Organic acid water treatment reduced *Salmonella* horizontal transmission in broiler chickens

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The purpose of the present study was to determine whether an organic acid water treatment could reduce the spread of *Salmonella* (SAL) to naïve birds when infected birds were part of the population. A total of one thousand eighty (1080), day-old Cobb X Cobb male chicks were allocated 60/pen to each of 18 pens by blocks and divided into three treatment groups: T1, unmedicated control; T2 , 0.04%; and T3, 0.08% of an organic acid blend (OAB; ACTIVATE® WD). The OAB was added to water from 0-14 days and 42-49 days. Half of the birds in each pen were orally dosed with Naladixic acid resistant-*S. heidelberg* on Day 0 (tagged) and housed with the remaining uninfected birds (not tagged). Salmonella status of ceca and crops (Day 49 only) was evaluated by random selection of 5 tagged and 5 untagged birds/pen on days 0, 14 and 49. Dragswabs of pens were also obtained on the same days as was mortality, weight gain, feed consumption and feed conversion. On day 49, 22% of T1 untagged birds were SAL+ compared to only 7% of T2 and T3 untagged birds. Similar results were obtained with untagged birds for % SAL+ dragswabs and crops on Day 49. There was no effect of OAB on % SAL+ for tagged birds. Feed conversion was significantly improved by OAB at 42 days of age (1.80 vs 1.77 and 1.77) and at 49 days similar trends were apparent (1.95 vs 1.90 and 1.91) but not different. These results demonstrated that the OAB treatment significantly reduced horizontal spread of SAL to uninfected birds and reduced environmental SAL contamination.

Keywords: organic acids; ACTIVATE WD; *Salmonella*

Introduction

Food safety is one of the top priorities in poultry production today. *Salmonella* is one of the major contributors to food-borne illnesses linked to poultry products like eggs and poultry carcasses (8,7,4). According to the Centers for Disease Control annual report (5), *Salmonella* Typhimurium (ST) and *Salmonella* Enteritidis (SE) are recognized as the first and second most common serotypes found in humans. As said by the editor of the National Chicken Council report dated January 27, 2006; *Salmonella* reduction in poultry is Food Safety and Inspection Service (FSIS) priority for 2006 (6). The US poultry industry needs to place its emphasis on farm interventions, *Salmonella* loads of birds arriving at the plant, and process and sanitary controls (6). Achievable strategies for *Salmonella* reduction must involve interventions at multiple points from the farm to the table. Different methods have been used to reduce *Salmonella* on the farm such as drug therapy and vaccination (13), competitive exclusion (9,11,12), and drug therapy and competitive exclusion (10). The use of organic acids in the water (1) or in the feed (2,3) have proven to be practical and efficacious farm interventions to reduce *Salmonella* incidence and colonization in broilers. Reduced levels of *Salmonella* in the intestine means less micro-organisms to be spread in the environment of the chicken house and during processing, and therefore less contaminated final product (2). Additional research in the effect of organic acids on *Salmonella* reduction in broilers is in progress.

The objective of this study was to determine whether a nutritional organic acid (ACTIVATE ® WD) water treatment could reduce the spread of *Salmonella* (SAL) to naïve birds when infected birds were part of the population.

Materials and methods

A total of one thousand eighty (1080), one-day-old Cobb X Cobb male chicks were used in the study. At the hatchery, the birds received routine vaccinations. Only healthy appearing chicks were used in the study. At study initiation sixty males were allocated to each of the 18 pens by blocks (0.77 sq. ft/ bird, stocking density). Half of the chicks (30) per pen were tagged for identification and then orally dosed with (2.2×10^7) Naladixic acid resistant-*Salmonella heidelberg* on Day 0 (prior to placement). No birds were replaced during the course of the study. Bird weights (kg) by pen were recorded at study initiation, Day 42, and termination (Day 49). All pens had approximately 4 inches of fresh pine shavings. There were 30 birds/hanging feeder and all birds consumed feed and water *ad libitum.*

Treatments:

Treatment	Activate WD %	Days of Treatment	Salmonella	Pens/Trt.
T1	Nonmedicated	0	YES	6
T2	0.04%	1 to 14 and 42 to 49	YES	6
T3	0.08%	1 to 14 and 42 to 49	YES	6

Broiler diets were fed as crumbles (Starter feed) or as pellets (all remaining feeds – Grower and Finisher). Diets were representative of local formulations and calculated analyses met or exceeded NRC standards. All starter feeds contained 125ppm Nicarbazin, and all grower feeds contained 100 ppm Coban, and 50 ppm 3-Nitro 20. All finisher feeds were nonmedicated.

Birds received feed appropriate to the treatment from Day 0 to Day 49. A change from starter to grower in which all previous feed was removed and weighed occurred on Day 21. Grower diet was removed and weighed from each pen on Day 35 and replaced with the finisher diet. At each feed change, feeders were removed from pens by block, weighed, emptied, and refilled with the appropriate treatment diet. On the final day of the study (Day 49), feed was weighed.

Birds from treatment 1 received nonmedicated water throughout the study. Birds from treatments 2 and 3 received Activate WD% at 0.04% and 0.08% respectively for the first 14 days of the study and from Day 42 to 49. The pH of of the treatment water and total water consumption were also measured.

Salmonella sampling

Drag swab samples were collected from all pens on Days 0, 14, and 49. Sampling by dragging sterile gauze (4 x 4) swab soaked in double strength skim milk across the birds bedding material is considered to be the most sensitive method of environmental sampling by the US National Poultry Improvement Plan (NPIP). All drag swabs were placed in individual sterile plastic sample bags and labeled.

Cecal and crop culture. On Days 14 and 49, ten birds per pen (5 tagged and 5 nontagged) were euthanized by cervical dislocation, weighed, and the ceca aseptically removed and placed into sterile plastic sampling bags for salmonella enumeration. To these samples, 100mls of tetrathionate brilliant green broth (TTB) with iodine were added and the samples put in a stomacher (Technar Company, Cincinnati, OH) for 1 minute. These samples were incubated at 41.5 C and then cultured for *Salmonella*. In addition to ceca collection on Day 49, crop samples were collected. Positive or negative presence of *Salmonella* was determined in these crop samples, no enumeration was preformed. All enrichments were performed on primary samples. TTB was added to all samples accordingly and incubated at 41.5 C for 18 h and isolation and identification was performed.

For the isolation and identification of Salmonella, 1 µl loop of the TTB was streaked onto XLT4 and BGN agar plates containing naladixic acid and incubated at 37 C overnight. The H_2S-positive isolated colonies were then placed onto triple sugar iron slants and incubated at 37 C overnight. Suspect *Salmonella* colonies were then confirmed with poly O *Salmonella*-specific antiserum. All *Salmonella* was stored for PCR comparison to the challenge strain. A representative number of the *Salmonella* isolates saved were DNA fingerprinted using the method described by Liu et al. (Avian Diseases, 2002).

Other than coccidiostat drugs, no concomitant drug therapy was used during the study. The pen was the unit of measure. Twice daily observations were recorded for general flock condition, temperature, lighting, water, feed, litter condition, mortality and unanticipated events for the house.

Results and Discussion

Results from the cultured ceca at day 49 (Figure 1) showed that on day 49 treatments with ACTIVATE WD significantly (P<0.05) reduced the horizontal spread of *Samonellal* from the infected seeders (tagged) to their penmates (nontagged) as evidenced from $\log_{10}$ 1.3 and 1.8 for tagged seeders and 0.7 for penmates.

Figure1

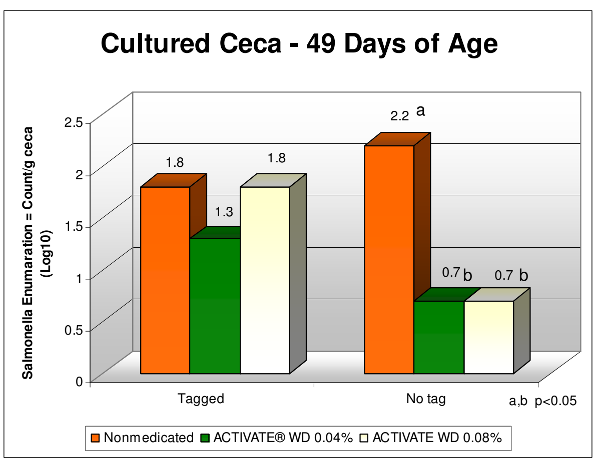
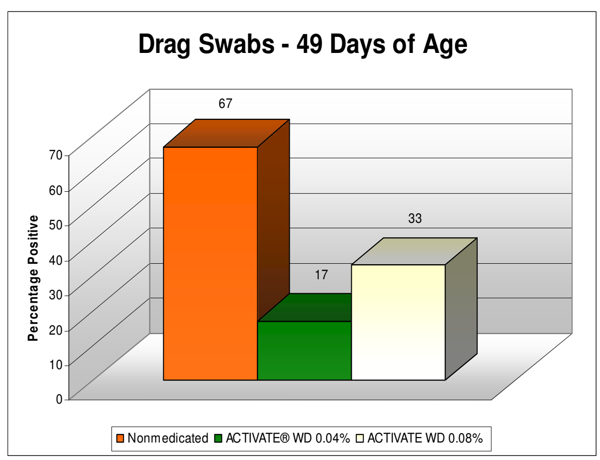


Figure 2



Additional evidence of this reduced level of *Salmonella* in the environment of the treated pens is the lower percent positive dragswabs; 67% for nonmedicated vs. 17% and 33% for the 0.04% and 0.8% treatments with ACTIVATE WD, respectively (Figure 2). The lower level of positive *Salmonella* in the crops, 7% for the nonmedicated vs 2% and 3% of the ACTIVATE WD treatments indicates that the observed reduction of Salmonella in the environment was was reflected in lower intake of Salmonella by the birds, i.e. the intestinal bacterial flora of the chicken reflects their environment.

Figure 3

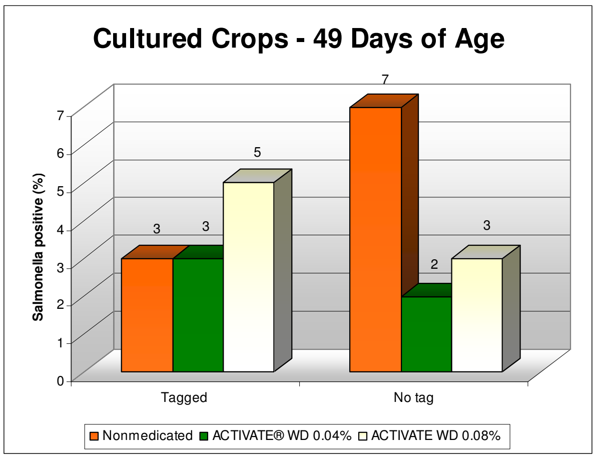
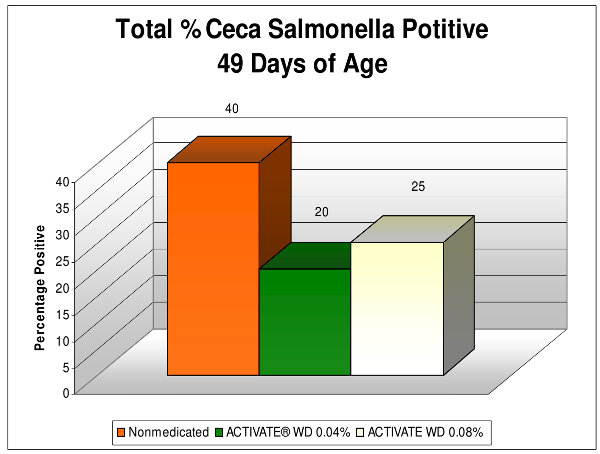


Figure 4



This study clearly demonstrated that lower *Salmonella* levels in the litter (dragswabs) resulted in lower levels in the crop and ceca of uninfected birds. It also demonstrated up to a 50% reduction in the total percentage of ceca *Salmonella* positive at 49 days (Figure 4). Performance results indicate that there was no significant difference between treatments in average live weight gain on both day 42 and 49; however, a significant improvement (P<0.05) of day 42 adjusted feed conversion was shown in both treatments using ACTIVATE WD. On day 49 this difference was still numerically in favor of the birds given ACTIVATE WD organic acid blend (Table 1). Thus ACTIVATE WD nutritional organic acid blend successfully interrupted the infection cycle of Salmonella in uninfected chickens which significantly reduced the number of Salmonella positive chickens at the end of the trial.

Table 1. Performance

	Day 42		Day 49	
Treatment	**Adj. Feed Conversion**	**Avg. Live Wt. Gain (kg)**	**Adj. Feed Conversion**	**Avg. Live Wt. Gain (kg)**
1. Nonmedicated	1.797 a	2.557 a	1.947 a	3.007 a
2. Activate WD 0.04%	1.768 b	2.556 a	1.900 a	3.013 a
3. Activate WD 0.08%	1.772 b	2.557 a	1.910 a	3.029 a

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References

1. **Byrd, J. A., Hargis, B. M., Caldwell, D. J., Bailey, R. H., Herron, K. L., McReynolds, J. L., Brewer, R. L., Anderson R. C., Bischoff K. M., Callaway T. R., and Kubena L.**

F. (2001) Effect of lactic acid administration in the drinking water during preslaughter feed withdrawal on *Salmonella* and *Campylobacter* contamination of broilers. Poultry Science 80:278-283.

2. **Cox N. A., McHan F., and Bailey J. S.** (1994) Effect of butyric or lactic acid on the in vivo colonization of *Salmonella typhimurium.* J. Appl. Poultry Res. 3:314-318.
3. **Hinton, M. and Linton, A. H.** (1988) Control of *Salmonella* infections in broiler chickens by the acid treatment of their feed. Veterinary record 123: 416-421.
4. **Mead, P.S., Slutsker, L., Dietz, V., McCaig, L.F., Bresse, J.S., Shapiro, C., Griffen, P.M., and Tauxe, R. V.** (1999) Food-related illness and death in the United States. Emerg. Infect. Dis. 5:607-625.
5. **NARMS** 2002 Annual report. http://www.cdc.gov/ncidod/dbmd/phlisdata.salmtb/2002/salmoellaintroduction2002.pdf.
6. **National Chicken Council Washington report.** (2006) Editor: Margaret Ernst Vol.43 No. 4.
7. **Olsen, S. J., MacKinnon, L. C., Goulding, J. S., Bean, N. H., and Slutsker, I.** (2000) Surveilance for food born-disease outbreaks – United Satates 1993-1997, Morb. Mortal. Wkly. Rep. 49:1-62.
8. **Orndorff, B.W., Novak, C. L., Pierson, F. W., Caldwell, D. J., and McElroy, A. P.** (2005) Comparison of prophylactic or trerapeutic dietary administration of capsaicin for reduction of Salmonella in broiler chickens. Avian Dis. 49:527-533.
9. **Salvat, G., Lalande, F., Humbert, F., and Lahellec, C.** (1992). Use of a competitive exclusion product (Broilact®) to prevent *Salmonella* colonization of newly hatched chicks. Int. J. Food Microbiol. 15:307–311.
10. **Seo, K. H., Holt, P. S., Gast, T. K., and Hofacre, C. L.** (2000) Elimination of early *Salmonella enteritidis* infection after treatment with competitive-exclusion culture and enrofloxacin in experimentally infected chicks. Poultry Science 79:1408-1413.
11. **Stavric, S.** (1987) Microbial colonization control of chicken intestineusing defined cultures. Food Technol. 41:93–98.
12. **Stavric, S.** (1992). Defined cultures and prospects. Int. J. Food Microbiol. 15:245–263.
13. **White, P. L., Baker, A. R., and James, W. O.** (1997) Strategies to control Salmonella and Campylobacter in raw poultry products. Rev. Sci. Tech. Off. Int. Epiz. 16:525-542.