

Prophylactic Supplementation of Caprylic Acid in Feed Reduces *Salmonella* Enteritidis Colonization in Commercial Broiler Chicks[†]

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ABSTRACT

Salmonella Enteritidis is a major foodborne pathogen for which chickens serve as reservoir hosts. Reducing *Salmonella* Enteritidis carriage in chickens would reduce contamination of poultry meat and eggs with this pathogen. We investigated the prophylactic efficacy of feed supplemented with caprylic acid (CA), a natural, generally recognized as safe eight-carbon fatty acid, for reducing *Salmonella* Enteritidis colonization in chicks. One hundred commercial day-old chicks were randomly divided into five groups of 20 birds each: CA control (no *Salmonella* Enteritidis, CA), positive control (*Salmonella* Enteritidis, no CA), negative control (no *Salmonella* Enteritidis, no CA), and 0.7 or 1% CA. Water and feed were provided ad libitum. On day 8, birds were inoculated with 5.0 log CFU of *Salmonella* Enteritidis by crop gavage. Six birds from each group were euthanized on days 1, 7, and 10 after challenge, and *Salmonella* Enteritidis populations in the cecum, small intestine, cloaca, crop, liver, and spleen were enumerated. The study was replicated three times. CA supplementation at 0.7 and 1% consistently decreased *Salmonella* Enteritidis populations recovered from the treated birds. *Salmonella* Enteritidis counts in the tissue samples of CA-treated chicks were significantly lower ($P < 0.05$) than those of control birds on days 7 and 10 after challenge. Feed intake and body weight did not differ between the groups. Histological examination revealed no pathological changes in the cecum and liver of CA-supplemented birds. The results suggest that prophylactic CA supplementation through feed can reduce *Salmonella* Enteritidis colonization in day-old chicks and may be a useful treatment for reducing *Salmonella* Enteritidis carriage in chickens.

Among the foodborne pathogens transmitted through poultry and poultry products, *Salmonella enterica* serovar Enteritidis is the most common serotype isolated from poultry products (4, 37, 41, 51), accounting for more than 1.4 million cases of nontyphoid salmonellosis in the United States (26, 39, 54). The total annual cost associated with salmonellosis in the United States is estimated at approximately \$3 billion (50). The primary colonization site of *Salmonella* Enteritidis in chickens is the cecum (1), with cecal carriage of *Salmonella* leading to horizontal transmission of the infection, contamination of eggshell with feces, and carcass contamination during slaughter (31). *Salmonella* Enteritidis colonization of the bird cecum can result in contamination of eggs (yolk, albumen, and shell membranes) by the transovarian route (12, 42, 49).

Because *Salmonella* Enteritidis can be transmitted to chickens from many sources, including feed, water, litter, equipment, feed trucks, rodents, insects, and service personnel (11, 18, 25, 27, 29), elimination of the pathogen by

cleansing and disinfection of the farm environment alone may be difficult (16, 36). *Salmonella* infection can be persistent (34), and birds can become reinfected from contaminated water, litter, and barn walls (11, 47). Therefore, farm sanitation combined with interventions targeting the birds would be a useful approach for controlling *Salmonella* Enteritidis carriage in chickens.

Because poultry and poultry products serve as vehicles for human infection (38), reduction of *Salmonella* populations in the chicken intestinal tract could reduce contamination of poultry meat and eggs. A variety of approaches including competitive exclusion bacteria (23, 46), bacteriophages (5), oligosaccharides (7, 22), and organic acids (2, 26) have been investigated for reducing *Salmonella* Enteritidis colonization in chickens, but success has been variable.

Despite progress in food safety through pathogen reduction programs, *Salmonella* Enteritidis remains one of the most common foodborne pathogens transmitted to humans through consumption of poultry products. Innovative on-farm strategies for preventing *Salmonella* Enteritidis colonization of birds are critical for preventing contamination of poultry products with this pathogen. An antimicrobial treatment that can be applied through feed represents the most practical and economically viable method

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TABLE 1. Five *Salmonella* Enteritidis strains used in this study

<i>Salmonella</i> Enteritidis strain no.	Phage type	Source
12	14b	Chicken liver
22	8	Chicken intestine
28	13a	Chicken ovary
31	13a	Chicken gut
90	8	Human

for pathogen reduction on the farm. A natural and safe antimicrobial will be better accepted by producers, including organic farmers without concerns for toxicity. The widespread use of antibiotics at therapeutic and subtherapeutic levels may contribute to the emergence of antibiotic-resistant bacteria (14, 15, 21). Therefore, caprylic acid (CA) was evaluated as a feed supplement for reducing *Salmonella* Enteritidis carriage in chickens.

Free fatty acids, especially medium-chain fatty acids, are bactericidal against gram-positive and gram-negative bacteria (17, 40). CA (octanoic acid) is a natural eight-carbon medium chain fatty acid present in breast milk, bovine milk (30), and coconut oil (45). CA is a food-grade chemical approved by the U.S. Food and Drug Administration (CFR 184.1025) as generally regarded as safe. Previous research conducted in our laboratory revealed that CA was effective for killing *Salmonella* Enteritidis in chicken cecal contents in vitro (53) and for killing *Escherichia coli* O157:H7 in rumen fluid (3). Recently we reported that feed supplemented with CA reduced *Campylobacter jejuni* counts in broiler chickens (43, 44). The objective of the present study was to investigate the prophylactic efficacy of CA as a feed supplement for reducing *Salmonella* Enteritidis populations in commercial broiler chicks.

MATERIALS AND METHODS

Experimental birds and housing. Day-old commercial broiler chicks (Pureline Genetics, Norwich, CT) were allocated into floor pens in the isolation farm equipped with provisions for age-appropriate temperatures and bedding. The birds had access to ad libitum feed (Blue Seal Feeds Inc., Londonderry, NH) and water. All the experiments were approved by the Institutional Animal Care and Use Committee at the University of Connecticut.

Bacterial strains and dosing. Five strains of *Salmonella* Enteritidis (Table 1) were used to colonize the birds. Each strain was preinduced for resistance to nalidixic acid (NA; Sigma-Aldrich, St. Louis, MO) at 50 µg/ml and for selective enumeration (2, 26). Strains were cultured separately in 10 ml of tryptic soy broth (Difco, Becton Dickinson, Sparks, MD) supplemented with 50 µg/ml NA and incubated at 37°C for 24 h with agitation (100 rpm). After three successive transfers, equal volumes of the cultures were combined and sedimented by centrifugation (3,600 × g for 15 min at 4°C). The pellet was resuspended in phosphate buffered saline (PBS; pH 7.0) and used as the inoculum. The bacterial count of the individual cultures and the five-strain mixture were confirmed by plating 0.1-ml portions of appropriate dilutions on xylose lysine desoxycholate agar (XLD; Difco, Becton Dickinson) plates containing NA (XLD-NA) and incubating the plates at 37°C for 24 h.

Experimental design. One hundred day-old broiler chicks (male and female) were weighed and randomly distributed into five groups of 20 birds each. The treatments included negative controls (no *Salmonella* Enteritidis, no CA), positive controls (*Salmonella* Enteritidis, no CA), CA control (no *Salmonella* Enteritidis, 1% CA), and a low dose (0.7%) and a high dose (1%) of CA (Sigma-Aldrich) supplemented in the feed for the entire 18-day trial period. On day 8, the birds were challenged with 1 ml of the inoculum (approximately 5.0 log CFU) by crop gavage. On days 1, 7, and 10 days postinfection (PI), six birds from each treatment were euthanized by carbon dioxide asphyxiation and dissected to collect organ samples for further bacteriological analysis. The feed consumption and body weight also were determined. The experiment was replicated three times.

Determination of *Salmonella* Enteritidis in organs. Cecum, small intestine, cloaca, and crop with their contents, liver, and spleen from each bird were collected in separate sterile 50-ml tubes containing 5 ml of PBS. The weighed samples were processed with a tissue homogenizer (Tissue Master, Omni International, Marietta, GA) and diluted 10-fold in sterile PBS. A 0.1-ml portion of appropriate dilutions was surface plated on duplicate XLD-NA plates. The colonies were enumerated after incubation at 37°C for 48 h. Representative colonies from XLD-NA plates were confirmed as *Salmonella* with a *Salmonella* rapid detection kit (Microgen Bioproducts Ltd., Camberley, UK). When colonies were not detected after direct plating, samples were tested for surviving cells by enrichment for 48 h at 37°C in 100 ml of selenite cysteine broth (Difco, Becton Dickinson) (22, 33) followed by streaking on XLD-NA plates. Representative colonies from the plates were confirmed as *Salmonella* with the *Salmonella* rapid detection kit.

Histological examination. Representative samples of liver and cecum from each group were collected at necropsy and fixed in 10% neutral buffered formalin. Duplicate sections (5 mm thick) were cut from each sample and processed for histological examination using standard hematoxylin and eosin staining (24). Tissues from birds that were not inoculated with *Salmonella* and were not treated with caprylic acid were used as negative controls.

Statistical analysis. Each sample was considered an experimental unit, and a completely randomized 5 × 6 × 6 × 3 factorial design was followed. Factors were five treatments (negative, positive, and CA controls and 0.7 and 1% CA) and six organ samples from six birds at three sampling points (days 1, 7, and 10 PI). The data for bacterial counts, feed intake, and body weight from three trials for the positive control and treatment groups were averaged and analyzed with the Proc-mixed version of the Statistical Analysis Software (SAS Institute Inc., Cary, NC). Differences among the means were considered significant at $P \leq 0.05$ and were detected using Fisher's least significance difference test with appropriate corrections for multiple comparisons.

RESULTS AND DISCUSSION

In chickens, the cecum is a major colonization site for *Salmonella* Enteritidis, and the pathogen usually is present in large numbers (13, 19, 52). *Salmonella* Enteritidis also colonizes the small intestine (32, 35) and cloaca (52), through which the pathogen is horizontally transmitted. In addition to these sites, *Salmonella* Enteritidis also has been recovered from the crop, although in lower numbers (6, 8, 20, 28). The pathogen reaches the liver and spleen by lymphatic or circulatory systems (13, 52). In the current study,

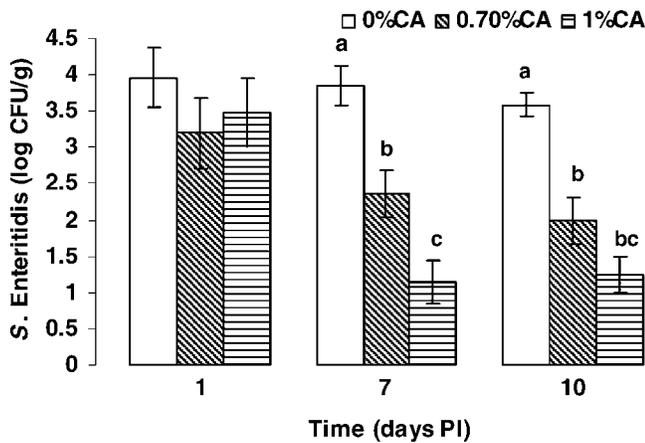


FIGURE 1. Effect of caprylic acid supplementation on *Salmonella* Enteritidis counts in cecum. Bacterial counts for the positive control and treatment groups from three trials were averaged, for six chicks per sampling point per treatment per trial. Columns with no common letters differ significantly ($P < 0.05$).

we investigated the efficacy of CA for reducing *Salmonella* Enteritidis populations in all of these organs.

No morbidity or mortality of birds was observed in any groups during the study. *Salmonella* was not detected in the unchallenged control groups (negative control and CA control), indicating that these birds stayed negative for *Salmonella* Enteritidis infection throughout the trials.

The effect of CA feed supplementation on *Salmonella* Enteritidis populations in various organs is depicted in Figures 1 through 6. *Salmonella* Enteritidis at approximately 4.0 log CFU/g was recovered from the cecal samples of positive control birds on day 1 PI (Fig. 1). In CA-treated birds, the pathogen loads in cecal samples on day 1 were not significantly different ($P > 0.05$) from those in samples from control chickens. However, on days 7 and 10 PI, both levels of CA reduced cecal *Salmonella* Enteritidis counts markedly compared with those recovered from control birds. At the end of the trial (day 10 PI), *Salmonella* Enteritidis cecal counts in birds treated with 1% CA were

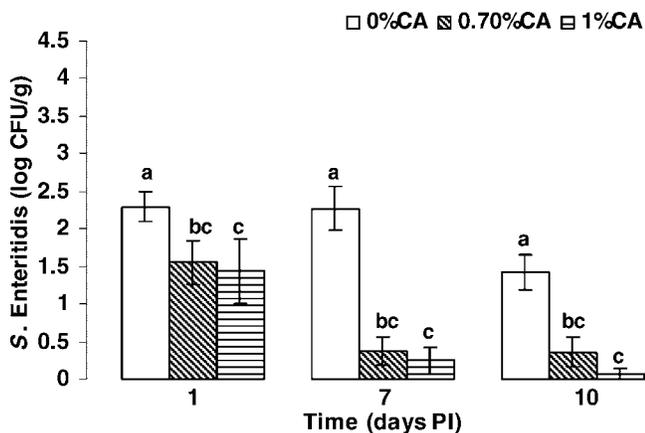


FIGURE 2. Effect of caprylic acid supplementation on *Salmonella* Enteritidis counts in small intestine. Bacterial counts for the positive control and treatment groups from three trials were averaged, for six chicks per sampling point per treatment per trial. Columns with no common letters differ significantly ($P < 0.05$).

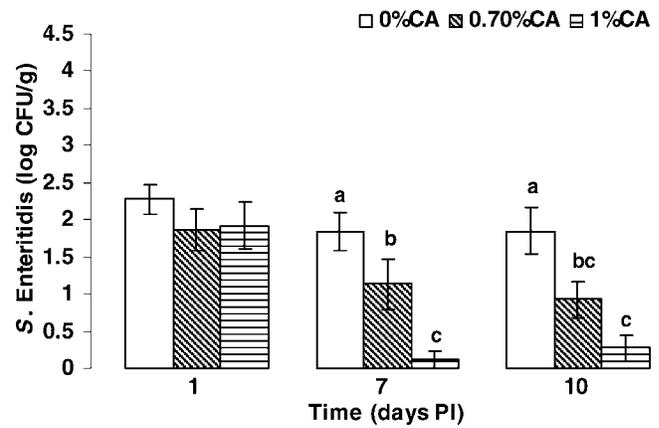


FIGURE 3. Effect of caprylic acid supplementation on *Salmonella* Enteritidis counts in crop. Bacterial counts for the positive control and treatment groups from three trials were averaged, for six chicks per sampling point per treatment per trial. Columns with no common letters differ significantly ($P < 0.05$).

reduced by approximately 2.5 log CFU/g compared with control chicks. In the small intestine, 0.7 and 1% CA decreased *Salmonella* Enteritidis populations by >2.0 log CFU/g at 7 days PI compared with controls ($P < 0.05$). On day 10 PI, 0.7 and 1% CA reduced the pathogen population to less than 0.5 log CFU/g, whereas approximately 1.5 log CFU/g was recovered from the control chicks (Fig. 2). Crop and cloaca results were similar to those for the cecal and intestinal samples. CA supplementation at both concentrations decreased *Salmonella* Enteritidis populations ($P < 0.05$) in the crop (Fig. 3) and cloaca (Fig. 4) of birds after 7 and 10 PI. However, on day 10 PI cloacal *Salmonella* Enteritidis counts of birds treated with 0.7% CA were not different from those recovered from control birds (Fig. 4).

In the liver, both concentrations of CA reduced ($P < 0.05$) *Salmonella* Enteritidis populations significantly compared with the controls at 7 and 10 days PI (Fig. 5). On day 7 PI, 1% CA decreased the pathogen counts in the liver by approximately 2.0 log CFU/g compared with the counts for the control birds. As observed for the liver, CA supple-

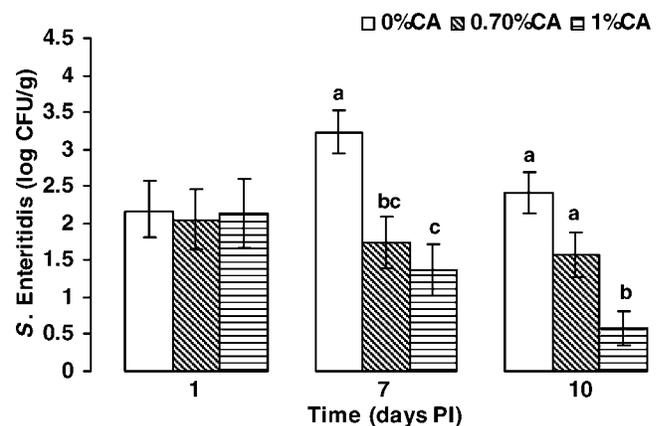


FIGURE 4. Effect of caprylic acid supplementation on *Salmonella* Enteritidis counts in cloaca. Bacterial counts for the positive control and treatment groups from three trials were averaged, for six chicks per sampling point per treatment per trial. Columns with no common letters differ significantly ($P < 0.05$).

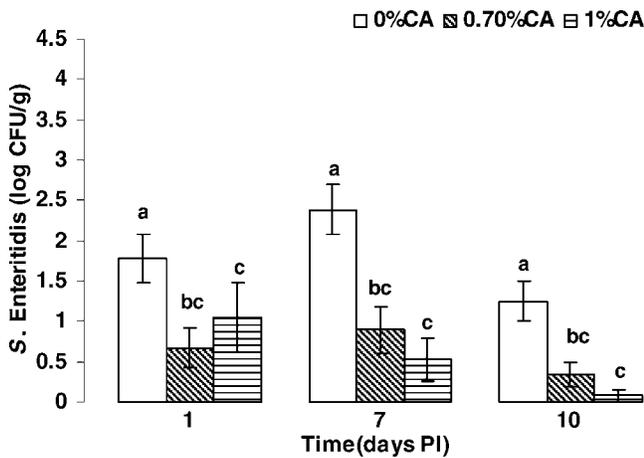


FIGURE 5. Effect of caprylic acid supplementation on *Salmonella Enteritidis* counts in liver. Bacterial counts for the positive control and treatment groups from three trials were averaged, for six chicks per sampling point per treatment per trial. Columns with no common letters differ significantly ($P < 0.05$).

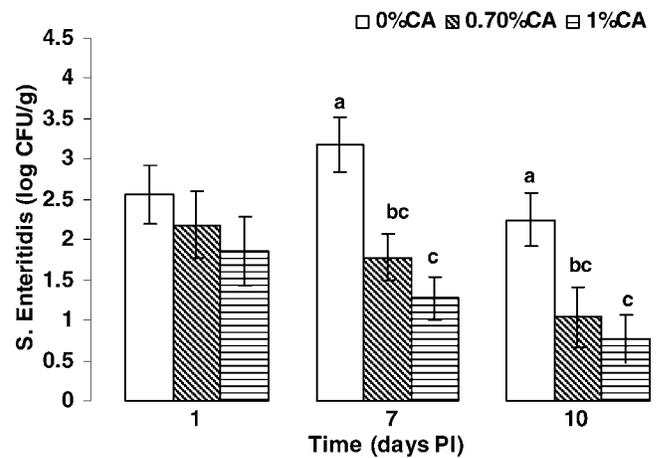


FIGURE 6. Effect of caprylic acid supplementation on *Salmonella Enteritidis* counts in spleen. Bacterial counts for the positive control and treatment groups from three trials were averaged, for six chicks per sampling point per treatment per trial. Columns with no common letters differ significantly ($P < 0.05$).

mentation at both concentrations significantly decreased ($P < 0.05$) the population of *Salmonella Enteritidis* recovered from the spleen on days 7 and 10 PI (Fig. 6).

These results indicate that CA supplementation at 0.7 and 1% consistently reduced *Salmonella Enteritidis* populations in chicks. Feeding of 1% CA was more effective for reducing *Salmonella Enteritidis* than was 0.7% CA. Both concentrations of CA were more effective for reducing ($P < 0.05$) *Salmonella Enteritidis* in chicks at 10 days than at 7 days PI. In a previous study in which we investigated the efficacy of CA on *C. jejuni* in 10-day-old chicks, we observed that CA supplementation at concentrations below 1.05% consistently reduced pathogen counts in the cecum ($P < 0.05$) (43). Similarly, Van Immerseel et al. (52) reported that supplementation with 0.3% caproic acid, another medium chain fatty acid, was effective for reducing *Salmonella Enteritidis* counts in chicken cecum, liver, and spleen, although the magnitude of pathogen reduction was smaller than that observed in the current study.

The body weights of birds from different treatment groups are provided in Table 2. In comparison to control chicks, CA at both levels did not reduce feed consumption and body weight of birds after 18 days of feeding ($P > 0.05$). Histological examination revealed no pathological

TABLE 2. Effect of prophylactic supplementation of caprylic acid on feed consumption and body weight of 18-day-old chicks^a

Treatment group ^b	Feed consumption (g)	Body wt (g) ^c
No SE, no CA	320.6 ± 31.9	844.8 ± 16.4
No SE, 1% CA	319.7 ± 22.2	864.8 ± 20.0
SE, no CA	327.5 ± 27.8	850.3 ± 32.2
SE, 0.7% CA	324.2 ± 27.8	841.1 ± 17.2
SE, 1% CA	321.4 ± 27.2	876.1 ± 16.3

^a Values are mean ± standard error of the mean. No significant difference was observed among the groups for both parameters.

^b SE, *Salmonella Enteritidis*.

^c Values are for 18-day-old chicks, six birds per group per trial.

changes in the cecum and liver of CA-supplemented birds when compared with control chicks (data not shown).

Although the mechanism behind CA-mediated *Salmonella Enteritidis* reduction in chicks is unclear, fatty acids can diffuse into bacterial cells in their undissociated form and dissociate in the protoplasm, leading to intracellular acidification (48). Fatty acids also can penetrate and become incorporated into the bacterial plasma membrane, thereby adversely affecting membrane permeability (9, 10). Another potential mechanism may involve an inhibitory effect of CA on the expression of virulence genes in *Salmonella Enteritidis*, which aid in pathogen colonization in the host. Van Immerseel et al. (52) found that medium chain fatty acids suppressed the expression of *hilA*, a key gene regulator involved in *Salmonella* invasion, thereby resulting in decreased *Salmonella* colonization in chicks. However, further investigation is needed to elucidate the exact mechanisms by which CA reduces *Salmonella Enteritidis* in chicks.

Prophylactic supplementation of 0.7 and 1% CA in the feed was effective for reducing *Salmonella Enteritidis* populations in chicks. No significant differences in feed consumption and body weight were observed between CA-treated and control birds. Histological examination revealed no pathological changes in the cecum and liver of CA-supplemented birds. When coupled with standard hygienic practices used on the farm, CA could be used as an antimicrobial feed additive to reduce *Salmonella Enteritidis* colonization in chickens. Future studies will be conducted to investigate the effect of CA on *Salmonella* carriage in market-age birds.

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