Litter aeration and spread of *Salmonella* in broilers

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ABSTRACT Litter quality in the poultry sector is one of the main parameters of health, productivity, and animal welfare. Therefore, innovative management methods have been developed to improve the quality of litter. One of them is litter aeration (LA) by tumbling. However, there is little information related to the effect of this technique on the spreading of pathogens of public health importance such as *Salmonella*. In this context, the objective of this study was to determine the epidemiology of *Salmonella* in poultry farms, when serial LA were implemented during the rearing cycle of broilers. For this purpose, an experimental broiler farm with 3 identical rooms was used in the study. Two rooms were assigned to the LA treatment, and the other one served as the control room. Environmental samples were taken in poultry houses after LA in 4 consecutive weeks at the end of the cycle. All samples collected were analyzed according to the standards of the International Organization for Standardization (ISO 6579:2002, Annex D). The results of this study showed that in the control and treated rooms, the percentage of positive samples for *Salmonella* decreased in the first 3 LA sessions (LA 1, LA 2, and LA 3). However, in the last LA session of rearing (LA 4), the percentage of positive samples increased from 8.2 to 33.2% in the control room instead the treated rooms where the positive samples decreased ($P = 0.017$). Thus, the aeration of the litter as litter management technique in poultry broiler production does not increase the shedding or the spread of *Salmonella* throughout broiler houses. In addition, it could be an effective technique to reduce the infective pressure of this bacterium in several areas of the farm or in certain moments of the rearing period with more risk of multiplication and spreading of *Salmonella*.

Key words: broiler, litter aeration, *Salmonella*, *Campylobacter*, poultry

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INTRODUCTION

Salmonellosis is considered as one of the most important problems for public health worldwide associated with food consumption (EFSA, 2012). Data published by the European Food Safety Authority (**EFSA**) in 2012 showed that *Salmonella* is one of the pathogens most often implicated in foodborne disease. Nevertheless, *Salmonella* continued to be an important threat to public health; recently published data (EFSA, 2012) reported 99,020 and 4,420 cases of human salmonellosis in the European Union and Spain, respectively.

There are numerous sources of human salmonellosis, although eggs and poultry meat are considered the most common source of human infection (EFSA, 2012). In 2001, the FAO-WHO Expert Consultation on Risk Assessment of the microbiological hazards in foods performed the "Risk characterization of *Salmonella* spp. in eggs and broiler chickens," which demonstrated that reducing the prevalence of salmonellosis in poultry farms corresponds to a proportional decrease in risk of contamination of prepared foods for consumption (FAO-OMS, 2001). This decline was especially significant in broiler production. In this context, legislators have been working to minimize *Salmonella* prevalence in poultry sectors with the introduction of a National Control Programme to reduce the incidence of the bacteria in poultry flocks. The program for broiler flocks in Spain set measures to reduce the prevalence of *Salmonella* Enteritidis and *Salmonella* Typhimurium, the strains which pose the highest human health risk, to 1.0% or less for December 2011 (EC, 2007). However, noncontaminated broiler meat has been sold since 2011 for human consumption. Many epidemiological studies have reported the wide variety of routes by which *Salmonella* can be disseminated within integrated poultry companies in Europe (Heyndrickx et al., 2002; Namata

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et al., 2008; Marin et al., 2011). Among the different environmental factors involved in the epidemiology of *Salmonella*, remains of feces of previous flocks (Marin et al., 2011), water and drinkers (Jung et al., 2012), feed and feeders (Berge and Wierup, 2012), boots of farmers (Namata et al., 2009), work tools (Gradel et al., 2002), and litter (Thakur et al., 2013) are highlighted.

The role of the litter in animal health, productivity, and welfare has been reported in several studies (Jones et al., 2005; Namata et al., 2008; Volkova et al., 2010). Different factors such as mishandling of the litter, inadequate ventilation of the facilities, as well as excessive moisture of the litter can predispose the development of the poor litter quality (ASABE, 2007). This is an important fact from an animal health point of view because bad quality of litter encourages the growth and spread of nonpathogenic and pathogenic microorganisms such as *Salmonella* on poultry production system (Marin and Lainez, 2009). In addition, the quality of the litter has also an impact on animal welfare resulting in pododermatitis problems (Berk, 2009), breast abrasions (Ekstrand et al., 1998), keratoconjunctivitis (Miles et al., 2006), and consequently decreases carcass quality (Bender and Mallinson, 1991; Al Homidan et al., 2003).

Therefore, due to the importance of the quality of litter on poultry sector, innovative management methods have been developed. Some of them are the use of probiotics and prebiotics (Badia et al., 2013), the implementation of forced ventilation techniques (Kaliste et al., 2004), and the use of absorbent materials for the composition of the litter (Cambra-López et al., 2009). It has also been demonstrated that litter aeration technique (**LA**) by tumbling (ASABE, 2007) is an important technique that increases the quality of the litter and reduces its humidity during the rearing period, increasing the quality of broiler production (ASABE, 2007). Litter aeration is a manure management method that can be used to break up and turn the litter during the rearing period leading to aeration and drying of the litter (van Middelkoop, 1994; Allen et al., 1998). However, there is little research related to the effect that this technique has on the spreading of pathogens of public health importance such as *Salmonella*. In this context, the objective of this study was to determine the epidemiology of *Salmonella* in poultry farms, when serial litter aerations were implemented during the rearing period.

MATERIALS AND METHODS

This experiment was performed in 2 consecutive rearing periods. The rearing was done inside an experimental poultry house in the Animal Research and Technology Centre (Instituto Valenciano de Investigaciones Agrarias, Segorbe, Spain) to mimic the real conditions of poultry production. The experimental house was tested for *Salmonella* before the experiment. In each rearing, 2,400 one-day-old chickens were received and divided equally in 3 experimental rooms. All rooms had the same dimensions and environmental conditions in which the birds were reared (ventilation, illumination, feeding, and so on). In 2 rooms, LA was carried out at weekly intervals (from wk 4 until wk 7) using a machine designed for this purpose (Benza, ER73AV, La Coruña, Spain, Figure 1). In the third room, LA was not performed, thus following the usual process of commercial farms (control room).

Salmonella Sampling

Samples were collected at different time points of the flock lifespan: before the arrival of the birds, the first day of rearing, the third week of the rearing cycle (the week before LA started), and weekly after LA (4 last weeks of rearing; Table 1).

Before the chicks were placed in the experimental rooms, environmental samples were taken from the rooms to assess the *Salmonella* contamination of the house (walls, feeders, and water dispensers). These samples were collected with sterile wet gauze pads (AES Laboratories, Bruz Cedex, France). Moreover, samples of water (500 mL) and feed (500 g) were also collected. Finally, one sterile jar of bedding was filled from 6 different points of the house (500 g).

To determine the *Salmonella* status of 1-d-old chick flocks in accordance with the Commission Regulation (EC, 2003), meconia were obtained by lightly press-

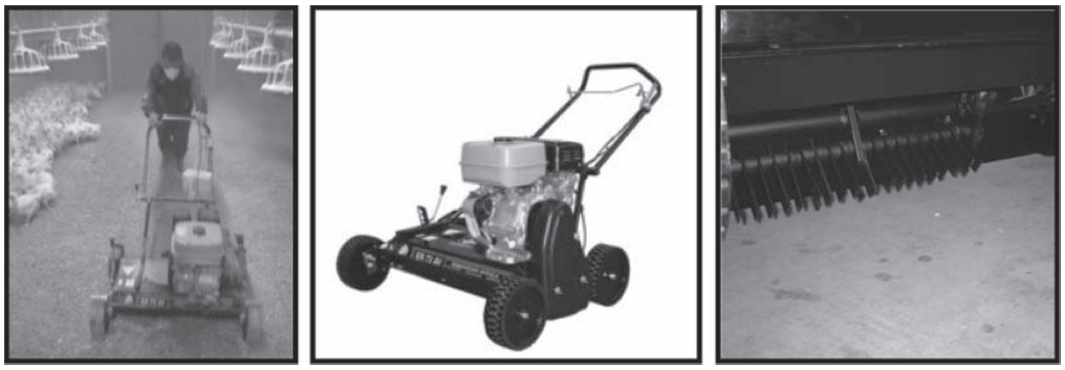

Figure 1. Machine used for litter aeration (LA) and detail of the rotative parts.

Table 1. Samples collected at different moments of the rearing period

	Rearing period (wk)						
	Nonlitter aeration			Litter aeration			
Sample type	1[1]	2	3	4	5	6	7
Surfaces	Walls	—	Walls	Walls	Walls	Walls	Walls
	Feeders	—	Feeders	Feeders	Feeders	Feeders	Feeders
	WD	—	WD	WD	WD	WD	WD
Flock	Meconia	—	Feces	Feces	Feces	Feces	Feces
	Box liners	—	Ceca	—	—	—	—
	Box surfaces	—	—	—	—	—	—

[1]On the first day of rearing, feed, water, and bedding were also collected. WD: water dispensers.

ing in the abdomen of 250 to 300 chicks. Moreover, 10 chick delivery-box liners were collected by placing the whole consignment into sterile bags. Further, delivery-box surface sample was collected with sterile wet gauze pads (AES Laboratories). Day-old chicks were declared infected if at least one of the samples taken tested positive.

The third week of rearing, 10 broilers from each room were euthanized and ceca contents were analyzed as a pool. Moreover, samples of surfaces (walls, feeders, and water dispenser) were collected from each room as reported above. Moreover, feces samples from each flock were collected from wk 4 until wk 7 with 3 pairs of sock swabs in accordance with EFSA guidelines (EC, 2005). First, the floor area of the houses was divided into 3 equal sectors and 1 pair of sock swabs was used in each sector for sampling. Samples were taken by walking over the chosen sector, and each pair of sock swabs with fecal material was analyzed as an individual sample. This sampling procedure will theoretically provide 95.0% confidence of detection of 1.0% within-flock prevalence assuming the test is 100% sensitive (EC, 2005).

Environmental samples were collected 24 h after LA treatments from aerated rooms and the control room (walls, feeders, water dispensers, and feces).

Salmonella Isolation

All samples were collected directly into sterile sample jars and analyzed according to ISO 6579:2002 (Annex D; International Organization for Standardization, 2002). First, the samples were preenriched in 1:10 vol/vol Buffered Peptone Water (BPW, Scharlau, Barcelona, Spain) and incubated at 37 ± 1°C for 18 ± 2 h. Then, preenriched samples were transferred into Semi-Solid Rappaport Vassiliadis (MSRV, Difco, Valencia, Spain) agar plate (100 μL), which was incubated at 41.5 ± 1°C for 24 to 48 ± 3 h. Suspicious plates were transferred to 2 different agar plates, ASSAP (AES Laboratories) and XLD (Xylose Lysine Deoxycholate agar, Liofilchem, Valencia, Spain), and incubated at 37 ± 1°C for 24 ± 3 h. After the incubation period, 5 suspect colonies of *Salmonella* were selected, and were transferred to a nutrient agar plate (Scharlab, Barcelona, Spain) and incubated at 37 ± 1°C for 24 ± 3 h. Then, urease test was performed for 4 h at 37°C. Finally, a biochemical test API-20E (API-20, bioMerieux, Madrid, Spain) was done to confirm *Salmonella* spp. All *Salmonella* strains isolated were serotyped according to the Kauffman-White-Le-Minor technique.

Statistical Analysis

The cross-contamination between the flock and the room environment at third week of rearing (before LA) were analyzed using a chi-squared test (version 5.1, Statgraphics Plus, STSC Inc., Rockville, MD). The influence of LA in the spread of *Salmonella* in broiler rooms as well as the evolution of the spread along the weeks of the reading period were analyzed by logistic regression using the GLIMMIX procedure of SAS (SAS Institute, 2009). The presence of *Salmonella* contamination according to the LA and type of sample collected was compared by a chi-squared test.

RESULTS AND DISCUSSION

The status of the house before placing the chicks (Rose et al., 2000; Marin et al., 2011) and *Salmonella* status of 1-d-old chicks (Kim and Kim, 2010) are 2 of the most important risk factors for *Salmonella* infection during the rearing period. During the study, a total of 408 samples were collected and analyzed for *Salmonella* and 29.4% were positive for the bacterium. All the environmental samples collected in the poultry house before the arrival of the 1-d-old chicks were negative (n = 36). Furthermore, when chicks arrived from the hatchery, delivery-box liners and surfaces of the chicks' transport cages and meconia were positive for *Salmonella* (n = 6). The infection of 1-d-old flocks could be transmitted vertically from infected parent flocks or horizontally transmitted during hatching, loading, and transport to the farm (Heyndrickx et al., 2002). When 1-d-old chick flocks are infected by the bacterium, a rapid spread of *Salmonella* throughout the feeding, drinkers, and environment of house has been observed leading to cross-contamination between birds and the environment (Marin et al., 2011). This coincides with a period when the animals are more susceptible to the infection by the

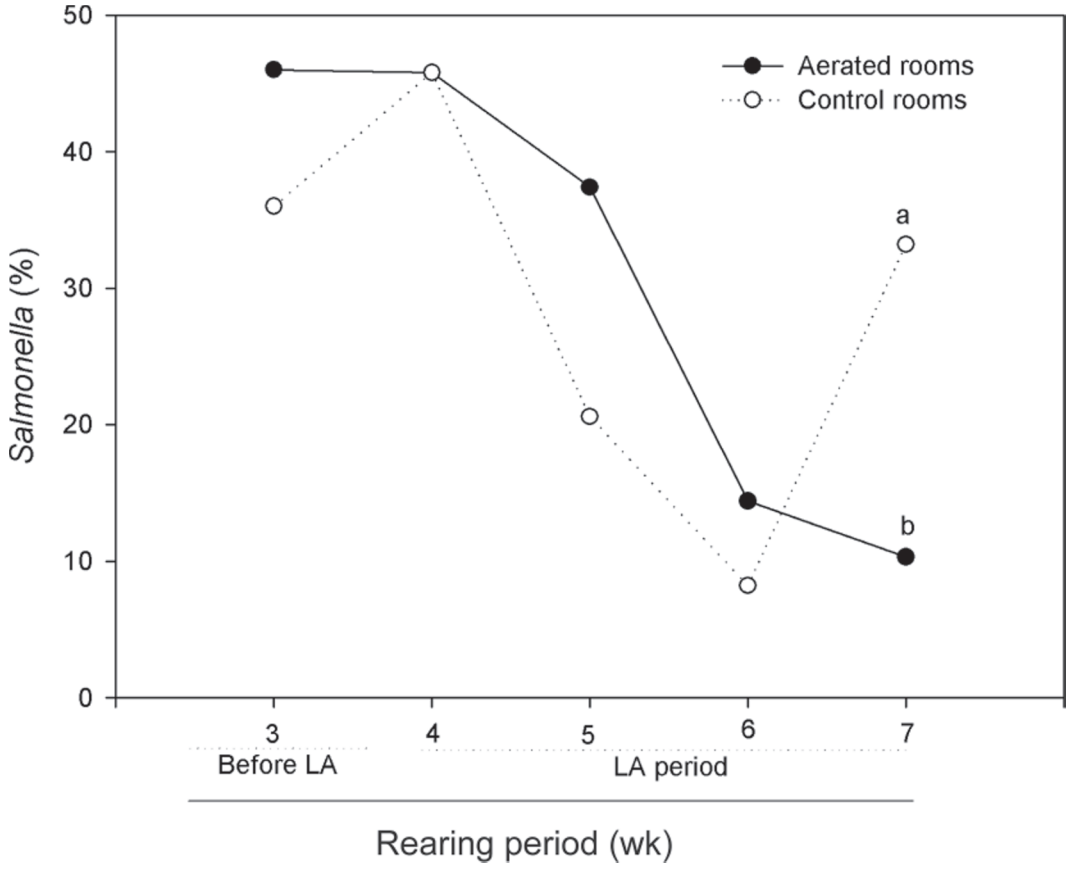

Figure 2. Comparison of the prevalence of *Salmonella* before and along litter aeration (LA, from wk 4 until wk 7 of rearing period). Different letters (a,b) represent significant differences ($P < 0.05$).

bacterium (Marin and Lainez, 2009), due to immaturity of the immune system resulting in the infection of the whole flock (Beal et al., 2004).

Before the LA started (wk 3), the cross-contamination between chicken feces and farm environment was demonstrated regardless of room (47.4%, $P = 0.111$) or sample collected (walls: 33.3%; feeders: 66.6%; water dispensers: 55.5%; and feces: 38.8%, $P = 0.239$).

In reference to the total number of samples analyzed during LA sessions on treated rooms, *Salmonella* was isolated in 23.9% of the samples collected (n = 268). In the control room the bacteria was isolated in 23.8% of the samples collected (n = 134). No statistically significant differences were found between treated and control rooms (Figure 3). These results agree with those obtained by Kwak et al. (2005) who studied the effect of LA in the amount of microbiota present in a broiler farm. In particular, the effect on the survival of enteric bacteria in both treated and untreated litter with LA was studied, and no differences were found between the concentrations of the enterobacteria.

The evolution of the prevalence of *Salmonella* along LA treatment showed that the percentage of positive samples for *Salmonella* decreased from 45.8 to 10.3% from the first to the last session of LA, respectively (Figure 2). In the control room, the same detection pattern was observed, except the last weeks of treatment (LA 4). At the end of the rearing period (LA 4), the percentage of positive samples increased from 8.2% to 33.2% in the control room, whereas the LA-treated room decreased from 14.4 to 10.3% ($P = 0.017$, Figure 2).

The natural pattern of excretion of *Salmonella* in poultry broiler shows that when the chicks arrive at the farm shedding the bacteria, they present the maximum shed at 14 d of life, reducing *Salmonella* shedding until their departure to the slaughterhouse (Van Immerseel et al., 2004). Nevertheless, several reports showed that the presence of adverse environmental conditions such as high stocking density, incorrect ventilation of the facilities, and presence of poor quality of litter for excessive moisture and droppings (Lange et al., 1997; Skov et al., 1999; Eriksson de Rezende et al., 2001; van de Giessen et al., 2006) may increase *Salmonella* shedding and create a favorable environment for the multiplication and spread of microorganisms such as *Salmonella* (Hayes et al., 2006). These conditions occur mainly at the end of the rearing period when birds have highest average bird weight, which leads to less space between the animals, resulting in difficult ventilation and an increase in litter moisture (Cobb-Vantress Inc., 2008). In addition, at the end of rearing there is an excessive accumulation of droppings, feathers, or remains of feed leading to a decline in quality of litter (Ritz et al., 2004). However,

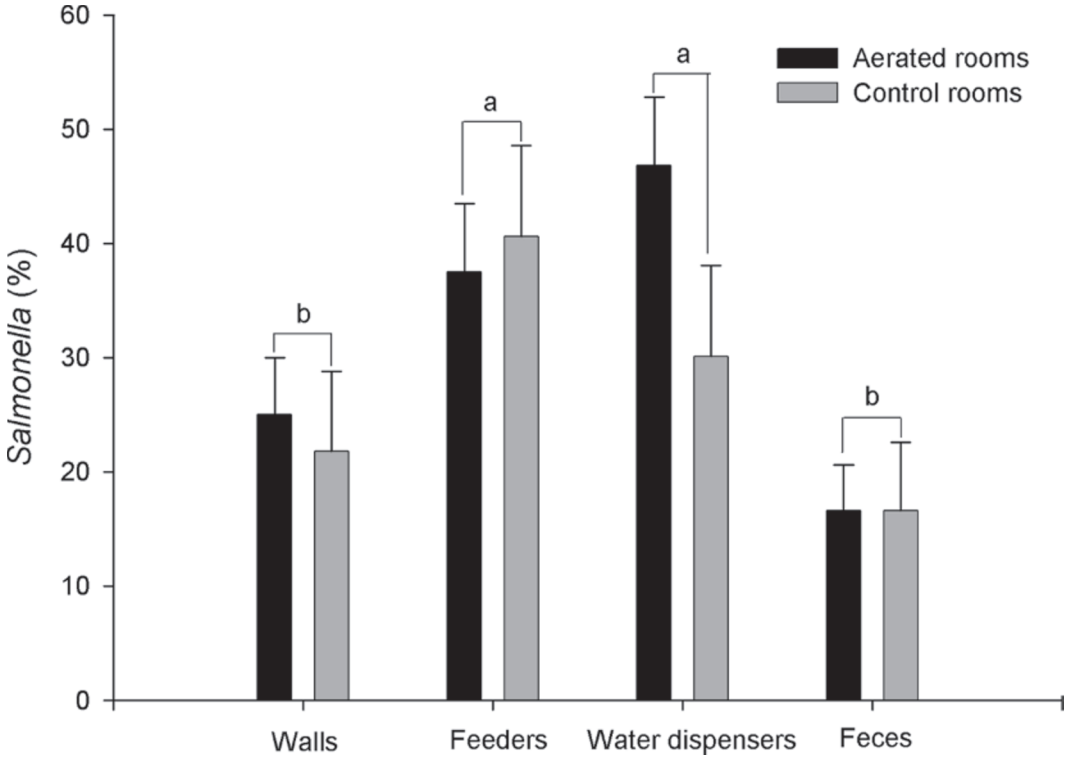

Figure 3. Percentage of *Salmonella* isolated during litter aeration (from the fourth until the seventh week of rearing period) in environmental samples. Different letters (a,b) represent significant differences between sample types ($P < 0.05$).

where LA was performed in treated rooms the results were different in the last LA session, because humidity levels were controlled throughout the rearing with this technique and less water is available for the microorganisms, which causes the dehydration and inactivation of many of them, improving litter characteristics (De la Rosa et al., 2002). Therefore, the implementation of the LA could control the development of adverse environmental conditions, preventing the multiplication and spreading of microorganisms such as *Salmonella* (Hayes et al., 2006).

In relation to the environmental samples collected, both treated and control rooms presented similar percentages of positive samples ($P = 0.769$, Figure 3). Additionally, there were statistically significant differences between the different environmental samples collected ($P = 0.023$, Figure 3). The highest percentage of positive samples were found in drinkers (40.6%), followed by feeders (38.5%), walls (23.9%), and feces (16.6%).

In accordance with the results obtained, several authors demonstrated that there are risk areas in poultry houses around drinkers, which present a greater water activity, creating favorable conditions which promote the multiplication of the bacteria (Eriksson de Rezende et al., 2001; Hayes et al., 2006; Payne et al., 2007). Consequently, the importance of the implementation of measures such as LA to reduce moisture (especially in areas such as drinkers) is an important tool to implement in broiler production (ASABE, 2007). In addition, advantages from this type of technique also involve an improvement of the sanitary quality of the litter (Koon et al., 1994), as well as an exponential reduction in the number of pads and conjunctiva injuries present in the birds (van Middelkoop, 1994; Allen et al., 1998; ASABE, 2007).

Nevertheless, several studies showed that the process of LA affects air quality, with an increase in particulate matter emissions of harmful gases and microorganisms (Quarles and Caveny, 1979). Thus, it is possible that broilers suffer different respiratory (Andersen et al., 2004) and cardiovascular diseases (Meluzzi et al., 2008). This implies that further studies are needed regarding this technique and its possible impact on the health of the birds.

In brief, LA, as litter management technique in poultry broiler production, does not increase the shedding or the spread of *Salmonella* throughout broiler houses. In addition, it could be an effective technique to reduce the infective pressure of this bacterium in several areas of the farm or in certain moments of the rearing period with more risk of multiplication and spreading of *Salmonella*. Nevertheless, further research is needed to clarify its effects on the birds as well as on the litter moisture content.

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