

## Therapeutic Supplementation of Caprylic Acid in Feed Reduces *Campylobacter jejuni* Colonization in Broiler Chicks<sup>∇</sup>

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**Poultry colonized with *Campylobacter* species are a significant source of human food-borne illness. The therapeutic use of the medium chain fatty acid caprylic acid consistently reduced enteric *C. jejuni* colonization in chicks by 3 to 4 logs in three separate trials. These results support caprylic acid's potential to reduce *Campylobacter* carriage in poultry.**

*Campylobacter* species are some of the most commonly reported bacterial causes of human food-borne illnesses in the United States, and epidemiological evidence indicates that poultry and poultry products are significant sources of human infection (6, 10, 18). Contamination with *Campylobacter* originates from environmental sources, including flies, rodents, and wild birds (11, 17), and spreads rapidly through the flock (14). Even with biosecurity measures, *Campylobacter* colonization is widespread in most poultry flocks (4, 15, 29).

Cecal carriage of *C. jejuni* results in horizontal transmission of the pathogen and in carcass contamination during slaughter. Therefore, interventional strategies implemented at the farms for reducing *C. jejuni* counts in the chicken intestinal tract are critical for delivering a microbiologically safer product. Fatty acids, especially medium chain fatty acids, were found to have antimicrobial properties for a wide range of microorganisms (2, 3, 16, 19, 24, 27). Recently, Thormar and coworkers (26) reported that monocaprin, the monoglyceride of capric acid, was effective in killing significant populations of *C. jejuni* in chicken feed. Caprylic acid is a medium chain fatty acid with eight carbons naturally found in breast milk, bovine milk, and coconut oil (12, 13, 16). It is a food-grade compound classified as generally recognized as safe (GRAS) by the U.S. Food and Drug Administration. We recently demonstrated the prophylactic efficacy of feed supplemented with caprylic acid against *Campylobacter* colonization in 10-day-old broiler chicks (23). This strategy provides a potentially important method for limiting *Campylobacter* colonization; however, strategies are needed to reduce *Campylobacter* populations after colonization in poultry has already occurred. Therefore, the objective of the present study was to determine the therapeutic effect of feed supplemented with caprylic acid on *C. jejuni* colonization in broiler chicks.

**Experimental design.** In three separate trials, day-of-hatch commercial broiler chicks ( $n = 60$  per trial; mixed sex) were

assigned to six treatment groups ( $n = 10$  per group): negative controls (no *Campylobacter*; no caprylic acid); positive controls (*Campylobacter*; no caprylic acid); and four caprylic acid treatment groups (based on caprylic acid doses of 0.35%, 0.7%, 1.4%, and 2.8%). These doses were selected since all doses were equally effective at killing *Campylobacter* within 1 h in vitro (data not shown). Caprylic acid (Sigma-Aldrich, St. Louis, MO) was supplemented in starter feed for the last 72 h of each 15-day trial. At 3 days of age, chicks were inoculated with five wild-type strains of *C. jejuni* (8), and at 15 days of age, chicks were euthanized, and cecal contents were collected for *Campylobacter* enumeration (7). Birds were individually weighed on days 12 and 15 to determine body weight differences, feed consumption, and feed conversion during the treatment period. There was no morbidity or mortality during the treatment period.

Based on the results from trials 1 and 2, intestinal samples from chicks fed with 0.7% and 1.4% caprylic acid and the positive control (0% caprylic acid) were collected for enteric morphometric analysis in trial 3 on day 15 of the experiment. Segments (2 cm) of the midpoint of the duodenum, jejunum, ileum, and cecum were prepared and evaluated (22). Twenty replicates for each variable studied (villus height, base, surface area, crypt depth, lamina propria thickness, and neutral goblet cell density) were measured for each sample. Data were analyzed by analysis of variance, using the GLM procedure of SAS, with means partitioned by LSMEANS analysis (STAT user's guide, release version 9.03; SAS Institute Inc., Cary, NC).

The results of this study demonstrate that select doses of caprylic acid, when fed for only 3 days, can consistently reduce enteric *Campylobacter* populations in young chickens already colonized with the bacterium (Fig. 1). In three separate trials, supplementation of a 0.7% and a 1.4% dose of caprylic acid reduced cecal *Campylobacter* counts substantially compared with those in the positive controls. Supplementation of caprylic acid at 0.35% and 2.8% levels had an inconsistent effect on the reduction of *C. jejuni* populations in the cecal content.

Since caprylic acid is a natural and GRAS-designated feed additive, it could be used immediately by poultry farmers to

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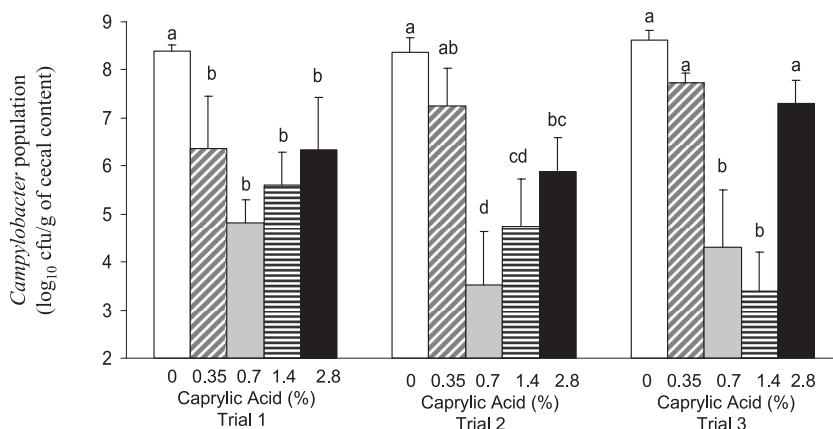


FIG. 1. Effect of caprylic acid on cecal *C. jejuni* counts in 15-day-old chickens. Values (means  $\pm$  standard errors of the means) per treatment ( $n = 10$  birds/treatment per trial) represent the population of *C. jejuni* in the cecal content of 15-day-old broiler chickens. In each trial, chicks (except for negative controls) were orally challenged 3 days posthatch with a mixture of five *C. jejuni* isolates ( $n = 10$  chicks/treatment per trial; average dose of  $2 \times 10^6$  CFU/ml). Caprylic acid was added to the feed at 72 h prior to necropsy. Columns within the same trial (1 to 3) with different letters (a, b, c, and d) denote significant differences between doses ( $P < 0.05$ ). All *Campylobacter* data were  $\log_{10}$  transformed for statistical analysis (5).

reduce *C. jejuni* carriage in chickens. Using the 0.7 and 1.4% doses, we observed a consistent 3- to 4-log reduction in cecal *Campylobacter* populations in chicks. This could have significant beneficial implications for food safety, since, during processing, enteric contents can contaminate the carcass, thereby resulting in food-borne transmission of *C. jejuni*. (1, 21). It has been estimated that a 2-log reduction in *C. jejuni* populations on poultry carcass contaminations could bring about a 30-fold reduction in human campylobacteriosis cases (20). The use of caprylic acid should be accepted by poultry producers, since it does not have any adverse effect on parameters important for production (profit) or health such as morbidity, mortality, body weight gain, or feed conversion (Table 1). The cost of treatment would be limited (\$2 to 3/kg; KIC Chemicals Inc., New Paltz, NY) because it has therapeutic efficacy and would be used only for the last 3 days prior to slaughter. This dosing strategy would have the added benefit of preventing the reestablishment of higher *C. jejuni* populations, since there would be no withdrawal period prior to slaughter.

The mechanism of caprylic acid-mediated *Campylobacter* reduction in chicks is not clear but may be due to the diffusion of caprylic acid into bacterial cells in the undissociated form and

dissociation within the protoplasm, thereby leading to intracellular acidification (25). A lower intracellular pH can lead to inactivation of intracellular enzymes (28) and inhibition of amino acid transport (9). Based on previous morphometric analysis, we postulated that another possible mechanism of action of caprylic acid may be a physical or functional alteration of the gastrointestinal colonization site of *C. jejuni* in chicks. Our previous research with poult fed bacteriocins found that the reduction in *Campylobacter* counts was associated with an alteration in the preferential gastrointestinal colonization sites for this organism (7). In the present study, caprylic acid supplementation showed either no effects or inconsistent effects on gastrointestinal morphology (data not shown), suggesting that other mechanisms are responsible for the reduction observed for cecal *C. jejuni* counts. Caprylic acid may compromise the outer membrane determinants in *Campylobacter* bacteria that are needed for bacterial adaptation to the host environment and colonization, or it may have a direct inhibitory effect on the expression of virulence factors necessary for *C. jejuni* colonization. However, additional research is necessary to confirm these hypotheses and to determine why the highest dose of caprylic acid (2.8%) was not consistently

TABLE 1. Effect of caprylic acid on feed consumption, body weight gain, and feed conversion in chicks<sup>a</sup>

Caprylic acid dose (%)	Feed consumption (g) (mean $\pm$ SEM)	Body weight gain (g) (mean $\pm$ SEM)	Feed conversion ratio $\pm$ SEM
0 ( <i>Campylobacter</i> -negative controls)	228.1 $\pm$ 4.7 AB	135.0 $\pm$ 6.6 A	1.69 $\pm$ 0.05 A
0 ( <i>Campylobacter</i> -positive controls)	240.9 $\pm$ 7.4 A	125.1 $\pm$ 3.9 A	1.93 $\pm$ 0.09 A
0.35	213.0 $\pm$ 2.7 BC	127.4 $\pm$ 5.5 A	1.67 $\pm$ 0.09 A
0.70	220.7 $\pm$ 5.0 B	135.1 $\pm$ 0.7 A	1.63 $\pm$ 0.04 A
1.4	215.3 $\pm$ 8.7 BC	134.3 $\pm$ 7.6 A	1.60 $\pm$ 0.08 A
2.8	197.2 $\pm$ 8.4 C	119.7 $\pm$ 13.6 A	1.70 $\pm$ 0.26 A

<sup>a</sup> Means within columns with no letters (A, B, C) in common differ significantly ( $P < 0.05$ ). Data, means  $\pm$  standard errors of the means (SEM), represent the overall means of feed consumption, body weight gains and feed conversion of 15-day-old chicks when averaged for each of three separate trials. In each trial, chicks (except for negative controls) were orally challenged at 3 days posthatch with a mixture of five *C. jejuni* isolates ( $n = 10$  chicks/treatment per trial). Caprylic acid was fed to the chicks from day 12 to day 15. Body weight values on day 12 were subtracted from body weight values on day 15 to determine the body weight gain during the 3-day caprylic acid-dosing period. Feed consumption was determined by subtracting the remaining feed value from the original amount of feed per treatment. Feed conversion was calculated by dividing feed consumption values by the body weight values during the dosing period.

effective. It is possible that higher doses of caprylic acid alter competing intestinal microflora, allowing higher *Campylobacter* carriage.

In conclusion, the results of the present study suggest that therapeutic supplementation of caprylic acid for 3 days reduced *Campylobacter* populations in the cecal content of chicks by 3 to 4 logs. Follow-up studies are currently under way to elucidate the mechanism(s) by which caprylic acid reduces enteric *Campylobacter* carriage in poultry.

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