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Effect of Poultry Guard Litter Amendment on Horizontal Transmission of *Salmonella enteritidis* in Broiler Chicks

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Abstract: To evaluate the effect of a litter acidifier (PGLA) on *Salmonella enteritidis* (SE) horizontal transmission, two experiments were conducted with broiler chicks grown on used (Exp. 1) and new (Exp. 2) litter. In each experiment, three hundred day-old broiler chicks from a commercial hatchery were obtained and divided into three litter treatments with four replicate pens each. The treatments were: control (no litter treatment); low dose of PGLA (LD: 815g/2.27m²); and high dose (HD: 1631 g/2.27m²). In Exp. 1, two hundred-forty chicks were placed in floor pens with pine shaving-based litter previously used for at least two prior growouts (20 chicks/pen). Another 60 chicks were challenged with 7.5×10³ cfu of SE (seeders), placed in a separate pen with clean new pine shaving-based litter for 24 hours, then 5 seeders (20%) were placed with the contact chicks in each respective treatment pen. *Salmonella* recovery from cecal tonsils of 10 chicks/ pen were evaluated on days 11 and 21. Application of PGLA at both LD and HD on used litter significantly reduced ($p<0.05$) SE recovery compared to controls (Control: 28%, LL: 0%; HL: 3% respectively) on day 11 after placement, but no difference was observed at day 21. However, a significant increase ($p<0.05$) in body weight was detected in the HD compared to the control group on d21, but not d11. Similarly, application of PGLA to clean pine shavings (Exp. 2) reduced ($p<0.05$) SE recovery from cecae of chicks cultured on day 11 (control: 46%; LD: 23%; HD: 18% respectively). Body weights through 21 days were unaffected by PGLA treatment of new litter. These data suggest that PGLA treatment of new or used litter may reduce early horizontal transmission of *Salmonella*. Enhanced 21-day performance of chicks on used litter treated with PGLA may suggest that other low-level pathogens were reduced by treatment, although further studies are necessary to confirm and extend these findings.

Key words: Litter treatment, *Salmonella enteritidis*, horizontal transmission, broiler chicks

Introduction

In the United States, it is estimated that 1.4 million humans contract salmonellosis and that the annual cost of this illness, including lost productivity, is \$3 billion annually (WHO, 2006). In the year 2004, surveillance data indicated that the greatest number of food borne illnesses were caused by *Salmonella*, comprising 42% of all laboratory diagnoses (FoodNet, 2005). Because poultry and poultry products often serve as the vehicle for human *Salmonellosis* (Kimura *et al.*, 2004; Marcus *et al.*, 2007), the poultry industry and governmental agencies are focused on eradicating *Salmonella* both in live birds and at the processing plant (Hargis *et al.*, 2001).

It is well known that *Salmonella* and other pathogens can survive for long time on the litter as well as some fungi and viruses that could potentially affect bird's health and performance (Srivastava *et al.*, 1972). *Salmonella*-contaminated litter is a potential source of cross contamination of the carcasses in the processing plant, as it may be carried into the plant on the feet and feathers (Line, 2002). Recently, the re-utilization of litter as bedding material has become a common practice in the poultry industry in the United States (Hess *et al.*, 2000). However, it sometimes represents a problem for perpetuation of pathogens on the farm. Therefore,

implementation of practices to break the transmission of pathogens like *Salmonella* between flocks and reduce the possibility of contamination before slaughter are required.

Many studies have been performed in the past years focused on litter treatments with different organic acids to reduce the emission of ammonia by modifying the pH of the litter. The effects of these organic acids (such as citric, tartaric and salicylic) (Ivanov, 2001) and other compounds like formalin (Williams, 1980), sodium bisulfate (Blake and Hess, 2001), sodium sulfate (Ivanov, 2001) and sulfuric acid (Asari *et al.*, 2004) on *Salmonella* viability and other microorganisms have been documented. Poultry Guard Litter Amendment (PGLA)® = Poultry Guard® litter amendment, Oil Dri Corporation of America, 410 North Michigan Ave. Chicago IL 60611 is a product based on sulfuric acid (40-50%), used to control ammonia volatilization by converting litter ammonium to ammonium sulfate, thereby decreasing litter pH and providing potent ionic effects that enhance acidification and reduction of pathogens on litter (Payne *et al.*, 2002; Asari *et al.*, 2004). The purpose of this study was to evaluate the effect of two levels of PGLA spread on the top of used and new litter on the horizontal transmission of *Salmonella enteritidis* and body weight of broiler chicks.

Table 1: Effect of two concentrations of PGLA spread on the top of used litter on *Salmonella enteritidis* horizontal transmission in broiler chicks

| Treatment | No. <i>Salmonella</i> -positive cecal tonsils samples / total (%) | |
|---------------------------------------|---|-------------------------|
| | Day 11 | Day 21 |
| Control | 11/40 (27.5) ^a | 1/40 (2.5) ^a |
| Low Dose (815g/2.27m ²) | 0/40 (0.0) ^b | 0/38 (0.0) ^a |
| High Dose (1631G/2.27m ²) | 1/40 (2.5) ^b | 2/37 (2.7) ^a |

^{a-b}Values within columns with different superscripts are significantly different (p<0.05)

Table 2: Body weight of chicks placed on used litter treated with PGLA

| Treatment | Body weight (grams±SEM) | | |
|---------------------------------------|-------------------------|--------------------------|----------------------------|
| | Day 1 | Day 11 | Day 21 |
| Control | 46.52±0.45 ^a | 202.86±5.65 ^a | 644.00±13.19 ^a |
| Low Dose (815g/2.27m ²) | 47.03±0.45 ^a | 205.40±5.01 ^a | 658.95±9.52 ^{a,b} |
| High Dose (1631G/2.27m ²) | 46.44±0.40 ^a | 211.19±4.77 ^a | 678.38±10.15 ^b |

^{a-b}Values within columns with different superscripts are significantly different (p<0.05)

Materials and Methods

Bedding material: Used pine shaving-based litter from at least two prior growouts used in experiment one was obtained from the Poultry Research Farm at the University of Arkansas. Before placement, the litter was blended and mixed to uniformity and level of moisture was calculated (23%). New pine shaving-litter was used in the second experiment. The depth of the litter in the first trial was ~10 cm and in the second trials was ~5 cm. Samples of litter (used and new) were cultured for *Salmonella* before the placement of chicks using described procedure (Andrews *et al.*, 1978). None was detected in either litter source.

Experimental design: In each trial, twelve pens were built (~2.27 m²/pen) in an isolation room of the Poultry Health Laboratory and a random block pattern was utilized for three different treatments: 1. Control; 2. Low dose (LD) of PGLA (815g); 3. High dose (HD) (1631g) of PGLA. Treatments were applied to the top of the litter manually 24h before placement of the chickens. Three hundred day-of-hatch broiler chicks from a commercial hatchery were obtained. Two hundred-forty chicks were randomly assigned to three groups with four replicates (20 chicks/pen). The other 60 chicks were wing banded, challenged with 7.5×10³ cfu of *Salmonella enteritidis* (seeders) and placed in a separate pen on clean shavings. Twenty-four hours later, 5 seeders (20%) were placed in each treated pen. Chicks received a balanced corn/soybean-based diet containing salinomycin (Coxistac®) at label-recommended concentration, formulated to meet or exceed age-appropriate National Research Council recommendations and water *ad libitum*.

***Salmonella* challenge:** A primary poultry isolate of *Salmonella enteritidis*, bacteriophage type 13A (SE), was obtained from the USDA National Veterinary Services Laboratory. This isolate was resistant to novobiocin¹ (NO) (25 µg mL⁻¹) and was selected for resistance to nalidixic acid² (NA) (20 µg mL⁻¹) in our laboratory. For these studies SE was grown overnight in tryptic soy broth³ (TSB) at 37°C. Cells were washed three times in sterile saline by centrifugation at 1864g and the concentration was estimated with a spectrophotometer to approximately 10⁹ cfu mL⁻¹ in sterile saline and then diluted to inoculated concentrations. Concentrations of SE were retrospectively determined by spread plating on brilliant green agar⁴ (BGA) plates containing NO (25 µg mL⁻¹) and NA (20 µg mL⁻¹) and enumeration for each experiment. Actual determined cfu for each experiment are reported.

***Salmonella* recovery and body weight:** Horizontal transmission of *Salmonella enteritidis* was evaluated at 11 and 21 days in both experiments. Ten chickens per pen were humanely killed and cecal tonsils were aseptically removed following enrichment in tetrathionate broth. After 24-hour of incubation at 37°C, the broth was agitated and streaked on Brilliant Green Agar (BGA) plates with (NA/NO) antibiotics. Plates were incubated for an additional 24 hours at 37°C and examined for the presence or absence of typical *Salmonella* lactose-negative and antibiotic resistant colonies.

Statistical analysis: Culture data were analyzed using chi-square analysis to determine significant differences in cecal colonization (Zar, 1984). Body weight was determined by one-way analysis of variance using General Linear Models procedure and significant differences between groups were further separated using Duncan's multiple range test (SAS Institute, 1988).

Results and Discussion

In experiment 1, the application of both, LD and HD doses of PGLA on used litter prior to placement of chicks significantly reduced (p<0.05) SE recovery compared to the control at 11 days. However, very low levels of *Salmonella* were recovered at 21 days (Table 1) in all the experimental groups. Interestingly, a significant increase in body weight (p<0.05) was detected in chicks placed on treated litter with high dose of PG compared with control chicks (Table 2).

In the second experiment, the number of *S. enteritidis* positive samples were statistically higher (p<0.05) in the control group (46%) compared to both treated groups (LD: 23% and HD: 18% respectively) at 11 days but no difference was detected at day 21 (Table 3). No differences in body weight were detected in any group at day 21 (data not shown).

Table 3: Effect of two doses of PGLA spread on the top of new litter on *Salmonella enteritidis* horizontal transmission in broiler chicks

| Treatment | No. <i>Salmonella</i> -positive cecal tonsils samples / total (%) | |
|---------------------------------------|--|---------------------------|
| | Day 11 | Day 21 |
| Control | 18/39 (46.1) ^a | 11/39 (28.2) ^a |
| Low Dose (815g/2.27m ²) | 9/40 (22.5) ^b | 5/39 (12.8) ^a |
| High Dose (1631G/2.27m ²) | 7/40 (17.5) ^b | 6/40 (15.0) ^a |

^{a,b}Values within columns with different superscripts are significantly different ($p < 0.05$)

In the present study, higher levels of *Salmonella enteritidis* was detected in broiler chicks placed on new litter than those raised on used litter. Gustafson and Kobland (1984) found similar results with *Salmonella typhimurium*-infected chicks placed on used and fresh litter but with no litter treatment. Olesuik *et al.* (1971) reported that *Salmonella typhimurium* could survive longer on new litter than used bedding material. Turnbull and Snoeyenbos (1973) have reported that high level of ammonia and pH generated from used litter has a salmonellacidal effect and it could be one of the factors of *S. enteritidis* horizontal transmission reduction observed in both used and new litter in the present report.

Low levels of *Salmonella enteritidis* recovered from chicks placed on both used or new litter in this study could be due to the reduction on the litter pH. Payne *et al.* (2002) reported that litter treated with different levels of sulfuric acid and sodium sulfate dramatically reduced the pH. Pope and Cherry (2000) reported that used litter treated with 2.27kg/9.29 m² of poultry litter amendment reduced significantly the pH and it was associated with a reduction of *Escherichia coli*. Also, Line (2002) found that litter treated with aluminum sulfate or sodium bisulfate reduced *Campylobacter* colonization and population in the ceca.

Bacteria are challenged by many environmental factors that can reduce their viability. These organisms are frequently confronted with more acid or alkaline conditions outside of their tolerable limits. *Salmonella typhimurium* and *E. coli* can grow in pH levels ranging from 5 to 9 (Foster, 1993), but *Salmonella* grows optimally between 6.5 and 7.5 (Chung and Goepfert, 1970). There are reports that in acidic environments, *Salmonella typhimurium* are able to generate an acid tolerance response by inducing synthesis of some defense mechanisms (Foster, 1993; Willmes-Riesenberg *et al.*, 1996; Hall and Foster, 1996). Foster and Spector (1995) observed minimum growth of *S. typhimurium* when it was exposed to 4.3 pH in minimal glucose medium. The present data indicate that the acidic environment in litter treated with PGLA indeed reduced *Salmonella* horizontal transmission, but it did not completely eliminate colonization of chicks in all groups, perhaps due to some resistance to acidic environments.

Reducing or controlling intestinal colonization and fecal shedding associated with *Salmonella* infection could be useful in several contexts. The reduction in the number of *Salmonella* cells shed in feces should help control the spreading of this pathogen within and between houses (Gast *et al.*, 1993). Our results suggest that to treat used or new litter with PGLA may reduce early horizontal transmission of *Salmonella enteritidis*, but by itself it is not enough to completely eliminate contamination. Perhaps combinations including litter acidification and other non-antibiotic alternatives may be synergistic for complete reduction of *Salmonella* in poultry. In addition, the enhancement of performance at 21 days in chicks over used litter treated with PGLA may suggest that other low-level pathogens were reduced, although further studies are necessary to confirm and extend these findings.

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