds. (D. No 3675, Dated 30-08-2021)

Synthesis of nanoparticles: The nanoparticles of copper, zinc and aluminium oxide were synthesized by using co-precipitation method (Manyasree *et al*., 2017; Manyasree *et al*., 2018).

Plan of study: The experimental trial was conducted at the poultry shed of Parasitology department, University of Agriculture Faisalabad in the month of January. The litter material used was rice husk. The average temperature outside the house was 20-22°C with humidity of 70%. So, the shed was prepared with the required conditions of temperature, humidity and ventilation to produce the ammonia in winter season.

A total 540 number of day-old broiler chicks were raised under suitable environmental conditions for the first 15 days and basal feed was provided to them. On the 15th day, the birds were randomly divided into 9 different groups (A-I) having 21 subgroups. The groups A and B were kept as control positive and control negative, respectively. The group C was treated with starch only. The groups D, E and F, each had further 3 subgroups, i.e., D1 (500mg/m^2 ZnO + 20g starch), D2 (1000mg/m^2 ZnO + 20g starch), D3 (1500mg/m^2 ZnO + 20g starch); E1 (100mg/m^2 CuSO4 + 20g starch), E2 (200mg/m^2 CuSO4 + 20g starch), E3 (400mg/m^2 CuSO4 + 20g starch); F1 (250mg/m^2 alum + 20g starch), F2 (500mg/m^2 alum + 20g starch) and F3 (1000mg/m^2 alum + 20g starch). Each subgroup had 20 birds. Every compartment was separated/sealed by polythene sheath. The litter material used for first 15 days was equally distributed in all the sealed grouped compartments with the additional bedding material. The ammonia was produced by increasing the level of humidity by sprinkling water (250 ml water/day for first week) in all the treatment groups except in the negative control. The control negative group was an open house compartment and was not sealed. The nanoparticles were sprayed on the litter material twice in a week on every Monday and Thursday. Total duration of the experiment was 42 days.

The doses were selected by reviewing different literature and keeping in view the results of previous M.Phil. research where three litter amendments were used to determine the effect of in-house ammonia in broiler.

To make it more clear and avoid confusion inserted a table of experimental design showing all groups and subgroups with treatments.

Parameters studied: Temperature and humidity levels were monitored by using digital hygrometer on daily basis. Microbial count was determined using sterilized nutrient rich petri dishes on weekly basis (Napoli *et al*., 2012). The pH of litter material was determined using pH meter. Samples of litter were collected and were processed for microbiological analysis as described by Terzich *et al.* (2000). Air samples for the number of cultural microorganism (NCM) were collected and analyzed for microbiological count as method demonstrated by Wójcik *et al.* (2010). Moisture level was also determined by performing proximate analysis (Odebunmi *et al*., 2010).

Statistical analysis: Data collected from above experiments were analyzed by using general linear model procedure by two-way analysis of variance technique ($P \leq 0.05$). Means were compared by Tukey's test using SAS statistical software (SAS, 2007).

RESULTS

Ammonia: The control positive group in this study, showed significantly ($P<0.05$) increased ammonia level than the control negative group in all four weeks. In week 1, groups 100, 400 mg/m^2 CuSO4 and 250 mg/m^2 alum showed significantly ($P<0.05$) higher ammonia levels, while groups 500, 1000 mg/m^2 alum showed significantly ($P<0.05$) lowest ammonia levels, respectively than the positive control group. In week 2, all the treatment groups showed significantly ($P<0.05$) lower ammonia level from that of control positive group except the 500 and 1000 mg/m^2 ZnO groups. In weeks 3 and 4, all the treatment groups showed significantly ($P<0.05$) lower ammonia level than control positive group (Table 2).

Temperature: Control positive group revealed non-significantly lower temperature after 1st week, while this group showed non-significantly higher temperature after 2nd and 3rd week.

Table 1: Layout of Experimental trial, 20g starch was added to the groups D1, D2, D3, E1, E2, E3, F1, F2 and F3

Group Name		Number of Birds	Treatment (15th day of age)
A		20	Positive Control (only H_2O @250 ml)
B		20	Negative Control
C		20	Starch sprayed @ 20grams
	D1	20	ZnO @ 500 mg/m^2
	D2	20	ZnO@1000 mg/ m^2
D	D3	20	ZnO @1500 mg/ m^2
	E1	20	CuSO4@100 mg/ m^2
	E2	20	CuSO4@ 200 mg/ m^2
E	E3	20	CuSO4@ 400 mg/ m^2
	F1	20	Alum @ 250 mg/ m^2
	F2	20	Alum @ 500 mg/ m^2
F	F3	20	Alum @ 1000 mg/ m^2

Table 2: Mean±SD values of ammonia level (PPM) and temperature (°C) are presented with percentage change

Groups		Ammonia				Temperature			
		Week1	Week2	Week3	Week4	Week1	Week2	Week3	Week4
A		20.58±0.56¥	15.91±0.65¥	20.29±0.20¥	19.62±0.38¥	26.00±1.00	24.00±1.00	22.00±1.00	22.00±1.00
		88.53%	78.63%	81.22%	90.98%	1.27%	-1.38%	-6.05%	3.05%
B		2.36±0.04	3.40±0.56*	3.81±0.39*	1.77±0.69*	25.67±0.58	24.33±0.58	23.33±1.15	21.33±0.58
C		19.36±0.15*¥	14.59±0.07*¥	18.38±0.64*¥	13.61±0.08*¥	27.00±1.00	24.00±1.00	22.67±0.58	21.67±1.15
		5.92%	8.29%	9.41%	30.63%	-3.85%	0%	-3.05%	1.5%
D	D1	18.29±0.33¥	15.35±0.15¥	10.46±0.22*¥	5.41±0.22*¥	25.00±1.00	24.33±0.58	23.00±1.00	21.33±1.15
		11.13%	3.52%	48.44%	72.43%	3.85%	-1.38%	-4.55%	3.05%
	D2	17.44±0.17¥	15.91±0.81¥	10.42±0.19*¥	4.43±0.19*¥	26.00±1.00	25.00±1.00	24.00±1.00	22.67±1.53
		15.23%	0%	48.64%	77.42%	0%	-4.17%	-9.09%	-3.04%
	D3	16.58±0.38¥	14.32±0.17*¥	12.31±0.18*¥	3.58±0.07*¥	26.67±0.58	25.00±1.73	23.00±1.00	23.00±1.00
		19.44%	9.99%	39.33%	81.75%	-2.58%	-4.17%	-4.55%	-4.55%
E	E1	22.52±0.23*¥	13.47±0.22*¥	11.32±0.18*¥	4.23±0.02*¥	26.00±1.00	24.00±1.00	23.67±1.53	21.33±1.53
		-8.61%	15.34%	44.20%	78.44%	0%	0%	-7.59%	0.03%
	E2	20.15±0.02¥	12.46±0.19*¥	12.55±0.06*¥	5.71±0.13*¥	25.00±1.00	26.00±1.00	24.00±1.00	23.00±1.00
		2.08%	21.68%	38.15%	70.88%	3.85%	-8.33%	-9.09%	-4.55%
	E3	21.58±0.07*¥	12.22±0.61*¥	11.48±0.25*¥	6.88±0.06*¥	25.00±1.00	25.00±1.00	23.00±1.00	21.00±1.00
		-4.86%	23.19%	43.42%	64.93%	4%	-4.17%	-4.55%	-4.55%
F	F1	23.55±0.06*¥	11.62±0.14*¥	12.61±0.08*¥	7.63±0.19*¥	26.67±1.53	24.33±0.58	22.33±1.15	22.33±1.15
		-14.43%	26.96%	37.85%	61.11%	-2.58%	-1.38%	-1.5%	-1.5%
	F2	18.75±0.68*¥	12.56±0.06*¥	11.38±0.24*¥	5.37±0.24*¥	27.00±1.00	24.00±1.00	23.67±1.15	23.33±1.15
		8.89%	21.06%	43.91%	72.63%	-3.85%	0%	-7.59%	-6.05%
	F3	18.53±0.49*¥	11.25±0.01*¥	10.46±0.22*¥	4.67±0.16*¥	28.00±1.00	24.00±1.00	22.33±1.53	20.67±0.58
		9.96%	29.29%	48.44%	76.19%	-7.69%	0%	-1.5%	6.04%

Data from 1st, 2nd, 3rd and 4th week depicted non-significant differences in all the treatment groups from both control positive and control negative groups as shown in Table 2.

Humidity level: The level of humidity was significantly ($P<0.05$) higher in control positive than the control negative group in all the 4 weeks, respectively. In the 1st week, groups 100 and 400 mg/m^2 CuSO4 depicted significantly ($P<0.05$) increased humidity level, while groups 500, 1000, 1500 mg/m^2 ZnO and 250 mg/m^2 alum showed significantly ($P<0.05$) lower level of humidity than the control positive group, respectively. In comparison with the control negative group, all the treatment groups showed significantly ($P<0.05$) higher humidity level in 1st week. In case of 2nd week, humidity level was significantly ($P<0.05$) lower in groups 1000 and 1500 mg/m^2 ZnO, 100, and 400 mg/m^2 CuSO4, 250, 500 and 1000 mg/m^2 alum, respectively than the control positive group. Data from 3rd and 4th week revealed significantly ($P<0.05$) lower humidity level in all the treatment groups from the positive control group as shown in Table 3.

Litter pH: The litter pH of control positive group was significantly ($P<0.05$) higher from that of the control negative group in 3rd week. In 1st, 2nd and 4th week control positive group showed non-significantly increased pH value than the control negative group as described in Table 2. In comparison with the control negative group, all the treatment groups except groups 500 mg/m^2 ZNO and 1000 mg/m^2 ZNO showed significantly ($P<0.05$) higher pH value.

Data from 3rd week depicted significantly ($P<0.05$) lower pH value in groups 500 mg/m^2 ZnO, 1000 mg/m^2 ZnO and 1500 mg/m^2 ZnO from the control positive group. In comparison with the control negative group, groups treated with 400 mg/m^2 CuSO4, 500 mg/m^2 alum and 1000 mg/m^2 alum revealed significantly ($P<0.05$) higher pH value in week 3 (Table 3).

Moisture level: The moisture level in the control positive group was significantly ($P<0.05$) increased from that of the control negative group in all the weeks as presented in Table 4.

In week 1, all the treatment groups except of 1000 mg/m^2 alum + 20g starch, showed significantly ($P<0.05$) lower moisture level from that of control positive group. In week 1, the groups treated with 500 mg/m^2 ZnO , 1000 mg/m^2 ZnO , 1500 mg/m^2 ZnO + 20g Starch), E2 (200 mg/m^2 CuSO4 + 20g Starch), E3 (400 mg/m^2 CuSO4 + 20g Starch), F1 (250 mg/m^2 alum+ 20g Starch), F2 (500 mg/m^2 alum + 20g Starch) and F3 (1000 mg/m^2 alum + 20g Starch), showed significantly ($P<0.05$) higher moisture level from that of the control negative group, while 100 mg/m^2 CuSO4 treated groups showed significantly ($P<0.05$) lower moisture levels from that of the control negative group.

In week 2, all the treatment groups showed ($P<0.05$) lower moisture level from that of control positive group except of 1500 mg/m^2 ZnO, 200 mg/m^2 CuSO4 and 1000 mg/m^2 alum treated groups. In week 2, all the treatment groups except of starch and 500 mg/m^2 ZnO showed significantly ($P<0.05$) higher moisture level from that of the control negative group (Table 4).

In week 3, all the treatment groups showed significantly ($P<0.05$) lower moisture level from that of control positive group except of starch and (200 mg/m^2 CuSO4 + 20g Starch) treated groups. In week 3, the groups treated with starch, 500 mg/m^2 ZnO, 1000 mg/m^2 ZnO, 1500 mg/m^2 ZnO, 200MG/M2 CuSO4 and 400mg/m^2 CuSO4, showed significantly ($P<0.05$) higher moisture level from that of the control negative group, while all other treatment groups showed non-significant difference (Table 4).

In week 4, all the treatment groups showed significantly ($P<0.05$) lower moisture level from that of control positive group except of starch and100 mg/m^2 CuSO4 treated groups. In week 4, the groups treated with 1500 mg/m^2 ZnO, 100 mg/m^2 CuSO4, 200 mg/m^2 CuSO4, 400 mg/m^2 ZnO and 1000 mg/m^2 alum, showed significantly ($P<0.05$) increased moisture level from that of

Table 3: Mean±SD values of Relative Humidity (%) and pH are presented with percentage change

Groups		Relative Humidity				PH			
		Week1	Week2	Week3	Week4	Week1	Week2	Week3	Week4
A		67.52±0.06¥ 7%	71.26±0.06¥ 7.11%	71.24±0.94¥ 9.69%	70.30±0.31¥ 7.24%	9.10±0.70 18.35%	8.80±1.100 15.91%	11.17±1.00¥ 34.91%	10.43±0.68 28.76%
B		62.52±0.61*	66.19±0.29*	64.33±0.19*	65.21±1.10*	7.43±0.25	7.40±0.30	7.27±0.21*	7.43±0.21
C		69.48±0.22*¥ -2.82%	70.36±0.13¥ 1.26%	68.57±0.57*¥ 3.75%	69.48±0.27*¥ 1.16%	9.63±0.74 -5.82%	10.90±1.54¥ -23.86%	9.27±0.81 17%	10.00±0.17 4.12%
D	D1	66.31±0.12*¥ 1.79%	70.30±0.17¥ 1.35%	69.29±0.21*¥ 2.74 %	69.22±0.11*¥ 1.54%	8.07±0.64 11.32%	10.10±0.17 -14.77%	8.07±0.64* 27.75%	9.17±1.27 12.08%
	D2	66.45±0.17*¥ 1.58%	65.19±0.06* 8.52%	69.39±0.16*¥ 2.59%	69.22±0.01*¥ 1.54%	8.80±1.10 3.29%	10.23±0.31 -16.25%	8.47±0.95* 24.17%	8.43±0.64 19.17%
	D3	65.76±0.68*¥ 2.74%	66.34±0.21* 6.90%	68.14±0.02*¥ 4.35%	69.22±0.01*¥ 1.54%	8.80±1.10 3.29%	11.23±1.05¥ -27.84%	8.53±1.24* 23.63%	8.43±0.64 19.17%
E	E1	68.60±0.06*¥ -1.59%	66.19±0.06* 7.11%	67.13±0.02*¥ 5.77%	68.22±0.01*¥ 2.95%	9.17±0.64 0.76%	11.25±1.03¥ -27.84%	8.80±1.42 21.22%	9.17±0.64 12.08%
	E2	67.77±0.69¥ -0.37%	72.37±1.85¥ -1.55%	67.27±0.24*¥ 5.57%	68.32±0.17*¥ 2.82%	8.70±1.11 4.39%	11.27±0.95¥ -28.07%	9.30±0.56 16.74%	9.17±0.64 12.08%
	E3	69.22±0.67*¥ -2.52%	69.47±0.37*¥ 2.51%	68.29±0.21*¥ 4.14%	69.22±0.01*¥ 1.54%	9.17±0.64 -0.77%	11.23±1.05¥ -27.61%	9.77±0.64¥ 12.53%	9.53±0.64 8.63%
F	F1	66.44±0.16*¥ 1.59%	68.95±0.70*¥ 3.24%	68.22±0.11*¥ 0.04%	69.22±0.01*¥ 1.54%	9.60±0.64 -5.49%	10.47±0.67¥ -18.97%	9.10±0.26 18.65%	10.13±0.21 2.87%
	F2	68.55±0.17¥ -1.53%	69.33±0.19*¥ 2.70%	69.26±0.17*¥ 2.77%	69.21±0.09* 1.55%	9.17±0.64 -0.76%	11.27±1.05¥ -28.07%	9.77±0.38¥ 12.54%	10.63±0.75 -1.92%
	F3	67.42±0.24¥ 0.15%	68.16±0.04*¥ 4.35%	69.19±0.06*¥ 2.87%	70.18±0.07* 0.17%	9.60±0.70 -5.49%	10.47±0.67¥ -18.98%	10.67±1.19¥ 4.47%	12.04±0.62 -15.05%

Table 4: Mean±SD values of Moisture (%) and Litter microbial count (CFU) are shown with percentage change

Groups		Moisture				Air Microbial Count			
		Week 1	Week2	Week3	Week4	Week1	Week2	Week3	Week4
A		66.93±1.45¥ 27%	73.67±2.75¥ 38%	77.20±1.31¥ 44%	76.53±1.50¥ 41%	45106.33±1888.08¥ 22%	40220.67±269.54¥ 18%	39949.67±73.66 11%	39232.33±611.70 15%
B		49.07±0.38*	45.93±0.06*	43.23±2.12*	44.93±4.24*	35055.33±38.84*	33071.33±935.94*	35430.33±1014.14	33297.33±1188.64
C		53.27±1.60* 20%	46.03±2.25* 38%	67.83±0.77¥ 12%	73.53±2.05¥ 40%	35298.00±1200.38* 21.74%	32957.33±1037.64* 18%	37258.67±42.89 7%	33123.00±1923.42 16%
D	D1	59.17±0.32*¥ 12%	50.90±0.10* 31%	56.77±1.70*¥ 26%	37.77±1.05*¥ 51%	37154.67±25.89* 18%	27127.00±3597.02* 32%	32306.67±880.98 19%	33408.33±2447.82 15%
	D2	58.63±1.09*¥ 12%	53.17±1.53*¥ 28%	64.63±1.21*¥ 16%	37.13±2.08*¥ 51%	36752.33±37.81* 18%	26906.00±4916.84* 33%	25551.33±6093.70* 36%	35064.67±43.08 11%
	D3	56.50±1.32*¥ 15%	73.50±0.10¥ 0.2%	65.70±1.82*¥ 14%	63.93±0.75*¥ 16%	35131.67±45.46* 22%	33443.00±2743.79 16%	26517.00±5573.0* 34%	35865.00±44.17 8%
E	E1	46.47±0.06* 30%	65.63±1.88*¥ 11%	48.43±0.55* 37%	75.27±3.22¥ 20%	37423.00±36.39* 17%	35225.00±2251.67 12%	30203.00±7772.27 24%	37930.00±16.70 3%
	E2	58.00±1.00*¥ 13%	77.63±0.06¥ -5%	74.40±0.92¥ 4%	62.47±0.95*¥ 18%	35050.33±40.10* 22%	35165.33±2350.68 12%	33673.33±1919.70 16%	28477.33±685.86* 27%
	E3	53.90±0.10*¥ 19%	64.27±3.22*¥ 13%	63.80±0.61*¥ 17%	55.60±2.82*¥ 27%	34151.00±26.23*¥ 24%	33540.67±2059.64 17%	33848.00±1318.11 15%	28865.67±109.37* 26%
F	F1	61.60±0.10*¥ 8%	62.63±0.06*¥ 15%	54.40±5.91*¥ 29%	41.20±1.00* 46%	38148.67±44.24* 15%	34304.00±1869.64 15%	31750.33±695.29 20%	29537.33±506.46* 25%
	F2	58.10±2.86*¥ 13%	64.57±2.72*¥ 12%	45.07±4.48* 41%	47.67±0.25* 38%	37258.67±42.89* 17%	32105.33±737.344* 20%	31653.33±458.48 21%	28206.33±684.35* 27.1%
	F3	65.63±0.06¥ 2%	75.60±0.10¥ -3%	54.47±6.66*¥ 29%	51.27±1.06*¥ 33%	35065.67±39.95* 22%	24928.33±468.23*¥ 38%	29639.67±6466.22 26%	28303.33±750.74* 28%

the control negative group, while the groups treated with 500 mg/m^2 ZnO and 1000 mg/m^2 ZnO showed significantly ($P<0.05$) lower moisture level as described in Table 4.

Air microbial count: The air microbial count in control positive group was significantly ($P<0.05$) increased from that of the control negative group in first 2 weeks, respectively while it showed non-significant difference in last two weeks. In week 1, all the treatment groups showed significantly ($P<0.05$) lower air microbial count, while in 2nd week, 500, 1000 mg/m^2 ZnO, 500, 1000 mg/m^2 alum showed significantly ($P<0.05$) lower air microbial count, while in week 3, the only group 1000 mg/m^2 ZnO showed significantly ($P<0.05$) lower air microbial count from that of control positive group. In week 4, the groups 200, 400 mg/m^2 CuSO4, 250, 500, 1000 mg/m^2 alum showed significantly ($P<0.05$) lower air microbial count, respectively from that of control positive group as shown in Table 4.

Litter microbial count: The litter microbial count in control positive group was significantly ($P<0.05$) increased from that of the control negative group in all the weeks, respectively. In week 1, the litter microbial count was significantly ($P<0.05$) lower in all the treatment groups from that of control positive group except of starch and 1000 mg/m^2 ZnO treated group. In week 2, all the treatment groups showed significantly ($P<0.05$) lower litter microbial count from that of control positive group except of starch, 1500 mg/m^2 ZnO and 100, 400 mg/m^2 CuSO4 and 1000 mg/m^2 alum treated groups. In week 3, the groups treated with 1500 mg/m^2 ZnO, 100, 200, 400 mg/m^2 CuSO4 and 1000 mg/m^2 alum showed significantly (P<0.05) lower litter microbial count by 52%, 44%, 17%, 22% and 54% from that of control positive group with a non-significant difference from the control negative group. In week 4, all the treatment groups showed significantly (P<0.05) lower litter microbial count from that of control positive group (Table 5).

DISCUSSION

Ammonia being a toxic gas is recognized as dominant in-house environmental contaminant of broilers chicken (David *et al.*, 2015). The control positive group in this study, showed significantly (P<0.05) increased ammonia level of 20.58, 15.91, 20.29 and 19.62 than the control negative group in all four weeks, which might be attributed to increased moisture level as well as environmental temperature. There was no increase observed over time owing to slaughtering of birds every week in the control positive group, so the birds number kept on decreasing and the space allocated remained the same, which might be the reason of a constant level of ammonia in the shed during the four-week period. High in-house ammonia concentration may be linked with increased moisture level and environmental temperature that has also been reported by Jiang *et al.* (2021). In week 1, groups 100, 400 mg/m^2 CuSO4 and 250 mg/m^2 alum showed significantly (P<0.05) higher ammonia levels of 22.52, 21.58 and 23.55 with a percent increase of 9.42%, 4.82 and 14.43 %, while groups 500, 1000 mg/m^2 alum showed significantly (P<0.05) lowest ammonia levels of 18.75 and 18.53 with percent improvement of 8.89 % and 9.96%, respectively than the positive control group. In week 2, all the treatment groups showed significantly (P<0.05) lower ammonia level from that of control positive group except the 500, 1000 mg/m^2 ZnO groups. In weeks 3 and 4, all the treatment groups showed significantly (P<0.05) lower ammonia level than control positive group. Low levels of ammonia in all the nanoparticles treated groups might be attributed to antibacterial effects of nanoparticles. Antibacterial activity of nanoparticles has also been reported by Menazea and Ahmed (2020) and Dat *et al.* (2021). Moore *et al*. (1996) investigated the effects of the alum on litter as alum reacts with the moisture of the litter, so ammonia volatilization is reduced along with reduction in the moisture contents of the litter.

The level of humidity was significantly (P<0.05) higher (67.52, 71.26, 71.24 and 70.30) with increased by 7, 7.11, 9.69 and 7.24% in control positive than the control negative group in all the 4 weeks, respectively. In the 1st week, groups 100, 400 mg/m^2 CuSO4 depicted significantly (P<0.05) increased humidity level (69.48, 68.60 and 69.22) with the increase by 2.82, 1.59 and 2.52 percent, while groups 500, 1000, 1500 mg/m^2 ZnO and 250 mg/m^2 alum showed significantly (P<0.05) lower level of humidity (66.31, 66.45, 65.76 and 66.44), with improvement by 1.79, 1.58, 2.74 and 1.59% than the control positive group, respectively. In comparison with the control negative group, all the treatment groups showed significantly (P<0.05) higher humidity level in 1st week. In case of 2nd week, humidity level of 65.19, 66.34, 66.19, 69.47, 68.95, 69.33, 68.16 was significantly (P<0.05) lower in groups 1000, 1500 mg/m^2 ZnO, 100, 400 mg/m^2 CuSO4, 250, 500, 1000 mg/m^2 alum with improvement by 1.35, 6.90, 7.11, 2.57, 3.24, 2.70 and 4.35%, respectively than the control positive group. Data from 3rd and 4th week revealed significantly (P<0.05) lower humidity level in all the treatment groups from the positive control group. Dunlop and Jim (2021) found that as relative humidity increased from 45 to 75%, and the ammonia levels became more variable and generally increased.

The litter pH was 11.17 in control positive group, which was significantly (P<0.05) higher from that of the control negative group in 3rd week. This higher pH

Table 5: Mean±SD values of Air microbial count (CFU) are presented with percentage change

Groups		Litter Microbial Count			
		Week 1	Week 2	Week 3	Week 4
A		293.00±5.57¥ 47%	299.00±1.00¥ 53%	301.00±1.00¥ 39%	280.00±21.93¥ 33%
B		157.67±22.81*	142.00±21.38*	183.33±100.17*	187.00±8.54*
C		242.00±13.74 17%	266.00±6.56¥ 11%	258.67±14.01* 14%	256.00±4.58¥ 9%
D	D1	188.67±9.61* 36%	140.33±13.87* 54%	156.00±22.27 48%	191.67±6.66* 31%
	D2	210.00±5.57 28%	141.33±14.57* 53%	151.67±3.51 49%	178.67±12.86* 36%
	D3	181.00±104.1* 38%	211.00±37.03 29%	145.33±8.33* 52%	142.00±21.38* 49%
E	E1	119.33±2.08* 59%	259.67±33.50¥ 13%	167.33±76.79* 44%	140.33±13.87* 49%
	E2	127.67±3.05* 56%	252.33±37.07¥ 16%	249.33±6.11* 17%	141.33±14.57* 49%
	E3	102.33±2.52* 65%	236.33±22.50 21%	235.00±20.52* 22%	144.33±40.05* 48%
F	F1	114.33±4.04* 61%	178.00±55.22* 40%	248.67±7.09 17%	159.67±35.50* 43%
	F2	109.67±7.57* 62%	148.33±44.52* 50%	131.33±0.26 56%	162.33±22.89* 42%
	F3	134.67±25.89* 54%	207.67±16.19 30%	138.33±22.23* 54%	136.33±22.50* 51%

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

facilitates the growth of various bacteria. Data from 3rd week depicted significantly (P<0.05) lower pH value (8.07, 8.47 and 8.53) in groups 500, 1000, 1500 mg/m^2 ZnO with improvement by 27.75, 24.17 and 23.63%, respectively from the control positive group. The release of ammonia is directly influenced by the pH of the litter (Chapman *et al.*, 2021). The decrease in pH might be attributed to the pH buffering capacity of ZnO nanoparticles. The pH buffering capacity of nanoparticles has also been reported by Singappuli-Arachchige and Slowing (2020), as they used silica nanoparticles in their study. Reece *et al*. (1979) reported that very little NH3 was released from litter with a pH below 7, whereas it was rapidly released from litter with a pH above 8.

The air microbial count in control positive group was significantly (P<0.05) increased by 22 and 18% from that of the control negative group in first 2 weeks, respectively while it showed non-significant difference in last two weeks. In week 1, all the treatment groups showed significantly (P<0.05) lower air microbial count, while in 2nd week, 500, 1000 mg/m^2 ZnO, 500, 1000 mg/m^2 alum showed significantly (P<0.05) lower air microbial count by 32, 33, 20 and 38%, while in week 3, the only group 1000 mg/m^2 ZnO showed significantly (P<0.05) lower air microbial count by 36% from that of control positive group. In week 4, the groups 200, 400 mg/m^2 CuSO4, 250, 500, 1000 mg/m^2 alum showed significantly (P<0.05) lower air microbial count by 27, 26, 25, 27 and 28%, respectively from that of control positive group. This also clearly indicated antibacterial activity of nanoparticles used in this study.

The litter microbial count in control positive group was significantly (P<0.05) increased by 47, 53, 39 and 33% from that of the control negative group in all the weeks, respectively. In week 1, the litter microbial count was significantly (P<0.05) lower in all the treatment groups from that of control positive group except of starch and 1000 mg/m^2 ZnO treated group. In week 2, all the treatment groups showed significantly (P<0.05) lower litter microbial count from that of control positive group except of starch, 1500 mg/m^2 ZnO and 100, 400 mg/m^2 CuSO4 and 1000 mg/m^2 alum treated groups. In week 3, the groups treated with 1500 mg/m^2 ZnO, 100, 200, 400 mg/m^2 CuSO4 and 1000 mg/m^2 alum showed significantly (P<0.05) lower litter microbial count by 52, 44, 17, 22 and 54% from that of control positive group with a non-significant difference from the control negative group. In week 4, all the treatment groups showed significantly (P<0.05) lower litter microbial count from that of control positive group. Thus, indicating antimicrobial activity of nanoparticles. The antibacterial activity linked with ZnO and CuO nanoparticles have also been reported by Paul *et al.* (2020).

Conclusions: It can be concluded that increased concentrations of ammonia adversely affect the in-house environment of the poultry house which is linked with moisture, humidity, air and litter microbes. To overcome the problem, nanoparticles of zinc oxide, copper oxide and aluminium sulphate proved to have an antibacterial effect to lessen the problems of poor in-house environment of the poultry house.

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REFERENCES

Al-Homidan I, Robertson J and Petchey A, 2003. Review of the effect of ammonia and dust concentrations on broiler performance. Worlds Poult Sci J 59:340-9.

Aziz T and Barnes HJ, 2009. Harmful effects of ammonia on birds. World Poult 26:28–30.

Blake JP and Hess JB, 2001. Sodium bisulfate as a litter treatment. Ala Coop Ext Serv ANR-1208.

Chapman T, Burt CD, Bachoon D, *et al.,* 2021. The effect of an acidified-gypsum mixture on broiler litter urease-producing bacteria and nitrogen mineralization. J Environ Qual https://doi.org/10.1002/jeq2.20229

Dat NM, Khang PT, Anh TNM, *et al.,* 2021. Synthesis, characterization, and antibacterial activity investigation of silver nanoparticle-decorated graphene oxide. Mater Lett 285:128993.

David B, Mejdell C, Michel V, *et al.*, 2015. Air Quality in Alternative Housing Systems may have an Impact on Laying Hen Welfare. Part II-Ammonia. Animals : an open access journal from MDPI 5:886-96.

Hallaj-Nezhadi S and Hassan M, 2013. Nanoliposome-based antibacterial drug delivery. Drug Delivery 22:581-9.

Jiang J, Stevenson DS, Uwizeye A, *et al.,* 2021. A climate-dependent global model of ammonia emissions from chicken farming. Biogeosciences 18:135-58.

Kon K and Rai M, 2013. Metallic nanoparticles: Mechanism of antibacterial action and influencing factors. J Comp Clin Path Res 2:160-74.

Kreuter J, 2007. Nanoparticles—A historical perspective. Inter J Pharm 331:1-10.

Maliselo PS and Nkonde GK, 2015. Ammonia production in poultry houses and its effect on the growth of *Gallus gallus domestica* (broiler chickens): a case study of a small scale poultry house in Riverside, Kitwe, Zambia. Int J Sci Technol Res 4:141-5.

Manyasree D, Kiran P and Ravikumar R, 2017. CuO nanoparticles: Synthesis, characterization and their bactericidal efficacy. International J Appl Pharm 9:71-4.

Manyasree D, Kiranmayi P and Venkata K, 2018. Characterization and antibacterial activity of ZnO nanoparticles synthesized by co precipitation method. Intern J Appl Pharm 10:224-8.

Menazea AA and Ahmed MK, 2020. Synthesis and antibacterial activity of graphene oxide decorated by silver and copper oxide nanoparticles. J Mol Struc 1218:128536.

Moore PA, Daniel TC, Edwards DR and Miller DM, 1996. Evaluation of Chemical Amendments to Reduce Ammonia Volatilization from Poultry Litter. Poult Sci 75:315-20.

Odebunmi E, Oluwaniyi O and Bashiru M, 2010. Comparative proximate analysis of some food condiments. J Appl Sci Res 6:272-4.

Patterson P and Adrizal A, 2005. Management Strategies to Reduce Air Emissions: Emphasis-Dust and Ammonia. J Appl Poult Res 14:638-50.

Paul D, Mangla S and Neogi S, 2020. Antibacterial study of CuO-NiO-ZnO trimetallic oxide nanoparticle. Mater Lett 271:127740.

Phiwdang K, Phensaijai M and Pecharapa W, 2013. Study of antifungal activities of CuO/ZnO nanocomposites synthesized by co-precipitation method. Adv Materials Res 802:89-93.

Raffi M, Mehrwan S, Bhatti TM, *et al.,* 2010. Investigations into the antibacterial behavior of copper nanoparticles against Escherichia coli. Annals of Microbiol 60:75–80.

Reddy KM, Feris K, Bell J, *et al.,* 2007. Selective toxicity of zinc oxide nanoparticles to prokaryotic and eukaryotic systems. Appl phys lett, 90:2139021-3.
Reece FN, Bates BJ and Lott BD, 1979. Ammonia control in broiler houses. Poultry Sci 58:754.
SAS, I.I. 2018. SAS/STAT® 15.1 User's Guide. Cary, NC: STAT Institute. Inc, USA.
Singappuli-Arachchige D and Slowing II, 2020. Control of interfacial pH in mesoporous silica nanoparticles via surface functionalization. J Chem Phy 152:034703.
Stoimenov P, Klinger RL, Marchin GL and Klabunde K, 2002. Metal oxide nanoparticles as bactericidal agents. Langmuir 18:6679-86.
Terzich M, Pope MJ, Cherry TE, Hollinger J, 2000. Survey of pathogens in poultry litter in the United States. J Appl Poult Res 9:287-91.
Ullman J, Saqib M, Ronald L and John C, 2004. A Review of literature concerning odors, ammonia, and dust from broiler production facilities: Remedial Management Practices. J Appl Poult Res 13:521-31.
Dunlop MW and Jim M, 2021. Direct surface wetting sprinkler system to reduce the use of evaporative cooling pads in meat chicken production: indoor thermal environment, water usage, litter moisture content, live market weights, and mortalities. Poult Sci 100 https://doi.org/10.1016/j.psj.2021.101078.
Wójcik A, Chorąży L, Mituniewicz T, Witkowska D, Iwańczuk-Czernik K and Sowińska J, 2010. Microbial Air Contamination in Poultry Houses in the Summer and Winter. Polish J Environ Studies 19:1045-50.
Yi B, Chen L, Sa R, *et al.,* 2016. Transcriptome profile analysis of breast muscle tissues from high or low levels of atmospheric ammonia exposed broilers (*Gallus gallus*). Plos One 11:e0162631.