

Effectiveness of Sodium bisulfate and Calcium carbonate litter amendments on the Microbial load of Broiler Built-up Litter

Elshaimaa Ismael^{1*}, Eman Mohamed Ismail¹

¹ Department of Veterinary Hygiene and Management, Faculty of Veterinary Medicine, Cairo University, Egypt, 1211.

Abstract

In the poultry industry, litter treatment is an effective tool used to control microbial growth, public health risk, and the environmental impact of built-up litter. Our study aimed to compare the efficacy of the two commercial litter amendments, calcium carbonate and sodium bisulfate, to reduce the total aerobic (TAC) and total fungal counts (TFC) of dry and wet-caked built-up broiler litters. Litter samples were obtained from 21 and 35 days two broiler houses, then each sample was divided into three sub-groups (500 g/group): control untreated groups, desiccant treated groups (40g calcium carbonate), and acidifier treated groups (40 g Sodium bisulfate). TAC and TFC and log reduction were determined at zero, 2, and 14 days post-application. Calcium carbonate was highly efficient when applied on the wet litter and achieved a 4.76 log reduction of TAC after 48 h of application. Sodium bisulfate was effective on both litter conditions, with a 3.4 log reduction of total aerobic count in wet litter samples, while higher than 2.47 in the dry litter samples after 48 hrs. Both litter amendments showed low efficacy after two weeks of application, as log reduction ranged from 0.50 to 1.86. The efficiency of calcium carbonate and sodium bisulfate on total fungal count was negligible and ranged from 0.00 to 0.10 log reduction. In conclusion, calcium carbonate and sodium bisulfate are efficient litter amendments that can be used as a preventive measure for minimizing bacterial growth in broiler built-up litter with periodical reapplication to maintain good litter quality.

Keywords: litter amendments, calcium carbonate, Sodium bisulfate, Total aerobic count, Broiler litter.

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INTRODUCTION

Poultry litter is composed of multiple organic and inorganic components as wood shaving, manure, feather, food remnants, and water. The level of these components depends on the birds' age as well as husbandry practices. The build-up of organic matter over time leads to the increase of pH levels of the litter to levels of 8.5 which is suitable for microbial activities (Moore et al., 2000; Miles et al., 2006). The presence of high litter moisture content favors the microbial action in degrading the organic components as uric acid, producing urea and ammonia which volatilize and compromise the birds' and human health. Litter urea has a painful chemical burning effect that damages birds' tissue and causes contact dermatitis (Dinev et al., 2019). Additionally, high levels of litter moisture increase the incidence of footpad dermatitis (Mayne et al., 2007). As poultry litter is usually applied to agricultural fields as a fertilizer, it may contaminate the surface and groundwater, spreads pathogens, and affects air quality with gas emissions (Bolan et al., 2010). Litter treatment is required to mitigate these effects.

In the broiler industry, litter treatment is recently a popular topic of interest in commercial broiler production regarding numerous environmental, economic, and veterinary as well as public health aspects. Application of litter amendments inside poultry houses could reduce microbial load and ammonia emissions (Oviedo-Rondón et al., 2013; Soliman et al., 2018; De Toledo, et. Al., 2020). Multiple strategies could be taken to control litter moisture levels and ammonia emission rates inside broiler houses such as good ventilation, maintenance of watering system, adjusting bird density, dietary manipulations, and good litter management. Additionally, litter

amendments are applied as a control method to reduce moisture, pH, ammonia, and microbial levels of poultry litter. Litter amendments are classified into 3 categories called: acidifiers, desiccants, and microbial inhibitors (Linhoss et al., 2019).

Litter acidifiers act by lowering the pH, improves ammonia volatilization inside poultry houses, and inhibiting the bacterial action in the conversion of nitrogen to ammonia (Ritz et al., 2005; Tiquia et al., 2000). In addition, acidifiers have shown effective results in reducing bacterial populations, besides creating an unfavorable environment for the development of pathogenic bacteria (Cook et al., 2008; Chung et al., 2015; Roll et al., 2011). However, the action of acidifiers is for a short time range and depleted within 14 to 21 days (Linhoss et al., 2019). Litter desiccants act by adsorbing moisture as well as reducing the emission of ammonia through inhibiting the activity of ammonia-producing bacteria, and therefore reducing litter pH (Oliveira et al., 2003; Ritz et al., 2005). The adsorption of water occurs through the hydration of cations that compensate the surface load by osmotic balance (Castaing and Arcillas, 1998). Lowering the water content of the litter and have shown effective impacts on litter quality characteristics, especially when combined with other litter treatments (Lee et al., 2013).

Proper farm preparation and appropriate litter management with the correct application of litter amendments have a great impact to achieve maximum benefits and effectiveness. Therefore, this study aimed to compare the efficacy of two commercial litter amendments, calcium carbonate and sodium bisulfate, to reduce the total aerobic count (TAC) and total fungal count (TFC) of dry and wet-caked built-up broiler litter.

MATERIALS AND METHODS

An in-vitro experimental study was designed during spring 2020, to compare the efficacy of two commercially used litter amendments on the microbial load of collected built-up broiler litter samples for 14 days. This study was conducted at the research laboratory of animal, poultry, and environmental hygiene in the Department of Veterinary Hygiene and Management, Faculty of Veterinary Medicine, Cairo University.

1. Litter samples

Two samples of built-up litter were obtained from 21 and 35-days broiler houses. Litter was relatively dry and friable at 21 day- old with a moisture content of 21.7%. While at 35 days, litter was wet and caked with a moisture content of 50.9%.

2. Litter amendments

Two commercial products were evaluated: calcium carbonate 85% (EGY- HOLLAND EGYPT®), as a litter desiccant, in addition to Sodium Bisulfate 10% (EGY- HOLLAND EGYPT®), as a litter acidifier (table 1).

Table 1. Litter amendments evaluated for microbiological effect on poultry litter

Amendment type	Manufacture source	Composition	Recommended dilution rate
Desiccant	EGY- HOLLAND EGYPT®	Calcium carbonate 85%	500 gm/ 25 m ²
		Eucalyptus oil 0.5%	
		Saw dust 14.5%	
Acidifier	EGY- HOLLAND EGYPT®	Sodium Bisulfate 10%	250 gm/ 20 m ²
		Eucalyptus oil 0.5%	
		Saw dust 89%	

3. Treatment groups

The trial consisted of 2 major sets: the dry litter group and the wet litter group. Each set of litter samples was thoroughly mixed and sub-divided into 3-sub-sets: a control sub-group with no amendments, a sodium bisulfate (acidifier) treated sub-group, and a calcium carbonate (desiccant) treated sub-group. Each sub-group contained 500 gm dry or wet litter in sterile containers (5 cm litter depth and 200 cm² surface area). calcium carbonate and sodium bisulfate amendments were added at a dilution rate of 40 g/500 g (8% w/w) to litter samples on day zero before incubation. Litters and amendments were thoroughly mixed, then incubated at room temperature 27± 2° C.

4. Total Aerobic and Fungal Counts:

For comparing the efficacy of calcium carbonate and sodium bisulfate before and after application, 10 gm litter samples from each sub-group were collected at zero, 2, and 14 days of amendments application. Each litter sample was diluted in 90 ml w/v sterile saline solution, then serially diluted till 10¹⁵. Then one ml, from 10¹⁰ to 10¹⁵ litter dilution, was transferred and streaked into nutrient agar plates for TAC and incubated at 37 °C for 24 h, as well as Sabouraud dextrose agar plates for TFC that incubated at 24 °C for 5-days. Total aerobic count (TAC) and Total fungal count (TFC) were determined according to methods described by the American public health association (A.P.H.A.) (1998).

5. Statistical analysis:

Total aerobic and total fungal counts were expressed as log₁₀ CFU/g of litter. The differences between log₁₀ counts between control and treated litter sub-groups were calculated to obtain Log reduction values. Simple linear regression and the Coefficient of Determination (*R*²) were calculated using PASW Statistics Version 18.0 software (SPSS Inc., Chicago, IL, USA), to test the

association between the period of incubation and the change of total aerobic count.

RESULTS

The initial microbial load of both litter types ranged from 13.36 to 14.44 log₁₀ CFU/g for the dry and wet litters; respectively. The initial fungal count ranged from 5.48 to 7.78 log₁₀ CFU/g for the dry and wet litters; respectively (table 2 and figure 1).

Table 2. Microbial load (Log₁₀ CFU/g) of control and treated litter sub-groups

	Log ₁₀ CFU/g litter					
	Control		Desiccant (Calcium carbonate)		Acidifier (Sodium bisulfate)	
	Dry Litter	Wet Litter	Dry Litter	Wet Litter	Dry Litter	Wet Litter
Total aerobic count						
Before application	13.36	14.44	13.36	14.44	13.36	14.44
After 48 h	12.78	13.90	12.89	9.15	10.31	10.41
After 2 weeks	16.58	12.78	14.72	11.54	14.95	12.28
Total fungal count						
Before application	5.48	7.78	5.48	7.78	5.48	7.78
After 48 h	0.00	4.77	3.00	4.67	0.00	4.72

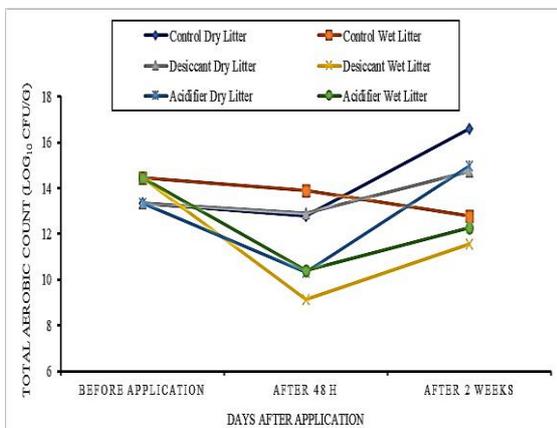


Fig. 1. The total aerobic counts (log₁₀ CFU/g) of dry and wet litter samples during 2 weeks after application of the calcium carbonate (desiccant) and sodium bisulfate (acidifier) litter amendments.

Regarding the efficacy of litter treatment with litter physical condition, data revealed that the wet litter sample showed the best

response to the treatment with calcium carbonate (desiccant), as its TAC was reduced from 14.44 to 9.15 log₁₀ CFU/g after 48 h of application. However, TAC re-increased after 2 weeks of application to 11.54 log₁₀ CFU/g, but still at a lower level than the initial count. Furthermore, the recorded log reduction for the wet litter treated with calcium carbonate (desiccant) was the highest (4.76) after 48 h of application but reduced to 1.23 after 2 weeks when compared to the corresponding control wet litter sample (table 2,3; Fig. 1).

Table 3. Log reduction of the microbial load in litter samples after application of each amendment.

	Desiccant (Calcium carbonate)		Acidifier (Sodium bisulfate)	
	Dry Litter	Wet Litter	Dry Litter	Wet Litter
	Total aerobic count			
After 48 h	0.00	4.75	2.47	3.49
After 2 weeks	1.86	1.24	1.63	0.50
Total fungal count				
After 48 h	+3.00*	0.10	0.00	0.05

(* In dry litter treated with calcium carbonate, no log reduction occurred after 48 h, but the log has increased)

For each 1-day increase in the incubation period of the calcium carbonate (desiccant) treated wet litter, we observed a 0.07 log₁₀ CFU decrease in litter aerobic count ($R^2 = 0.04$); as demonstrated in Fig. (3). The dry friable litter sample poorly responded to the desiccant (Calcium carbonate), as it demonstrated a similar TAC (12.89 log₁₀ CFU/g) after 48 h of application. However, after 2 weeks the calcium carbonate treated dry litter showed TAC (14.47 log₁₀ CFU/g) lower than the control dry one (16.58 log₁₀ CFU/g), with a log reduction of 1.86 (Table 2,3; Fig. 1). For each 1-day increase in the incubation period of the calcium carbonate (desiccant) treated dry litter, we observed a

0.12 log₁₀ CFU increase in litter aerobic count ($R^2 = 0.86$); as demonstrated in Fig. (4).

The application of sodium bisulfate (acidifier) lowered the TAC of both the wet (10.41 log₁₀ CFU/g) and dry (10.31 log₁₀ CFU/g) litter samples after 48 h, with log reduction values of 3.49 and 2.47; respectively. While after 2 weeks, a slight increase of the TAC of the wet litter treated with sodium bisulfate (acidifier) was observed (12.28 log₁₀ CFU/g) with 0.50 log reduction when compared with the corresponding control litter sample (table 2, 3; Fig. 2, 3).

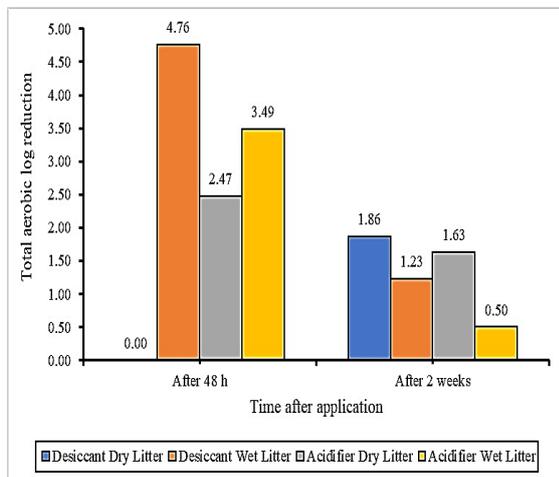


Fig. 2. Log reduction of the total aerobic count of dry and wet litter samples after application of calcium carbonate (desiccant) and sodium bisulfate (acidifier) litter amendments.

For each 1-day increase in the incubation period of the sodium bisulfate (acidifier) treated wet litter, we observed a 0.05 log₁₀ CFU decrease in litter aerobic count ($R^2 = 0.03$); as demonstrated in Fig. (3). Additionally, the dry litter treated with sodium bisulfate (acidifier) showed a relatively high increase in TAC (14.95 log₁₀ CFU/g) and recorded a log reduction value of 1.63 (Table 2,3; Fig. 2, 3). For each 1-day increase in the incubation period of the sodium bisulfate (acidifier) treated dry litter, we observed a 0.21 log₁₀ CFU

increase in litter aerobic count ($R^2 = 0.45$); as demonstrated in Fig. (4).

However, the total fungal count (TFC) of the control wet caked litter showed a higher count (7.78 log₁₀ CFU/g) than the control dry litter (5.48 log₁₀ CFU/g). After incubation of both litter samples for 48 h, the TFC declined to 0.0 and 4.77 log₁₀ CFU/g for the untreated dry and the wet litter samples, respectively. The addition of either calcium carbonate or sodium bisulfate didn't change the TFC compared to the respective control samples (table 2,3).

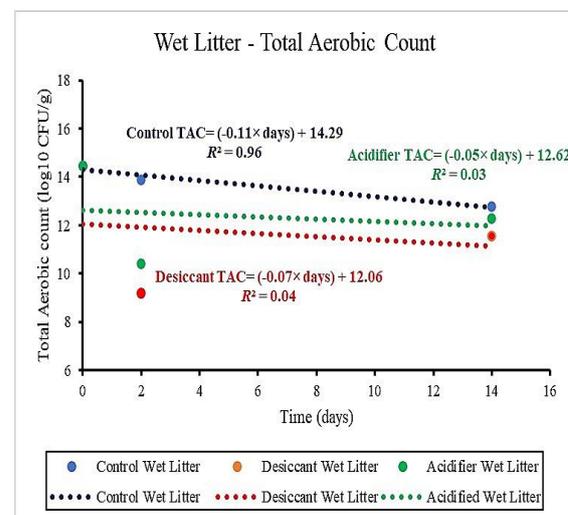


Fig. 3. The association between the total aerobic count (log₁₀ CFU/g) of the wet litter sample and the time of incubation in control and treated litter groups. TAC: total aerobic count; Control: wet litter without treatment; Desiccant: wet litter treated with calcium carbonate; Acidifier: wet litter treated with sodium bisulfate; R^2 : Coefficient of determination.

On the side of control litter samples, the TAC of control litter samples showed a decreased microbial load of the wet litter sample during the 2 weeks of the trial. Also, the TAC of control wet litter declined from 14.44 to 13.90 log₁₀ CFU/g (log reduction = 0.54) after 48 h of incubation and declined to 12.78 log₁₀ CFU/g (log reduction = 1.66) after 2 weeks of incubation (Tables 2, 3). So, for each 1-day increase in the incubation period of the control wet litter, we observed

a 0.11 \log_{10} CFU decrease in litter aerobic count ($R^2 = 0.96$); as demonstrated in Fig. (3). On the other hand, the dry litter samples first showed a simple decline in TAC count from 13.36 to 12.78 \log_{10} CFU/g (log reduction = 0.58) after 48 h of incubation but re-increased to 16.58 \log_{10} CFU/g (log increase = 3.22) after 2-weeks of incubation (Tables 2, 3). So, it was observed that for each 1-day increase in the incubation period of the control dry litter, a 0.26 \log_{10} CFU increase in litter aerobic count ($R^2 = 0.93$); as demonstrated in Fig. (4).

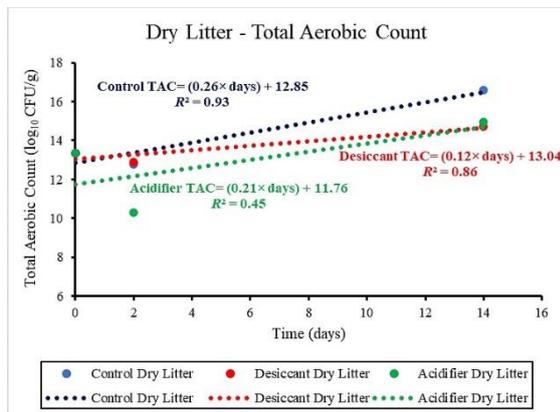


Fig. 4. The association between the total aerobic count (\log_{10} CFU/g) of the dry litter sample and the time of incubation in control and treated litter groups. TAC: total aerobic count; Control: dry litter without treatment; Desiccant: dry litter treated with calcium carbonate; Acidifier: dry litter treated with sodium bisulfate; R^2 : Coefficient of determination.

Overall, wet caked litter samples showed a better response to the effect of both calcium carbonate (desiccant) and sodium bisulfate (acidifier) than observed for the dry friable litter sample.

DISCUSSION

The initial microbial load of both litter types was high for both dry and wet litter. During the broiler production cycle, it is important to maintain the physical environment in optimum conditions to achieve maximum birds' survival rates. Though, the litter conditions as pH, temperature, and moisture content provide suitable media for

microbial growth and pathogens that cause various diseases. Additionally, some types of bacteria produce ureolytic enzymes that decompose uric acid and lead to ammonia emission problems inside broiler houses (McWard and Taylor, 2000).

In the current study, two types of commercial litter amendments were evaluated, calcium carbonate as a desiccant and sodium bisulfate as a litter acidifier. In a trial for resembling the real litter conditions in broiler houses, the two amendments were evaluated for their efficacy to reduce the microbial load of built-up litter inside broiler houses. Hence, the action of both products was evaluated against two different litter types: dry and wet caked broiler litters. Poultry litter is subjected to moisture increase, resulted from birds' excreta, drinkers, and atmospheric humidity (Toppel et al., 2019). Caked wet litter is commonly observed around and underneath the water drinkers, while dry litter is mostly found in free areas and corners (Dunlop et al., 2016).

Results of this study indicated that calcium carbonate had a better efficacy when applied on the wet caked litter and achieved a 4.76 log reduction of TAC after 48 h of application. Calcium carbonate is a Hygroscopic compound that tends to absorb water from the surrounding environment. When added to a moistened litter it reduces its moisture content and makes it unsuitable for microbial growth and activity. Our finding agreed with Zhang et al., (2005); Oliveira et al., (2015); Taherparvar et al., (2016); Avcilar et al. (2018) who reported amending litter with adsorbents improves litter quality without affecting broiler performance through lowering pH, microbial load, and pathogenic microbiota, especially when combined with other litter treatments (McWard and Taylor, 2000; Lee et al., 2013; De Toledo, 2020).

Regarding acidifier action, the current study revealed that sodium bisulfate achieved a good efficacy when applied to either the dry or the wet litter. Sodium bisulfate reported 2.47 and 3.49 log reduction of TAC after 48 h when applied to dry and wet litter, respectively. These findings agreed with other studies that reported sodium bisulfate as an effective litter acidifier for improving the characteristics of litter and broiler performance (Pope and Cherry, 2000; Li et al., 2013; Purswell et al., 2013). Sodium bisulfate (SBS) (NaHSO_4) is an acidifier litter amendment commonly applied in poultry houses. Sodium bisulfate has hygroscopic properties, it absorbs water then dissociates into Na^+ , H^+ , and SO_4^- ions. The H^+ ion is the responsible ion for the acidification characteristic of sodium bisulfate, which lowers litter pH making the litter pH unsuitable for bacterial growth and activity (Johnson and Murphy, 2008). Appropriate application of acidifiers as sodium bisulfate could significantly reduce the pH and water activity of litter, making litter conditions unsuitable for the survivability and activity of bacteria (Line, 2002).

The follow up of the litter microbial load after 2 weeks of application revealed that the efficacy of both amendment products has diminished and reported log reduction ranged from 0.50 to 1.86. from this finding, it is better to re-apply the litter amendments frequently during the production cycle. Increasing the application rate of litter treatment products could delay the onset of microbial colonization, but it may not be economically reasonable (Line and Bailey, 2006). A previous study suggested the use of amendment blends to enhance the efficacy of the products and to decrease the costs of application of a single amendment (Lee et al., 2013).

Regarding the control litter samples, data illustrated a 0.11 \log_{10} CFU decrease in litter aerobic count with each 1-day increase in the incubation period of the control wet litter, this could be attributed to the desiccation effect of environmental conditions and the absence of wet excreta add-up, the evaporation of litter moisture reduced the total aerobic bacterial count. Therefore, further evaluation studies in field conditions are recommended in the different environmental conditions with different application rates.

CONCLUSION

This study provided knowledge about the efficacy of calcium carbonate and sodium bisulfate amendments on broiler litter with different moisture conditions. Data indicated that calcium carbonate and sodium bisulfate are efficient litter amendments, especially with high moisture levels. For better efficacy, they can be re-applied periodically for minimizing bacterial survivability in built-up litter inside poultry houses. Further studies on the use of calcium carbonate and sodium bisulfate in poultry litter should be followed to test their effects on other litter qualities and pathogens.

CONFLICT OF INTEREST

The authors declare that they do not have any conflict of interest.

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