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Mitigation of Environmental and Health Impact of Spreading Some Pathogenic Bacteria Through Poultry Litter Using Litter Amendments: an Epidemiological Study

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Abstract

LITTER is an important component of poultry production, that can affect health, productivity. This study aimed to highlight pathogenic bacteria from litter and compare the efficacy of two amendments (alum at 7% concentration and calcium carbonate at 10% concentration). A total of 140 poultry litter samples were collected equally (n=20) throughout cycle (42 days) and transported to the laboratory for physico-chemical and microbiological analysis. Results revealed increase in all physico-chemical parameters throughout the study (p-value ≤ 0.01) including pH, temperature (℃) , moisture content (%), NH_3-N (g Kg^{-1}, Kg^{-1}, wb),and NO_3-N (mg Kg^{-1}, wb) at the end of the cycle (8.0 ± 1.3, 30.54 ± 2.8, 60.13 ± 3.8, 6.80 ± 1.1, and 29.75 ± 6.1 respectively) as compared to litter at beginning of the cycle (7.08 ± 0.06, 20.6 ± 1.2, 25.0 ± 1.8, 0.18 ± 0.03, and 1.84 ± 0.02 respectively).The predominant pathogens were Proteus spp. (32.47%), E. coli (23.07%), and Klebsiella spp. (22.22%), and 20 samples showing mixed infection (17.09%) and the least one was Salmonella spp (1.70%). The addition of alum (7%) to broiler litter resulted in t reduction in all physical parameters (8.06 ±0.4, 24.6±3.2, and 32.7±4.0 for pH, temperature(℃), moisture content (%) respectively), and 4.76±1.2 for ammonia volatilization. Alum had lethal effect (100%) against all isolates with lower efficacy of 80% against Proteus species compared to calcium carbonate. litter could pose a pathogenic risk for the environment and animal health. Regular usage of litter amendments like alum is one of the indispensable management.

Keywords: poultry litter, physico-chemical parameters, bacterial pathogens, environmental mitigation, Alum treatment.

Introduction

Poultry litter is a serious agricultural waste because of the water, soil, and air pollutants (greenhouse gases, unfortunate odour, emission of NH_3, H_2S, etc.) and the environmental issues that have arisen from its storage and disposal. Additionally, the poultry litter has higher levels of calcium (Ca), phosphorus (P), nitrogen (N), and a variety of other elements (including K, Mg, Fe, Cu, Zn, Mn, B, P_2O_5, and K_2O) [1] It is essential for absorbing fecal moisture and for maintaining carcass quality because it lowers the risk of footpad and breast lesions and offers a warm, smooth, and spongy surface for the birds' maximum comfort [2]. Typically, broiler chicks are grown on the ground with different kinds of litter [3]. Poultry excrement, water, feathers, spilt feed, and bedding material utilized in poultry operations make up the final result of the production cycle [4]

The most popular bedding materials used worldwide involve sawdust, pine wood shavings, chopped straw, peanut and nut hulls, rice hulls, shredded paper, sand, and grasses like switch grass). However, the availability of these materials used in commercial poultry houses differs by region [5] Frequently, the chicken litter comes into contact with various surroundings, including soil, water, and microorganisms. In many places, chickens' litter is specifically reused for the subsequent flock after they defecate, which may lead to cross-contamination. There is a significant likelihood of bacterial transmission due to the environment's numerous points, which could cause infections to spread among humans and animals [6]. Different bedding materials'

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physical and chemical characteristics have an impact on NH_3 emissions and litter quality. As a result, altering the bedding could be one way to cut NH_3 at its source [7] In accordance with [8], the optimal litter material should have a moisture content of 20–25%, a pH of 8–10, and an ammonia content of no more than 25 ppm. If the litter moisture content increases, the pH, NH_3 concentration, and caking level will also increase. Increased total ammonia nitrogen (TAN) build up due to elevated moisture levels in the litter might disrupt microbial metabolism, which in turn can affect avian productivity and welfare [9].

In poultry production systems, the quality of the litter is a significant concern since it may serve as a reservoir and a vehicle for the spread of infections, in addition to having an impact on the productivity and health of the flock [10]. Using poultry litter on agricultural land can have a number of negative effects on the environment. It acts as a conduit for the spread of bacterial species with various antibiotic resistance genes (ARGs) to the environment, despite its function in enhancing soil fertility[11]. Broiler litter has been found to include enteric infections[12], increasing the possibility of horizontal transmission and pathogen carryover effects between subsequent batches raised on the recycled litter. As a result, the litter material used in chicken houses needs to meet sanitary and hygienic requirements as well as the allowable ammonia level for the duration of the rearing period [13]

Ammonia emissions and the microbiological load within chicken houses may be decreased by applying litter amendments[14]. As a control measure, amendments are used to lower the poultry litter's moisture, pH, ammonia and microbial levels. Three types of litter amendments are distinguished: microbiological inhibitors, desiccants, and acidifiers [15]. Acid amendments based on sulphate are used to remove litter between chicken flocks and to reduce ammonia indoors during grow-out. By changing ammonia into non-volatile ammonium, these amendments lower the pH of litter and prevent ammonia volatilization [16]. Microbial populations in the poultry litter were significantly impacted by the addition of alum. The community of microbes in poultry litter was significantly altered by acidification; certain groups were less prevalent, and others were more widespread [17]

Therefore, the study was aimed to determine the physicochemical parameters (pH, temperature, moisture content, ammonia-N, and nitrate-nitrogen) and bacteriological characteristics (total coliform count, *E.coli*, *Klebsiella* spp., *Pseudomonas* spp., and *Proteus* spp.) of litter used throughout the production cycle, as well as the efficiency of aluminium ammonium sulphate $NH_4Al(SO_4)_2.12H_2O$ (7%) and calcium carbonate (10%) as a litter amendments on the physicochemical parameters and isolated bacteriological pathogens

Material and Methods

Study location and period

The study was conducted in three broiler poultry farms in the Beni-suef province, Egypt (coordinates: 29E04'N 31E05'E), during the period from May to September 2024. Ten thousand birds in each farm were housed in two different semi-controlled facilities on deep litter with a stocking rate of 10/m². Chopped straw with an average depth of 5 cm made up the used litter, which was merely routinely removed of its moist sections beneath water drinkers without any kind of treatment. Water was available from manual drinkers. At the final stage of the production cycle, the used litter is disposed of by either selling it, utilizing it as a soil amendment, and/or reusing it untreated. Sanitary practices are fair within the farms under investigation.

Litter sampling

Litter samples (n= 140) were gathered from fresh bedding materials and then once a week during the production cycle on days 0, 7, 14, 21, 28, 35, and 42 of the study. Using self-sealed plastic bags and sterile gloves, representative litter samples were taken from each pen from the four corners and the center (around the feeders and drinkers). For additional analysis, the collected litter samples were transported in an ice box to the laboratory of Hygiene, Zoonoses and Epidemiology department, Faculty of Veterinary Medicine. Beni-Seuf University. For physico-chemical and bacteriological analyses, each sample was homogenized and separated into two portions in sterile plastic bags in a completely aseptic laboratory. [18]

Physico-chemical parameters of poultry litter

Litter pH

The pH of the litter was measured and recorded using a pH meter (AD1030 Professional pH-ORP-TEMP Bench Meter) after calibration with pH 4, 7, and 10 buffers. Randomly selected litter samples from the center and four corners of each pen were mixed once a week; thereafter 20 g of the sample taken from this mixture was mixed thoroughly with 30 mL of distilled water in a sterile container and left for 30 min. Thereafter used for measuring litter pH [19]

Litter moisture content and temperature

The moisture content of the litter was ascertained by placing 10 g of each litter sample on tared aluminium drying dishes and drying them in a drying oven set at 100°C for 24 hours. Samples were taken out of the oven, weighed, put back in for an hour, and then weighed once again to be sure there had been no more weight loss. Next, using the difference between the sample's initial and final weights, litter

moisture was computed [18]. The litter temperature (°C) was measured once a week using a digital thermometer (Testo 110, Alton Hampshire, UK) [20]

NH_3-N emission

Using the micro-diffusion approach, litter NH_3-N emissions were ascertained as follows: The amount of sulphuric acid used (A) was multiplied by its normality and the molecular weight of ammonia to calculate volatilised NH_3 (in mg/100 g of litter): $NH_3 = A \times 0.1 \times 17$ [21]. A 500 mL cylindrical flask was filled with 100 g of litter sample, levelled, and then covered with a 50 mL beaker containing 10 mL of 2% (m/v) boric acid. The flask was then sealed and incubated for 20 hours at 30°C.

Nitrate-nitrogen (NO_3-N)

Nitrate-nitrogen (NO_3-N) was measured by suspending 2.0 g of poultry litter in 100 mL of distilled water, shaking it in a horizontal shaker for 5 minutes, and then letting it rest for12 hours. The quantities of nitrate were then obtained by distilling a 20 mL aliquot with 0.2 g of Devarda's alloy [22]

Isolation of litter bacterial pathogens

To isolate *E.coli* and *Klebsiella* spp., each sample was inoculated independently into buffer peptone water and cultured for 18 to 24 hours at 37°C in an aerobic condition. Each sample's broth was streaked onto a loopful of MacConkey's agar (HiMedia, MH081, India) and incubated for 18 to 24 hours at 37°C. The pure, distinct pink colonies were then collected and cultivated on Eosin Methylene Blue (HiMedia, M317, India) and incubated for 24 hours at 37°C [23].All samples were cultivated in peptone water (Oxoid) and incubated for 24 hours at 37ºC to isolate *Salmonella* spp. After that, it was streaked on XLD agar (HiMedia, M031, India) and incubated for another 24 hours at 37ºC [24] after being inoculated at a 1:10 ratio on Rappaport-Vassiliadis broth (HiMedia, MH1491, India) and stored at 42±1ºC for 24±2 hours. Additionally, *Pseudomonas* was isolated by inoculating litter samples in nutritional broth and then incubating them for 24 hours at 37°C. After streaking a loopful of the broth over MacConkey agar and *Pseudomonas* agar base, the mixture was cultured for 24 hours at 37°C. A tryptic soy agar plate was used to subculture the suspicious colonies in order to observe the pigmentation [23, 25] To determine the total coliform count (TCC), the collected litter samples were diluted in 100 mL of distilled water and then filtered through a membrane. The membrane was then placed in incubator for 24 hours at 37°C using M-FC agar (EM Science, Gibbstown, NJ) [26].

Identification of litter bacterial pathogens

The isolated litter pathogens were biochemically confirmed using the Indole reaction, Citrate Utilization test , Methyl red test , Voges Proskauer test and Triple Sugar Iron Agar) for suspected green metallic colonies of *E.coli* and suspected pink with black center colonies of *Salmonella* spp [23, 27], respectively, as well as the urease test for *Salmonella* spp identification [28].The oxidase test, colony and cellular morphology, pigment synthesis, fruity odour detection, and Gram staining were used to identify *Pseudomonas* spp [23, 25]. To verify isolates, the urease, methyl red, gelatin liquefaction, oxidase, catalase, and arginine hydrolysis tests were used [29]. After that, the pure culture of the isolated organisms was inoculated in tryptone soy broth (HiMedia, M011, India) and incubated for 24 hours at 37°C before being stored in 80% sterile glycerin to maintain the stock culture for the isolated bacteria. For later usage, a bacterial culture and an equivalent volume of 80% glycerin were combined, sealed with paraffin wax, and kept in a refrigerator at 4°C [30]

Sensitivity pattern of litter bacterial pathogens to litter amendments

According to[31], the sensitivity profile of 36 strains of bacterial pathogens isolated from poultry litter to the litter amendments, including alum [aluminum ammonium sulphate dodecahydrate $NH_4Al(SO_4)_2.12H_2O$ (Oxford,M.W.453.32)] at 7% concentration and calcium carbonate (calcium carbonate 85% (EGY-HOLLAND EGYPT®)) at 10% concentration, was evaluated using the agar well diffusion assay. Each testing isolate's suspension was made in accordance with the McFarland standard (0.5). Distribute evenly a volume of the bacterial inoculum across the whole agar surface on the Muller Hinton agar medium. After using a sterile well puncher to aseptically punch a hole 5 mm in diameter, 50 µL of each tested amendment (alum and calcium carbonate) were added to individual wells on the agar surface. After18 to 24 hours of incubation at 37ºC, the zone of inhibition was measured in accordance with the interpretation of the zone diameter. Bacterial isolates were classified as susceptible and resistant based on their sensitivity to the tested litter amendment.

Statistical analysis

All the data were collected, and prepared for analysis using SPSS (Statistical Package for Social Sciences Software, version 22). The Chi-Square test (a non-parametric test) was used to analyze the frequent distribution of isolated bacterial pathogens from examined poultry litter throughout the production cycle. The one-way ANOVA test was used to determine the mean values (± SE) of estimated physico-chemical parameters as well as *total coliform count* (TCC $\times 10^3$) in the examined poultry litter throughout the production cycle.

Results

The results illustrated in Table (1) the mean values (±) SE of estimated phsico-chemical

parameters of examined poultry litter throughout the production cycle showed significant increase in all physico-chemical parameters of the litter toward the end of the production cycle comparing it to the start of the cycle regarding pH that increases from 7.08±0.06 at zero day of the production cycle to 8.0±1.3 at 42 day of the cycle (mean 7.5±0.92) at P-value ≤ 0.5. Mean value of temperature 27.23°C±2.02 where it increases from 20.6°C±1.2 at zero day of the cycle to 30.54°c ±2.8 (P ≤0.04). Referring to moisture content (mean 42.45±2.8) increased from 25.0% ±1.8 at the start to 60.13%± 3.8 at the end of the cycle (P ≤ 0.02), meanwhile mean content of the ammonia nitrogen NH_3-N 2.95±0.39 it showed an increase from 0.18g/kg-1 ±0.03 at zero day to 6.80g/kg-1±1.1 at 42 day of the production cycle (P ≤ 0.05), while nitrates nitrogen increased from 1.84g/kg-1±0.02 at the start of the cycle to 29.75g/kg-1±at the end of the production cycle (P ≤ 0.01).

The aforementioned results in Table (2) frequent distribution of isolated bacterial pathogens from poultry litter throughout the study period revealed that out of 140 litter sample 117(83.57%) were positive for bacteriological isolation and the most predominant bacterial isolate was *Proteus* spp. (32.47%) followed by *E.coli* and *Klebseilla* spp. (23.07 and 22.22%, respectively). Surprisingly and unexpectedly the least bacterial isolates to be recovered were *pseudomonas* spp. followed by *Salmonella* spp. (3.41 and 1.7%, respectively). Generally, isolation rates significantly increase toward the end of production cycle at P ≤0.05. Mixed infection was detected at 17.09% of the obtained isolates that refers to two or more bacterial pathogens were recovered from the same sample.

Referring to the mean values ±SE of total coliform count (TCC×10^3) in the examined poultry litter throughout the production cycle (Table 3) it was noticed that there was a significant increase in TCC throughout the cycle ranging from less than 10 ×10^3 at the beginning of the cycle to 23.28×10^3±3.1 in the end of the production at P value ≤ 0.02.

Regarding Table (4) in vitro evaluation of the effectiveness of adding alum (7%) and calcium carbonate (10%) on physicochemical parameters of litter results showed that at the end of the cycle at 42^{th} day all physicochemical parameters were to some extent elevated especially with no treatment of any kind to the litter throughout the cycle and this is probably due to the bacterial action on the organic matter in the litter that break down into ammonia which raise pH, temp. and nitrogen content (pH 8.0±12, temp. 30.54±2.6°c, moisture content 60.13±3.8%, NH_3-N 6.80±1.1and NO_3N 29.75±6.1, respectively). But when treating the litter with alum (7%) results showed a significant decrease in some of the parameters including temp 28.6±1.2°c at P value ≤ 0.05, moisture content 36.7± 2.8% (P value ≤ 0.03), NH_3-N 3.06±1.2 at P value ≤ 0.4 and NO_3N 14.85±1.4 (P value ≤ 0.02), suggesting the improvement of litter characteristics and increasing its value as a soil amendment. On the other hand, addition of 10% calcium carbonate also lower some of the estimated physicochemical parameters but to lesser degree than alum 7% including temp., moisture content, NH_3-N and NO_3-N (28.0±1.8, 47.1±1.7, 6.43±1.6 and 27.83±1.7, respectively).

Referring to the results in (Table 5) concerned with evaluating the efficacy of alum (7%) and calcium carbonate (10%) on total coliform count and isolated bacterial pathogens, there was a high isolation rate before the treatment where total coli-form at 42^{th} of production cycle was 6.290±0.359 also *E.coil*, *Klebseilla* spp, *Proteus* spp. and *Pseudomonas* spp. were recovered from litter samples. Meanwhile treatment of the litter with alum (7%) had nearly bactericidal effect (100%) on all recovered pathogens except for *Proteus* spp. (80%) also significantly lowered TCC to 2.097±0.041. Calcium carbonate to some extent reduced TCC (5.255±0.098) but all isolated pathogens showed complete resistance to it (100%).

Discussion

The results in Table (1) are to much extent in harmony that could be explained as a result of increase of moisture content of the litter, it leads to increase in litter pH and volatilization of NH_3-N to ammonia gas emission into the environment and decreased nitrogen content of the litter, which provides a favorable media for the microbial growth in the litter and elevates litter's temp. [32] These results are variable compared to those obtained by [33] who detected that pH 8.43±0.057 which is significantly affected by the type of diet the poultry are fed, while litter moisture content are much lower than the results in this study (5.41 and 42.15%). Also nitrogen content in the litter is much higher than the results obtained in this study without differentiation between ammonia and nitrates nitrogen (ranged between 4.5 and 36.6). These results were slightly higher than those reported by [34] that recorded moisture content in poultry litter 1 and 2 were (39.1 and 30.2%, respectively), meanwhile pH recorded by him (PL1 8.86 and PL2 8.49, respectively) were higher than those recorded in this study. Also ammonia nitrogen was variable to that obtained in this study (6.12±0.14 for PL1 and1.84±0.02 for PL2). Results of moisture content in the litter were in agreement to those recorded by [35] who recorded moisture content 44 to 47.7%, meanwhile the results recorded by [36] were greater than thoses obtained by this study (54, 78.2% , respectively).

On the contrary to the results obtained in this study in Table (2) , [37] revealed that the highest isolation was *Salmonella* followed by *Enterococcus* and *E.coli* . As well and unlike this study [38]

reported 38 sample out 44 ones were positive for bacteriological isolation and the main recovered bacterial pathogen were *E.coli* 24 (46%) followed by *S. aureus* 10 (19%), CNS 7 (13.5 %), *Enterobacteraerogenes* 3(5.8%), *Enterobacter cloacae* 2 (4%), *Serratia* spp., 2(4%) and others 4(7.7%). But similar to our finding [38] were not able to isolate *Salmonella* at any percentage which might be attribted to the dominance of coliforms that can grow over *Salmonella* spp. and hinder their growth [39].

Referring to in-vitro evaluation of the effectiveness of adding alum (7%) and calcium carbonate (10%) on physicochemical parameters, [44] proved that daily ammonia emission was reduced by 42% in treated house while the overall ammonia emission was reduced by 47% also proved that alum addition has a role in reducing CO_2 emission and increasing N content of the litter. Conversely [45] recorded decreased pH of the litter in groups treated with alum where control group (8.82±0.01), group T1 (8.60±0.03) & group T3 (8.47±0.02) and the least pH of litter material was of T2 group (8.34±0.17). Similarly, [44] showed significant decrease in moisture content of litter treated with alum (T1 34.13±0.57, T2 31.90±0.06, T3 28.27±0.18) compared to control group (35.79 ± 0.32). In contrast [46] found that addition of alum significantly decreased pH of the litter additional NH_4+-N contents followed by increase in NH_4+-N contents with the gradual decrease of litter's pH.[47]reported that ammonia emission from swine manure treated with alum (2.5%) was decreased by 84% during 18 days of composting. Inversely [48]proved that addition of calcium carbonate reduced the moisture content of the litter due to its hygroscopic nature. Although pH value in this study did not decrease but the alum treatment still had nearly 100% bactericidal effect this might be attributed to the direct effect of alum and its ability to deteriorate bacterial cell wall rather than providing unfavourable condition to their growth when shifting litter pH into acidic [49]

Concerning Table (5) [50]recorded that litter acidifiers significantly reduce gram positive bacteria as *Clostridium perfringens*, *Enterococcus* spp., and *Lactobacillus* spp. Unlike the results recorded in this study [51]proved that alum treatment reduced total bacterial counts (50%) and urease producing bacteria (90%) in 4 weeks that might be referred to low pH that inhibit the growth of bacteria in the litter. [17] mentioned that alum treatment for one month reduced *E*.coli and *Campylobacter jejuni* to 3 log and 2 log, respectively, meanwhile *Salmonella* spp were not detectable throughout the study. [49] recorded that *E.coli* was sensetive (50%) to alum (1 and 2%) concentration meanwhile *S. aureus* was 100% resistant to alum (1%) and 50% resistant to alum (2%). [52] proved that alum had antibacterial efficacy against gram negative bacteria more than gram positive ones as *E. coli* (20 mm) and *Pseudomonas aeruginosa* (21mm). [53] proved that Alum had bactericidal effect on *Campylobacter spp.* that is reduced with time through the production cycle. Reciprocally [54,55] and [48]proved that adding calcium carbonate to the litter improve quality and lowering its microbial load the subsequently improve broiler performance.

Conclusion

Since poultry litter considers an important soil amendment that plays an important role in environmental contamination with serious pathogens such as *E. coli*, *Proteus* spp., *Klebseilla and Salmonella* that can find their way to human food chain. Therefore, it became a necessity to control these pathogens and increasing the value of litter as a soil amendment. By Proper management of poultry litter through adding alum (7%) before disposal is indispensable to mitigate these negative environmental and health impacts.

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Conflict of interest

The authors do not have any competing interests.

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Consent to Publish

All the authors have given their consent for publish this manuscript.

Ethical of approval

Not needed in this study

TABLE 1. The mean values (± SE) of estimated physico-chemical parameters of examined poultry litter throughout the production cycle.

Parameters Age/day	pH	Temperatue (℃)	Moisture (%)	NH_3–N (g Kg-1,wb)	NO_3N (mg Kg-1,wb)
0	7.08±0.06	20.6±1.2[b]	25.0±1.8[ab]	0.18±0.03	1.84±0.02[ab]
7	7.26±0.7	24.92±1.7	29.7±2.1	0.28±0.07[ab]	3.8±0.05
14	7.3±0.9	26.71±1.8	36.8±2.6[b]	1.36±0.11	14.0±2.1[b]
21	7.48±1.1	28.62±2.1[ab]	40.8±2.8	1.78±0.41[b]	23.64±4.9[c]
28	7.6±1.2	29.18±2.0	48.7±3.1	4.16±0.06	25.16±5.8
35	7.8±1.2	30.08±2.6	56.03±3.1	6.12±1.0	26.77±5.6
42	8.0 ±1.3	30.54±2.8[a]	60.13±3.8[a]	6.80±1.1[a]	29.75±6.1[a]
Mean ±SE	7.5±0.92	27.23±2.02[c]	42.45±2.8	2.95±0.39	17.85±3.5
P-value	0.5	0.04	0.02	0.05	0.01

TABLE 2. Frequent distribution of isolated bacterial pathogens from examined poultry litter throughout the production cycle.

Bacterial findings Age/day	Total Examined	positive	*E.coli*	*Sal.* spp.	*Pseudomonas* spp	*Klebsiella* spp.	*Proteus* spp.	Mixed infection
0	20	4(20.0)	0.0	0.0	0.0	2(50.0)	2(50.0)	0.0
7	20	13(65.0)	1(7.69)	0.0	0.0	4(30.76)	7(53.8)	1(7.69)
14	20	20(100.0)	3(15)	0.0	1(5.0)	5(25.0)	7(35.0)	4(20.0)
21	20	20(100.0)	5(25)	0.0	1(5.0)	4(20.0)	6(30.0)	4(20.0)
28	20	20(100.0)	5(25)	0.0	0.0	3(15.0)	7(35.0)	5(25.0)
35	20	20(100.0)	6(30.0)	1(5.0)	1(5.0)	4(20.0)	5(25.0)	3(15.0)
42	20	20(100.0)	7(35.0)	1(5.0)	1(5.0)	4(20.0)	4(20.0)	3(15.0)
Mean±SE	140	117(83.57)	27(23.07)	2(1.70)	4(3.41)	26(22.22)	38(32.47)	20(17.09)
P-value				0.05				

TABLE 3. Mean values (±SE) of total coliform count (TCC ×10^3) in the examined poultry litter throughout the production cycle.

Total Coliform count Age/ days	Log (CFU/ml) (Mean ± SE)
0	<10
7	2.06±0.8[ab]
14	3.7±1.1
21	5.92±2.0[c]
28	11.48±2.10[b]
35	20.80±2.8
42	23.28±3.1 [a]
P-value	0.03

TABLE 4. In vitro evaluation of the effectiveness of adding alum (7%) and calcium carbonate (10%) on physicochemical parameters of litter.

Parameters estimated	pH	Temperature (℃)	Moisture content (%)	NH3–N (g Kg-1,wb)	NO3-N (mg Kg-1, wb)
Poultry Litter					
(Soon before disposal) No treatment	8.0±12	30.54±2.6	60. 3±3.8[a]	6.80±1.1	29.75±6.1[a]
After treatment with					
Alum treatment (7%)	8.06 ±0.4	28.6±1.2	36.7± 2.8[ab]	3.06±1.2	14.85±1.4[b]
Calcium carbonate (10%)	8.32± 0.6	28.0±1.8	47.1± 1.7[b]	6.43±1.6	27. 83±1.7[a]
P-value	0.7	0.5	0.03	0.6	0.05

TABLE 5. In-vitro evaluating the efficacy of alum (7%) and calcium carbonate (10%) on total coliform count and litter bacterial isolates in examined poultry farms.

Tested bacterial isolates / **Poultry Litter**	***E. coli*** **(n=12)**		***Pseudomonas*** **spp. (n=4)**		***Klebsiella*** **spp (n=10)**		***Proteus*** **spp (n=10)**		**Log TCC (CFU/ml)**
(Soon before disposal) No treatment	+ve		+ve		+ve		+ve		6.290±0.359
After treatment with	S	R	S	R	S	R	S	R	
Alum treatment (7%)	12(100)	0.0	10(100)	0.0	8(80)	2(20)	10(100)	0.0	2.097±0.041
Calcium carbonate (10%)	0.0	12(100)	0.0	10(100)	0.0	10(100)	0.0	10(100)	5.255±0.098
P-value				> 0.05					

References

1. Kacprzak, M., Malińska, K., Grosser, A., Sobik-Szołtysek, J., Wystalska, K., Dróżdż ,D., Jasińska, A. and Meers, E. Cycles of carbon, nitrogen and phosphorus in poultry manure management technologies−environmental aspects- A Critical Reviews. *Environmental Science and Technology*, **53**(4), 914–938 (2023). doi: 10.1080/10643389.2022.2096983.
2. Karthiga, S. and Sharmilaa, G. Methodologies for re-use of poultry litter in broiler farms – A Review. *International Journal of Civil Engineering and Technology*, **9**(4), 230–233(2018).
3. Sharma, G., Khan, A., Singh, S. and Anand, A.K. Efficacy of pine leaves as an alternative bedding material for broiler chicks during summer season. *Veterinary World,* **8**(10), 1219-1224 (2015) doi: 10.14202/vetworld.2015.1219-1224
4. Koli, S.S., Dhumal, M.V., Ingle, V.D. and Koli, R.S. Influence of quality characteristic parameters with use of different litter material combinations on performance of broilers. *International Journal of Science, Environment and Technology* **6**(1), 50-63 (2017).
5. Watson, K. and Wiedemann, S. G. Review of Fresh Litter Supply, Management and Spent Litter Utilisation. *AgriFutures Chicken Meat*, no. 19-001. (2018).
6. Kumar, S., Anwer, R., Mehra, N., Devi, T., Yadav, M., Sehrawat, N. and Sharma, A .K. Assessment of bacterial diversity in the chicken litter: A potent risk to environmental health. *Journal of Experimental Biology and Agricultural Sciences*, **11**(4),640 – 649 (2023). doi: 10.18006/2023.11(4).640.649.
7. Brink, M., Janssens, G.P.J., Demeyer, P., Bağci, Ö. and Delezie, E. Ammonia concentrations, litter quality, performance and some welfare parameters of broilers kept on different bedding materials. *British Poultry Science*, **63**(6), 768-778(2022).
8. Gençoglan, S. and Gençoglan, C. The effect of the litter materials on broiler chicken's welfare and performance. *Turkish Journal of Agriculture-Food Science and Technology*, **5**(12), 1660–1667(2017).doi: 10.24925/turjaf.v5i12.1660-1667.1736
9. Diarra, S., Lameta, S., Amosa, F. and Anand S. Alternative bedding materials for poultry: Availability, efficacy, and major constraints. *Frontiers in Veterinary Science,* **8**, 669504 (2021).doi: 10.3389/fvets.2021.669504.
10. Bjedov, S., Žikić, D., Perić, L., ĐukićStojčić, M. and Milošević, N. Effect of different litter treatments on production performance of broiler chickens. *Biotechnology in Animal Husbandry,* **29** (4), 625-630 (2013). DOI:10.2298/BAH1304625B
11. Kubasova, T., Faldynova, M., Crhanova, M., Karasova, D., Zeeman, M., Babak, V. and Rychlika, I. Succession, Replacement, and Modification of Chicken Litter Microbiota. *Applied and Environmental Microbiology*, **88** (24) e0180922, (2022). doi:10.1128/aem.01809-22.
12. Wei, S., Gutek, A., Lilburn, M. and Yu, Z. Abundance of pathogens in the gut and litter of broiler chickens as affected by bacitracin and litter management. *Veterinary Microbiology*, **166**, 595–601(2013). DOI:10.1016/j.vetmic.2013.06.006
13. Villagra, A., Olivas, I., Benitez, V. and Lainez, M. Evaluation of sludge from paper recycling as bedding material for broilers. *Poultry Science,* **90**(5), 953–957 (2011). doi: 10.3382/ps.2010-00935.
14. De Toledo, T. D. S., Roll A. A. P., Rutz, F., Dallmann, H. M., Dai Prá, M. A., Leite, F. P. L. and Roll, V. F. B. An assessment of the impacts of litter treatments on the litter quality and broiler performance: A systematic review and meta-analysis. *PLOS ONE*, **15**(5), e0232853 (2020). doi: 10.1371/journal.pone.0232853.
15. Linhoss, J. E., Purswell, J. L., Street, J. T. and Rowland, M. R. Evaluation of biochar as a litter amendment for commercial broiler production. *Journal of Applied Poultry Research*, **28**(4), 1089 -1098 (2019).doi: 10.3382/japr/pfz071.

16. Johnson, J., Zwirzitz, B., Oladeinde, A., Milfort, M., Looft, T., Chai, L., Zock, G.,Sommers, M., Tunim, S. and Aggrey, S. E., Succession patterns of the bacterial community in poultry litter after bird removal and sodium bisulfate application. *Journal of Environmental Quality*, **50**(4), 923–933 (2021),doi: 10.1002/jeq2.20248.

17. Rothrock Jr, M. J., Cook, K. L., Warren, J. G. and Sistani, K. The Effect of Alum Addition on Microbial Communities in Poultry Litter. *Poultry Science***,** **87**(8),1493-503(2008), doi: 10.3382/ps.2007-00491.

18. Hussein, M.A., Khattak, F., Vervelde L., Athanasiadou, S. and Houdijk, J. G. M. Growth performance, caecal microbiome profile, short-chain fatty acids, and litter characteristics in response to placement on reused litter and combined threonine, arginine and glutamine supplementation to juvenile male broiler chickens. *Animal Microbiome*, **5**(1), 18 (2023).

19. Pope, M. J. and Cherry, T. E. An evaluation of the presence of pathogens on broilers raised on poultry litter treatment-treated litter. *Poultry Science*, **79**(9), 1351–1355(2000).doi: 10.1093/ps/79.9.1351.

20. Bilgili, S. F., Montenegro, G. I., Hess, J. B. and Eckman, M. K. Sand as litter for rearing broiler chickens. *Journal of Applied Poultry Research*, **8**(3), 345–351(1999). doi: 10.1093/japr/8.3.345.

21. Hernandes, R. and Cazetta, J.O. Simple and accessible method to determine liberated ammonia from the broiler litter. *Rev. Bras. Zootec.*, **30**(3), 824–829 (2001).doi: 10.1590/S1516-35982001000300030.

22. Tedesco, M.J., Gianello, C., Bissani, C.A., Bohnen, H. and Volkweiss, S.J. *Análise, de solo, plantas e outros materiais*.2nd Edition, Universidade Federal do Rio Grande do Sul, Porto Alegre (1995)

23. Quinn, P.J., Markey, B.K., Carter, M.E., Donnelly, W.J. and Leonard, F.C. *Veterinary Microbiology and Microbial diseases*. Oxford, London: Blackwell Scientific Publication (2002).

24. ISO 6579 *Microbiology of Food and Animal Feeding Stuffs*: Horizontal Method for the Detection of Salmonella spp. ISO, Geneva, Switzerland (2002).

25. Shukla, S. and Mishra, P. Pseudomonas aeruginosa infection in Broiler Chicks in Jabalpur. *International Journal of Extensive Research*, **6**, 37-39, (2015).

26. American Public Health Association. *Standard Methods for the Examination of Water and Wastewater* .22nd ed., (2012)

27. Cruickshank, R., Duguid, J. P., Marmian, B.P. and Swain, R.H.A. Medical Microbiol. *The practice of Medical Microbiol* 12th ed., Churchill Livingstone, Edinburgh, London (1979).

28. Edwards, P.R. and Ewing W.H. *Identification of Enterobacteriaceae*, 3rd ed., pp.:67–107, Burgess Publishing Co., Minneapolis(1972)

29. Cheesbrough, M. Pseudomonas and related organisms. *Biochemical test to identify bacteria. Antibiotic susceptibility testing*. In: District Laboratory Practice in tropical countries. Cambridge University Press, New York, USA, pp. 1933- 1943. (2000)

30. Chowdhury, M.A., Rahman, K.M., Miah, M.R. and Haq, J.A. Transferable drug resistance (R-factor) among the enterobacteriaceae in urinary tract infections: a study at an urban hospital in Bangladesh. *Journal of Tropical Medicine and Hygiene*, **97** ,161-166. (1994).

31. European Committee on Antimicrobial Susceptibility Testing (EUCAST): http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/Breakpoint_ table_v_**2.0**_120221.pdf. (2015).

32. Ashworth, A. J., Chastain, J. P. and Moore, Jr, P. A. Nutrient characteristics of poultry manure and litter, In*: Animal Manure: Production, Characteristics, Environmental Concerns and Managements*, H. Waldrip, P. H. Pagliari, and Z. He (Ed.), Soil Science Society of America Monongraph. John Wiley & Sons, Ltd., Madison, WI, 63-87(2020).

33. Maruthamuthu, T., Karuppusamy, S., Ramesh, V., Nagarajan, M., Ragavan, P.M., Santiago, M., Nallathambi, B., Dharmalingam, A.P.S, Radhakrishnan, K., Ramasamy, A., Ramasamy, S. R.S. and Kannan, T.A. Physicochemical Characterization of Broiler Poultry Litter from Commercial Broiler Poultry Operation in Semiarid Tropics of India. *Agriculture*, **14**(10),1708 (2024). doi: 10.20944/preprints202408.1343.v1.

34. Katuwal, S., Rafsan, N.A.S., Ashworth, A.J. and Kolar, P. Poultry litter physicochemical characterization based on production condition for circular system. *BioResources*, **18** (2), 3961–3977(2023). doi.10.15376/biores.18.2.3961-3977.

35. Sistani, K.R., Brink, G.E., McGowen, S.L., Rowe, D.E. and Oldham, J.L. Characterization of broiler cake and broiler litter, the by-products of two management practices. *Bioresource Technology*, **90**(1), 27-32 (2003). doi:10.1016/S0960-8524(03)00096-8

36. Ogunwande, G.A., Ogunjimi, L. A.O. and Osunade, J.A. Fate of compost nutrients as affected by co-composting of chicken and swine manures. *International Agrophysics*, **28**(2),177–184(2014). DOI:10.2478/intag-2014-0006

37. Plumblee Lawrence, J.R., Cudnik, D. and Oladeinde, A. Bacterial Detection and Recovery From Poultry Litter. *Journal Frontiers in Microbiology*, **12**, 803150(2022). doi: 10.3389/fmicb.2021.803150

38. Eyasu, A., Moges, F. and Alemu, A. Bacterial isolates from poultry litters and their antimicrobial susceptibility patterns in Gondar, Northwest Ethiopia. *International Journal of Microbiology Research and Reviews*, **6** (2),197-204(2017)

39. El-Jalil, M.H., Zinedine, A. and FAID, M. Some microbiological and chemical properties of poultry wastes manure after lactic acid fermentation. *International Journal of Agriculture and Biology*, **10**(4),1560-8530 (2008)

40. Martin, S. A., McCann, M. A. and Waltman II, W. D. Microbiological survey of Georgia poultry litter. *The Journal of Applied Poultry Research*, **7**(1) 90–98(1998).doi:10.1093/japr/7.1.90

41. Abreu, V.M.N., De Abreu, P.G., Jaenisch ,F.R.F., Coldebella, A., Paiva, D.P. Effect of Floor Type (Dirt or Concrete) on Litter Quality, House Environmental Conditions, and Performance of Broilers. *Brazilian Journal of Poultry Science*, **13** (2),127-137 (2011). doi:10.1590/S1516-635X2011000200007

42. Hartel, P. G., Segars, W. I., Summer, J. D., Collins, J. V., Phillips, A. T. and Whittle, E. Survival of fecal coliforms in fresh and stacked broiler litter. *The Journal of Applied Poultry Research*, **9**(4),505-512(2000). DOI:10.1093/japr/9.4.505

43. Milanov, D., Knežević,S., Vidaković,S., Pajić, M., Živkov-Baloš, M.and Aleksić, N. Microbial contamination of poultry litter during fattening period. *Biotechnology in Animal Husbandry*, **35** (3), 253-265(2019). doi :10.2298/BAH1903253M.

44. Eugene, B., Moore, Jr, P.A., Li, H., Miles, D., Trabue, S., Burns, R. and Buser, M. Effect of alum additions to poultry litter on in-house ammonia and greenhouse gas concentrations and emissions. *Journal of Environmental Quality*, **44**(5), 1530-1540(2015). doi:10.2134/jeq2014.09.0404.

45. Chakrvarti, R., Pramanik, P.S., Panday, G., Kumar, S., Gautam, P., Gupta, V. and Singh, B. Effect of alum treated litter in reduction of ammonia, pH, and moisture level of poultry litter and its effect on the Broiler Performance. *Journal of Pharmacognosy and Phytochemistry*, **7**(1),1084-1087(2018).

46. Liu, S., Zhuang, X. and Wang, C. Application of polyaluminium chloride coagulant in urban river water treatment influenced the microbial community in river sediment. *Water (Switzerland)*, **13**(13), 1791 (2021). doi.org/10.3390/w13131791

47. Bautista, J.M., Kim, H., Ahn, D.-H, Zhang, R. and Oh, Y.-S. Changes in physicochemical properties and gaseous emissions of composting swine manure amended with alum and zeolite. *Korean Journal of Chemical Engineering*, **28(**1),189-194(2011). doi:10.1007/s11814-010-0312-6

48. Ismael , E. and Ismail, E.M. Effectiveness of Sodium bisulfate and Calcium carbonate litter amendments on the Microbial load of Broiler Built-up Litter. *International Journal of Veterinary Sciences*, **4**(2), 1-10 (2021). DOI: 10.21608/svu.2021.56458.1095.

49. AL-Khikani, F. H. O., Zaraa, D. M., Abbas, H. S., Musa, H.S., Dahir, H. A., Musa, H. A. M. and Alhusayni, A. A. Evaluating the antibacterial activity of potassium aluminium sulphate (alum) combined with other antibiotics. *Microbes and Infectious Diseases*, **5**(3), 1190-1197(2024). doi: 10.21608/MID.2023.206322.1514.

50. Garrido, M. N., Skjervheim, M., Oppegaard, H. and Sørum, H. Acidified Litter Benefits the Intestinal Flora Balance of Broiler Chickens. *Applied And Environmental Microbiology*, **70**(9),5208–5213(2004). DOI: 10.1128/AEM.70.9.5208–5213.2004.

51. Cook, K.L., Rothrock Jr, M.J., Warren, J.G., Sistani, K.R. and Moore, Jr .P.A. Effect of Alum Treatment on the Concentration of Total and Ureolytic Microorganisms in Poultry Litter. *Journal of Environmental Quality*, **37**,2360–2367 (2008). doi: 10.2134/jeq2008.0024.

52. Amadi, L.O., Wanabia, D. and Amadi, V. Synergistic effects of alum and guava (PsidIumguajava) leaf extracts on some pathogens from clinical samples. *International Journal of Current Research*, **08** (05), 31354- 31358(2016).

53. Arsi, K., Moore Jr, P.A., Donoghue, A.M., Dirain, M.L. and Donoghue, D.J. Litter Treatment with Aluminum Sulfate (Alum) Produced an Inconsistent Reduction in Horizontal Transmission of Campylobacter in Chickens. *International Journal of Poultry Science*, **6** (2),31-36.doi: 10.3923/ijps.2017.31.36.

54. Taherparvar, G., Seidavi, A., Asadpour, L., Payan-Carreira, R., Laudadio, V. and Tufarelli,V. Effect of litter treatment on growth performance, intestinal development, and selected cecum microbiota in broiler chickens. *Revista Brasileira de Zootecnia*, **45**(5), 257-264 (2016). doi:10.1590/S1806-92902016000500008

55. Avcılar, O.V., Kocakaya, A., Onbasılar, E.E.and Pirpanahi, M., Influence of sepiolite additions to different litter materials on performance and some welfare parameters of broilers and litter characteristics. *Poult. Sci. Journal*, **97** (9), 3085–3091(2018.doi:10.3382/ps/pey185 PMID: 29800332

التخفيف من الأثر البيئي والصحي لانتشار بعض البكتيريا المسببة للأمراض من خلال فرشة الدواجن باستخدام محسنات الفرشة: دراسة وبائية

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الملخص

الفرشة مكون مهم في الإنتاج الداجني الذي من الممكن ان يؤثرعلى الصحة والإنتاجية. تهدف هذه الدراسة إلى القاء الضوء على الميكروبات البكتيرية التي يمكن عزلها من الفرشة ومقارنة اثنين من محسنات الفرشة في القضاء عليها (الشبّة بتركيز 7% وكربونات الكالسيوم بتركيز 10%). بتجميع عدد 140 عينة من الفرشة (عدد عشرون) خلال الدورة (42 يومً) وقد تم نقلها الي المعمل لإجراء الفحص الفيزيائي- الكيميائي والبكتيرولوجي.اظهرت النتائج زيادة في كل المعايير الفيزيائية والكيميائية خلال الدراسة (نسبة مؤثرة P اكبر من او يساوي 0.01) وذلك يشمل الرقم الهيدروجيني,درجة الحرارة (℃) ,نسبة الرطوبة (%),والنتيروجين في الامونيا ووالنيتروجين في النيتريت (ملجم / كجم) في نهاية الدورة بالمقارنة مع بداية الدورة (0.02±1.84,0,18±0.03 ,25±1.8 ,6.1± 29.75 ,1.1 ± 60.80 , 60.13±3.8 ,2.8± 30.54 ,1.3 ± 8.0 ,1.2 ± 20.6 ,0.06 ± 7.08).اكثر المعزولات التي كانت متوفرة هي البروتيس (32.47٪) ,ايشريشيا كولاي(23.07٪) ,الكلبسيلا (22.22٪) وعشرون عينة اظهرت عدوي مختطلة بنسبة (17.09٪) بينما اقل المعزولات كانت السالمونيلا بنسبة (1.70٪) , اضافة الشبة (7٪) الي الفرشة ادت الي نقص في كل المعايير الفيزيائية (4.0±32.7,3.2±24.6,0.4±8.06) بالنسبة للرقم الهيدروجيني ,درجة الحرارة والرطوبة بالترتيب 4.76± 1.2لتطاير الامونيا .الشبة له تاثير قاتل (100%) علي كل المعزولات وبنسبة (80%) علي ميكروب البروتيس بالمقارنة مع كربونات الكالسيوم .الفرشة لها تاثير خطير علي صحة الحيوان والبيئة. الاستخدام المنتظم لمحسنات الفرشة مثل الشبة يعتبر من الاجراءات التي لا يمكن الاستغناء عنها.

الكلمات المفتاحية: فرشة الدواجن، المعايير الفيزيائية والكيميائية، مسببات الأمراض البكتيرية، التأثير البيئي، المعالجة بالشبه .