Medium-chain fatty acids and plant-derived antimicrobials to prevent *Campylobacter* colonization in broiler chickens

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Aims

***Campylobacter jejuni* is the most common cause of bacterial-mediated diarrheal disease worldwide. Because poultry products are a major source of *C. jejuni* infections in humans, efforts should be taken to develop strategies to decrease *Campylobacter* colonization of poultry during primary production. Medium-chain fatty acids (MCFA) and plant-derived antimicrobial compounds possess marked bactericidal activity toward *C. jejuni in vitro* and might therefore have potential as feed or drinking water additives to control *C. jejuni* colonization in broiler chickens. For this purpose, the *in vitro* and *in vivo* anti-*Campylobacter* properties of a selection of compounds were analyzed.**

Experimental design and results

1. MCFA and *trans*-cinnamaldehyde show marked anti-*C. jejuni* activity *in vitro*

Mueller-Hinton broth was supplemented with the test compounds. Solutions were adjusted to pH 6 and inoculated with *C. jejuni*. Determination of minimal inhibitory concentrations (MIC, **Table 1**) and bacterial counts over a 24-h period (**Fig. 1**) revealed marked anti-*C. jejuni* activity especially for *trans*-cinnamaldehyde[2] and capric acid[1].

Table 1 Minimal inhibitory concentration (MIC) of selected compounds at pH 6 against *C. jejuni* strain KC 40

Compound		MIC (μM)
MCFA	Caproic acid	500
	Caprylic acid	500
	Capric acid	500
Caffeic acid		> 1000
Vanillic acid		1000
Gallic acid		2000
Protocatechuic acid		> 2000
Epigallocatechin gallate		125
Trans-cinnamaldehyde		125
Thymol		1000

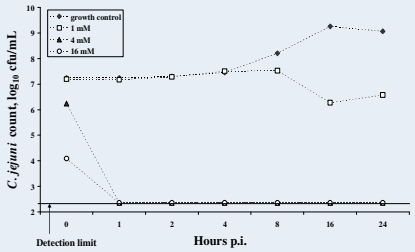

Fig. 1 Time-kill curve of capric acid at pH 6.0 against *C. jejuni* strain KC 40. Bacterial counts are represented as $\log_{10}$ cfu/mL.

2. MCFA and *trans*-cinnamaldehyde reduce neither *C. jejuni* transmission nor cecal *C. jejuni* colonization in broiler chicks

To examine the effect of MCFA and *trans*-cinnamaldehyde on *C. jejuni* transmission the compounds were added to the feed or drinking water from day-of-hatch. At two weeks of age, 3 out of 10 birds per group were orally inoculated with *C. jejuni*. Five days after inoculation, cecal *C. jejuni* numbers were determined. Neither MCFA, nor *trans*-cinnamaldehyde[2] (**Fig. 2A**) significantly ($P > 0.05$) reduced bacterial counts.

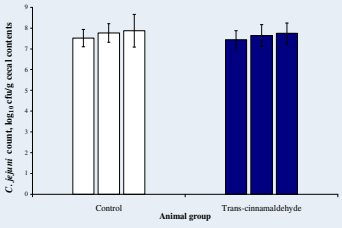

Fig. 2A Cecal *C. jejuni* counts in 21-day-old broilers (n = 9 chicks/group) fed un-supplemented feed or feed supplemented with *trans*-cinnamaldehyde from day-of-hatch.

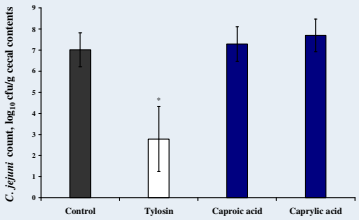

Fig. 2B Cecal *C. jejuni* counts in 27-day-old broilers (n = 10 chicks/group) fed standard feed or feed supplemented with 1% (wt/wt) tylosin, caproic or caprylic acid from 3 days before euthanization.

To analyze the therapeutic effect of MCFA, all animals were orally inoculated with *C. jejuni* at two weeks of age. After inoculation, the test compounds were added to the feed or drinking water for the last 72 h of the trial and cecal *C. jejuni* numbers were determined. Both MCFA-treated drinking water and in-feed (**Fig. 2B**) MCFA failed to significantly ($P > 0.05$) reduce cecal *C. jejuni* counts.

3. MCFA and *trans*-cinnamaldehyde fail to target *C. jejuni* in the broiler chicken cecum

A cecal loop model (**Fig. 3A**) allows direct comparison of *C. jejuni* numbers in both ceca of the same animal[1,2]. In this study, MCFA and *trans*-cinnamaldehyde (**Fig. 3B**) reduced bacterial counts compared to the control ceca.

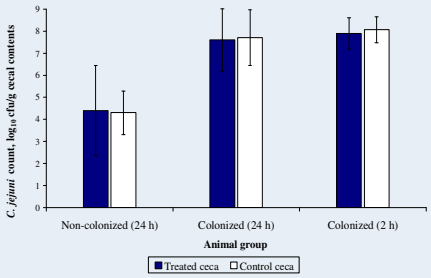

Fig. 3A Application and injection of a cecal loop

Fig. 3B *C. jejuni* counts in cecal loops of 19- (n = 2 chicks/treatment) and 21-day-old (n = 3) broilers. In the left group, one cecum of a *Campylobacter*-free chick was injected with *trans*-cinnamaldehyde, the other with HBSS. Both ceca were injected with *C. jejuni* and bacterial counts were determined 24 h later. In the middle and right group, one cecum of a *C. jejuni*-colonized chick was injected with *trans*-cinnamaldehyde, the other with HBSS. *C. jejuni* counts were determined 24 h (middle) or 2 h (right) later.

When setting up time-kill curves in the presence of chicken intestinal mucus, the bacteriostatic and bactericidal properties of capric acid against *C. jejuni* were reduced (results not shown), indicating that mucus plays an important role in the unresponsiveness of *C. jejuni* to the antibacterial properties of capric acid in the broiler cecum[1].

4. MCFA supplementation increases *C. jejuni* colonization treshold of broiler chicks

Day-old broiler chicks (n = 60) were randomly assigned to 6 groups and housed individually. Birds received control or MCFA-supplemented drinking water. After two weeks, chicks were orally inoculated with $2.2 \times 10^2 - 3.5 \times 10^4$ cfu *C. jejuni* KC 40. After 24 h all birds were euthanized and their cecal colonization status was determined (**Table 2**).

Table 2 Colonization status of 15-day-old broilers 24 hours after inoculation with *C. jejuni*

Treatment group	Dose *C. jejuni* KC 40	Number of colonized birds
CDW	2.8×10^2	2/10
	2.2×10^3	6/10
	2.5×10^4	5/10
MSDW	2.8×10^2	0/10
	2.2×10^3	0/10
	2.5×10^4	1/10

CDW = control drinking water; MSDW = MCFA-supplemented drinking water at a 0.4% (vol/vol).

A *C. jejuni* dose of 2×10^3 cfu colonized significantly ($P = 0.03$) more birds receiving control drinking water (60%) than birds receiving MCFA-supplemented drinking water, 24 h after inoculation.

Conclusion

Despite the marked bactericidal effect of medium-chain fatty acids and *trans*-cinnamaldehyde *in vitro*, supplementing these compounds to the feed or drinking water did neither prevent *C. jejuni* transmission nor reduce cecal *C. jejuni* colonization in broilers. These compounds are not capable to target *C. jejuni* in its cecal niche, probably by the protective effect of intestinal mucus. In contrast, preventive MCFA supplementation reduced the colonization treshold of broiler chicks, probably by exerting a bactericidal effect in the crop. Therefore, MCFA supplementation might be a valuable tool to prevent *Campylobacter* colonization of broiler chicken flocks.

References:

[1]Hermans *et al*. Intestinal mucus protects *Campylobacter jejuni* in the ceca of colonized broiler chickens against the bactericidal effects of medium-chain fatty acids. 2010. Poultry Science, 89:1144-1155.

[2]Hermans *et al*. The cinnamon-oil ingredient *trans*-cinnamaldehyde fails to target *Campylobacter jejuni* in the broiler chicken cecum despite marked *in vitro* activity. 2011. Journal of Food Protection. In Press.

Acknowledgements: This study was sponsored by grant RT-08/8 CAMPOUL of the Belgian Federal Public Service for Health, Food Chain Safety and Environment. Research in the author's laboratory was further sponsored by Sanluc International NV (Gijzenzele, Belgium).

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