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Presence of *Salmonella* spp. in reused broiler litter¤

Presencia de Salmonella spp. en cama reutilizada de pollos de engorde

Presença de Salmonella spp. em cama reutilizada de frangos de corte

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Summary

Background: reutilization of poultry litter for multiple broiler flocks is a common practice in modern production systems due to the increasing scarcity and cost of bedding materials, and the necessity to reduce environmental impact. However, this practice has been associated with sanitary risks, such as the presence of *Salmonella* spp. in broiler meat. **Objective:** a study was conducted to detect the presence of *Salmonella* spp. in reused litters. **Methods:** 1,280 litter samples from Midwestern Brazilian poultry farms were analyzed during seven consecutive flocks. Samples were collected from flocks aged 28 to 32 days. Disposable shoe covers were used for sample collections. Presence of *Salmonella* spp. was determined by microbiological isolation. During the interval period between flocks the litter was fermented prior to its reuse by covering it with a black plastic canvas for 7 days. **Results:** positive samples for *Salmonella* spp. decreased when the number of litter reuses increased compared with the first reuse of the litter. An anaerobic digestion process with biological and physicochemical changes in the litter material and microbial communities may explain the low survival of pathogenic bacteria such as *Salmonella* spp. **Conclusions:** our study demonstrates that litter reused after the fermentation process is a safe and recommended practice to reduce the presence of *Salmonella* spp.

Key words: *ammonia, anaerobic digestion, composting, fermentation, microbiota.*

Resumen

Antecedentes: la reutilización de la cama de pollos de engorde es una práctica común en el sistema moderno de producción avícola, sustentada por la reducción en el impacto ambiental, escasez de este material y disminución de costos de producción. Sin embargo, esta reutilización se ha asociado con riesgos

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sanitarios, tales como presencia de *Salmonella* spp. en los lotes de pollo. **Objetivo:** se realizó un estudio con el fin de detectar la presencia de *Salmonella* spp. en camas reutilizadas y fermentadas de pollos de engorde pertenecientes a granjas comerciales. **Métodos:** se analizaron 1280 muestras de cama de diversas granjas avícolas ubicadas en el centro oeste de Brasil durante siete lotes consecutivos de pollos. Las muestras de cama fueron tomadas de galpones con aves entre los 28 y 32 días de edad, utilizando polainas. La presencia de *Salmonella* spp. se determinó mediante aislamiento microbiológico. Durante el intervalo entre lotes, la cama fue fermentada antes de cada reutilización cubriendo la superficie entera de la cama con una lona de plástico negra por siete días. **Resultados:** fue observada una disminución en las muestras positivas para *Salmonella* con la reutilización y fermentación de las camas entre lotes, significativa con respecto al primer reuso. Esto indica que puede estar ocurriendo un proceso de digestión anaeróbica que conduce a que los procesos biológicos y físico-químicos entre el material de la cama y la comunidad microbiana allí presentes, estén afectando la supervivencia de bacterias patógenas como *Salmonella*. **Conclusiones:** nuestro estudio demuestra que la reutilización de la cama es una práctica segura y recomendable cuando se realiza después del proceso de fermentación, debido a que reduce la presencia de *Salmonella* spp.

Palabras clave: *amonio, compostaje, digestión anaeróbica, fermentación, microbiota.*

Resumo

Antecedentes: a reutilização de cama aviária por vários lotes é uma prática moderna do sistema de produção de aves, baseada na redução do impacto ambiental, escassez de este material e diminuição nos custos de produção. Porém, dita prática é associada com riscos sanitários como a presença de patógenos como *Salmonella* spp. nos lotes de frango. **Objetivo:** uma pesquisa foi realizada para detectar a presença de *Salmonella* spp. na cama reutilizada e fermentada de produtores de frango. **Métodos:** foram analisadas 1280 amostras de cama de diferentes produtores do Centro-oeste do Brasil durante sete lotes consecutivos. As amostras de cama foram coletadas com aves na idade entre 28 e 32 dias usando pró-pés descartáveis e a presença de *Salmonella* spp. foi determinada por isolamento bacteriológico. Durante o intervalo dos lotes a cama foi tratada antes da reutilização por meio da cobertura através de uma lona plástica preta em toda a superfície interna do aviário por sete dias. **Resultados:** foi observada uma diminuição no número de amostras positivas de *Salmonella* spp. com a reutilização e fermentação das camas entre os lotes, significativa em relação ao primeiro reuso. Isto indica que o processo de reutilização, seguido de fermentação anaeróbia do material da cama pela comunidade de microrganismos afetou a sobrevivência de bactérias patogênicas como *Salmonella* spp. **Conclusões:** este estudo evidencia que o reuso da cama é seguro e recomendado quando realizado após o processo de fermentação no intervalo do lote, devido a que diminui a presença de *Salmonella* spp.

Palavras chave: *amônia, compostagem, digestão anaeróbica, fermentação, microbiota.*

Introduction

Multiple broiler flocks are commonly reared on a single batch of litter in intensive poultry production systems. In these systems, birds are placed into grow-out houses one day after hatching, directly on litter bedding (Volkova *et al.*, 2009). Management and processing is required to decrease the microbial load before reusing the litter (Vizzier Thaxton *et al.*, 2003). With this purpose, fermentation has been proposed as an optimal alternative to ensure the microbiological quality of the litter (Macklin *et al.*, 2006). However, some doubts regarding potential sanitary risks associated to this practice have been also posed.

Many microorganisms in poultry litter originate in bird excrement, including Enterobacteriaceae and other bacteria with zoonotic capacity (Cook *et al.*, 2012; Fries *et al.*, 2005). Continuous exposure to undesirable bacteria from litter can increase contamination of the birds' digestive tract. Even though enterobacteria do not cause health problems in chickens, it may become a human sanitary

problem during slaughtering due to contamination when the carcass accidentally come in contact with contents from infected crop or intestine, compromising food safety and public health (Haapapuro *et al.*,1997).

Transference of pathogens into the food chain may also occur when litter is applied to soil as an organic fertilizer, resulting in the contamination of fresh produce (Lovanh *et al.*, 2007; Volkova *et al.*, 2009). Contaminated litter can promote pathogen perpetuation from one flock to another when it is reused more than once. For this reason, reuse of litter is not recommended when sanitary episodes have occurred.

Independent of litter destination (reuse for subsequent flocks or used as fertilizer), treatment to reduce or inactivate bacteria is vital for decreasing animal and human health risks. Thus, litter treatment is considered a needful condition in good poultry production practices (Larrison *et al.*, 2010; Pope and Cherry, 2000). Total replacement of the litter after every flock results in considerable environmental impact due to the high amounts of substrate required (e.g. wood shavings, straw or sawdust) and the destination of this residue in the environment (Pandey and Soupir, 2011; Pote *et al.*, 2011, Watts *et al.*, 2011). Furthermore, changing the litter after every flock represents a significant cost in poultry production.

The purpose of this study was to evaluate the presence of *Salmonella* after fermenting the litter by covering it with a canvas prior to each reuse.

Materials and methods

Experiments

A total of 1,280 litter samples from several poultry farms in Midwestern Brazil were analyzed during seven consecutive flocks. The population corresponds to 196 producers.

Litter management

The procedure performed between flocks (Figure 1) is briefly described as follows:

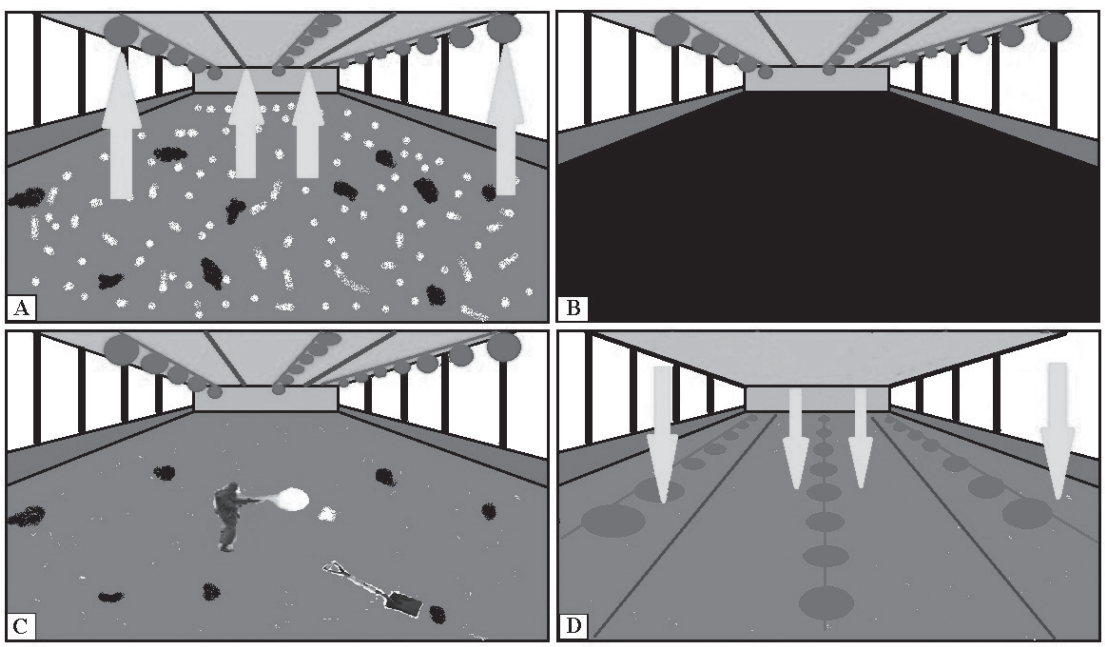

Figure 1. Process used for litter management. **A.** Poultry house after depopulation. Feathers and crusts can be seen on the litter. Feeders and drinkers are cleaned and raised-up. **B.** The entire surface of the litter is covered with plastic canvas for seven days. **C.** Crusts are removed, feathers are burnt with a flame-thrower, and the litter is mixed. Ventilation is provided for two days. **D.** The litter is ready to receive a new flock.

1. Cleaning and rising of the equipment (feeders, drinkers, etc.) with soap and water immediately following depopulation (Figure 1A).
2. Watering the litter (20 liters/m^2).
3. All columns inside the shed were covered with plastic canvas (approximately one square meter) to protect them from the fermentation process.
4. Displacing the litter from the shed sides to create a space between the walls and the litter.
5. Covering the litter with plastic canvas avoiding air entrance (Figure 1B).
6. Removing the canvas after seven days of fermentation, discarding the crusts and mixing the litter.
7. Applying the flame-thrower uniformly to the entire surface to burn residue such as feathers (Figure 1C).
8. Ventilating the house for two days before placing the new flock of birds (Figure 1D).

After this process was completed, one-day-old chicks were housed directly on the reused litter.

Sample collection

Litter samples were collected when birds were between 28 and 32 days of age. Collectors cleaned their hands carefully before. Plastic boots with shoe cover swabs were used for collection. Collectors walked on the litter for about 10 min, focusing placement of steps between feeders and drinkers, given that these sites maintain a high concentration of animals and therefore a greater amount of feces. The sample was collected on the shoe cover surface in contact with the litter. The shoe cover swabs were placed inside sterile bags containing 1% buffered peptone water solution and stored in cool boxes or coolers with ice while microorganism samples solubilized. Sample bags were sent to laboratory after collection.

Laboratory processing

Shoe cover swabs were discarded. Two aliquots of the solution were transferred to selective enrichment broths: 0.5 ± 0.05 mL into 10 mL Tetrathionate Broth and 0.1 ± 0.02 mL into 10 mL Rappaport-Vassiliadis. Both were incubated separately at 35 ± 2 °C and 42 ± 2 °C for 18 h and 24 h, respectively, and carefully mixed by vortexing. Then, each culture was streaked both in Brilliant Green agar and MacConkey agar plates using 10 µL inoculum for each. Agar plates were incubated overnight at 35 ± 2 °C and examined for the presence of *Salmonella* spp. colonies (BRASIL, 1995).

Statistical analysis

The chi-squared test was used to verify whether the frequency of positive samples for *Salmonella* spp. was related to the number of litter reuses. The criterion for statistical significance was $p<0.05$.

Results

Detection of *Salmonella* spp. in litter reused up to seven times is shown in table 1. A decrease in positive samples was observed when comparing the new litter with all subsequent reused litters after the covering process, significant between the first flock with new litter and the second flock with the first reuse.

Table 1. Number of positive and negative samples to *Salmonella* in reused litter.

Number of flocks reusing litter	1	2	3	4	5	6	7	Total
Salmonella positive	43[a]	19[b]	28	28	22	20	11	171
Salmonella negative	164	177	133	163	190	166	116	1109
Total	207	196	161	191	212	186	127	1280

[a, b] Different letters in the same line indicate statistical difference in Chi-Square ($p<0.05$).

Besides the observed *Salmonella* reduction and beyond the aim of this report, a noteworthy decrease of darkling beetle (*Alphitobius diaperinus*) infestation was also observed after the covering process. Although it was not measured, we consider this an interesting observation for further studies since the covering and fermentation processes could also eliminate most insects and larvae without requiring the use of chemicals.

Discussion

The farms included in this study used the litter-covering method between flocks as a strategy for

poultry waste management. Ammonia concentration increased during the anaerobic digestion and fermentation process that probably occurred. We believe this leads to a microbicidal action in different populations, including *Salmonella* spp. These results are in agreement with Roll *et al.* (2011) who analyzed the presence of *Salmonella* in reused litters of 14 consecutive flocks and observed a reduction in the presence of *Salmonella* after treatment with lime.

The fermentation process consists of the hydrolysis of complex components, including fats, proteins and polysaccharides, which are broken down by microorganisms to their component subunits with the posterior production of collectable biogases (mainly methane and CO_2) (Chen *et al.*, 2008; Kelleher *et al.*, 2002). Poultry waste is composed of litter, bird manure, and other residues. Due to high protein and amino acid metabolism, poultry manure is rich in organic nitrogen in the form of urea, which is mostly converted into ammonia by microbial activity, consequently undergoing a nitrification process (i.e. conversion to nitrate) (Kelleher *et al.*, 2002). In this pathway ammonia exists as either a gas (NH_3) or as ammonium (NH_4^+), which is a hydrophobic, water-soluble and highly permeable molecule to biological membranes.

Ammonia is proposed as an anaerobic digestion inhibitor by the leak of proton-motive forces or interference with the tricarboxilic acid cycle. The first requires both a pH and electron gradient. The second involves the amination of α-ketoglutarate, an intermediate needed for the metabolism of organic compounds (Chen *et al.*, 2008; Krylova *et al.*, 1997).

Additional abiotic factors should be considered; the temperature reached during fermentation may also play a microbicidal role. The maximum temperature in our study was 60 °C (data not shown). Kim *et al.* (2012) observed that reduction of *Salmonella* can be achieved by exposing fresh chicken litter to 70 °C for 80.5 to 100.8 min. Wilkinson *et al*. (2011) reported that *Salmonella typhimurium* in fresh chicken litter was completely eliminated in 1 h at 55 to 65 °C under laboratory conditions. Other abiotic parameters that regulate microbiological conditions are pH (Payne *et al.*, 2007) and moisture (Eriksson de Rezende *et al.*, 2001). Different litter management and treatment methods can modify those factors (Miles *et al.*, 2011; Torok *et al.*, 2009).

Microorganisms and their complex microbial communities are responsible for most biochemical transformations. Using a combination of culture and molecular detection in intestinal chicken, Lu *et al.* (2003a) reported that Gram-positive bacteria and proteobacteria were the predominant populations (including *Lactobacillus, Clostridium, Staphylococcus* and *Streptococcus*) involved in the decomposition of organic material, including wood. This may explain the absence of important animal and human pathogens from the environment. Our study was conducted in Midwestern Brazil. Composition of litter microbiota depends on the geographical region (Cressman *et al.*, 2010) and environmental conditions (Dumas *et al.*, 2011). It should be noted that most of the studies have been conducted in North America.

It has been demonstrated that diversity of intestinal bacterial populations increases as birds age (Lu *et al.*, 2003b). Deposition of excreta onto the litter rapidly alters biotic and abiotic environments simultaneously as litter conditions affect the intestinal microbiota and immune responses (Chapman and Rayavarapu, 2007; Lee *et al.*, 2011). This supports our hypothesis that reusing the litter under proper management conditions may improve intestinal microbiota composition, important for the growth and health of the bird.

In conclusion, by reusing litter we observed a reduction in the presence of *Salmonella* spp., which could contribute to reducing costs and usage of raw litter material. Further studies are needed to evaluate the impact of reusing the litter on the presence of other relevant poultry pathogens such as *Clostridium* and *Eimeria*, as well as its effect on other physical traits, production cost, and its impact on natural resources.

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