

Research on *Salmonella* in broiler litter reused for up to 14 consecutive flocks

V. F. B. Roll,¹ M. A. Dai Prá, and A. P. Roll

Federal University of Pelotas, Faculty of Agronomy Eliseu Maciel, Department of Animal Science,
PO Box 354, 96010-900 Pelotas, RS, Brazil

ABSTRACT The reuse of poultry litter is a common practice in the Brazilian poultry industry for flocks of healthy chickens, due to 2 fundamental aspects: production cost and environmental sustainability. Litter is a potentially important source of infection for *Salmonella*, which requires characterization by microbiological analysis in different aspects of management and reuse. The objective of this study was to verify the occurrence of *Salmonella* in broiler litters reused up to 14 times in Brazilian poultry farms. From January 2008 to November 2010, 8,877 samples of litter on disposable shoe covers were analyzed from broiler farms located in southern Brazil. At the laboratory, samples were pro-

cessed for isolation and identification of *Salmonella*. Of the total 8,877 samples analyzed, only 2.5, 5.27, and 2.08% were positive for *Salmonella* in the years 2008, 2009, and 2010, respectively. Linear regression models indicate that there is a significant decrease ($P < 0.05$) in the count of samples positive for *Salmonella* with the reuse of litter. After the sixth reuse of the litter, values of samples positive for *Salmonella* are significantly ($P < 0.0001$) lower than expected (chi-squared test). Results show that the reuse of treated broiler litter is a safe practice and contrary to expectations, it substantially decreases the bacterial load of *Salmonella*.

Key words: *Salmonella*, broiler, reuse, poultry, litter

2011 Poultry Science 90:2257–2262
doi:10.3382/ps.2011-01583

INTRODUCTION

Broiler litter is a mixture of a substrate with the feces of birds where many undesirable bacteria may develop, such as *Salmonella* spp., *Campylobacter* spp., *Escherichia coli*, *Clostridium perfringens*, and *Staphylococcus aureus*. The accumulation of these pathogens raises concerns about the flock itself and, especially, about consumer health. For this reason, it is important to know the microfloral composition present in poultry litter (Lu et al., 2003).

The reuse of broiler litter is a common practice in the Brazilian poultry industry for flocks of healthy chickens, due to 2 fundamental aspects: the cost of production and environmental sustainability. The reuse avoids the acquisition cost of material to prepare litter in sufficient quantity to cover 5 to 10 cm of length over the entire extension of broiler house floors.

Perhaps for this reason, Thaxton et al. (2003) have observed that the practice of reusing litter, just removing the moist litter that has been transformed into a

cake, has become very common in the North American poultry industry.

Likewise, companies produce programs for biosecurity, in which one of the key steps is the disinfection of the facilities, capable of destroying microorganisms that are pathogenic to birds (Bermudez and Stewart-Brown, 2003).

Inside the house, there are factors that inactivate pathogenic bacteria present in the litter: the elapsed time, antibiotics, physical agents (temperature and ammonia), water activity, humidity, and acidification.

However, according to the European rules for the protection of broiler chickens, the broiler litter should be changed for each flock (Council of the European Union, 2007). Brazilian companies must meet certifying requirements of certifiers to export their poultry products to other countries. Certifiers have different rules and do not specify the number of flocks that can be raised on the litter, requiring however, treatment for litter reuse. For this reason, Brazilian export companies meet the requirements of importing countries, ensuring that the reused litter is treated and tested against microbiological risks. The justification for not reusing litter is based solely on the aspect of health and welfare of the birds. However, several studies have shown that the use of substances or methods that promote decon-

©2011 Poultry Science Association Inc.

Received April 29, 2011.

Accepted July 3, 2011.

¹Corresponding author: roll98@ufpel.edu.br

tamination of material are viable alternatives to be applied in the reuse of litter for several subsequent flocks (Corrier et al., 1992; Jeffrey et al., 1998; Hartel et al., 2000; McWard and Taylor, 2000; Pope and Cherry, 2000; Kwak et al., 2005; Vicente et al., 2007; Larrison et al., 2010; Macklin and Krehling, 2010; Stringfellow et al., 2010).

Indeed, the reuse of litter has been used in poultry for a long time, with performance results that did not differ from chickens reared on new litter (Kennard and Chamberland, 1951; McCartney, 1971; Jones and Hager, 1983).

Moreover, it was demonstrated that the reuse of the litter has an inhibitory effect on the development of *Salmonella* (Olesiuk et al., 1971).

Finally, Lu et al. (2003) assert that poultry litter microflora comparison from farms with different management practices can identify the conditions that decrease or eliminate pathogenic bacteria. Therefore, the objective of this study was to verify whether the reuse of broiler litter, for up to 14 consecutive times, affects the occurrence of *Salmonella* in Brazilian poultry farms.

MATERIALS AND METHODS

From January 2008 to November 2010, 8,877 samples of litter from disposable shoe covers from broiler farms located in southern Brazil were analyzed. All samples were collected from broiler litters from the same integrator company whose overall management was as follows: the new bed is only submitted to thermic treatment at the moment of preparation. It passes through a cylinder at 280°C before being placed in houses. For litter reuse, after the depopulation of the first flock, a treatment with quicklime is done. The procedure is standard, being done likewise up to the fourteenth flock.

Depopulation, cleaning, and disinfection of the house were performed between 15 to 20 d prior to the housing of different flocks, which involved burning feathers, removal of the moist litter that had turned into a cake, leveling, mixing, and incorporation of 600 g of quicklime per square meter of litter 5 d before housing a new flock. No new litter was added to replace that removed in the moist litter. Only in the brooding chamber (25% of the house area), a layer of a new litter made of pine wood shavings 2-cm thick was placed. Litter was again leveled and feathers burnt. Equipment for lodging was prepared. A total of 430 integrated producers with houses of 2,400 m², which produce an average of 6 flocks per year, with a 42-d slaughtering average were sampled. Cobb progeny birds originating from the same hatchery were used during the study. The procedures for sampling and research of *Salmonella* were performed according to the protocol recommended by the Standards for Accreditation of Diagnosis Laboratories and Monitoring of Avian Salmonellosis (*Salmonella* Enteritidis, *Salmonella* Gallinarum, *Salmonella* Pullorum, and

Salmonella Typhimurium), ANNEX I, Animal Health Protection Legislation—Poultry, Ministry of Agriculture and Food Supply of the Federative Republic of Brazil, and the National Avian Health Program (Ministério da Agricultura e do Abastecimento, 2002).

The positive and negative controls to verify the effectiveness of the methodology used were made from the weighing of a routine litter sample, adding 1% peptone water to a 1:10 ratio, and the 10⁻¹ dilution was then made adding 1 mL of standard strain (*Salmonella baireli*) grown according to the Ministério da Agricultura e do Abastecimento (2002), using the following methodology.

Collection of Samples

All samples were collected by a field technician and sent to the laboratory in sterile bags of the Nasco (Whirl-Pak Bag, Fort Atkinson, WI) type. After putting on disposable shoe covers, the technician walked from one end to the other end of the house close to the nipple line and returned to the original point where the litter was collected from the disposable shoe covers. Samples were collected from flocks with ages between 19 and 25 d in 100% of the houses.

Preparation of Samples

The opening of the packaged samples was done inside a laminar flow chamber, along with their weighing and hydration to a 1:10 ratio (1 part sample to 9 parts 1% peptone water made in a gravimetric diluter; Dilumat 4 AES Chemunex, Combourg, France). In 60 s, the diluter was able to make a precise dilution of the shoe cover sample with an indeterminate weight of sample in a sterile polyethylene bag, reaching the final target weight. Immediately the material was placed in an oven at 37°C for 18 to 24 h. After this period, 1 mL from the culture was used to inoculate tetrathionate broth media and this was incubated for 24 h at 36°C; 0.1 mL was used for inoculation of Rappaport broth bottle media and incubated for 24 h at 42 to 43°C.

Isolation

From the selective enrichment broths, 0.1 mL was used to inoculate plates of xylose lysine deoxycholate (XLD) agar, brilliant green, and MacConkey agar and incubated at 36 (±1)°C for 18 to 24 h.

Preliminary Biochemistry

From the isolation of characteristic colonies on XLD agar, brilliant green agar, and MacConkey agar, 1 to 3 colonies from each plate with characteristics of *Salmonella* were subcultured in triple sugar iron (TSI) agar and lysine iron agar (LIA). Inoculation of the medium was made by picking a portion of an isolated colony

with an inoculating needle and stabbing into the TSI and LIA media to the appropriate depth and then streaking across the slant. The tubes were inoculated and incubated at 36 (± 1)°C for 18 to 24 h.

The inoculation of the sulfide indole motility agar medium was done by stabbing the media 1 cm in depth and incubating it at 36 (± 1)°C for 18 to 24 h. The inoculation of the urea broth medium was made by shaking the needle into the broth and incubating it at 36 (± 1)°C for 18 to 24 h. The inoculation of the nutrient agar medium was made by fine streaks on the steep surface of the medium and incubating it at 36 (± 1)°C for 18 to 24 h.

Identification of *Salmonella*

The principle of the serological identification of *Salmonella* involved mixing the suspected organism with antiserum containing specific *Salmonella* antibodies. The bacteria agglutinate in the presence of homologous antiserum.

Statistical Analysis

Polynomial regression analysis was used to estimate the influence of the reuse of litter for the percentage of samples of reused litters infected with *Salmonella* spp. To verify if the frequency of samples positive for *Salmonella* is related to the number of reuses of the litter, the chi-squared test was used. To verify if the season of the year interfered with the count of *Salmonella*, the Kruskal Wallis test was used. All statistical comparisons were made considering a $P < 0.05$ significance level.

RESULTS AND DISCUSSION

A total of 8,877 samples of litter from different poultry farms of an important Brazilian broiler company were analyzed during 3 consecutive years. The sample size in this study is highly significant and represents well the population about which inferences were made.

Of the total 3,233 samples in the period from January to December 2008, only 2.5% were positive for *Salmonella*. Among the isolated serotypes, 71 were *Salmonella* Enteritidis, 4 *Salmonella* Bredney, 1 *Salmonella*

Anatun, 2 *Salmonella* Agona, 2 *Salmonella* Senftenberg, and 1 *Salmonella* Saint Paul. Of the total 2,852 samples analyzed in the period from January to December 2009, 5.27% were positive for *Salmonella*. Among the analyzed serotypes, 5 were *Salmonella* Enteritidis, 116 *Salmonella* spp., 2 *Salmonella* Typhimurium, 15 *Salmonella* Bredney, 1 *Salmonella* Anatun, 3 *Salmonella* Agona, 4 *Salmonella* Senftenberg, and 1 *Salmonella* Havana. Of the total 2,792 samples analyzed in the period from January to November 2010, 2.08% were positive for *Salmonella*. Among the analyzed serotypes, 21 were *Salmonella* Enteritidis, 3 *Salmonella* spp., 6 *Salmonella* Bredney, 1 *Salmonella* Anatun, 14 *Salmonella* Agona, 7 *Salmonella* Senftenberg, 2 *Salmonella* Ovakan, and 1 *Salmonella* Genovar. Therefore, the serotype with the highest prevalence among the positive samples in 3 years of evaluation was *Salmonella* Enteritidis.

As shown in Table 1, the results of the F-test for regression models were all significant ($P < 0.05$) in the 3 yr evaluated, demonstrating that the decrease in the count of samples positive for *Salmonella* is not due to chance and that most of it can be explained by the reuse of the litter. This can be explained probably by the increased presence of feces, ammonia, and moisture in the litter. Our hypothesis is that litter reuse causes an increase in humidity and denitrifying bacteria that intensify urate degradation present in feces and litter, intensifying the production of ammonia, which inhibits the development of *Salmonella*.

These data are in agreement with those reported by Terzich et al. (2000), who, after checking the average incidence of each category of bacteria in various regions of the United States, identified that *Staphylococcus* was more frequent in new litters.

The use of 5% litter used by adult chickens as part of the diet significantly decreased the colonization of the cecum and other organs in Leghorn chicks, but not in adult hens, indicating that it is possible to increase resistance to *Salmonella* through exposure to the intestinal contents of adult chickens (Corrier et al., 1993). Similarly, Corrier et al. (1992) observed that chicks reared on reused litter showed higher levels of volatile fatty acids in the cecum and increased resistance to intestinal colonization by *Salmonella* than chickens reared on new litter.

Table 1. Regression equations to predict the percentage of samples contaminated with *Salmonella* according to the number of times of reuse of the broiler litter

Year	Model	Equation	R ²	P-value
2008	Linear	$y = 5.40 - 0.481x$	43.3	0.002
	Quadratic	$Y = 8.21637 - 1.53590x + 0.0703x^2$	55.1	0.0122
	Cubic	$Y = 10.9614 - 3.41571x + 0.373077x^2 - 0.0135x^3$	60.3	0.0217
2009	Linear	$y = 10.0 - 0.868x$	81.2	0.0001
	Quadratic	$Y = 13.2292 - 2.06177x + 0.0796x^2$	89.9	0.0000034
	Cubic	$Y = 13.7647 - 2.42847x + 0.138615x^2 - 0.00262x^3$	90.0	0.0000259
2010	Linear	$y = 3.98 - 0.275x$	38.0	0.001
	Quadratic	$Y = 6.19687 - 1.10762x + 0.0555x^2$	57.8	0.00875
	Cubic	$Y = 9.51564 - 3.38033x + 0.421543x^2 - 0.0163x^3$	78.2	0.00121

Table 2. Association between number of reuses of broiler litter and counts of samples positive for *Salmonella*¹

Sample	Number of times that the litter was reused													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Positive	101 ²	49	27	24	21	30	9	7	8	4	4	2	3	0
	30 ³	29	25	21	20	19	19	20	21	18	20	19	13	9
Negative	831 ²	857	745	648	615	572	578	618	644	551	621	607	407	294
Total	932	906	772	672	636	602	587	625	652	555	625	609	410	294

¹Chi squared = 272.25; df = 13; $P < 0.0001$; n = 8,877.

²Observed number.

³Expected number.

Linear regression models presented in Table 1 estimate the expected percentage of samples positive for *Salmonella*. In all cases, it is possible to infer that the more times the litter is used, the lower the count of positive samples. This result seems to be controversial, given that the European Union demands changing the litter in each flock of broilers as a way of improving the microbiological quality and welfare of the birds (Council of the European Union, 2007).

The reuse of litter, in fact, has already been used in poultry for a long time with performance results that did not differ from those in chickens reared on new litter (Kennard and Chamberland, 1951; McCartney 1971; Jones and Hagler, 1983). Vieira and Moran (1999) found that the accommodation in used litter caused a decrease in initial weight gain of chickens. But the BW at slaughter was similar to that of chickens reared on new litter, due to compensatory gain. Moreover, it was demonstrated before that the reuse of the litter has an inhibitory effect on the development of *Salmonella* (Olesiuk et al., 1971).

The contact of birds with litter rich in bacterial remnants from a previous flock, beginning with the arrival in the house, facilitates the early development of the intestinal flora. Colonization of intestinal mucous by a great and diversified number of bacteria is normal in several animal species, including chickens (Lee, 1985). Susceptibility of chickens to intestinal *Salmonella* spp. colonization is higher during the first days of life, being decreased afterwards as the normal intestinal microbiota grows (Bailey, 1988). Ingestion of litter microbiota can confer some protection to chickens against the colonization of some pathogens, particularly *Salmonella* spp. Intestinal protection in chickens is attributed to competitive exclusion by adherence sites and to the production of small-chain volatile acids, starting with lactose, for example, with cecal pH reduction (Ziprin et al., 1991). Some lactic acid bacteria have been reported to produce soluble antimicrobial peptides, called bacteriocins, which are postulated to contribute to their ability to improve intestinal health (Higgins et al., 2008)

Another possible explanation for the decrease in counts may be because of increased immunity due to exposure to litter with low levels of initial contamination (Corrier et al., 1992, 1993).

The litter is a reservoir of *Salmonella* and its source can be the chicks themselves or the vectors that remain in the facility during the fallow period. Santos et al. (2005) showed that the population of *Salmonella* in litter is positively correlated with the population of *Salmonella* in bird feces, indicating that the sampling in litter is a good indicator of the microbiological status of the feces.

These results have great practical importance, opposed to what is routinely recommended, as the change of litter at each flock does not present a microbiological status better than when litters are reused.

The presence of *Salmonella* in the litter of broilers decreases as the number of flocks raised on it increases. A probable explanation is that the reuse of litter probably promotes the exclusion of pathogenic bacteria by competition. Lu et al. (2003) found that many species identified in the microbiology of poultry litter are actively involved in composting organic matter, which would explain the absence of several pathogens of veterinary importance and dangerous to human health. Farms that place one layer of new litter over the used one for each new flock of chickens allow the microbial activity of the litter to be sufficient to promote composting (Jeffrey et al., 1998).

These data are in accordance with those of Thaxton et al. (2003), who found no significant correlation between the number of reuses and the aerobic and anaerobic bacteria present in the litter. According to the authors, because the population of bacteria is established, it remains relatively constant over time, regardless of the number of birds that were housed on it. Therefore, Thaxton et al. (2003) conclude that the microbial population does not increase with increasing reuse of litter and argue that there is no microbiological reason for changing the litter after each use.

Using nonparametric statistical analysis (Kruskal-Wallis), no effect was detected for season on the count of samples positive for *Salmonella* [Wilcoxon score (rank sum) mean scores are: summer = 261.59, autumn = 253.0, winter = 240.39, and spring = 255.0; $P = 0.5066$].

One can observe that there is a negative association ($P < 0.0001$) between the number of times that the litter was reused and the percentage of samples positive

for *Salmonella* (Table 2). In the first and second flocks using the litter, the observed frequency of positive samples is much greater than expected. After the fifth reuse of the litter the values of positive samples observed are much lower than expected. This means that farms with litter reused more than 6 times show lesser probability of having positive results for *Salmonella*.

The reuse of litter for several subsequent flocks is economically profitable for the poultry industry. However, the use of some kind of treatment to decrease the pathogenic bacterial load is critical so that it does not become the cause of contamination of the flocks. There are several methods available for achieving this purpose. Vicente et al. (2007) found that the use of a litter acidifier decreased the recovery of *Salmonella* in cecal tonsils of chickens reared on new or reused litter, concluding that the horizontal transmission of these bacteria can be decreased.

Acidification of the litter with pH that can go under 4 promotes a decrease in the concentration of viable bacteria in litter and improves the environmental conditions inside the house (Ivanov, 2001). This can be achieved with the use of products based on aluminosilicates, which are minerals that contain aluminum oxide (Al_2O_3) and silica or silicon dioxide (SiO_2), diatomaceous earth (inert dust from grinding of fossilized deposits of phytoplanktonic algae), gypsum ($CaSO_4$), which is a result of the production of phosphoric acid, or chemicals such as sodium bisulfate ($NaHSO_4$) or aluminum sulfate [$Al_2(SO_4)_3$].

Aluminum sulfate lowers the pH of the litter. Burgess et al. (1998) observed that its addition in a dose of 10% of the weight of the litter causes a decrease in pH from 7.47 to 4.43 in litter composed of rice shells.

Alkalinization of the litter with the pH reaching above 11 allows a decrease in the concentration of bacteria. The use of quicklime (CaO) or hydrated lime ($CaOH$) provides these levels with relative ease and low cost. Stanush et al. (2000) observed a decrease in cfu of total bacteria in litter treated with $CaOH$, starting at a dose of 0.2% of the weight of the litter.

The treatment of the litter with any of the methods mentioned has action over the control of pathogenic bacteria. However, the method chosen must obligatorily meet certain criteria. First, and most important, is to ask the integrated producer if he has the necessary conditions to carry out the proposed methodology or, in other words, if the method can be applied on that property. The second criterion is to be effective in controlling pathogenic bacteria. The third criterion is that it be accepted by the audits that the company receives.

According to Santos et al. (2005), research should be conducted to determine the critical points on farms for reducing or eliminating *Salmonella*. However, due to time consumption and costs of analysis, few researchers have evaluated *Salmonella* in broiler litter reused in consecutive flocks. In conclusion, the reuse of broiler litter is a safe practice and that, contrary to expecta-

tions, substantially reduces the bacterial load of *Salmonella* along the time of use.

REFERENCES

- Bailey, J. S. 1988. Integrated colonization control of *Salmonella* in poultry. *Poult. Sci.* 67:928-932.
- Bermudez, A. J., and B. Stewart-Brown. 2003. Principles of disease prevention diagnosis and control: Disease prevention and diagnosis. Pages 17-55 in *Diseases of Poultry*. 11th ed. Y. M. Saif, H. J. Barnes, A. Fadly, J. R. Glisson, L. R. McDougald, and D. E. Swayne, ed. Iowa State University Press, Ames.
- Burgess, R. P., J. B. Carey, and D. J. Shafer. 1998. The impact of pH on nitrogen retention in laboratory analysis of broiler litter. *Poult. Sci.* 77:1620-1622.
- Corrier, D. E., B. M. Hargis, and A. Hinton Jr. 1992. Effect of used litter from floor pens of adult broilers on *Salmonella* colonization of broiler chicks. *Avian Dis.* 36:897-902.
- Corrier, D. E., B. M. Hargis, A. Hinton Jr., and J. R. DeLoach. 1993. Protective effect of used poultry litter and lactose in the feed ration on *Salmonella enteritidis* colonization of leghorn chicks and hens. *Avian Dis.* 37:47-52.
- Council of the European Union. 2007. Laying down minimum rules for the protection of chickens kept for meat production. Council Directive 2007/43/EC. Official Journal of the European Union L 182/19. Accessed March 7, 2011. http://ec.europa.eu/food/animal/welfare/references_en.htm.
- Hartel, W., I. Segars, J. D. Summers, J. V. Collins, A. T. Phillips, and E. Whittle. 2000. Survival of fecal coliforms in fresh and stacked broiler litter. *J. Appl. Poult. Res.* 9:505-512.
- Higgins, S. E., J. P. Higgins, A. D. Wolfenden, S. N. Henderson, A. Torres-Rodriguez, G. Tellez, and B. Hargis. 2008. Evaluation of a *Lactobacillus*-based probiotic culture for the reduction of *Salmonella* Enteritidis in neonatal broiler chicks. *Poult. Sci.* 87:27-31.
- Ivanov, I. E. 2001. Treatment of broiler litter with organic acids. *Res. Vet. Sci.* 70:169-173.
- Jeffrey, J. S., J. H. Kirk, E. R. Atwill, and J. S. Cullor. 1998. Prevalence of selected microbial pathogens in processed poultry waste used as dairy cattle feed. *Poult. Sci.* 77:808-811.
- Jones, F. T., and W. M. Hagler. 1983. Observations on new and reused litter for growing broilers. *Poult. Sci.* 62:175-179.
- Kennard, D. C., and J. D. Chamberlin. 1951. Growth and mortality of chickens as affected by the floor litter. *Poult. Sci.* 30:47-54.
- Kwak, W. S., J. W. Huh, and T. A. McCaskey. 2005. Effect of processing time on enteric bacteria survival and on temperature and chemical composition of broiler poultry litter processed by two methods. *Bioresour. Technol.* 96:1529-1536.
- Larrison, E. L., J. A. Byrd, and M. A. Davis. 2010. Effects of litter amendments on broiler growth characteristics and *Salmonella* colonization in the crop and cecum. *J. Appl. Poult. Res.* 19:132-136.
- Lee, A. 1985. Neglected niches. The microbial ecology of the gastrointestinal tract. *Adv. Microb. Ecol.* 8:115-162.
- Lu, J., S. Sanchez, C. Hofacre, J. J. Maurer, B. G. Harmon, and M. D. Lee. 2003. Evaluation of broiler litter with reference to the microbial composition as assessed by using 16S rRNA and functional gene markers. *Appl. Environ. Microbiol.* 69:901-908.
- Macklin, K. S., and J. T. Krehling. 2010. The use of metam-sodium to reduce bacteria in poultry litter. *J. Appl. Poult. Res.* 19:274-278.
- McCartney, M. G. 1971. Effect of type of housing and litter on production of broilers. *Poult. Sci.* 50:1200-1202.
- McWard, G. W., and D. R. Taylor. 2000. Acidified clay litter amendment. *J. Appl. Poult. Res.* 9:518-529.
- Ministério da Agricultura e do Abastecimento. 2002. Ministério da Agricultura e do Abastecimento, Secretaria de Defesa Agropecuária. Legislação de defesa sanitária animal avicultura: Programa nacional de sanidade avícola. Brasília: Ministério da Agricultura e do Abastecimento [citado 2005 out. 18]. Accessed March 3, 2011. <http://www.agricultura.gov.br/animal/sanidade-animal>.

- Olesiuk, O. M., G. H. Snoeyenbos, and C. F. Smyser. 1971. Inhibitory effect of used litter on *Salmonella typhimurium* transmission in the chicken. *Avian Dis.* 15:118–124.
- Pope, M. J., and T. E. Cherry. 2000. An evaluation of the presence of pathogens on broilers raised on poultry litter treatment-treated litter. *Poult. Sci.* 79:1351–1355.
- Santos, F. B. O., X. Li, J. B. Payne, and B. W. Sheldon. 2005. Estimation of most probable number *Salmonella* populations on commercial North Carolina turkey farms. *J. Appl. Poult. Res.* 14:700–708.
- Stanush, D. D., R. Beltron, C. M. Corsiglia, D. J. Caldwell, and B. M. Hargis. 2000. Effect of hydrated lime on selected litter microflora and poultry growth performance. *Poult. Sci.* 79(Suppl. 1):1. (Abstr.)
- Stringfellow, K., D. Caldwell, J. Lee, A. Byrd, J. Carey, K. Kessler, J. McReynolds, A. Bell, R. Stipanovic, and M. Farnell. 2010. Pasteurization of chicken litter with steam and quicklime to reduce *Salmonella Typhimurium*. *J. Appl. Poult. Res.* 19:380–386.
- Terzich, M., M. J. Pope, T. E. Cherry, and J. Hollinger. 2000. Survey of pathogens in poultry litter in the United States. *J. Appl. Poult. Res.* 9:287–291.
- Thaxton, Y. V., C. L. Balzli, and J. D. Tankson. 2003. Relationship of broiler flock numbers to litter microflora. *J. Appl. Poult. Res.* 12:81–84.
- Vicente, J. L., S. E. Higgins, B. M. Hargis, and G. Tellez. 2007. Effect of poultry guard litter amendment on horizontal transmission of *Salmonella enteritidis* in broiler chicks. *Int. J. Poult. Sci.* 6:314–317.
- Vieira, S. L., and E. T. Moran. 1999. Effects of delayed placement and used litter on broiler yields. *J. Appl. Poult. Res.* 8:75–81.
- Ziprin, R. L., M. H. Elissalde, A. Hinton Jr., R. C. Beier, G. E. Spates, D. E. Corrier, T. G. Benoit, and J. R. DeLoach. 1991. Colonization control of lactose-fermenting *Salmonella typhimurium* in young broiler chickens by use of dietary lactose. *Am. J. Vet. Res.* 52:833–837.