

The influence of caprylic acid on counts of salmonellas and coliforms in chickens experimentally infected with *Salmonella enteritidis*

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Medium-chain fatty acids are efficient antimicrobial agents. In our *in vitro* experiments, caprylic acid (C 8:0) was found the most effective fatty acid against salmonellas. The aim of this study was to evaluate the effect of caprylic acid on counts of salmonellas and coliforms in chickens experimentally infected with *Salmonella enteritidis*. Fourteen days old chickens were fed a commercially available diet. Treatment groups received a diet supplemented with 0.25 % and 0.5 % of caprylic acid. The feed of treated birds was infected with 5 ml of overnight-grown bacterial culture per one kilogram of feed. On the eighth day of the experiment, chickens were slaughtered and crop and cloaca contents sampled for microbiological analyses. Differences between control and treated samples were evaluated by the Student's t-test. Caprylic acid at both concentrations significantly decreased counts of salmonellas and coliforms in all samples, the effect of caprylic acid in the crop contents, however, was more pronounced. Antibacterial activity of caprylic acid was dose-dependent. It can be concluded that caprylic acid is able to reduce numbers of salmonellas in the gastrointestinal tract of chickens and has a potential to improve health status of infected animals.

Keywords: chicken; salmonella infection; caprylic acid; inhibition

Introduction

Salmonellas represent one of the most frequent pathogens in poultry production. Contaminated feed is the major source of *Salmonella* infections in poultry (Al-Natour and Alshwabkeh, 2005). Poultry meat and eggs have been reported to be the major sources of *Salmonella* infection in humans. Reducing of the incidence of salmonellas in chickens should reduce the appearance of foodborne outbreaks of this pathogen and decrease economic losses of the poultry industry. Most *Salmonella* infections in poultry arise from the ingestion of this pathogen (Suzuki, 1994).

Fatty acids and their derivatives are reported to have bacteriostatic and bactericidal properties against a wide range of microorganisms. In our *in vitro* experiments, caprylic acid (C 8:0) was the most effective fatty acid against *Salmonella enteritidis*, *S. infantis* and *S. typhimurium* (Skrivanova et al., 2004). Similar results were observed by Vasudevan et al. (2005), who observed a 5 Log₁₀ CFU/g reduction of salmonellas in autoclaved caecal contents of chickens. Antibacterial activity of monocaprin (glycerol monocaproate) emulsion to *Campylobacter* spp. was confirmed by Hilmarsson et al. (2006). Addition of monocaprin to drinking water and feed reduced counts of *Campylobacter* in the caecum of naturally infected chickens.

Many studies were concerned with the use of organic acids in poultry production. Al-Natour and Alshwabkeh (2005) observed a reduction of *S. gallinarum* in the crop, caecal and intestinal contents of chickens reared on feed that was artificially contaminated with this pathogen. Approximately 1-2

Log₁₀ CFU/g reduction was observed. Significant reduction in growth rates of *S. typhimurium* in tryptic soy broth with buffered propionic acid was observed by Ha et al. (2004).

The aim of this study was to evaluate the effect of caprylic acid on counts of salmonellas and coliforms in chickens which were reared on feed that was artificially contaminated with *Salmonella enteritidis*.

Materials and methods

Two experiments were carried out. Fourteen days old Ross 308 chickens were used in both experiments. Chickens were purchased from a commercial hatchery and were randomly assigned in two groups of ten animals. Chickens were housed individually in metabolic cages. Animals were fed a commercially available diet. Animals of the control group received the diet with no supplements. Chickens of treated groups received the diet supplemented with 0.25 or 0.5 % of caprylic acid (Sigma Aldrich). On the second day of the experiment, the feed was contaminated with 5 ml of overnight-grown culture of *S. enteritidis* ATCC 13076. Bacterial culture was mixed slowly and thoroughly until complete homogenisation. The feed and water were freely available during the experiment. The feeders and drinkers were cleaned routinely to avoid reinfection. Animals were checked daily for morbidity and mortality.

On the eighth day of the experiment, animals were slaughtered. Immediately after the slaughter, the crop and cloaca contents were taken for microbiological analyses. Plating technique was used for evaluating of the number of bacteria in samples. The number of viable bacteria was determined by streaking 0.1 mL of an appropriate dilution on XLD and MacConkey agar plates (Oxoid). Inoculated plates were incubated aerobically at 37 °C for 48 h. Typical colonies were counted and means and SD calculated. Results were statistically evaluated using the t-test.

Results and discussion

Viable numbers of salmonellas and coliforms in crop and cloacal contents of chickens receiving diet with 0.25 % of caprylic acid are shown in Table 1. There was a significant reduction ($P < 0.05$) in Log₁₀ CFU of coliforms and salmonellas in the crop and cloaca contents of treated animals compared with control group. Numbers of salmonellas decreased below the detection limit.

Table 1. The influence of caprylic acid (0.25 %) on numbers* of coliforms and salmonellas (log₁₀ CFU/g) in crop and cloaca contents of infected chickens

	Crop contents		Cloaca contents	
	Positive control	C ₈ (0.25 %)	Positive control	C ₈ (0.25 %)
Coliforms	4.9 ± 0.5 ^a	3.2 ± 0.5 ^b	6.3 ± 0.4 ^a	4.9 ± 0.4 ^b
Salmonellas	4.7 ± 0.3 ^a	< 2 ^b	< 2 ^b	< 2 ^b

*Means ± SD

^{a, b}Treated group differs significantly from the control ($P < 0.05$)

Colonization numbers of salmonellas and coliforms in crop and cloacal contents of chickens receiving diet with 0.5 % of caprylic acid are shown in Table 2. The dose-dependent inhibition was observed, 0.5 % of caprylic acid inhibited bacterial growth in higher extent.

Table 2. The influence of caprylic acid (0.5 %) on numbers* of coliforms and salmonellas (log₁₀ CFU/g) in crop and cloaca contents of infected chickens

	Crop contents		Cloaca contents	
	Positive control	C ₈ (0.5 %)	Positive control	C ₈ (0.5 %)
Coliforms	7.1 ± 0.2 ^a	< 2 ^b	8.4 ± 0.2 ^a	6.5 ± 0.6 ^b
Salmonellas	6.0 ± 0.2 ^a	< 2 ^b	4.1 ± 0.3 ^a	< 2 ^b

*Means ± SD

^{a, b}Treated group differs significantly from the control (P < 0.05)

The results of this experiment confirm the antibacterial activity of caprylic acid under *in vitro* conditions. Caprylic acid reduced salmonellas in the crop and caecum below the detection limit. The antibacterial effect was more pronounced in the crop; the 0.25 % and 0.5 % concentration of caprylic acid reduced coliforms by more than 4 and 6 orders, respectively.

Antibacterial effect of caprylic acid was observed in our previous *in vitro* observations (Skrivanova et al., 2004), where the susceptibility of *Salmonella* spp. to 15 fatty acids was determined. Caprylic acid was the only acid inhibiting bacterial growth. Inhibitory activity of caprylic acid on *S. enteritidis* in autoclaved chicken caecal contents was studied by Vasudevan et al. (2005). Concentrations of 50 and 100 mM reduced bacterial population by 5 orders within 5 minutes of incubation. Emulsions of 1.25 mM monoglyceride of capric acid at pH 4 – 5 caused a 6 – 7 Log₁₀ reduction of *Salmonella* spp. in 10 minutes, however no reduction was observed at neutral pH (Thormar et al., 2005).

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