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Use of nanotechnology can improve the in-house environment and enhance the growth indicators of broilers

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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 ammonia production. For this purpose, this study was performed for 42 days, including 540-day-old broiler birds. The birds were randomly divided into six groups A-E and X, while the groups C, D and E were further divided into four sub-groups at 15th day. First two groups were kept as a positive and negative control. Birds were treated with combination of three types of nanoparticles, i.e., alum, zinc oxide and copper oxide in combination along with starch as an excipient powder. 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 250ml per day for first week of treatment trial. Ammonia, Temperature, moisture, humidity, litter pH were recorded daily throughout the experiment. Air and litter microbial count were recorded on weekly basis. Birds from each group were slaughtered at the 22nd and 42nd day of the final age of the birds. The results showed that positive control group showed significantly ($P<0.05$) increased levels of ammonia, humidity, litter moisture, litter pH level, air and litter microbial count as compared to the treatment groups. However, the combination of lower dose nanoparticles of copper oxide, zinc oxide and alum showed better effect in improving the in-house environment as compared to the negative control group. The study showed that Copper oxide, zinc oxide and alum nanoparticles can be used as possible substitute to overcome the in-house environment problems in broiler birds. However, further studies are suggested at commercial setting to see the real impact of these nanoparticles.

Keywords: Ammonia, air microbes, litter microbes, nanoparticles, broilers

INTRODUCTION

Poultry production faces many problems that include dust, bacterial and viral diseases and the production of many odorous chemical compounds from litter material. The ammonia in poultry houses is the most important air contaminant, its irritating nature causes respiratory distress of the broiler birds that effect the immune system of the birds. It also effects the lungs and air sacs by disrupting the cilia resulting in infection (Aziz and Barnes 2010; 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 content of the poultry house depends on ventilation, temperature and relative humidity (Ullman et al. 2004). Ammonia levels above 25 ppm have been found problem for poultry houses. Temperature also plays important role in increasing the bacterial activity and production of ammonia (Mahmoud et al. 2020). Exposure of chicken to high temperature has been reported to hinder the performance in chicken production. it could be further compounded by increased relative humidity for its negative impact on evaporative cooling (Zarnab et al. 2019). The symptoms of ammonia toxicity are sneezing, tracheal irritation, air sac inflammation, conjunctivitis, and dyspnea. The high ammonia level causes reduction in weight gain, feed efficiency and causes damage to the respiratory system leading to mortality. Furthermore, it increases the chances of occurrence of ND and mycoplasmosis (Patterson and Adrizal, 2005).

However, to face this emerging challenge to treat bacterial infections in poultry, nanotechnology has been introduced to conquer the microbial resistance due to its wide range of antibacterial activity (Hallaj-Nezhadi et al. 2013). Different metal oxides like copper, titanium, iron and zinc oxide have proved of remarkable importance in different environmental and biomedical field of studies. Nanoparticles have antibacterial activity against both gram positive and negative bacteria, and fungi (Hallaj-Nezhadi et al. 2013). "Nanoparticles" (NPs) have been described as solid colloidal particles ranging in size from 1-100 nm (Kreuter, 2007).

Different environmental modifiers like zinc, copper sulfate and alum can be used to improve the poultry house environment (Blake et al. 2001). Different types of litter modifiers, including acidifiers, alkaline materials, and different enzymatic or microbial treatments and feed modifications are also effective to reduce the ammonia production (Meda et al. 2011). These modifiers are very effective to decrease the bacterial or enzyme activity in the litter and reduces the volatilization of ammonia. It was 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 (Zarnab et al. 2019).

The nanoparticles of copper have shown an important role in elimination of different microbial infections. The nanoparticles treated in wastewater disrupts the bioactivity of microbes and affect. Copper in the form of $CuSO_4$ is known to have antibacterial activity (Raffi et al. 2010). Copper has also been reported as an antifungal agent (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 (Stoimenor 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 rate, to have efficient poultry production, while preserving the consumer health by avoiding antibiotic residues. There is considerable work being done in evaluating the use of 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 and to observe their effect on live, carcass weights and feed conversion ratio (FCR).

MATERIALS AND METHODS

Ethical approval:

The proposed design of study was approved by ethical committee of UAF ensuring comfort and welfare of the birds.

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).

The nanoparticles of copper sulphate /zinc oxide /alum will be synthesized by using respective chemicals of copper sulphate (Manyasree et al. 2017) / zinc chloride (Manyasree et al. 2018) and aluminium sulphate, while sodium hydroxide were used as precursor in all the three preparations. Complete dissolution of chemicals was made and then under constant stirring an amount of 2M of sodium hydroxide was added drop by drop touching the beaker walls. The reaction procedure was carried further for two hours. The solution was allowed to settle overnight. After this the supernatant was discarded and the precipitate was washed several times by using distilled water. After washing the precipitate was dried overnight at 80 °C. The precipitate was grinded further to very fine particles and at end placed in furnace at 500 °C. Then it was weighed and characterized.

Plan of Study

The experimental trial was conducted at the poultry shed of Parasitology Department, University of Agriculture Faisalabad in January, 2021. The litter material used was rice husk. The average temperature outside the house was 20-22℃ with humidity 70%. So, the shed was prepared with the required conditions of temperature, humidity and ventilation to produce the ammonia in winter season.

A total 300 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 15^{th} day, the birds were randomly divided into different groups (A-E and X), where C, D and E having 4 subgroups. The groups A and B were kept as control positive and control negative, respectively. The group X was treated with starch only. The groups C, D and E, each have further 4 subgroups, i.e., C1 (500mg/m^2 ZnO + 20g starch), C2 (1000mg/m^2 ZnO + 20g starch), C3 (1500mg/m^2 ZnO + 20g starch); D1 (100mg/m^2 CuSO4 + 20g starch), D2 (200mg/m^2 CuSO4 + 20g starch), D3 (400mg/m^2 CuSO4 + 20g starch); E1 (250mg/m^2 alum + 20g starch), E2 (500mg/m^2 alum + 20g starch) and E3 (1000mg/m^2 alum + 20g starch). Each subgroups carry 20 birds as described in Table 1. 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.

Table 1: Layout of Experiment

Group Name		Number of Birds	Treatment (15th day)
A		20	Positive Control (only H_2O @250 ml)
B		20	Negative Control
X		20	Starch sprayed @ 20 grams (H_2O @250 ml by creating wet litter conditions)
C	C1	20	ZnO @500 mg/ m^3+ CuSO4@ 100 mg/ m^2
	C2	20	ZnO @ 1500 mg/ m^2 + CuSO4 @ 400 mg/ m^2
	C3	20	ZnO @ 500 mg/ m^2 + CuSO4 @ 400 mg/ m^2
	C4	20	ZnO @ 1500 mg/ m^2+ CuSO4 @ 100 mg/ m^2
D	D1	20	Alum @250 mg/ m^2+ ZnO @ 500mg/ m^2
	D2	20	Alum @1000 mg/ m^2+ ZnO @ 1500 mg/ m^2
	D3	20	Alum @250 mg/ m^2 + ZnO @ 1500mg/ m^2
	D4	20	Alum @1000 mg/ m^2 + ZnO @ 500 mg/ m^2
E	E1	20	CuSO4 @100 mg/ m^2 + Alum @ 250 mg/ m^2
	E2	20	CuSO4 @400 mg/ m^2 + Alum @ 1000 mg/ m^2
	E3	20	CuSO4 @100 mg/ m^2 + Alum @ 1500 mg/ m^2
	E4	20	CuSO4 @400 mg/ m^2 + Alum @ 250 mg/ m^2

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 materials 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 Wojcik et al. (2010). Moisture level was also determined by performing proximate analysis (Odebumni 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 results of ammonia levels in different groups are presented in Table 1. The control positive group showed significantly ($P<0.05$) increased ammonia level than the control negative group in all four weeks.

In week 1, the ammonia level was significantly ($P<0.05$) lower in the groups C2 (ZnO @ 1500 mg/ m^2 + CuSO4 @ 400 mg/ m^2), C4 (ZnO @ 1500 mg/ m^2+ CuSO4 @ 100 mg/ m^2), E2 (CuSO4 @400 mg/ m^2 + Alum @ 1000 mg/ m^2) and E3 (CuSO4 @100 mg/ m^2 + Alum @ 1500 mg/ m^2) as compared to the positive control group.

In week 2, the level of ammonia was significantly ($P<0.05$) lower in the treated groups including C1 (ZnO @500 mg/ m^3+ CuSO4@ 100 mg/ m^2), C2 (ZnO @ 1500 mg/ m^2 + CuSO4 @ 400 mg/ m^2), C4 (ZnO @ 1500 mg/ m^2+ CuSO4 @ 100 mg/ m^2), D1 (Alum @250 mg/ m^2+ ZnO @ 500mg/ m^2), D4 (Alum @1000 mg/ m^2 + ZnO @ 500 mg/ m^2), E1 (CuSO4 @100 mg/ m^2 + Alum @ 250 mg/ m^2), E2 (CuSO4 @400 mg/ m^2 + Alum @ 1000 mg/ m^2) and E4 (CuSO4 @400 mg/ m^2 + Alum @ 250 mg/ m^2) than the control positive group.

In week 3, the ammonia level was significantly ($P<0.05$) lower in all the treatment groups as compared to the positive control group.

In week 4, the level of ammonia was significantly ($P<0.05$) lower in all the treatment groups than the positive control group.

1.3.2. Temperature:

The results on temperature in different treatment groups and control groups are presented in Table 2.

The results in all the four weeks of were non-significantly different between control positive and negative groups.

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

Humidity:

The results on humidity levels in the environment at bird level in different treatment groups and control groups are presented in Table 3.

Th level of humidity was significantly ($P<0.05$) higher in control positive group than the control negative group in all the 3 weeks.

Table 2: The effect of different combination of nanoparticles on In-House Ammonia levels

Groups		Ammonia (PPM) W1 Mean ±SD	W2 Mean ±SD	W3 Mean ±SD	W4 Mean ±SD
A (Control Positive)		20.58¥ ±0.56	15.91¥ ±0.65	20.29¥ ±0.20	19.62¥ ±0.38
B (Control Negative)		2.36 ±0.04	3.40* ±0.56	3.81* ±0.39	1.77* ±0.69
X (Starch)		19.36*¥ ±0.15	14.59*¥ ±0.07	18.38*¥ ±0.64	13.61*¥ ±0.08
C	C1 (ZnO @500 mg/ m^3+ CuSO4@ 100 mg/ m^2)	19.76¥ ±0.18	10.43*¥ ±0.18	12.43*¥ ±0.28	6.58*¥ ±0.27
	C2 (ZnO @ 1500 mg/ m^2 + CuSO4 @ 400 mg/ m^2)	18.56*¥ ±0.08	12.64*¥ ±0.01	11.32*¥ ±0.18	5.57*¥ ±0.07
	C3 (ZnO @ 500 mg/ m^2 + CuSO4 @ 400 mg/ m^2)	17.56¥ ±0.08	15.39¥ ±0.21	10.35*¥ ±0.16	4.58*¥ ±0.31
	C4 (ZnO @ 1500 mg/ m^2+ CuSO4 @ 100 mg/ m^2)	18.75*¥ ±0.17	14.32*¥ ±0.17	9.67*¥ ±0.17	5.44*¥ ±0.211
D	D1 (Alum @250 mg/ m^2+ ZnO @ 500mg/ m^2)	16.45¥ ±0.12	14.28*¥ ±0.22	9.62*¥ ±0.06	6.43*¥ ±0.18
	D2 (Alum @1000 mg/ m^2+ ZnO @ 1500 mg/ m^2)	17.53¥ ±0.11	16.17¥ ±0.06	9.79*¥ ±0.13	6.59*¥ ±0.38
	D3 (Alum @250 mg/ m^2 + ZnO @ 1500mg/ m^2)	18.39¥ ±0.34	15.26¥ ±0.05	8.61*¥ ±0.08	6.75*¥ ±0.68
	D4 (Alum @1000 mg/ m^2 + ZnO @ 500 mg/ m^2)	16.53¥ ±0.31	14.45*¥ ±0.11	10.03*¥ ±0.33	6.58*¥ ±0.38
E	E1 (CuSO4 @100 mg/ m^2 + Alum @ 250 mg/ m^2)	17.73¥ ±0.24	12.26*¥ ±0.06	10.04*¥ ±0.17	7.63*¥ ±0.19
	E2 (CuSO4 @400 mg/ m^2 + Alum @ 1000 mg/ m^2)	18.56*¥ ±0.07	14.46*¥ ±0.11	10.07*¥ ±0.27	2.50* ±0.16
	E3 (CuSO4 @100 mg/ m^2 + Alum @ 1500 mg/ m^2)	18.43*¥ ±0.22	15.64 ±0.01¥	9.82*¥ ±0.39	3.61*¥ ±0.75
	E4 (CuSO4 @400 mg/ m^2 + Alum @ 250 mg/ m^2)	17.56¥ ±0.08	13.21*¥ ±0.01	9.02*¥ ±0.72	4.55*¥ ±0.11

Table 3: The effect of different combination of nanoparticles on Poultry House Environmental Temperature

Groups		Temperature (℃) W1 Mean ±SD	W2 Mean ±SD	W3 Mean ±SD	W4 Mean ±SD
A (Control Positive)		26.00 ±1.00	24.00 ±1.00	22.00 ±1.00	22.00 ±1.00
B (Control Negative)		25.67 ±0.58	24.33 ±0.58	23.33 ±1.15	21.33 ±0.58
X(Starch)		27.00 ±1.00	24.00 ±1.00	22.67 ±0.58	21.67 ±1.15
C	C1 (ZnO @500 mg/ m^3+ CuSO4@ 100 mg/ m^2)	27.00 ±1.00	24.67 ±1.53	22.00 ±1.00	22.00 ±1.00
	C2 (ZnO @ 1500 mg/ m^2 + CuSO4 @ 400 mg/ m^2)	27.00 ±1.00	24.00 ±1.00	22.33 ±1.53	21.33 ±1.15
	C3 (ZnO @ 500 mg/ m^2 + CuSO4 @ 400 mg/ m^2)	26.33 ±0.58	24.00 ±1.00	22.00 ±1.00	22.33 ±1.15

	C4 (ZnO @ 1500 mg/ m^2+ CuSO4 @ 100 mg/ m^2)	26.67 ±1.53	25.00 ±1.00	21.67 ±0.58	22.00 ±1.00
D	**D1 (Alum @250 mg/ m^2+ ZnO @ 500mg/ m^2)**	25.00 ±1.00	25.00 ±1.00	23.33 ±0.58	21.67 ±0.58
	D2 (Alum @1000 mg/ m^2+ ZnO @ 1500 mg/ m^2)	25.00 ±1.00	24.33 ±0.58	22.00 ±1.00	21.00 ±1.73
	D3 (Alum @250 mg/ m^2 + ZnO @ 1500mg/ m^2)	26.67 ±0.58	25.00 ±1.00	22.33 ±1.15	22.33 ±1.53
	D4 (Alum @1000 mg/ m^2 + ZnO @ 500 mg/ m^2)	27.00 ±1.00	24.67 ±0.58	22.33 ±0.58	21.67 ±0.58
E	**E1 (CuSO4 @100 mg/ m^2 + Alum @ 250 mg/ m^2)**	27.33 ±1.15	25.67 ±0.58	22.00 ±1.00	22.00 ±1.00
	E2 (CuSO4 @400 mg/ m^2 + Alum @ 1000 mg/ m^2)	27.00 ±1.00	24.00 ±1.00	22.33 ±0.58	23.33 ±1.58
	E3 (CuSO4 @100 mg/ m^2 + Alum @ 1500 mg/ m^2)	25.00 ±1.00	24.67 ±0.58	21.67 ±0.58	21.33 ±1.53
	E4 (CuSO4 @400 mg/ m^2 + Alum @ 250 mg/ m^2)	27.00 ±1.00	25.00 ±1.00	22.33 ±1.15	21.33 ±1.15

In week 1, level of humidity was significantly ($P<0.05$) higher in the treatment groups D1 (Alum @250 mg/ m^2+ ZnO @ 500mg/ m^2), D2 (Alum @1000 mg/ m^2+ ZnO @ 1500 mg/ m^2), D3 (Alum @250 mg/ m^2 + ZnO @ 1500mg/ m^2), D4 (Alum @1000 mg/ m^2 + ZnO @ 500 mg/ m^2) and E4 (CuSO4 @400 mg/ m^2 + Alum @ 250 mg/ m^2) as compared to the positive control group.

In week 2, the level of humidity was significantly ($P<0.05$) lower in treated groups C1 (ZnO @500 mg/ m^3+ CuSO4@ 100 mg/ m^2), C2(ZnO @ 1500 mg/ m^2 + CuSO4 @ 400 mg/ m^2), C3(ZnO @ 500 mg/ m^2 + CuSO4 @ 400 mg/ m^2), D2(Alum @1000 mg/ m^2+ ZnO @ 1500 mg/ m^2), D3(Alum @250 mg/ m^2 + ZnO @ 1500mg/ m^2) and D4 (Alum @1000 mg/ m^2 + ZnO @ 500 mg/ m^2) than that of positive control group.

In week 3, the humidity level was significantly ($P<0.05$) lower in the treatment groups C2 (ZnO @ 1500 mg/ m^2 + CuSO4 @ 400 mg/ m^2), C4 (ZnO @ 1500 mg/ m^2+ CuSO4 @ 100 mg/ m^2), D2 (Alum @1000 mg/ m^2+ ZnO @ 1500 mg/ m^2), D3 (Alum @250 mg/ m^2 + ZnO @ 1500mg/ m^2), D4 (Alum @1000 mg/ m^2 + ZnO @ 500 mg/ m^2), E1 (CuSO4 @100 mg/ m^2 + Alum @ 250 mg/ m^2), E2 (CuSO4 @400 mg/ m^2 + Alum @ 1000 mg/ m^2), E3 (CuSO4 @100 mg/ m^2 + Alum @ 1500 mg/ m^2) and E4 (CuSO4 @400 mg/ m^2 + Alum @ 250 mg/ m^2) than the positive control group.

In 4th week, the humidity level was significantly ($P<0.05$) lower in all the treated groups except C1 (ZnO @500 mg/ m^3+ CuSO4@ 100 mg/ m^2), D1 (Alum @250 mg/ m^2+ ZnO @ 500mg/ m^2), D2 (Alum @1000 mg/ m^2+ ZnO @ 1500 mg/ m^2), E1 (CuSO4 @100 mg/ m^2 + Alum @ 250 mg/ m^2) and E2 (CuSO4 @400 mg/ m^2 + Alum @ 1000 mg/ m^2) than the control positive group.

Litter pH:

The results on litter pH in different treatment groups and control groups are presented in Table. 4. The litter pH of control positive group was significantly ($P<0.05$) higher from that of control negative group in 3rd week. In 1st, 2nd and 4th week control positive group showed non-significantly difference in pH value than the control negative group.

In week 1, all the treatment groups showed non-significant difference from both control positive and control negative group except the group D1 (Alum @250 mg/ m^2+ ZnO @ 500mg/ m^2), which showed significantly ($P<0.05$) higher level of pH than the control positive group.

In week 2, all the treatment groups showed non-significant ($P>0.05$) difference than the control positive group.

In week 3, a significantly ($P<0.05$) lower pH value was recorded in group D1 (Alum @250 mg/ m^2+ ZnO @ 500mg/ m^2) from than of the control positive group.

In week 4, all the treatment groups showed non-significant difference from both the control positive and control negative groups.

Moisture Level:

The results on moisture levels in the litter in different treatment groups and control groups are presented in Table. 5. The moisture level in the control positive group was significantly ($P<0.05$) higher from that of control negative group in all the four weeks.

In week 1, all the treatment groups except groups C4 (ZnO @ 1500 mg/ m^2+ CuSO4 @ 100 mg/ m^2) and D1 (Alum @250 mg/ m^2+ ZnO @ 500mg/ m^2) showed significantly ($P<0.05$) higher level of moisture than the control positive group.

In week 2, all the treated groups showed ($P<0.05$) lower moisture level from that of control positive group except groups C1 (ZnO @500 mg/ m^3+ CuSO4@ 100 mg/ m^2), C4 (ZnO @ 1500 mg/ m^2+ CuSO4 @ 100 mg/ m^2) and D2 (Alum @1000 mg/ m^2+ ZnO @ 1500 mg/ m^2).

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Table 4; The effect of different combination of nanoparticles on Poultry House Humidity

Groups		Humidity % W1 Mean±SD	W2 Mean ±SD	W3 Mean±SD	W4 Mean ±SD
A (Control Positive)		67.52¥ ±0.06	71.26¥ ±0.06	71.24¥ ±0.94	70.30¥ ±0.31
B (Control Negative)		62.52* ±0.61	66.19* ±0.29	64.33* ±0.19	65.21* ±1.10
X (Starch)		69.48*¥ ±0.22	70.36¥ ±0.13	68.57*¥ ±0.57	69.48*¥ ±0.27
C	C1 (ZnO @500 mg/ m^3+ CuSO4@ 100 mg/ m^2)	68.36¥ ±0.19	68.32*¥ ±0.17	71.18¥ ±0.06	70.33¥ ±0.15
	C2 (ZnO @ 1500 mg/ m^2 + CuSO4 @ 400 mg/ m^2)	68.07¥ ±0.63	67.16* ±0.06	70.19*¥ ±0.07	69.29 ±0.13*¥
	C3 (ZnO @ 500 mg/ m^2 + CuSO4 @ 400 mg/ m^2)	67.64¥ ±0.18	67.29* ±0.11	71.29 ±0.12¥	68.28 ±0.11*¥
	C4 (ZnO @ 1500 mg/ m^2+ CuSO4 @ 100 mg/ m^2)	67.30¥ ±0.13	70.18¥ ±0.06	70.19*¥ ±0.06	69.18 ±0.05*¥
D	D1 (Alum @250 mg/ m^2+ ZnO @ 500mg/ m^2)	69.43*¥ ±0.18	70.18¥ ±0.06	70.42¥ ±0.17	70.22¥ ±0.01
	D2 (Alum @1000 mg/ m^2+ ZnO @ 1500 mg/ m^2)	69.35*¥ ±0.15	69.34*¥ ±0.18	69.26*¥ ±0.06	70.34¥ ±0.19
	D3 (Alum @250 mg/ m^2 + ZnO @ 1500mg/ m^2)	69.53*¥ ±0.07	69.38*¥ ±0.15	68.65*¥ ±0.52	69.31*¥ ±0.17
	D4 (Alum @1000 mg/ m^2 + ZnO @ 500 mg/ m^2)	69.41*¥ ±0.16	68.29*¥ ±0.23	68.22*¥ ±0.01	69.22*¥ ±0.10
E	E1 (CuSO4 @100 mg/ m^2 + Alum @ 250 mg/ m^2)	68.31¥ ±0.12	68.30¥ ±0.19	69.39*¥ ±0.12	70.25¥ ±.06
	E2 (CuSO4 @400 mg/ m^2 + Alum @ 1000 mg/ m^2)	68.51¥ ±0.15	70.22¥ ±0.01	68.28*¥ ±0.11	70.42¥ ±0.40
	E3 (CuSO4 @100 mg/ m^2 + Alum @ 1500 mg/ m^2)	68.34¥ ±0.18	70.28¥ ±0.21	68.29*¥ ±0.11	68.23*¥ ±0.01
	E4 (CuSO4 @400 mg/ m^2 + Alum @ 250 mg/ m^2)	69.44*¥ ±0.15	70.19¥ ±0.06	68.19*¥ ±0.06	68.39*¥ ±0.16

In week 3, all the treatment groups showed significantly (P<0.05) lower moisture level from that of control positive group except X (starch) treated group.

In week 4, all the treatment groups showed significantly (P<0.05) lower moisture level from that of control positive group except of group X (starch), C4 (ZnO @ 1500 mg/ m^2+ CuSO4 @ 100 mg/ m^2), D1(Alum @250 mg/ m^2+ ZnO @ 500mg/ m^2) and E2 (CuSO4 @400 mg/ m^2 + Alum @ 1000 mg/ m^2) groups

Air Microbial Count:

The results on air microbial count in different treatment and control groups are presented in Table 6. The air microbial count in control positive group was significantly (P<0.05) higher from that of control negative group in the first 2 weeks, while it showed non-significant difference in last two weeks.

In week 1, all the treatment groups revealed significantly (P<0.05) lower air microbial count from that of control positive group.

In week 2, the treatment groups X (starch), C1 (ZnO @500 mg/ m^3+ CuSO4@ 100 mg/ m^2), C3 (ZnO @ 500 mg/ m^2 + CuSO4 @ 400 mg/ m^2), C4 (ZnO @ 1500 mg/ m^2+ CuSO4 @ 100 mg/ m^2), D2 (Alum @1000 mg/ m^2+ ZnO @ 1500 mg/ m^2), D3 (Alum @250 mg/ m^2 + ZnO @ 1500mg/ m^2), D4 (Alum @1000 mg/ m^2 + ZnO @ 500 mg/ m^2), E1 (CuSO4 @100 mg/ m^2 + Alum @ 250 mg/ m^2), E3 (CuSO4 @100 mg/ m^2 + Alum @ 1500 mg/ m^2) and E4 (CuSO4 @400 mg/ m^2 + Alum @ 250 mg/ m^2) showed significantly (P<0.05) lower air microbial count from that of control positive group.

In week 3, the treatment group C3 (ZnO @ 500 mg/ m^2 + CuSO4 @ 400 mg/ m^2) showed significantly (P<0.05) lower air microbial count from that of control positive group.

In week 4, the treatment groups C2 (ZnO @ 1500 mg/ m^2 + CuSO4 @ 400 mg/ m^2), C3 (ZnO @ 500 mg/ m^2 + CuSO4 @ 400 mg/ m^2), C4 (ZnO @ 1500 mg/ m^2+ CuSO4 @ 100 mg/ m^2), D1 (Alum @250 mg/ m^2+ ZnO @ 500mg/ m^2), D2 (Alum @1000 mg/ m^2+ ZnO @ 1500 mg/

m^2), E1 ($CuSO_4$ @100 mg/ m^2 + Alum @ 250 mg/ m^2), E2 ($CuSO_4$ @400 mg/ m^2 + Alum @ 1000 mg/ m^2) and E3 ($CuSO_4$ @100 mg/ m^2 + Alum @ 1500 mg/ m^2) showed significantly ($P<0.05$) lower air microbial count from that of control positive group.

Litter Microbial Count:

The results on litter microbial count in different treatment groups and control groups are presented in Table 7. The litter microbial count in control positive group was significantly ($P<0.05$) increased from that of control negative group in all the four weeks.

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 X (starch) and D2 (Alum @1000 mg/ m^2+ ZnO @ 1500 mg/ m^2) groups.

In week 2, all the treatment groups showed significantly ($P<0.05$) lower litter microbial count from that of control positive group except groups C1(ZnO @500 mg/ m^3+ $CuSO_4$@ 100 mg/ m^2), C2 (ZnO @ 1500 mg/ m^2 + $CuSO_4$ @ 400 mg/ m^2) and D4 (Alum @1000 mg/ m^2 + ZnO @ 500 mg/ m^2).

In week 3, the treatment groups C1 (ZnO @500 mg/ m^3+ $CuSO_4$@ 100 mg/ m^2), C2 (ZnO @ 1500 mg/ m^2 + $CuSO_4$ @ 400 mg/ m^2) and D4 (Alum @1000 mg/ m^2 + ZnO @ 500 mg/ m^2) showed significantly ($P<0.05$) lower litter microbial count from that of control positive 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: The effect of different combination of nanoparticles on poultry house litter pH

Groups		pH			
		W1 Mean ±SD	W2 Mean ±SD	W3 Mean ±SD	W4 Mean±SD
A (Control Positive)		9.10± 0.70	8.80± 1.100	11.17±¥ 1.00	10.43± 0.68
B (Control Negative)		7.43 ±0.25	7.40 ±0.30	7.27* ±0.21	7.43 ±0.21
X(Starch)		9.63 ±0.74	10.90¥ ±1.54	9.27 ±0.81	10.00 ±0.17
C	**C1 (ZnO @500 mg/ m^3+ $CuSO_4$@ 100 mg/ m^2)**	10.10 ±0.17	9.63 ±0.74	11.07¥ ±0.76	11.70 ±1.04
	C2 (ZnO @ 1500 mg/ m^2 + $CuSO_4$ @ 400 mg/ m^2)	9.53 ±0.64	10.50¥ ±0.79	9.17 ±0.64	11.53 ±1.16
	C3 (ZnO @ 500 mg/ m^2 + $CuSO_4$ @ 400 mg/ m^2)	9.67 ±0.78	10.87¥ ±0.84	9.40 ±0.95	11.67 ±1.27
	C4 (ZnO @ 1500 mg/ m^2+ $CuSO_4$ @ 100 mg/ m^2)	9.17 ±0.64	10.50¥ ±0.79	9.07 ±1.12	9.27 ±0.81
D	**D1 (Alum @250 mg/ m^2+ ZnO @ 500mg/ m^2)**	9.63 ±0.74*	11.93¥ ±0.64	8.77* ±0.55	9.23 ±0.75
	D2 (Alum @1000 mg/ m^2+ ZnO @ 1500 mg/ m^2)	9.16 ±0.64	11.50¥ ±1.13	9.17 ±0.64	9.17 ±0.64
	D3 (Alum @250 mg/ m^2 + ZnO @ 1500mg/ m^2)	9.53 ±0.64	10.93¥ ±1.09	9.53 ±0.64	9.27 ±0.81
	D4 (Alum @1000 mg/ m^2 + ZnO @ 500 mg/ m^2)	9.67 ±0.77	11.23¥ ±1.01	9.53 ±0.64	10.10 ±0.17
E	**E1 ($CuSO_4$ @100 mg/ m^2 + Alum @ 250 mg/ m^2)**	9.90 ±0	10.97¥ ±1.08	10.00¥ ±0.17	9.80 ±0.87
	E2 ($CuSO_4$ @400 mg/ m^2 + Alum @ 1000 mg/ m^2)	10.00 ±0.17	10.60¥ ±0.53	10.00¥ ±0.17	10.60 ±0.61
	E3 ($CuSO_4$ @100 mg/ m^2 + Alum @ 1500 mg/ m^2)	10.00 ±0.17	11.03¥ ±1.18	9.97¥ ±0.21	9.90 ±0.52
	E4 ($CuSO_4$ @400 mg/ m^2 + Alum @ 250 mg/ m^2)	10.00 ±0.17	10.23 ±1.05	10.00¥ ±0.26	10.03 ±0.23

Table 6: The effect of different combination of nanoparticles on poultry house litter moisture

Groups		Moisture %			
		W1 Mean ±SD	W2 Mean ±SD	W3 Mean ±SD	W4 Mean ±SD
A (Control Positive)		66.93¥ ±1.45	73.67 ±2.75¥	77.20 ±1.31¥	76.53¥ ±1.50
B (Control Negative)		49.07* ±0.38	45.93* ±0.06	43.23* ±2.12	44.93* ±4.24
X (Starch)		53.27* ±1.60	46.03* ±2.25	67.83¥ ±0.77	73.53¥ ±2.05
C	C1 (ZnO @500 mg/ m^3+ CuSO4@ 100 mg/ m^2)	45.40* ±0.10	75.17¥ ±1.59	61.63*¥ ±2.74	62.17*¥ ±2.08
	C2 (ZnO @ 1500 mg/ m^2 + CuSO4 @ 400 mg/ m^2)	61.13*¥ ±2.39	57.37*¥ ±0.15	65.27*¥ ±3.05	53.47*¥ ±1.00
	C3 (ZnO @ 500 mg/ m^2 + CuSO4 @ 400 mg/ m^2)	55.60*¥ ±0.10	53.83*¥ ±2.51	58.37*¥ ±0.51	62.83*¥ ±1.15
	C4 (ZnO @ 1500 mg/ m^2+ CuSO4 @ 100 mg/ m^2)	64.00¥ ±0.10	68.50¥ ±0.10	46.40* ±2.19	73.17¥ ±2.56
D	D1 (Alum @250 mg/ m^2+ ZnO @ 500mg/ m^2)	63.60¥ ±0.69	65.23*¥ ±1.98	39.67* ±3.48	74.63¥ ±1.20
	D2 (Alum @1000 mg/ m^2+ ZnO @ 1500 mg/ m^2)	47.83* ±0.57	68.87¥ ±1.10	34.07* ±2.29	66.03*¥ ±1.57
	D3 (Alum @250 mg/ m^2 + ZnO @ 1500mg/ m^2)	33.23*¥ ±0.06	66.20*¥ ±2.07	45.27* ±2.25	52.70*¥ ±1.38
	D4 (Alum @1000 mg/ m^2 + ZnO @ 500 mg/ m^2)	32.83*¥ ±0.06	56.27*¥ ±2.40	43.67* ±4.47	44.60* ±1.31
E	E1 (CuSO4 @100 mg/ m^2 + Alum @ 250 mg/ m^2)	37.70*¥ ±0.10	54.83*¥ ±3.81	36.03* ±2.34	33.90*¥ ±0.78
	E2 (CuSO4 @400 mg/ m^2 + Alum @ 1000 mg/ m^2)	39.90*¥ ±0.10	66.40*¥ ±0.10	43.07* ±5.13	78.30¥ ±0.43
	E3 (CuSO4 @100 mg/ m^2 + Alum @ 1500 mg/ m^2)	47.80* ±1.00	57.43*¥ ±1.58	38.00* ±0.43	63.13*¥ ±1.48
	E4 (CuSO4 @400 mg/ m^2 + Alum @ 250 mg/ m^2)	37.47*¥ ±5.22	53.27*¥ ±1.66	40.30* ±3.65	64.50*¥ ±1.00

Table 7: The effect of different combination of nanoparticles on poultry house air microbial count

Groups		Air Microbial Count (cfu/m^3)			
		W1 Mean ±SD	W2 Mean ±SD	W3 Mean ±SD	W4 Mean ±SD
A (Control Positive)		45106.33¥ ±1888.08	40220.67¥ ±269.54	39949.67 ±73.66	39232.33 ±611.70
B (Control Negative)		35055.33* ±38.84	33071.33* ±935.94	35430.33 ±1014.14	33297.33 ±1188.64
X(Starch)		35298.00* ±1200.38	32957.33* ±1037.64	37258.67 ±42.89	33123.00 ±1923.42
C	C1 (ZnO @500 mg/ m^3+ CuSO4@ 100 mg/ m^2)	37052.67* ±40.02	29107.67* ±4885.62	32150.33 ±56.08	28635.67* ±281.95
	C2 (ZnO @ 1500 mg/ m^2 + CuSO4 @ 400 mg/ m^2)	36166.00* ±28.58	35196.67 ±2299.01	30187.00 ±4101.51	29403.33* ±654.74
	C3 (ZnO @ 500 mg/ m^2 + CuSO4 @ 400 mg/ m^2)	35064.67* ±43.09	33206.00* ±1139.41	25214.00* ±6365.3	28008.00* ±1523.35
	C4 (ZnO @ 1500 mg/ m^2+ CuSO4 @ 100 mg/ m^2)	35881.67* ±351.42	32182.33* ±578.811	34092.33 ±2492.27	28506.00* ±1144.20
D	D1 (Alum @250 mg/ m^2+ ZnO @ 500mg/ m^2)	35374.67* ±555.35	31669.33* ±755.69	34784.00 ±2023.17	28994.67* ±140.63
	D2 (Alum @1000 mg/ m^2+ ZnO @ 1500 mg/ m^2)	35059.67* ±954.09	31733.00* ±1392.79	32085.00 ±721.04	28840.00* ±131.19

	D3 (Alum @250 mg/ m^2 + ZnO @ 1500mg/ m^2)	34127.33*¥ ±2264.59	32256.67* ±610.12	31419.33 ±151.66	34829.33 ±289.14
	D4 (Alum @1000 mg/ m^2 + ZnO @ 500 mg/ m^2)	35058.00* ±2540.05	31649.00* ±1226.15	33313.67 ±2815.70	34862.33 ±2894.14
E	**E1 (CuSO4 @100 mg/ m^2 + Alum @ 250 mg/ m^2)**	36130.00* ±795.02	32095.00* ±730.07	33859.33 ±2307.72	29103.67* ±5746.37
	E2 (CuSO4 @400 mg/ m^2 + Alum @ 1000 mg/ m^2)	35153.33* ±2173.68	33860.33 ±1559.55	33206.00 ±1139.41	28885.00* ±6356.23
	E3 (CuSO4 @100 mg/ m^2 + Alum @ 1500 mg/ m^2)	35135.33* ±2178.36	33243.67* ±2629.69	32192.33 ±587.55	26523.00* ±5582.73
	E4 (CuSO4 @400 mg/ m^2 + Alum @ 250 mg/ m^2)	35166.00* ±2351.26	33370.33* ±1546.85	38184.67 ±44.24	30753.33 ±8075.57

Table 8: The effect of different combination of nanoparticles on poultry house litter microbial count

Groups		**Litter Microbial Count (cfu/m^3)**			
		W1 Mean ±SD	**W2 Mean ±SD**	**W3 Mean ±SD**	**W4 Mean ±SD**
A (Control Positive)		293.00¥ ±5.57	299.00 ±1.00¥	301.00¥ ±1.00	280.00¥ ±21.93
B (Control Negative)		157.67* ±22.81	142.00 ±21.38*	183.33* ±100.17	187.00* ±8.54
X (Starch)		242.00 ±13.74	266.00 ±6.56¥	258.67* ±14.01	256.00¥ ±4.58
C	**C1 (ZnO @500 mg/ m^3+ CuSO4@ 100 mg/ m^2)**	168.33* ±37.87	211.33 ±5.51	210.33 ±78.05*	106.67*¥ ±9.81
	C2 (ZnO @ 1500 mg/ m^2 + CuSO4 @ 400 mg/ m^2)	172.67* ±18.15	215.33 ±43.50	147.67 ±10.21*	119.67* ±11.59
	C3 (ZnO @ 500 mg/ m^2 + CuSO4 @ 400 mg/ m^2)	176.33* ±22.81	188.67* ±5.03	144.00 ±6.93	143.33* ±47.34
	C4 (ZnO @ 1500 mg/ m^2+ CuSO4 @ 100 mg/ m^2)	170.33* ±17.04	156.00* ±28.16	132.67 ±16.17	170.00* ±19.29
D	**D1 (Alum @250 mg/ m^2+ ZnO @ 500mg/ m^2)**	129.67* ±26.08	187.67* ±23.00	252.00 ±6.93	179.33* ±16.29
	D2 (Alum @1000 mg/ m^2+ ZnO @ 1500 mg/ m^2)	177.00* ±28.16	184.00* ±30.00	208.67 ±6.81	154.33* ±13.05
	D3 (Alum @250 mg/ m^2 + ZnO @ 1500mg/ m^2)	150.00 ±7.81*	139.67* ±21.96	182.67 ±7.09	150.67* ±35.12
	D4 (Alum @1000 mg/ m^2 + ZnO @ 500 mg/ m^2)	118.67* ±22.50	214.33 ±87.00	180.67 ±16.26*	158.33* ±29.57
E	**E1 (CuSO4 @100 mg/ m^2 + Alum @ 250 mg/ m^2)**	137.67* ±40.28	117.33* ±2.52	173.33 ±17.47	120.33* ±6.81
	E2 (CuSO4 @400 mg/ m^2 + Alum @ 1000 mg/ m^2)	118.00* ±5.29	124.33* ±3.05	185.67 ±11.5	120.67* ±6.03
	E3 (CuSO4 @100 mg/ m^2 + Alum @ 1500 mg/ m^2)	126.33* ±18.77	112.00* ±16.64	200.00 ±15.52	121.00* ±4.00
	E4 (CuSO4 @400 mg/ m^2 + Alum @ 250 mg/ m^2)	127.67* ±31.78	111.67* ±8.50	140.67 ±14.01	119.33* ±16.86

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 groupis from the negative control group and the percent difference of all treatment groups is from thepositive control group.)

DISCUSSION

Ammonia

In this study, the control positive group showed significantly ($P<0.05$) higher levels of ammonia (20.58, 15.91, 20.29 and 19.62) than the control negative group in all the four weeks, respectively. The constant amount or little drop in ammonia production over the time was due to gradual decrease in number of birds in each group due to slaughtering of the birds as the area provided to the birds remained the same, due to this reason the level of ammonia remained constant in the shed during the whole trial. High ammonia levels were probably due to inadequate ventilation in poultry house, as the shed was sealed and is same in commercial setting as well, especially in winter season.

In week 1, the ammonia was lower significantly ($P<0.05$) in treatment groups of ZnO @ 1500 mg/ m^2 + $CuSO4$ @ 400 mg/ m^2, ZnO @ 1500 mg/ m^2+ $CuSO4$ @ 100 mg/ m^2, $CuSO4$ @400 mg/ m^2 + Alum @ 1000 mg/ m^2 and $CuSO4$ @100 mg/ m^2 + Alum @ 1500 mg/ m^2 than the control positive group. In week 2, the treatment groups ZnO @ 500 mg/ m^2 + $CuSO4$ @ 400 mg/ m^2, Alum @1000 mg/ m^2+ ZnO @ 1500 mg/ m^2, Alum @250 mg/ m^2 + ZnO @ 1500mg/ m^2 and $CuSO4$ @100 mg/ m^2 + Alum @ 1500 mg/ m^2 was lower as compared to the positive control group. In week 3 and 4, all the treatment groups showed significantly ($P<0.05$) lower ammonia level from that of control positive group. Antibacterial activity of nanoparticles has also been described by Menazea and Ahmed (2020) and Dat *et al.* (2021). Moore *et al*. (1996) evaluated the effects of the alum on litter as it reacts with the moisture of the litter, so ammonia volatilization is reduced along with reduction in the moisture contents of the litter.

1.4.2. Humidity:

The level of humidity was significantly ($P<0.05$) higher (67.52, 71.26, 71.24 and 70.30) in control positive than the control negative group in all the 4 weeks, respectively.

In 2nd week, the humidity was lower in most of the treatment groups (ZnO @500 mg/ m^3+ $CuSO4$@ 100 mg/ m^2, ZnO @ 1500 mg/ m^2 + $CuSO4$ @ 400 mg/ m^2, ZnO @ 500 mg/ m^2 + $CuSO4$ @ 400 mg/ m^2, Alum @1000 mg/ m^2+ ZnO @ 1500 mg/ m^2, Alum @250 mg/ m^2 + ZnO @ 1500mg/ m^2 and Alum @1000 mg/ m^2 + ZnO @ 500 mg/ m^2) than the control positive group. In 3rd week, humidity was also lowered in addition by Alum @1000 mg/ m^2+ ZnO @ 1500 mg/ m^2, Alum @250 mg/ m^2 + ZnO @ 1500mg/ m^2, $CuSO4$ @100 mg/ m^2 + Alum @ 250 mg/ m^2, $CuSO4$ @400 mg/ m^2 + Alum @ 1000 mg/ m^2, $CuSO4$ @100 mg/ m^2 + Alum @ 1500 mg/ m^2 and $CuSO4$ @400 mg/ m^2 + Alum @ 250 mg/ m^2 than the positive control group. In 4th week, in all the treatment groups showed lower level of humidity except ZnO @500 mg/ m^3+ $CuSO4$@ 100 mg/ m^2, Alum @250 mg/ m^2+ ZnO @ 500mg/ m^2, Alum @1000 mg/ m^2+ ZnO @ 1500 mg/ m^2, $CuSO4$ @100 mg/ m^2 + Alum @ 250 mg/ m^2 and $CuSO4$ @400 mg/ m^2 + Alum @ 1000 mg/ m^2 treatment group than the positive control group. Weaver and Meijerhof (1991) found that as relative humidity increased from 45 to 75% that raised the levels of the ammonia in the shed. The results of the present study showed that the treatments not only reduced the ammonia level but also the humidity as well, which is quite understandable that humidity and ammonia have a direct correlation as has also been reported by Weaver and Meijerhof (1991).

Litter pH:

The litter pH was recorded 11.17 in the control positive group, which was significantly ($P<0.05$) higher from that of the control negative group in 3rd week. This higher pH facilitates the growth of various bacteria as the bacterial count was also higher, discussed in future section.

In 3rd week the treatment group Alum @250 mg/ m^2+ ZnO @ 500mg/ m^2 showed significantly ($P<0.05$) lower pH value from control positive group. Rothrock *et al.* (2008) stated that alum decreases the litter pH, in-turn pathogen reduction with performance improvement. The lower level of the pH in the all the treated groups of zinc oxide is the reason of buffering ability the ZnO nanoparticles. This was stated by Singappuli-Arachchige and Slowing (2020), as they used silica nanoparticles in their research. Reece *et al*. (1979) found that due to low level of pH (7), the release of ammonia is decreased, however when the pH reaches to 8, the release of ammonia also increases. So, the release is directly linked with the pH of the litter. These results are not ideal though, as litter pH, humidity and ammonia are more or less interlinked, but the results are little different, but if we look at the overall trend, the treatment did cause similar effect though on some parameters the effect is more significant but on other the effect is not significant. There may exist some other factors in the environment contributing to this variation seen.

Litter Moisture:

The level of moisture in the control positive group was significantly ($P<0.05$) increased from that of the control negative group during all the weeks.

In week 1, all the treatment groups except of ZnO @ 1500 mg/ m^2+ $CuSO4$ @ 100 mg/ m^2 and Alum @250 mg/m^2+ ZnO @ 500mg/m^2 showed significantly ($P<0.05$) lower moisture level from that of control positive group. In week 2, all the treatment groups showed ($P<0.05$) lower moisture level from that of the control positive group except of ZnO @500 mg/m^3+ $CuSO4$@ 100 mg/m^2, ZnO @ 1500 mg/m^2+ $CuSO4$ @ 100 mg/m^2 and Alum @1000 mg/ m^2+ ZnO @ 1500 mg/m^2 treatment groups. 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 Alum @ 500 mg/m^2 treated groups.

In week 4, all the treatment groups showed significantly ($P<0.05$) lower moisture level from that of control positive group except of ZnO @ 1500 mg/ m^2+ $CuSO_4$ @ 100 mg/m^2, Alum @250 mg/m^2+ ZnO @ 500mg/m^2 and $CuSO_4$ @400 mg/m^2 + Alum @ 1000 mg/m^2 treated groups. Carr *et al*. (1990) reported that if the moisture level is kept below 30 percent, then there are less chances of ammonia production in the sheds. In our case the level of moisture in treatment groups was between 33-64%, which was 76% in control positive group. However, among the combination, $CuSO_4$ 100 + alum 250 produced the best results.

Air microbial count:

The air microbial count in control positive group was significantly ($P<0.05$) higher from the control negative group in first 2 weeks, respectively, while it showed non-significant difference in last two weeks. This decrease in last two weeks in positive control group again can be linked with number of birds left in the group, while the size of the compartment remained the same. Now, why this actually decreased and why not remained constant could not be explained here.

In week 1, all the treatment groups showed significantly ($P<0.05$) lower air microbial count from that of control positive group. In week 2, most of the treatment groups (ZnO @500 mg/ m^3+ $CuSO_4$@ 100 mg/ m^2, ZnO @ 500 mg/ m^2 + $CuSO_4$ @ 400 mg/ m^2, ZnO @ 1500 mg/ m^2+ $CuSO_4$ @ 100 mg/ m^2, Alum @1000 mg/ m^2+ ZnO @ 1500 mg/ m^2, Alum @250 mg/ m^2 + ZnO @ 1500mg/ m^2, Alum @1000 mg/ m^2 + ZnO @ 500 mg/ m^2, $CuSO_4$ @100 mg/ m^2 + Alum @ 250 mg/ m^2, $CuSO_4$ @100 mg/ m^2 + Alum @ 1500 mg/ m^2 and $CuSO_4$ @400 mg/ m^2 + Alum @ 250 mg/ m^2) showed significantly ($P<0.05$) lower air microbial count from that of control positive group. In week 3, also most of the treatment groups (ZnO @ 500 mg/ m^2 + $CuSO_4$ @ 400 mg/ m^2 showed significantly ($P<0.05$) lower air microbial count from that of control positive group. In week 4, the groups treated with ZnO @ 1500 mg/ m^2 + $CuSO_4$ @ 400 mg/ m^2, ZnO @ 500 mg/ m^2 + $CuSO_4$ @ 400 mg/ m^2, ZnO @ 1500 mg/ m^2+ $CuSO_4$ @ 100 mg/ m^2, Alum @250 mg/ m^2+ ZnO @ 500mg/ m^2, Alum @1000 mg/ m^2+ ZnO @ 1500 mg/ m^2, $CuSO_4$ @100 mg/ m^2 + Alum @ 250 mg/ m^2, $CuSO_4$ @400 mg/ m^2 + Alum @ 1000 mg/ m^2 and $CuSO_4$ @100 mg/ m^2 + Alum @ 1500 mg/ m^2) showed significantly ($P<0.05$) lower air microbial count from that of control positive group. Scantling *et al.* (1995) reported that the *E. coli* and total coliform counts were significantly reduced when poultry litter was treated with alum. This study indicated that the use of nanoparticles as multiple compound has a role in reducing the air microbial count.

Litter microbial count:

The litter microbial count in control positive group was (293, 299, 301 and 280) significantly ($P<0.05$) higher from that of the control negative group in all the weeks, respectively. These results and those of change in pH to alkaline, high moisture level all can be related in positive control group and is quite expected.

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, ZnO @ 1500 mg/ m^2 + $CuSO_4$ @ 400 mg/ m^2 and Alum @1000 mg/ m^2+ ZnO @ 1500 mg/ m^2 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 ZnO @500 mg/ m^3+ $CuSO_4$@ 100 mg/ m^2, ZnO @ 1500 mg/ m^2 + $CuSO_4$ @ 400 mg/ m^2 and Alum @1000 mg/ m^2 + ZnO @ 500 mg/ m^2 treated groups. In week 3, the groups treated with ZnO @500 mg/ m^3+ $CuSO_4$@ 100 mg/ m^2, ZnO @ 1500 mg/ m^2 + $CuSO_4$ @ 400 mg/ m^2 and Alum @1000 mg/ m^2 + ZnO @ 500 mg/ m^2 showed significantly ($P<0.05$) lower litter microbial count from that of control positive group. In week 4, all the treatment groups showed significantly ($P<0.05$) lower litter microbial count from that of control positive group. It may be mentioned here that the results though in some cases were statistically non-significant but there was reduction in overall bacterial count. Thus, it shows that the nanoparticles have antimicrobial activity. The antibacterial activity linked with ZnO and CuO nanoparticles have also been described by Paul et al. (2020). Dobrzanski et al. (2010) also reported that nanoparticles being a microbicidal preparation decreases the number of *E. coli*, streptococcus and other harmful bacteria like mesophilic in the litter.

CONCLUSION

It can be concluded that increased concentrations of ammonia adversely affect the in-house environment of the poultry house which is linked with increase in litter moisture, pH, humidity, air and litter microbial count. 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. This study showed that the use of nanoparticles as multiple compounds has better role in improving the chicken in-house environment, better the production parameters and immune status, as well as the organs/tissue health.

CONFLICT OF INTEREST

The authors had no conflict of interest.

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AUTHOR CONTRIBUTIONS

SZ, MTJ contributed in planning the research, data analysis and manuscript writing. STG and MSM helped in proof reading

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