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M. J. Pope
Stephen F Austin State University

T. E. Cherry
Stephen F Austin State University

An Evaluation of the Presence of Pathogens on Broilers Raised on Poultry Litter Treatment®-Treated Litter[1]

M. J. Pope* and T. E. Cherry*,[2]

*Stephen F. Austin State University, Department of Agriculture, PO Box 13000, Nacogdoches, Texas 75962

ABSTRACT Two trials were conducted to evaluate the presence of salmonella, campylobacter, and generic *Escherichia coli* on broilers raised on Poultry Litter Treatment (PLT®)-enhanced litter in comparison with those raised on untreated litter. Two Company A farms included three houses on each farm as the treated group and three houses per farm as controls. Two complete growouts were evaluated on each farm. The Company B study included 10 farms with two paired houses per farm, one house as the treated group and one house as the control. One growout was evaluated per farm. The pathogen sampling consisted of litter sampling and whole bird rinses on the farm and in the processing plant. Litter pH, ammonia concentration, total litter bacteria, temperatures, and humidity were also recorded. The study with Company A resulted in lower mean levels of pH, ammonia concentration, total litter bacteria, litter *E. coli*, and bird rinse counts for salmonella and *E. coli* in houses treated with PLT.® The results for Company B closely resembled those for Company A, but also included campylobacter data, which showed no difference between treated and control groups. The data indicate that PLT® may be a beneficial component for on-farm pathogen reduction.

(*Key words*: pathogen, broiler, litter, Poultry Litter Treatment,® microbiology)

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INTRODUCTION

Food safety is not only an industry responsibility, but also a major consumer concern. As a result of society's heightened awareness about food safety, the poultry industry has recently been faced with producing the same high-quality, cost-efficient product using Hazard Analysis Critical Control Point (HACCP) guidelines. The HACCP procedures are relatively new to the industry, but the idea of supplying consumers with chicken that is safe for consumption is an old philosophy. In an effort to help the poultry industry accomplish its production and HACCP goals, a pathogen reduction trial was conducted involving the use of Poultry Litter Treatment (PLT®)[3] on commercial broiler farms.

In order to fully appreciate why an integrated poultry industry is so concerned about meeting HACCP requirements, one must first realize the purpose of HACCP. In short, HACCP is a preventive system of food safety control for identifying and controlling potential hazards. In general, a hazard in food processing is considered to be a physical, chemical, or biological entity that causes a food to be unsafe (Bryan, 1992; The National Advisory Committee on Microbiological Criteria for Foods, 1992). The HACCP is used to target these hazards to the food supply, and is a tool in the control, reduction, and prevention of pathogens in meat and poultry. Although the food supply is considered safe according to food safety experts, 6.5 to 81 million individuals have cases of microbial foodborne illnesses each year (Archer and Kvenberg, 1985; Archer and Young, 1988). The responsibility for producing and marketing products that are safe for the consumer is that of the poultry industry, whereas the government's role is to set performance standards or criteria and ensure that the industry is meeting its food safety responsibilities. As of January 26, 1998, the HACCP plan became mandatory in most processing plants.

The HACCP as a component of a total quality assurance effort does not rely on simply finished product testing (Troutt et al., 1995). The National Advisory Committee on Microbiological Criteria for Foods (1992) has emphasized the need for control of food safety risks at all levels, and has endorsed HACCP systems as a goal to control these risks. In keeping with this line of reasoning, Bains and MacKenzie (1974) tracked the transmission of salmonella through an integrated poultry organization. From the results of their study, it appears that salmonella may be transmitted continuously through the grains to the

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[2]To whom correspondence should be addressed: tcherry@sfasu.edu.

[3]Jones-Hamilton Co., PLT® Division, Salisbury, MD 21801.

Abbreviation Key: HACCP = Hazard Analysis Critical Control Points; PLT® = Poultry Litter Treatment.

breeder feed, to breeder parent stock, to day-old chicks, and thence to the finished product. In order for any system to effectively reduce risk on all levels, all segments must work together to identify hazards and assess potential risk, develop science and technology for intervention, and communicate appropriately with industry, government regulators, and consumers (Harris et al., 1995).

As previously mentioned, this study was directed toward reducing pathogenic populations of bacteria associated with commercial broiler farms. Although HACCP guidelines are not yet required for this particular sector, they may be in the future. The objective of this study was to evaluate the number or presence of pathogens, specifically salmonella, generic *E. coli*, and campylobacter, on carcasses of broilers raised on PLT®-treated litter compared with those raised on untreated litter.

Poultry Litter Treatment® is a dry, granular acid composed of sodium bisulfate, and is used extensively by the poultry industry for poultry house ammonia control, litter acidification, on-farm pest management, and, in this instance, HACCP programs for pathogen reduction (Terzich, 1997). The major advantages of using PLT® are derived from its ability to acidify poultry house litter. A previous trial found that using formaldehyde flakes as an agent for the control of microorganisms in built-up litter caused total bacterial counts in the treated pens to be reduced to about 10% of the control values. Simultaneously, the litter became more acidic (Veloso et al., 1974). The idea behind the present study was that acidifying the litter would reduce total bacteria, *E. coli*, and salmonella in the litter and, in turn, their presence on the live bird and broiler carcass. Bacterial contamination of processed broiler carcasses originates from many sources, including live broilers, plant equipment, environment, and plant employees (Izat et al., 1988; De Boer and Hahne, 1990; Jones et al., 1991). Contamination of the live animal may occasionally originate from the internal tissues, such as air sacs, but the two major sources are the bacteria present in the gastrointestinal tract (internal) and those on feathers and skin (external) (Musgrove et al., 1997). Some research suggests that surface contamination of the bird (feet, feathers, skin, adhering matter) is the major factor responsible for the introduction of salmonella into processing plants (Rigby and Pettit, 1980; Rigby et al., 1980a,b; Lillard, 1989; Izat et al., 1990). Commercially raised broilers are in constant contact with the litter upon which they are raised. Therefore, litter is one of the many variable factors that could influence salmonella contamination of the external surface of the bird (Reiber et al., 1990). This study was carried out to determine whether this type of contamination was not exclusive to salmonella, but could also be applied to *E. coli*, campylobacter, and other bacteria.

MATERIALS AND METHODS

The research was conducted on two Company A farms and 10 Company B farms. For Company A, three houses on each farm served as the treated group, and three houses per farm served as the negative control. Two complete growouts on each farm were evaluated. The Company B trial involved two houses per farm, with one serving as the PLT®-treated group and the other as the control. One growout per farm was evaluated. Houses were paired based on housing design and equipment. Wood shaving litter was used in treated and control houses. The number of flocks that had been previously grown on the litter was not consistent among all farms, but all houses on each individual farm had the same number of flocks grown in them. The PLT® was applied on top of the litter in the half-house brooding area according to manufacturer's recommendations[4] at the rate of 2.27 kg (5 lbs)/9.29 m^2 (100 ft^2), 12 to 24 h prior to the arrival of chicks. The PLT® was applied using a 91-kg (200 lb) capacity push spreader. The control houses were left untreated.

Litter pH, ammonia, and litter bacterial samples were taken before and after PLT® treatment, as well as from the control houses. Litter pH testing consisted of scraping approximately 20 g of litter from the top surface of the litter and placing it in a sterile cup, where it was combined with approximately 30 mL of sterile distilled water. The litter and water solution was mixed thoroughly and allowed to stand for 1 to 2 min. A pH reading[5] was then taken and recorded. Ammonia readings were taken at floor level in the center of the houses with a calibrated ammonia gun.[6] Ammonia testing tubes were not reused. Litter was collected at various locations within the house, including areas adjacent to feed lines and water lines, and in the center of the house. Approximately one composite 100-g sample of litter was collected for bacterial testing by scraping the heel of the hand along the top of the litter surface while wearing a sterile latex glove and placing the sample in a sealed, sterile plastic bag. Samples were shipped overnight to Virginia-Maryland Regional College of Veterinary Medicine, Center for Molecular Medicine and Infectious Diseases (Blacksburg, VA 24061), where they were tested for total bacteria, salmonella, and *E. coli*. Litter pH, ammonia, and litter bacterial sampling continued once per week in Weeks 1 and 2. Just prior to the time at which birds were permitted to migrate from the half-house brooding area into the entire house, PLT® was applied to the off chamber of the treated houses at the rate of 2.27 kg (5 lb)/9.29 m^2 (100 ft^2). The off chamber of the treated houses received its first application of PLT® just before bird migration so that the litter amendment would be fresh when the chicks were moved into the area.

All treated houses then received an application of PLT® to the entire house at the previous rate 7 d prior to processing the broilers. The control houses remained un-

[4]PLT® Research and Technical Information Notebook: Application Instructions, Jones-Hamilton Co., Salisbury, MD 21801.

[5]pHTestr 1, OAKTON,® model #35624-00, Davis Instruments, Baltimore, MD 21215.

[6]Toxic Gas Detector, Matheson,® model 8014KA, Matheson Safety Products, East Rutherford, NJ 07073.

treated. At that time, drag swabs were taken in the control houses, and before and after PLT® treatment in the treated houses. Drag swabs were designed as two 10 × 10-cm gauze strips attached to twine, with one strip 1.5 m (5 ft) from the holding end and the other strip 1.8 m (6 ft) from the end. Utilizing sterile techniques, the assemblies were placed in sealed, sterile plastic bags with 5 to 10 mL of sterile skim milk medium and kept frozen until time of use. At time of use, the drag swab was removed from the plastic bags by touching only the end loop of the twine with latex gloves. The swabs, four per house, were then dragged through the houses for at least 15 min. Upon completion, the drag swabs were carefully placed back in their original plastic bag, at which time the twine was cut from the gauze strips. The plastic bags were kept in coolers in the field while sampling was being completed, then immediately taken to the company laboratory for testing for the presence of salmonella.

On-farm whole bird rinses were conducted 12 to 24 h prior to processing. Approximately 20 birds from each house were sampled for salmonella and *E. coli* in the Company A trial, whereas in the Company B trial, campylobacter was also sampled. The birds were euthanized, and carcass rinses were performed as described by Stern et al. (1995). Birds that were not sampled were transported to the company processing facility in live-haul cages that were unused for at least 12 h. Four bacterial swabs of the live-haul cages were taken from each trailer before birds were loaded. Swabs were identical to drag swabs used for the litter sampling 7 d prior to processing. Instead of being pulled, the swabs were randomly wiped across the live-haul cages. Once the birds arrived at the processing plant, treated birds were first to be processed. Twenty birds per house were randomly taken from the transfer table to be sampled by the whole carcass rinse method (Stern et al., 1995). Carcasses at the sample site, which was the transfer table just prior to evisceration, were scalded and picked, and the feet were removed. Samples were analyzed for salmonella and *E. coli* (AOAC, 1980; AOAC, 1980). Samples collected from the Company B trial were also analyzed for campylobacter (Stern et al., 1995).

Statistical Analysis

Statistical analyses of the data were performed with an analysis of variance using the general linear models of SAS with separation of the means by Duncan Multiple Range (SAS Institute, 1988). Significance was accepted at $P < 0.05$. The analysis of total bacteria and *E. coli* in the litter was derived from a total of 24 samples in each of the three weeks' litter that was collected. In the on-farm bird rinses for *E. coli* and salmonella, there were 119 samples obtained from the control houses and 179 samples from the PLT®-treated houses. The data for the in-plant bird rinses were obtained from 170 samples from the control houses and 150 samples from the PLT®-treated houses.

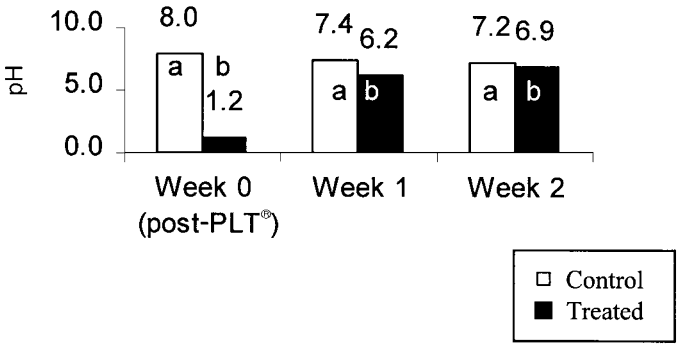


FIGURE 1. pH analysis during Weeks 0 [after Poultry Litter Treatment® (PLT®) treatment], 1, and 2 for Company A based on mean levels. Statistical differences ($P < 0.05$) were evaluated within each week between the PLT® treatment and control. Differences were not evaluated between weeks.

RESULTS AND DISCUSSION

The comparison of mean pH values from PLT®-treated houses and control houses for all flocks showed that pH readings were significantly lower in the treated houses, especially at the time of chick placement (Figure 1). Lower pH levels are beneficial for many reasons, one of which is lower levels of ammonia. Ammonia concentration increases with increasing pH (Carr et al., 1990). Ammonia release from litter is negligible when litter pH is below 7; release starts when the pH is near 7.0 and reaches high levels at 8.0 and above (Reece et al., 1979). Additionally, as the litter pH decreases from an average of 8.0 to 9.0, down to 3.0, the bacterial load declines as well (Hardin and Roney, 1989).

Poultry Litter Treatment® also proved to be effective in significantly reducing ammonia early in the flock's life (Figure 2). The ability of PLT® to effectively reduce atmospheric ammonia levels is attributed to a combination of mechanisms, including 1) direct chemical interaction with uric acid, 2) reduction in litter pH, and 3) reduction in populations of bacteria that generate ammonia gas from uric acid excreta (Terzich et al., 1998). High ammonia levels damage the bird's respiratory system and allow

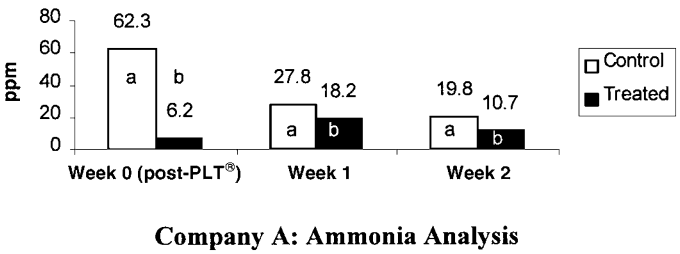


FIGURE 2. Ammonia analysis during Weeks 0 [after Poultry Litter Treatment® (PLT®) treatment], 1, and 2 for Company A based on mean levels. Statistical differences ($P < 0.05$) were evaluated within each week between the PLT® treatment and control. Differences were not evaluated between weeks.

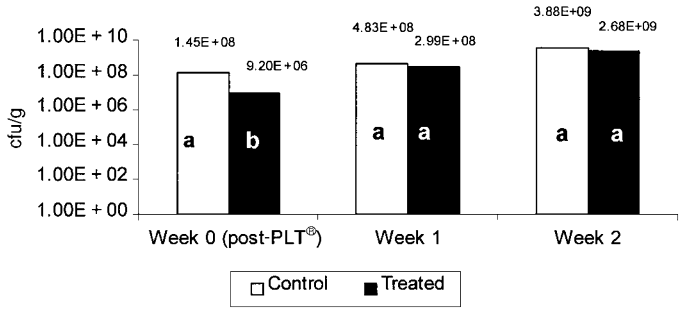
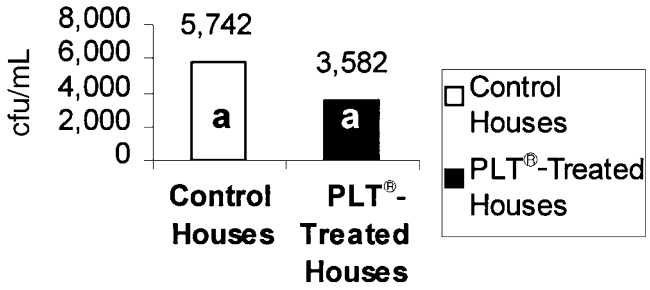


FIGURE 3. Total litter bacteria analysis during Weeks 0 [after Poultry Litter Treatment® (PLT®) treatment], 1, and 2 for Company A based on mean levels. Statistical differences ($P < 0.05$) were evaluated within each week between the PLT® treatment and control. Differences were not evaluated between weeks.



Company A: Farm Bird Rinses for *Escherichia coli*

FIGURE 5. On-farm whole bird rinses for *Escherichia coli* for Company A based on mean levels. Statistics were calculated where $P < 0.05$. PLT® = Poultry Litter Treatment.®

viruses and bacteria to cause infection, leading to declining flock health and performance (Terzich, 1997).

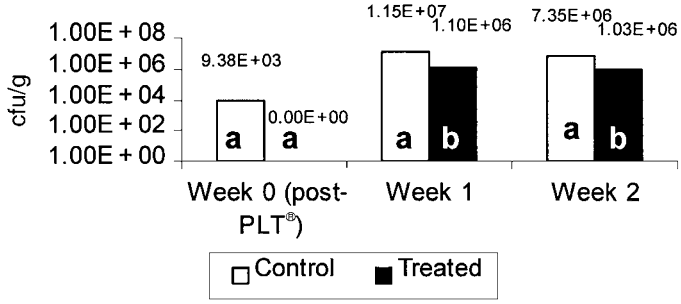
A significant difference in total litter bacteria was observed between treated and control houses at Week 0, but not at Weeks 1 or 2 (Figure 3). Also, a comparison of total bacterial levels with pH data could be used to support findings that bacterial load decreases as pH decreases.

Standard enumeration procedures were used to identify *E. coli*, which do not differentiate between pathogenic and nonpathogenic *E. coli*. The analysis of *E. coli* in the litter, like total bacteria, resulted in treated house litter having lower mean levels of colony-forming units than control house litter (Figure 4). Significant differences were seen for Weeks 1 and 2, but not for Week 0.

The sampling of salmonella in the litter during Weeks 0, 1, and 2 showed that treated and control groups consistently tested negative for the presence of the bacteria. Data collected on the farm seemed to show that the use of PLT® in broiler house litter could prove to be a beneficial component of a possible on-farm HACCP program and improve the birds' overall environment.

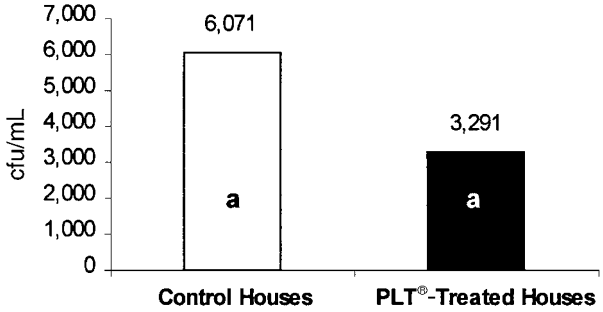
Trends similar to those evaluated in the litter during Weeks 0, 1, and 2 were also seen at the time of on-farm whole bird rinses; however, when comparing mean colonies of *E. coli*, the difference was not statistically significant (Figure 5). Nor were the mean number of colonies of *E. coli* on whole bird rinses in the processing plant, postscald and prior to evisceration, statistically different from the control group (Figure 6).

Salmonella data were not as promising for PLT® as the *E. coli* results seemed to be; however, there were some differences in the treated and control groups. As previously stated, treated and control groups tested negative for the presence of salmonella in the litter during Weeks 0, 1, and 2. On-farm bird rinses for salmonella were slightly lower for the PLT®-treated houses compared with the negative control houses, but the two groups were not statistically different. Similar findings were also seen in the processing plant. There were limited drag swab data available, but findings revealed no difference in the treated and control groups. Additionally, at the conclusion of the study, it was realized that drag swabs that were passed across housing equipment probably did not accurately portray salmonella found in the litter. Trailer swab data were too few to draw any scientific conclusions. However, it was noticed that trailers and live-haul cages that were washed and not completely dried provided a



Company A: Litter *Escherichia coli* Analysis

FIGURE 4. Analysis of litter *Escherichia coli* analysis during Weeks 0 [post-Poultry Litter Treatment® (PLT®) treatment], 1, and 2 for Company A based on mean levels. Statistical differences ($P < 0.05$) were evaluated within each week between the PLT® treatment and control. Differences were not evaluated between weeks.



Company A: Plant Bird Rinses for *Escherichia coli*

FIGURE 6. Plant bird rinses for *Escherichia coli* for Company A based on mean levels. Statistics were calculated where $P < 0.05$. PLT® = Poultry Litter Treatment.®

better environment for bacterial growth than those that were only out of service for approximately 12 h.

The campylobacter data from the Company B trial presented are also based on descriptive statistics or mean levels, and revealed no difference on the farm between the treated and control groups and only very marginal differences in the processing plant. The processing plant data were collected both before the inside and outside bird wash and postchill.

The data seem to indicate a reduction of bacteria on the farm, but this did not carry through statistically to the processing plant. Based on the results of this trial, it was concluded that PLT® has an inhibitory effect on the survival of *E. coli* and salmonella in broiler house litter. Although the compound cannot completely eliminate infection, it is presumed that under commercial conditions, PLT® may have the potential to reduce the prevalence of *E. coli* and salmonella entering the processing plant on live broilers.

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