Research Article

An Evaluation of Some Disinfectants Effectiveness in Reducing Nest Bedding Contamination in Broiler Breeder Farms

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Abstract | The purpose of this study was to assess whether two chemicals, paraformaldehyde, and potassium peroxy-monosulfate were effective in controlling the microbial load of nest bedding material as a first step in reducing contamination of broiler breeder hatching eggs. In a closed broiler breeder farm where birds were housed on built-up litter, the two disinfectants were used for the disinfection of nest bedding (sawdust). Paraformaldehyde powder was mixed manually with nest bedding (5 grams per nest), while potassium peroxy- monosulfate 0.5% based disinfectant was sprayed in each nest. Random samples were collected before and after treatment by 24-, 72- and 120-hours intervals and were tested for total bacterial, coliform, and fungal counts to evaluate the reduction percent achieved. The obtained results illustrated that neither disinfectant could achieve a complete reduction of the microbial load of nest bedding material. However, both disinfectants recorded a significant ($p \leq 0.05$) log reduction in total bacterial, coliform, and fungal count after 24, 72 and 120 hours, gradually. Conclusively, paraformaldehyde and potassium peroxy-monosulfate based disinfectants may be recommended for disinfection of nest bedding material to reduce the microbial contamination of hatching eggs.

Keywords | Broiler breeders, Nest bedding, Disinfection, Farm contamination.

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INTRODUCTION

Poultry producers are always trying to increase productivity by improving egg fertility, hatchability, number, and quality of day-old chicks. A clean, high-quality egg can achieve those characteristics (Ahamed et al., 2019). Laying systems impact the quality of chicken eggs; about 75% of commercial layers in the world are kept in cages as they are suitable for housing a high density of birds within a limited space. Furthermore, it can be used for breeders, but artificial insemination is required. In Egypt, breeding houses are both equipped with deep litter systems or with a combination of deep litter systems and slatted floors (Bhadhauria, 2016). In comparison with other housing systems, deep litter systems had twenty to thirty times more bacteria on the eggshell than slatted floor houses and fifteen times more bacteria and spoilage organisms than battery cage systems (Al-Shammari et al., 2015).

The nest box is considered an integral part of the breeder house in non-cage breeder farms (Ahammed et al., 2014). Still, some hens lay their eggs on the floor, raising the risk of bacterial contamination from floor litter (Singh et al., 2009).

Although eggshells can become contaminated during egg exit from the vent, many studies suggested that the primary contamination occurred within a short period after laying due to contact with dirty surfaces such as soiled nests, floors, or slats. Several bacteria can penetrate the eggshell through shell pores or cracks, multiply, and produce toxins that can kill the developing embryo, reduce chick hatchability, and negatively affect chick productivity after hatching (Bialka et al., 2004). However, microorganisms that are present on a few eggs can spread throughout the hatchery, thus potentially contaminating all of the eggs(Moustafa, 2009). Specific egg and hatcher-related infections such as aspergillosis and omphalitis are related to deficiencies in procedures used to identify and suppress microbial infection in the chain of production extending from laying to chick delivery (Shane,1993). Reducing eggshell contamination may reduce bacterial infection incidences in developing embryos and newly hatched chicks (Oviasogie et al., 2016). Chemical disinfectants are applied at recommended concentrations, or contamination is reduced on egg surfaces and throughout the surrounding environment, including nest materials.

Different nest bedding materials are available, including rice hulls, almond shells, silica pellets, hay, sugar cane, and wood shavings which are commonly used in Egypt. Nest materials can affect the frequency of floor eggs ranging from 2% for wood shavings to 10% for corn cobs as reported by Brake, (1985).

Several hatching egg sanitizers were recommended by many investigators like iodine preparations (Barbour et al., 1985), chlorine preparations (Bialka et al., 2004), H_2O_2 (Moustafa, 2009), potassium peroxy-monosulfate and glutaraldehyde (Cadirci, 2009).

Moreover, formaldehyde is used regularly in the poultry industry for disinfecting poultry houses, poultry litter, and hatching eggs (Fabrizio et al., 2002; Bialka et al., 2004). Paraformaldehyde is the powdered form of formalin that is mixed with methanol to limit the extent of polymerization (Dresser, 1973). It is used in poultry hatcheries to reduce the number of pathogens as *Salmonella* spp., *Escherichia coli*, and *Pseudomonas* spp. as reported by Al-Shammari et al., (2015). Formaldehyde has several disadvantages, including eye irritation and an unpleasant odor (Cadirci, 2009). Because of its suspected carcinogenicity, the Environmental Protection Agency regulated the use of formaldehyde fumigation under the Toxic Substances Control Act (Badran, 2018). Other safe and effective disinfectants that can replace formaldehyde (Fichet et al., 2007) includes a mix of peroxy oxygen molecules (potassium peroxy-monosulfate and other ingredients), organic acids, and surfactants, that may kill a wide range of microorganisms. Efficacy is limited when organic material is present (Dvorak, 2008). Applying peroxy compounds to surfaces, including floors, equipment, and walls, by spraying, fogging, or immersion can eliminate bacteria, fungi, and viruses (Kunanusont et al., 2020).

However, relatively few papers have discussed the use of chemicals for disinfecting nest bedding material as a method for controlling eggshell contaminants. So, the aim of this study was to determine whether two chemical disinfectants (paraformaldehyde powder and potassium peroxymonosulfate) could reduce the microbial load of egg nest bedding materials so that eggshells would be less likely to be contaminated.

MATERIALS AND METHODS

Examination Of Poultry Houses

Ethical approval was not sought for the present study as it is not applicable, because this article does not contain any studies with direct intervention with animal subjects. The current study was conducted at a broiler breeder's farm in Fayoum governorate, Egypt, where 57-week-old Ross breed birds were kept in closed houses with a fully controlled environment. The egg-laying system consisted of metal nests attached to the walls along with the house at a height of 15cm above the litter surface. Every nest had eight identical chambers arranged into two rows, and each row was equipped with wooden perches on the front to allow birds' easy access to the nests. Each nesting chamber was designed to accommodate one laying bird at a time, and it could accommodate up to five different laying birds per day. Sawdust was used as bedding in the nesting chambers to increase the comfort of the birds and decrease the risk of cracked eggs. The nest chamber's roof was sloped to reduce light intensity. During egg-laying, chamber ventilation was maintained by side openings to allow air movement and temperature control.

Use Of Disinfectants

Two commercial disinfectants were used in the present study; disinfectants' composition and dilutions are shown in Table 1.

Table 1: Chemical disinfectants and their dilutions used in the study.

Dilution	Active ingredients
5 g/ nest	Paraformaldehyde 92%
(1:200) 0.5% solution	Penta potassium peroxy-monosulfate 49.7% Sodium hexametaphosphate 21.5% Sodium dodecylBenzenesulphonate 13.3% Other ingredients

For the experiment, two metal egg nests inside the poultry breeder's house were selected and marked. The bedding material of the first nest was mixed by hand with 5 g of paraformaldehyde for 30 seconds to ensure an even distribution of the powder throughout the nest (Cadirci, 2009). In the other nest, potassium peroxy- monosulfate was sprayed using a hand sprayer then mixed by hand for 30 seconds to ensure a uniform distribution of the solution (Kunanusont et al., 2020).

Table 2: Effect of disinfectants use on total bacterial count (TBC) of nest bedding (CFU x 10^6/g).

Disinfectant	Sample	TBC before application		TBC after 24 h		TBC after 72 h		TBC after 120 h	
		No.	Mean±SD	No.	Mean±SD	No.	Mean±SD	No.	Mean±SD
Paraformaldehyde	n1	115	190.3±65.3	14	*50.0±32.1	37	*38.6±32.5	164	*129.0±31.0
	n2	231		60		7		118	
	n3	225		76		72		105	
Potassium peroxy-monosulfate	n1	66	108.6±80.0	26	*35.3±17.9	9	*15.0±15.8	13	*6.3±6.1
	n2	201		56		33		5	
	n3	59		24		3		1	

* Means of any time interval with asterisks in comparison with means of TBC before application are significantly different at ($p \leq 0.05$)

Table 3: Effect of disinfectants use on total coliform count (TCC) of nest bedding (CFU x 10^6/g).

Disinfectant	Sample	TCC before application		TCC after 24 h		TCC after 72 h		TCC after 120 h	
		No.	Mean±SD	No.	Mean±SD	No.	Mean±SD	No.	**Mean±SD**
Paraformaldehyde	n1	197	151.6±43.1	4	*35.6±48.9	1	*17.0±28.5	3	*20.0±32.0
	n2	111		11		0		0	
	n3	147		92		50		57	
Potassium peroxy-monosulfate	n1	25	44.0±24.7	1	*1.0±1.0	0	*4.6±8.0	0	*1.3±2.3
	n2	72		2		14		4	
	n3	35		0		0		0	

* Means of any time interval with asterisks in comparison with means of TCC before application are significantly different at ($p \leq 0.05$)

Table 4: Effect of disinfectants use on total fungal count (TFC) of nest bedding (CFU x 10^6/g).

Disinfectant	Sample	TFC before application		TFC after 24 h		TFC after 72 h		TFC after 120 h	
		No.	Mean±SD	No.	Mean±SD	No.	Mean±SD	No.	Mean±SD
Paraformaldehyde	n1	67	36.3±31.5	47	*18.6±24.5	4	*12.6±16.7	5	*2.7±2.5
	n2	38		5		32		3	
	n3	4		4		2		0	
Potassium peroxy-monosulfate	n1	20	8.3±10.1	1	1.6±1.1	6	2.3±3.2	2	*1.6±0.5
	n2	2		1		0		2	
	n3	3		3		1		1	

* Means of any time interval with asterisks in comparison with means of TFC before application are significantly different at ($p \leq 0.05$)

Collection Of Samples

In sterile plastic bags, three nest bedding samples were collected from three different chambers of each marked egg nest before treatment with disinfectants to determine the initial microbial load. After application of disinfectants at 24, 72 and 120 hours intervals, nest bedding was mixed then 1 g of the nest bedding of each chamber was received into a sterile glass flask containing 99 mL of a disinfectant neutralizing solution consisting of 3% Tween 80, 0.3% Lecithin, 1% Histidine, 0.5% Sodium thiosulphate and 3% Saponin, to deactivate disinfectants and prevent the inhibitory concentration of the used disinfectant from being transferred to the recovery medium after each contact time (Espigares et al., 2003). Samples were then marked and transported to the laboratory in an ice tank for microbiological examination with a minimum delay (Rosario Cortés et al., 2004).

Microbiological Examination Of Nest Bedding Material

In the laboratory, under complete aseptic conditions, each collected nest bedding sample before disinfectant application was mixed for even distribution of contaminants then, 1 g was weighed using an electrical balance and added to a sterile flask containing 99 mL of sterile normal saline solution 0.9% to obtain 1/100 dilution. Hand mixing of flasks containing samples before and after disinfection was applied for 1 minute to permit suspension of contaminants and left to allow large particles to settle to the bottom of the flask for ease of pipetting. A series of tenfold dilutions were prepared from the samples collected before and after disinfection by transferring 1 mL of saline or neutralizing solution suspension into sterile test tubes containing 9 mL of sterile saline solution 0.9%. For determination of total bacterial, coliform, and fungal counts, previously prepared plates of plate count agar, MacConkey's agar and Sabouraud's dextrose agar were inoculated each with 0.1 mL of each dilution. The inoculated plate count agar and MacConkey's agar plates were incubated at 37°C for 24-48 hours. However, Sabouraud's dextrose agar plates were incubated at 25°C for 3-5 days then the colonies were enumerated and recorded as Colony Forming Unit per gram (CFU/g) of nest bedding material (Williams et al., 2016).

Statistical analysis

All data were analyzed with SAS (SAS 9.2, 2009) by which a pairwise comparison was performed between the mean of total bacterial count before application of two different disinfectants and the mean of total bacterial count after application of each disinfectant at different time intervals at 24, 72, 120 hours separately. The same comparison was performed for the mean of total coliform count and total fungal count before and after application of disinfectants at the same time intervals.

RESULTS

The results of this study were recorded after using two different disinfectants applied to different samples of nest bedding material (saw dust) separately. The values presented in the tables, illustrate the total bacterial counts (TBCs), total coliform counts (TCCs) and total fungal counts (TFCs) before the application of disinfectant and after the application at different time intervals at 24, 72 and 120 hours.

In comparison to values before disinfectant application the TBCs (Table 2), TCCs (Table 3) and TFCs (Table 3) significantly ($p \leq 0.05$) reduced after disinfection. Paraformaldehyde (Table 2) decreased TBC from 190.33± 65.31 to 50± 32.1 after 24hr, 38.6± 32.5 after 72hr, and the count increased again to 129± 31 after 120hr however, potassium peroxy-monosulfate (Table 2) decreased TBC from 108.66± 80 to 35.3± 17.93 after 24hr, 15± 15.8 after 72hr, and 6.3± 6.1 after 120hr. The effect of the two different disinfectants in reducing TCCs and TFCs, takes nearly the same pattern as TBC and persist at all time intervals and most of these results are statistically significant ($p \leq 0.05$).

Paraformaldehyde decreased TCC from 151.6± 43.1 to 35.6± 48.9 after 24hr, 17± 28.5 after 72hr, and the count increased again to 20± 32 after 120hr however, potassium peroxy-monosulfate decreased TCC from 44± 24.75 to 1± 1 after 24hr, 4.6± 8 after 72hr, and 1.33± 2.33 after 120hr (Table 3).

Paraformaldehyde decreased TFC from 36.3± 31.5 to 18.6± 24.5 after 24hr, 12.6± 16.7 after 72hr, and 2.7± 2.5 after 120hr however, potassium peroxy-monosulfate decreased TFC from 8.33± 10.11 to 1.67± 1.16 after 24hr, 2.33± 3.2 after 72hr, and 1.67± 0.58 after 120hr (Table 4).

DISCUSSION

Results of TBCs, TCCs, and TFCs in the tested nest bedding sample before applying disinfectants (paraformaldehyde and potassium peroxy-monosulfate) were higher than after application. Our obtained results are in accordance with those of Cadirci (2009) who found that all litter samples before formalin treatment contained high levels of TBCs, TCCs, and TFCs, and Chen and Jiang (2014) who reported that the litter contains large and diverse populations of microorganisms, which can reach up to 10^{10} CFU/g, and Gram-positive bacteria count for nearly 90% of the microbial load. However, Barker et al. (2010) stated that litter contamination is increased by the deposit of fecal droppings that contain high levels of intestinal bacteria on the litter surface. On the other hand, Ngogang et al., (2021) reported that chicken litter can contain a variety of pathogens, including viruses, bacteria, parasites, and fungi. After applying paraformaldehyde to nest bedding material, the results obtained indicated that there is marked reductions of TBCs, TCCs and TFC after 24, 72, and 120 hours of contact.

The obtained results are in agreement with those of Veloso et al. (1974), who recorded that paraformaldehyde flakes slowly decompose into formaldehyde gas in poultry litter due to the moisture content. As a result, both the bacterial and mold counts were reduced, after which they began to increase again over time. Some researchers attributed this increase to the accumulation of poultry manure and the elevation of temperature, which may have affected the rate of decomposition of formaldehyde flakes into gaseous formaldehyde. Cadirci (2009) also reported that after litter treatment with formalin the contamination may build up again. Moreover, Moustafa (2009) demonstrated that spraying litter material with formaldehyde solution 40% revealed a complete reduction of the TCCs and 94.6 and 95% reduction for the TBCs and the TFCs respectively. According to Chen and Jiang (2014), paraformaldehyde was effective in reducing coliform and total bacteria to less than 2000 CFU/g in broiler litter. They mentioned that the addition of paraformaldehyde powder to fresh chicken feces (in a dose of 1, 3, and 7 g paraformaldehyde per 100 g feces), resulted in reduction of TBCs from 2.2×10^9 CFU/g to 1.6×10^8, 10^3, and zero CFU/g, respectively.

Formaldehyde is used regularly in the poultry industry for disinfecting poultry houses, poultry litter, and hatching eggs (Fabrizio et al., 2002; Bialka et al., 2004). It is a very potent disinfectant (Cadirci, 2009). However, it has many disadvantages including eye irritation and allergic contact dermatitis, it is considered an occupational carcinogen by the Environmental Protection Agency (Smith, 1992). Moreover, it is harmful to the respiratory tract of newly hatched chicks (Cadirci, 2009) and is considered as embryo-otoxic for chicken embryos (Magras, 1996).

Regarding treatment of egg nest material with potassium peroxy-monosulfate, the recorded results in Tables 2, 3, and 4 showed that the reduction percentages in TBCs were 67.5, 86.19, and 94.2 after 24, 72, and 120 hours of contact, respectively. However, reduction percentages in TCCs and TFCs were; 97.7, 89.5 & 96.97 and 79.9, 72 & 79.9 after the same contact times, respectively.

The obtained results are in agreement with those of Payne et al. (2005), who observed that potassium peroxy-monosulfate resulted in significant reductions in total aerobic bacterial populations when applied at high concentrations to the broiler house floors (0.7 log reductions). Furthermore, Payne et al. (2005) observed a significant reduction in yeast and mold populations (0.17 log reduction). Payne et al. (2005) recorded that potassium peroxy-monosulfate could inactivate coliform bacteria within minutes in the presence or absence of organic matter.

From the previously mentioned results, it can be noticed that neither disinfectant could achieve a complete reduction of the microbial load of nest bedding material. But both disinfectants recorded satisfactory results. On the other hand, Payne et al. (2005), proved that disinfectants should be applied at a high rate to obtain a significant reduction in aerobic bacterial populations on soil surfaces during a field trial. In conclusion, disinfectant paraformaldehyde and potassium peroxy-monosulfate may be recommended for disinfection of nest bedding material in order to reduce the microbial contamination of hatching eggs.

CONCLUSION

In a closed broiler breeder farm where birds were housed on built-up litter, broiler's breeders nest bedding material (sawdust) is highly reliable to increasing microbial load so must use appropriate disinfectants to decrease this load and to reduce contamination of broiler breeder hatching eggs. Frequent use of paraformaldehyde powder 5g / nest and potassium peroxy- monosulfate 0.5% sprayed on nest bedding is recommended as it helps to reduce total bacterial, *coliform,* and fungal counts of nest bedding material so probably will reduce the microbial contamination of hatching eggs.

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CONFLICT OF INTEREST

The authors of current work declared no conflict of interest.

NOVELTY STATEMENT

This work shows the importance of nest bedding disinfectant application in improving hatching egg quality. Our work shows the effect of different disinfectants on nest bedding quality.

AUTHORS CONTRIBUTIONS

All authors except first one had supervision contribution. The first to fourth author had the major contribution in paper writing, editing, and reviewing, in addition the role of the first author in practical part implementation and finally corresponding by the first author.

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