Caprylic Acid Supplemented in Feed Reduces Enteric \textit{Campylobacter jejuni} Colonization in Ten-Day-Old Broiler Chickens$^{1,2}$

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ABSTRACT \textit{Campylobacter} is one of the leading causes of human foodborne illness in the United States, and epidemiological evidence indicates that poultry and poultry products are a significant source of human \textit{Campylobacter} infections. Reducing \textit{Campylobacter} in the intestinal tract would reduce contamination of poultry products and eggs. Caprylic acid, an 8-carbon medium-chain fatty acid has been shown to be bactericidal against several pathogenic bacteria. It has, however, not been tested in the control of \textit{Campylobacter} in chickens. Four trials were carried out to evaluate the efficacy of caprylic acid against cecal \textit{Campylobacter jejuni} colonization in 10-d-old chicks. In the first 2 trials, day-of-hatch chicks (n = 40 per trial) were assigned to negative controls (no \textit{Campylobacter}, no caprylic acid), positive controls (\textit{Campylobacter}, no caprylic acid), and a low (0.7%) and a high (1.4%) dose of caprylic acid supplemented in regular chick starter feed (n = 10 chicks/treatment). Two more trials were carried out to evaluate a wider range of caprylic acid doses on cecal \textit{Campylobacter} counts, in which day-of-hatch chicks (n = 90 per trial) were assigned to 9 treatments: negative controls (no \textit{Campylobacter}, no caprylic acid) and caprylic acid doses of 0 (positive controls), 0.35, 0.525, 0.7, 0.875, 1.05, 1.225, and 1.4% (n = 10 chicks/treatment). Except for the negative controls, chicks were orally gavaged with approximately $1 \times 10^6$ cfu \textit{Campylobacter} on d 3. On d 10, cecal contents were collected and \textit{Campylobacter} concentrations were determined in each trial. In all 4 trials, the 0.7% dose of caprylic acid consistently reduced \textit{Campylobacter} content counts compared with the positive control. In trials 3 and 4, doses less than 1.05% consistently reduced cecal \textit{Campylobacter} content in both trials. At the higher doses, caprylic acid reduced feed consumption and body weight, but did not affect feed conversion when compared with the positive controls. These data suggest that low-dose supplementation with caprylic acid in feed may reduce \textit{Campylobacter} colonization in young chickens.

Key words: \textit{Campylobacter jejuni}, body weight gain, caprylic acid, broiler chick

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INTRODUCTION

\textit{Campylobacter} is one of the most commonly reported bacterial causes of human foodborne illness in the United States, and epidemiological evidence indicates poultry and poultry products as significant sources of human \textit{Campylobacter} infection (Oosterom et al., 1984; Friedman et al., 2004; CDC, 2007). Colonization of poultry by \textit{Campylobacter} is widespread and difficult to prevent, even with proper biosecurity measures (Wallace et al., 1998; Berrang et al., 2000; Loc Carillo et al., 2005).

Reducing \textit{Campylobacter} in the intestinal tract would reduce contamination of poultry products. A variety of approaches for reducing the colonization of \textit{Campylobacter} in poultry has been explored, but with varying degrees of success. These include feeding chicks or poults competitive exclusion bacteria (Mead et al., 1996; Stern et al., 2001; Mead, 2002), enzymes such as xylanase (Fernandez et al., 2000), bacteriophage (Loc Carrillo et al., 2005; Wagenaar et al., 2005), bacteriocins (Cole et al., 2006), noncultured cecal content (Mead et al., 1996; Stern et al., 2001), fructooligosaccharides and mucin-utilizing coliforms (Schoeni and Wong, 1994), organic acids (Byrd et al., 2001; Heres et al., 2004), and antibiotics (Farnell et al., 2005).

Fatty acids, especially medium-chain fatty acids, have been reported to possess antimicrobial activities against a wide range of microorganisms (Bergsson et al., 1998,
Caprylic acid is a medium-chain fatty acid with 8 carbons and is naturally found in breast milk, bovine milk (Jensen, 2002), and coconut oil (Jensen et al., 1990; Sprong et al., 2001). It is a food-grade compound generally recognized as safe (GRAS) by the Food and Drug Administration. Caprylic acid has been shown to be effective in killing a variety of bacterial pathogens such as *Salmonella Enteritidis* in chicken cecal contents (Vasudevan et al., 2005), and *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus agalactiae* in milk (Nair et al., 2005). In preliminary studies, caprylic acid killed *Campylobacter* in vitro (data not shown). The present study was carried out to investigate whether caprylic acid supplemented in feed would reduce *Campylobacter* in the intestines of 10-d-old broiler chickens.

**MATERIALS AND METHODS**

**Experimental Birds and Housing**

One-day-old male broiler chicks were obtained from a commercial hatchery. These birds were placed into floor pens with dimensions of 3.06 m² (33 ft²) previously equipped with fresh litter (pine shavings) and heaters. Birds had ad libitum access to both feed and water during all trials.

**Experimental Design**

Four trials were conducted in the present study. In the first 2 trials, day-of-hatch chicks (n = 40/trial) were randomly assigned to 1 of 4 treatment groups (n = 10 per treatment group). The treatments groups included negative controls (no *Campylobacter*, no caprylic acid), positive controls (*Campylobacter*, no caprylic acid), and a low (0.7%) and a high dose (1.4%) of caprylic acid (Sigma-Aldrich, St. Louis, MO) supplemented in the feed for the entire 10-d trial. To evaluate the effect of additional doses of caprylic acid on *C. jejuni* in birds, 2 follow-up trials were conducted using day-of-hatch chicks (n = 90). The chicks were assigned to 1 of 9 treatments, including negative controls (no *Campylobacter*, no caprylic acid) and 0 (positive controls), 0.35, 0.525, 0.7, 0.875, 1.05, 1.225, or 1.4% of caprylic acid supplemented in starter feed (n = 10 birds per treatment group). In all trials, the caprylic acid treatments were supplemented in feed and supplied to chicks for the entire 10-d experimental period. At 10 d of age, chicks were killed by CO₂ and cecal contents collected for *Campylobacter* determination (see below). Feed consumption was determined by subtracting the amount of feed remaining at the end of each trial from the amount given on d 1.

**Bacterial Strains and Dose**

Five strains of wild-type *Campylobacter jejuni* isolated from chickens were used to colonize the birds as described previously by this laboratory (Farnell et al., 2005). Briefly, 10-μL loops of each frozen strain were cultured into 5 mL of *Campylobacter* enrichment broth (CEB; International Diagnostics Group plc, Lancaster, UK) and incubated for 48 h at 42°C. For the second passage, 10-μL loops from each strain were transferred into 5 mL of fresh CEB to be incubated for 24 h at 42°C under microaerophilic conditions (5% O₂, 10% CO₂, and 85% N₂). Following incubation, the strains were pooled in a tube and 3 mL was transferred into a glass tube to be read in a spectrophotometer to determine the concentration of bacteria present in the culture. After measuring the absorbance (optical density), the tube containing the culture was centrifuged at 3,500 × g for 10 min. The supernatant was discarded and the pellet was resuspended in an equal amount of Butterfield’s phosphate diluent (BPD; 6.8% KH₂PO₄; 7.2 pH). The culture was serially diluted to obtain an inoculum with a desired concentration of 4 × 10⁶ cfu/mL. On d 3 of age, chicks were orally challenged individually with 250 μL of inoculum using a 1-mL syringe connected to a sterile stainless steel cannula.

**Cecal Campylobacter Determination**

Cecal *Campylobacter* concentrations were enumerated by the procedure of Cole et al. (2006). Briefly, ceca from each bird were transferred to a sterile plastic bag and the contents squeezed into 15-mL tubes and serially diluted (1:10) with BPD and inoculated on labeled Campy Line agar (Line, 2001) plates. The Campy Line agar (Line, 2001) plates were incubated for 48 h at 42°C under microaerophilic conditions. Direct bacterial counts were recorded and converted to colony-forming units per milliliter of the cecal content. *Campylobacter* colonies were confirmed by latex agglutination test (Panbio Inc., Columbia, MD) and further identified as *Campylobacter jejuni* using API Campy (Biomerieux, Durham, NC; ATCC 33291 strain used as control).

**Statistical Analysis**

Data were analyzed by ANOVA using the GLM procedure of SAS (SAS Institute, 2002). The number of *Campylobacter* colonies was logarithmically transformed (log₁₀ cfu/mL) before analysis to achieve homogeneity of variance (Byrd et al., 2003). Treatment means were partitioned by LSMEANS analysis (SAS Institute, 2002). A probability of *P* < 0.05 was required for statistical significance.

**RESULTS**

**Cecal Campylobacter Concentrations**

The 0.7% dose of caprylic acid in the feed consistently reduced (*P* < 0.05) cecal *Campylobacter* counts in chicks compared with the positive control treatment in all 4 trials (Figure 1, Table 1). Moreover, *C. jejuni* counts recovered from the cecal contents of birds fed 0.7% caprylic acid were lower (*P* < 0.05) than that from birds fed 1.4% caprylic acid. The 1.4% caprylic acid treatment reduced...
In each trial, chicks were orally challenged 3 d posthatch with approximately 1 × 10^6 cfu/mL of a mixture of 5 Campylobacter jejuni isolates. The treatment groups were fed a diet containing the caprylic acid treatments; the control group (0% caprylic acid) was fed the same commercial diet without caprylic acid. a, b Columns with no common superscript differ significantly (P < 0.05).

Table 1. The effect of various doses of caprylic acid on cecal Campylobacter colonization in 10-d-old chicks (mean ± SEM)

<table>
<thead>
<tr>
<th>Caprylic acid dose, %</th>
<th>Trial 3</th>
<th>Trial 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>8.3 ± 0.2 a</td>
<td>7.6 ± 0.6 a</td>
</tr>
<tr>
<td>0.35</td>
<td>5.8 ± 0.6 c</td>
<td>2.6 ± 0.4 c</td>
</tr>
<tr>
<td>0.527</td>
<td>6.3 ± 0.6 bc</td>
<td>2.8 ± 0.5 bc</td>
</tr>
<tr>
<td>0.7</td>
<td>6.3 ± 0.5 bc</td>
<td>4.5 ± 0.9 cde</td>
</tr>
<tr>
<td>0.875</td>
<td>6.1 ± 0.7 c</td>
<td>3.6 ± 0.8 cde</td>
</tr>
<tr>
<td>1.05</td>
<td>7.1 ± 0.6 cde</td>
<td>5.3 ± 0.8 bc</td>
</tr>
<tr>
<td>1.225</td>
<td>7.8 ± 0.7 bde</td>
<td>5.6 ± 0.9 b</td>
</tr>
<tr>
<td>1.4</td>
<td>7.3 ± 0.7 bde</td>
<td>2.8 ± 0.1 bde</td>
</tr>
</tbody>
</table>

a–e Means within columns with no common superscript differ significantly (P < 0.05).

All Campylobacter data were log_{10} transformed for statistical analysis. In each trial, chicks were orally challenged 3 d posthatch with approximately 1 × 10^6 cfu/mL of a mixture of 5 Campylobacter jejuni isolates (n = 10 chicks/treatment per trial). Caprylic acid was fed for the entire 10-d study.

The BW, feed consumption, and feed conversion were averaged for all trials. Feed consumption was reduced at the 1.4% dose and BW were reduced at 1.225 and 1.4% doses when compared with the positive controls (Table 2). However, the corresponding feed conversions were not affected by any of the caprylic acid doses compared with the positive controls (Table 2).

**DISCUSSION**

The results of the present study demonstrate that select doses of caprylic acid in the feed can consistently reduce enteric Campylobacter colonization in young chickens. In 4 separate trials, the 0.7% dose of caprylic acid reduced cecal Campylobacter concentrations compared with the positive controls (Figure 1, Table 1). When additional doses of caprylic acid were tested in trials 3 and 4, the lower doses (<1.05%) of caprylic acid were more consistent than the higher doses in reducing Campylobacter concentrations.

The mode of action of caprylic acid on Campylobacter reduction in chickens is unknown, but may be due to either a direct antimicrobial effect or an indirect effect via alterations in the enteric microflora or environment. Fatty acids such as caprylic acid directly penetrate and incorporate into bacterial plasma membranes altering the membrane permeability of the bacteria (Bergsson et al., 1998, 2001). Fatty acids may also diffuse into bacterial cells in the undissociated form and dissociate within the protoplasm leading to intracellular acidification (Sun et al., 1998). A lower intracellular pH can lead to inactivation of enzymes (Viegas and Sa-Correia, 1991), and inhibition of amino acid transport (Freese et al., 1973). Caprylic acid may also conceivably reduce enteric Campylobacter content by altering the amounts or ratios of other enteric microflora or change some other physical characteristic of the intestine. Similar to a pro- or prebiotic, supplementation with caprylic acid could potentially alter the enteric environmental conditions making it less favorable for Campylobacter colonization.

At the highest dose, caprylic acid supplementation reduced feed consumption, and it could be assumed that this reduction in feed consumption resulted in these birds consuming less total caprylic acid. This may explain why the highest doses of caprylic acid were not consistent in reducing Campylobacter concentrations. When total caprylic acid intake was compared, however, it was apparent that even with the reduced feed consumption at higher doses, the total caprylic acid intake by birds was greater for the higher doses. For example, at the 1.4% dose, birds consumed a total of 3.7 g of caprylic acid (265.3 g of feed consumed × 1.4% = 3.7 g; Table 2) vs. 2.0 g for the 0.7% dose. It is unclear why the higher doses of caprylic acid were less effective than the lower doses in reducing enteric Campylobacter counts. When caprylic...
acid was evaluated in vitro, all doses were equally effective at killing Campylobacter (data not shown).

The ability of caprylic acid at the highest dose to reduce feed consumption may be due to its effect on the satiety center as suggested by Cave (1982). In other words, consumption of high amounts of caprylic acid makes the bird feel full, although it has not consumed as much feed as control birds. Similar to our study results, reductions in feed consumption following caprylic acid consumption were reported by Cave (1982) and Traul et al. (2000). It is doubtful that reduced feed consumption is associated with toxicity because medium-chain fatty acids have a high degree of safety with no reported toxicity in acute toxicity tests performed orally, parenterally, or by the dermal route in several animal species, including chickens (Traul et al., 2000). These researchers reported that medium-chain triglycerides caused no risk of toxicity when ingested in a balanced diet at levels as high as 50% of dietary fat. Supplementation of Miglyol 812 (caprylic/capric acid preparation) at 16% level in the diet for 3 wk was found to be nontoxic in chickens, although the birds consumed less feed compared with control birds (Traul et al., 2000). Furthermore, caprylic acid is a food-grade chemical approved by the FDA as GRAS (FDA, 1981). Although a reduction in feed consumption and BW at the higher doses occurred in this study, the feed conversion ratio was not different (Table 2). Therefore, these birds were as efficient in feed utilization as control birds, but would take longer to reach market age. Although this would be a concern for producers, the problem could be avoided by using lower doses of caprylic acid. Moreover, the only dosing period tested in this study was 10 d and it may be possible to shorten the dosing period, thereby potentially preventing any decline in feed consumption while maintaining efficacy against Campylobacter. Studies are underway in our laboratory to test these possibilities.

In the present study, the dose of 0.7% caprylic acid consistently reduced cecal Campylobacter counts in 4 separate trials in 10-d-old broiler chickens. To our knowledge this is the first study showing that caprylic acid supplementation decreases the intestinal colonization of Campylobacter in broiler chickens. Follow-up studies are ongoing to evaluate the efficacy of caprylic acid in market-aged birds.

**REFERENCES**


