

In Vitro Study on the Effect of Organic Acids on *Campylobacter jejuni/coli* Populations in Mixtures of Water and Feed

P. Chaveerach,^{*1} D. A. Keuzenkamp,^{*} H. A. P. Urlings,[†] L. J. A. Lipman,^{*} and F. van Knapen^{*}

^{*}Department of the Science of Food of Animal Origin, Faculty of Veterinary Medicine, Utrecht University, The Netherlands; and [†]DLO-Institute for Animal Science and Health, Lelystad, The Netherlands

ABSTRACT Gastroenteritis caused by *Campylobacter* spp. infection has been recognized as one of the important public health problems in the developed countries. Outbreaks mostly originate from the consumption of contaminated poultry or infected water.

The aim of this study was to determine the bactericidal activity on *Campylobacter* spp. of organic acids individually and in combinations at different pH levels and times and to compare bactericidal activities with activities of commercially available products. Ten strains of *Campylobacter* spp. were added in a mixture of water with commercial broiler feed, separately adjusted by four acids: formic, acetic, propionic, and hydrochloric acids, into pH 4.0, 4.5, 5.0, and 5.5. A combination of three organic acids was used in two different formulation ratios: formic:acetic:propionic at 1:2:3 and 1:2:5, at pH 4.0, 4.5, 5.0,

and 5.5. All organic acids showed the strongest bactericidal effect on *Campylobacter* at pH 4.0.

In contrast, at pH 5.0 and 5.5, the bactericidal activity of the four acids was low. The combination of organic acids showed a synergistic bactericidal activity at pH 4.5. Interestingly, the effect of the combined organic acids was stronger than the commercial products. Morphological cell changes were studied by transmission electron microscopy to determine the effect of the organic acids on the cell structure of *Campylobacter*. Some loss of outer membranes of the bacteria could be found in treated groups.

Therefore, it can be concluded that organic acids, individually or in combination, have a strong bactericidal effect on *Campylobacter* spp. Routine application of organic acids to the water supply on poultry farms could prevent or diminish *Campylobacter* transmission.

(Key words: *Campylobacter jejuni/coli*, organic acid, broiler feed, water, bacteria)

2002 Poultry Science 81:621–628

INTRODUCTION

Gastroenteritis due to *Campylobacter* spp. is one of the diseases that plays an important role in public health in developing and developed countries. There are an increasing numbers of illnesses caused by *Campylobacter jejuni*. The elderly and the young are particularly vulnerable. Consumption of contaminated chicken meat is often the source of infection. Poultry is initially infected by these bacteria at the farm level (Shanker et al., 1982; Jacobs-Reitsma et al., 1995). Also, water as a vehicle plays an important role in the horizontal transmission route on broiler farms (Pearson et al., 1993). Previous results have shown that *Campylobacter* is able to survive or multiply in natural surface water (Blaser et al., 1980), public water supplies and in wastewater from slaughterhouses (Koenraad et al., 1995). Thus, poultry are likely to become in-

fectured by *Campylobacter* from rearing-water sources on the farm.

It has been reported that the disinfectants, e.g., phenolic, iodophor, quaternary ammonium compound, ethyl alcohol, and glutaraldehyde solutions used in hospitals could dramatically decrease *Campylobacter* within a short time (Wang et al., 1983). However, these products are too toxic to add to the drinking water of chickens. Therefore, substances added to water to control transmission of *Campylobacter* should have a strong bactericidal effect on *Campylobacter* but should be also simple to use, inexpensive, nonerosive, and nontoxic.

Short-chain organic acids (SCA) have been widely used to preserve food products in western European countries, because they can be used safely without creating residue problems. Their bactericidal effect can also prevent spoilage and kill potential pathogenic organisms. SCA have also been studied and used to eliminate pathogenic bacteria under different conditions, e.g., decontamination of

©2002 Poultry Science Association, Inc.

Received for publication May 29, 2001.

Accepted for publication December 28, 2001.

¹To whom correspondence should be addressed: chaveerach@vvd.vet.uu.nl.

Abbreviation Key: A-1 = formic:acetic:propionic acids at 1:2:3; A-2 = formic:acetic:propionic acids at 1:2:5; SCA = short-chain organic acids; TEM = transmission electron microscopy.

chicken carcasses using acetic or lactic acids reduced *Campylobacter* on carcasses or meat (Cudjoe and Kapperud, 1991; Van Netten et al., 1994). Hinton and Linton (1988) found that giving feed with formic and propionic acids could reduce *Salmonella* colonization in broilers. Thomson and Hinton (1997) showed that formic and propionic acids caused sublethal damage to *Salmonella*, causing incomplete colonization in broilers. However, the bactericidal activities of SCA as a disinfectant in rearing water on farm are rarely documented. Consequently, increasing the hygiene barrier by reducing the level of infection in chickens by decontaminated water could be one of the methods for keeping chickens free of *Campylobacter* infection.

The purpose of the present study was to determine whether organic acids individually, e.g., formic, propionic, or acetic acid, or combinations thereof, at different pH levels, had an influence on 10 strains of *Campylobacter jejuni/coli* in a mixture of water and feed. To determine the morphological cell changes in *Campylobacter* caused by these acids, the bacteria were studied by transmission electron microscopy (TEM). The effects of two commercial products containing three organic acids and chlorine were evaluated on the same 10 strains and compared with the effects of the organic acids.

MATERIALS AND METHODS

Bacterial Strains

Campylobacter jejuni C144, C186, C350, C591, C690, C2146, and C2150 and *Campylobacter coli* C4596, C4601, and C4602 isolated from chickens were provided from Institute for Animal Science and Health.² They were maintained in glycerol broth and stored at -70 C before beginning the experiment. One hundred microliters of each strain from a thawed vial was inoculated into 10 mL of Brucella broth,³ containing 0.02% cysteine HCl⁴, and placed on a shaker plate at 37 C for 24 h under microaerophilic conditions.⁵ All strains were subcultured onto *Campylobacter* blood-free agar⁶ containing 32 mg of cefoperazone⁷ and 100 mg of cyclohexedine⁸ under microaerophilic conditions. One colony was transferred into Brucella bouillon and was incubated at 37 C on the shaker overnight. One milliliter of each Brucella broth was transferred into 9 mL of the physiological salt solution. The result was a stock solution, having approximately 10^6 cfu of *Campylobacter*/mL, for use in this experiment.

²ID-Lelystad, 8200AB, Lelystad, The Netherlands.

³BBL, 11088, Becton Dickinson Co., MD 21030.

⁴L-cysteine 102839, Merck Co., Darmstadt 64293 Germany.

⁵GasPak, Becton Dickinson Co., MD 21030.

⁶Oxoid CM 739, Oxoid, Co., Hampshire, RG24 8PW UK.

⁷Product 62893-203, Sigma Co., St. Louis, MO 63178.

⁸Product c6255, Sigma Co., MO 63178.

⁹Coppens Hoogeloon, 5548AZ, The Netherlands.

¹⁰Merck KgaA. Co., D-64271, Darmstadt, Germany.

¹¹Hyalus, Envirotec. Co., Hereford, HR4 9UN UK.

¹²Selko-DWB, Selko Co., 5048AZ, Tilburg, The Netherlands.

Feed Preparation

A commercial broiler feed in the form of granules⁹ was used. It was ground and sterilized by hot-dry sterilization for 3 h at 160 C . A 6.25-g feed sample was put into a bottle containing 250 mL of tap water. This mixture of feed and water was sterilized at 121 C for 15 min.

Mixture and Acidity Preparation

All organic acids, formic (98 to 100% wt/wt), acetic (100% wt/wt), propionic (99% wt/wt), and hydrochloric (37% wt/wt) were analytical grade.¹⁰ To compare the bactericidal potential of each organic acid and their combinations on *Campylobacter jejuni/coli*, different amounts of hydrochloric, formic, acetic, and propionic acids were put into the prepared mixture bottle to reach a pH of 4.0, 4.5, 5.0, or 5.5, measured with an electric pH meter. The control mixture contained no organic acids, and its pH was approximately 6.0. All bottles were kept in a water bath at 32 C overnight. The stocks of combined organic acids were made with two formulas: formic:acetic:propionic acids at 1:2:3 (A-1) and 1:2:5 (A-2).

General Method

One milliliter from the current stock solution was put into the prepared mixture bottles with pH at 4.0, 4.5, 5.0, or 5.5, as well as into a bottle with control mixture. Bottles were kept in a water bath at 32 C until the end of experiment. The survival of *Campylobacter* was determined by a direct plate count method of 10-fold dilutions of *Campylobacter* on blood-free agar plates. Samples were taken at 0, 1, 2, 4, 6, and 8 h after adding the bacteria to the different organic acids. The experiments were done in triplicate. All cultures were kept under microaerophilic conditions by using a GasPak at 37 C for 42 h. Typical colonies were counted and expressed as \log_{10} colony-forming units per milliliter. Examination of screw-like motility by light microscope was used when necessary.

Individual Strain Test

One milliliter of each strain of *Campylobacter* from the stock solution was transferred into 250 mL of prepared mixture with the combined organic acid, A-2 as described above, at pH 4.0. As described in the general method, survival of *Campylobacter* was checked after 0, 0.5, 1, 2, and 4 h. The evaluation of each strain was performed in triplicate.

Testing Commercial Products

Commercial products tested were product A (Hyalus¹¹), based on chlorine dioxide and product B (Selko¹²), based on organic acids. The bactericidal effect of both commercial products in different concentrations on *Campylobacter* populations, with and without feed, was measured. Therefore, three bottles (No. 1, 2, and 3) with

250 mL of sterile water and 0.2 or 5% of Hyalus solution and 0.2% of Selko solution and three bottles (No. 4, 5, and 6) with 250 mL of water and commercial feed as described in the previous experiment and 0.2 and 5% Hyalus solution and 0.2% of Selko solution were made. One milliliter of active stock solution of 10 strains of *Campylobacter* was prepared and introduced into Bottles 1, 2, 3, 4, 5, and 6 as previously described.

Culturability of *Campylobacter* populations from each bottle was tested three times by direct plate count on charcoal-cefoperazone-deoxycholate agar (CCDA) plates by sequential time, as in the first experiment. All cultures were incubated under microaerophilic conditions at 37 C for 48 h.

TEM

The *Campylobacter* strains C4596 and C4601 were studied. One milliliter of each culture was transferred into 4 mL of Mueller Hinton¹³ broth balanced at pH 4 by HCl or formic acid. A control was performed at pH 5.8. The cultures were kept in a dark-light box under microaerophilic conditions at 37 C for 2 h. Then the cultures were centrifuged at 5,000 rotations per minute for 10 min, and the supernatant was discharged. The pellets were washed with PBS solution. The suspensions were recentrifuged at 5,000 rotations per minute for 10 min. Pellets were collected and fixed by immersion fixation in Karnovsky fixative (2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M Na-cacodylate buffer, pH 7.3), and postfixed for 3 h in 2% OsO₄ in the same buffer. The samples were stained *en bloc* with 2% aqueous uranylacetate for 3 h, followed by dehydration in graded series of acetone and embedded in Durcupan ACM.¹⁴ Ultrathin sections (silver-gold) were prepared with a diamond knife on a LKB V microtome and mounted on formvar-carbon-coated copper grids. After staining with lead citrate, the sections were examined and photographed with a transmission electron microscope¹⁵ operated at 80 kV.

Statistical Methods

Differences in bacterial counts among treatments were determined by ANOVA with SPSS software (1997). Values of $P < 0.05$ were considered significant. Undissociated acid concentrations were calculated with the Henderson-Hasselbach equation, $\text{pH} = \text{pK}_a + \log [A^-]/[HA]$, where $[A^-]$ is the dissociated acidic form and $[HA]$ is the undissociated acidic form.

RESULTS

Effect of Organic Acids on Cell Viability

There were no great differences among organic acids, but there were significant pH differences. However, at pH

4.0 with formic, propionic, and acetic acids, the survival of *Campylobacter* rapidly declined from the beginning doses of 3.44 ± 0.33 , 3.21 ± 0.16 , and $3.17 \pm 0.03 \log_{10}$ cfu/mL, respectively, to below detection limits within 1 h of incubation (Figure 1b, c, and d). On the other hand, when HCl was used at pH 4.0, survival rates decreased much more slowly. It took 4 h of incubation with HCl to go from the beginning incubation dose of $3.27 \pm 0.20 \log_{10}$ cfu/mL to a nondetectable level (Figure 1a and b).

At pH 4.5, the bactericidal effects of propionic, formic, acetic, and hydrochloric acids on *Campylobacter* population gave reductions of 2.55, 2.38, 2.02, and 1.21 \log_{10} cfu/mL at 1 h of incubation time, respectively. At pH 4.5 and 2 h incubation, *Campylobacter* numbers were below detection limits for the organic acid solutions and remained so through the 8-h test period, whereas for HCl, *Campylobacter* numbers did not decline below detection limits until 6 h of incubation.

At pH 5.0 and 5.5 propionic, formic, acetic and hydrochloric acids, *Campylobacter* showed was culturable, respectively, for 3.04 ± 0.73 , 3.07 ± 0.04 , 2.85 ± 0.28 , and 2.77 ± 0.23 and 2.93 ± 0.42 , 3.39 ± 0.14 , 3.24 ± 0.12 , and $2.66 \pm 0.23 \log_{10}$ cfu/mL after 1 h of incubation. Culturability consistently decreased until the end of the study. *Campylobacter* survival in the control samples was high compared with the treated samples. However, there was a slight decrease over time (Figure 1).

Effect of the Combination of Organic Acids on Cell Viability

When organic acid combinations were used, A-1 and A-2, the bactericidal effect on *Campylobacter* survival yielded a kinetic characteristic graph, which was similar to that of the individual acids (Figure 2). At pH 4.0, however, the organic acid combinations (A-1 and A-2) caused *Campylobacter* numbers to decline to below detection limits within 1 h.

Surprisingly, at pH 4.5 and 1 h of incubation, A-1 and A-2 gave a high reduction rate at 3.03 and $3.22 \log_{10}$ cfu/mL, respectively ($P < 0.05$). These rates were much higher when compared to rates for the individual organic acids. At pH 5.5, A-1 and A-2 gave continuous reduction rates of *Campylobacter* numbers over the corresponding time. After that, the culturability was below detection limits by 8 h of incubation (Figure 2).

Effect of Combined Organic Acid (A-2) on Individual Strain Viability

Only strains C690, C591, C144, C350, C186, and C2146 were above the detection limit after 0.5 h of incubation time. No *Campylobacter* viability could be detected after 1 h incubation time (Table 1).

Bactericidal Effect of a Commercial Product on Cell Viability

Both commercial products, when used in water, lowered *Campylobacter* viability below the detection limit

¹³Oxoid, CM 405 Unipath Co., Hampshire, RG24 8PW UK.

¹⁴Fluka, Buchs, 3330AA, Switzerland.

¹⁵CM 10 Philips, Philips Co., 5627GB, Eindhoven, The Netherlands.

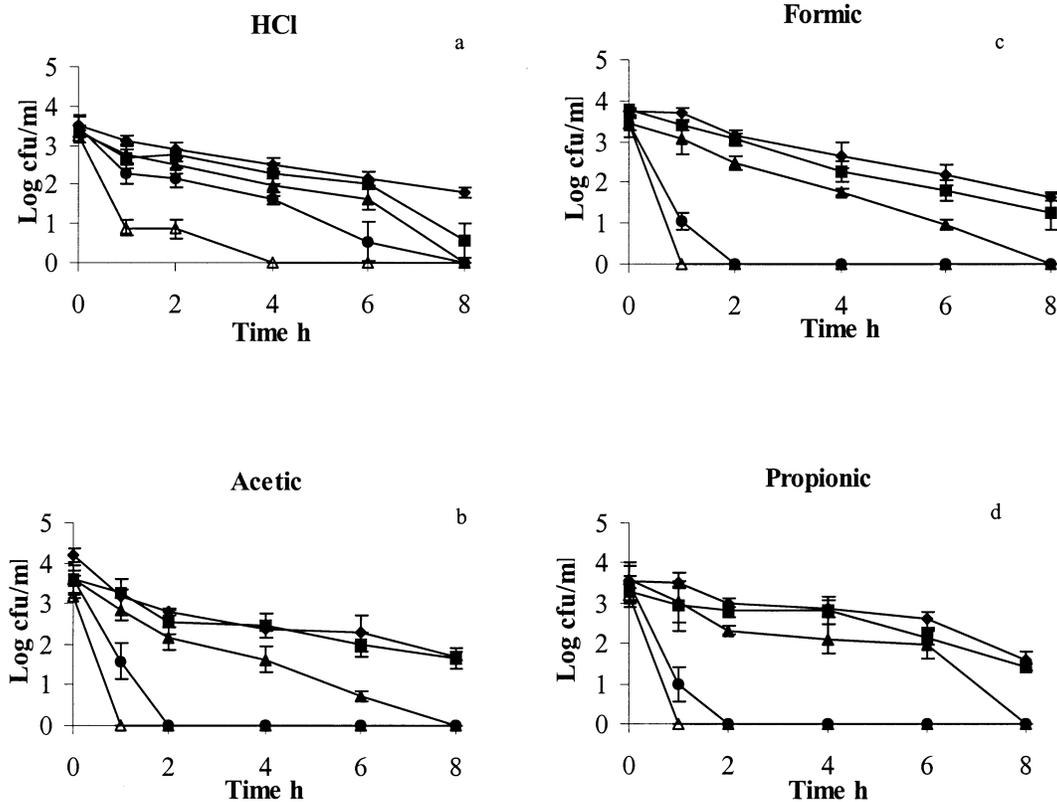


FIGURE 1. The survival of 10 strains of *Campylobacter jejuni/coli* after being exposed organic acids at different pH levels and times at 37 C; ◆, control; ■, pH 5.5; ▲, pH 5.0; ●, pH 4.5; and Δ, pH 4.0. a) Hydrochloric acid; b) acetic acid; c) formic acid; d) propionic acid.

within 1 h (Table 2). When the low concentration of product A (0.2%) was mixed with feed (Bottle 4, Table 2) *Campylobacter* numbers were consistently high ranging from $4.31 \pm 0.22 \log_{10}$ cfu/mL at 1 h to $2.52 \pm 0.23 \log_{10}$ cfu/mL at 8 h. When the high concentration of product A was mixed with feed (Bottle 5, Table 2) *Campylobacter* numbers declined from $4.03 \pm 0.31 \log_{10}$ cfu/mL at inoculation to $1.40 \pm 0.50 \log_{10}$ cfu/mL by 2 h and were below detection limits by 4 h. Adding 0.2% of product B to feed (Bottle 6, Table 2) reduced *Campylobacter* numbers below detection limits by 1 h of inoculation.

Effect of Acids on Cell Integrity

No significant differences were observed on the TEM pictures of the two strains C4596 and C4601 when grown in HCl or formic acid compared with growth at pH 5.8. Generally, the inner membrane stayed intact (Figures 3 and 4).

DISCUSSION

A previous study showed that the appropriate pH range for growth of *Campylobacter jejuni* was 5.5 to 7.5

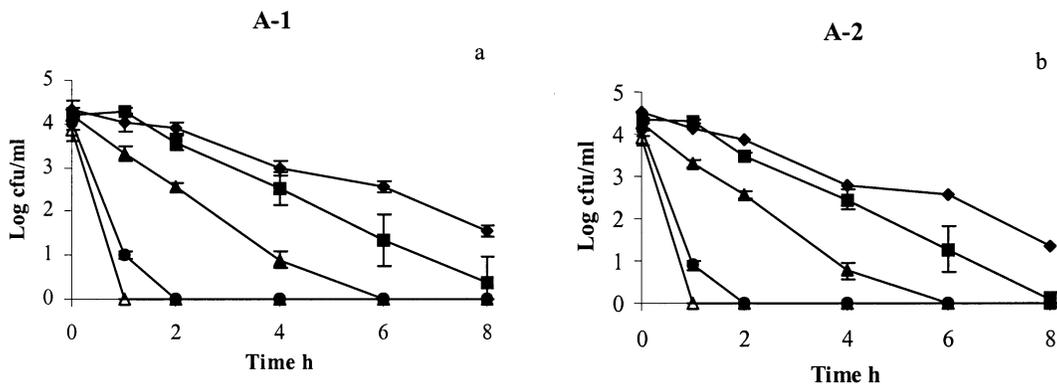


FIGURE 2. The survival of 10 strains *Campylobacter jejuni/coli* after being exposed combined organic acids at different pH levels and times at 37 C; ◆, control; ■, pH 5.5; ▲, pH 5.0; ●, pH 4.5; and Δ, pH 4.0. a) A-1 = formic:acetic:propionic acids at 1:2:3; b) A-2 = formic:acetic:propionic acids at 1:2:5.

TABLE 1. The effect of the organic acid combination A-2 (formic:acetic:propionic acids at 1:2:5) on individual *Campylobacter* spp. on different time (h)

	Incubation time under the acid condition (h)					
	0	0.5	1	1.5	2	4
C144 ¹	3.42 ± 0.21 ³	1.33 ± 1.15 ³	UD	UD	UD	UD
C690 ¹	3.47 ± 0.42	1.87 ± 0.67	UD	UD	UD	UD
C591 ¹	3.62 ± 0.13	1.77 ± 0.40	UD	UD	UD	UD
C4602 ²	3.36 ± 0.28	UD ⁴	UD	UD	UD	UD
C4596 ²	3.26 ± 0.65	UD	UD	UD	UD	UD
C350 ¹	3.13 ± 0.12	0.67 ± 1.15	UD	UD	UD	UD
C2150 ¹	5.12 ± 0.08	UD	UD	UD	UD	UD
C186 ¹	2.94 ± 0.15	1.59 ± 0.36	UD	UD	UD	UD
C2146 ¹	3.41 ± 0.14	0.10 ± 0.17	UD	UD	UD	UD
C4601 ²	2.90 ± 0.29	UD	UD	UD	UD	UD

¹*Campylobacter jejuni*.²*Campylobacter coli*.³Log₁₀ cfu/mL ± SD.⁴UD; under detection limit of 1 cfu/mL.

(Fletcher et al., 1983). Blaser et al. (1980) has also demonstrated that *Campylobacter jejuni* was not able to survive in extremely acidic conditions, e.g., below 3.0, but it was stated that *Campylobacter* was not affected at pH higher than 3.6. In weak acid conditions with pH between 4.0 to 6.0, *Campylobacter* could survive well and showed just a slight decrease in culturability (Blaser et al., 1980; Doyle and Roman, 1981; Rotimi et al., 1990; Watterman and Small, 1998). The effect of organic acids has already been shown in the control of *Campylobacter* contamination on poultry carcasses during slaughter (Stern et al., 1985; Cudjoe and Kapperud, 1991; Epling et al., 1993). Unfortunately, there were no suitable criteria to standardize substance or acidity, which affect inhibition of *Campylobacter* viability in their experiments.

In this study, we compared the bactericidal effect of organic acids on *Campylobacter jejuni/coli* at different pH levels with the effect of HCl use. Our results demonstrated that at low pH levels (4.0 and 4.5) HCl had only a slight inhibitory effect on the *Campylobacter* population, but it was not effective at high pH levels (5.0 and 5.5). Rotimi et al. (1990) has demonstrated that the bactericidal activity of HCl on *Campylobacter jejuni* was pH dependent. From the results using organic acids, it is quite clear that

at low pH (4.0 and 4.5) *Campylobacter jejuni/coli* rapidly die. The reduction rate was much higher compared to the use of HCl. However, at pH 5.0 and 5.5 *Campylobacter* could survive. Our results show that organic acids have a very strong bactericidal effect on *Campylobacter jejuni/coli* culturability at low pH. No individual differences between the different strains of *Campylobacter jejuni/coli* were found.

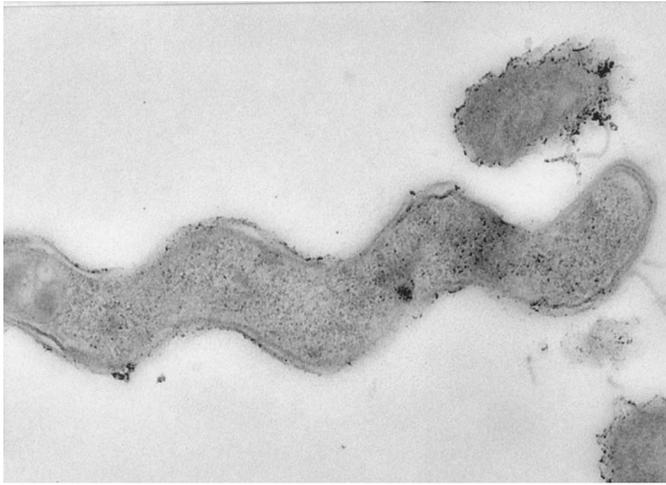
The inhibitory mechanism of short chain organic acids on bacteria has not been elucidated. Investigators have reported that growth inhibition of acids on bacteria is caused by two explicit components. One is the specific inhibition of an unidentified metabolic function by undissociated acid, whereas the other is diffusion of undissociated acid into the cell, which releases a proton that acidifies the cytoplasm (Eklund, 1983; Baronofsky et al., 1984; Salmond et al., 1984; Kroll and Patchett, 1991).

The degree of bactericidal activity of the different acids on the bacterial cell will most probably depend on the presence of the organic compounds, acid concentration, structure of the acid, and capacity of a cell to alkalize the cytoplasm. We found that a small excess of undissociated formic acid gave a stronger bactericidal effect on the culturability of *Campylobacter jejuni/coli* compared to a large

TABLE 2. Comparison of the survival of 10 strains of *Campylobacter jejuni* treated by commercial products A and B at different times

Time (h)	Commercial product					
	No. 1 ¹ (0.2% product A)	No. 2 ¹ (5% product A)	No. 3 ¹ (0.2% product B)	No. 4 ² (0.2% product A)	No. 5 ³ (5% product A)	No. 6 (0.2% product B)
0	UD ⁴	UD	3.70 ± 0.23 ⁵	4.02 ± 0.10 ⁵	4.03 ± 0.31 ⁵	3.82 ± 0.13 ⁵
1	UD	UD	UD	4.31 ± 0.22	2.60 ± 0.14	UD
2	UD	UD	UD	4.01 ± 0.10	1.40 ± 0.50	UD
4	UD	UD	UD	3.51 ± 0.11	UD ¹	UD
6	UD	UD	UD	3.52 ± 0.20	UD	UD
8	UD	UD	UD	2.52 ± 0.23	UD	UD

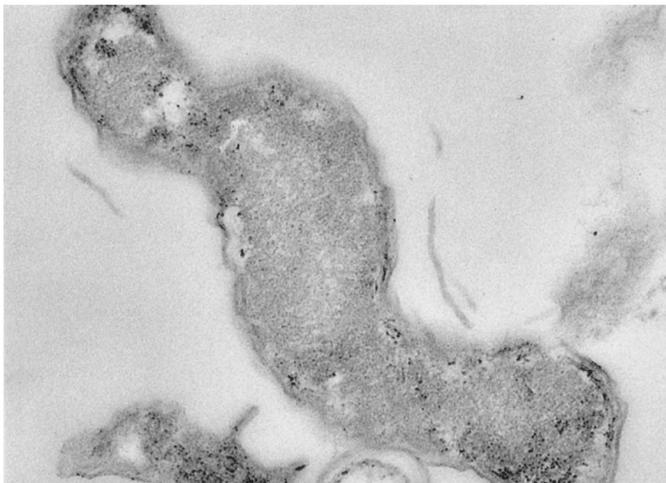
¹No. 1, 2, and 3 bottles contained only water. No. 4, 5, and 6 bottles contained water and feed.²Product A was Hyalus (Envirotect. Co., Hereford, HR4 9UN UK).³Product B was Salko (Selko Co., 5048AZ, Tilburg, The Netherlands).⁴UD = under detection limit of < 1 cfu/mL.⁵*Campylobacter* survival in log₁₀ cfu/mL ± SD.



a

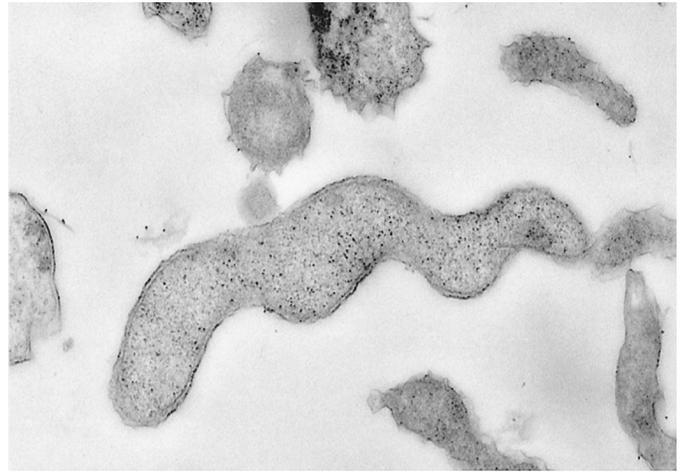


b

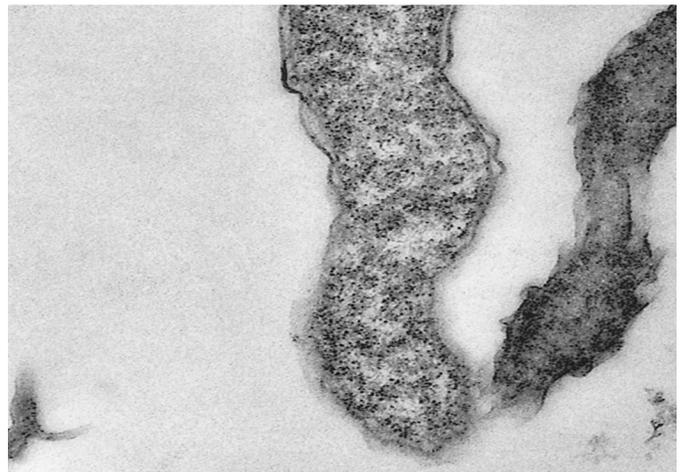


c

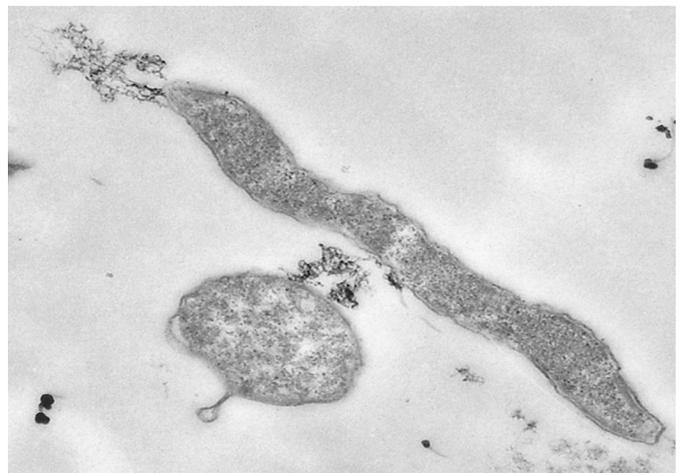
FIGURE 3. Transmission electron microscopic pictures of C4601 strain incubated for 2 h (a) control pH 5.8, (b) with HCl acid at pH 4, or (c) with formic acid at pH 4.



a



b



c

FIGURE 4. Transmission electron microscopic pictures of C4596 strain incubated for 2 h (a) control pH 5.8, (b) with HCl acid at pH 4, or (c) with formic acid at pH 4.

excess of undissociated propionic or acetic acid at the same pH levels. It is possible that the structure of acids is the most important in the inhibition of *Campylobacter jejuni/coli* viability. Formic acid is the shortest-chain organic acid, which could be beneficial process for diffusion into the cell and cause acidification of the cytoplasm.

No differences were observed with the TEM pictures between the bacteria grown in pH 5.8 and the acidic solutions. The inner and outer membranes of the bacteria were not damaged. There was a trend for some loss of the outer membrane in the bacteria grown in the acid solutions. Cell death initiated by formic acid, therefore, is probably caused by diffusion into the cell of the undissociated form of this organic acid that probably results in invisible denaturation of enzyme activity or DNA syntheses (Cherrington et al., 1991). Further experiments need to be investigated for this phenomenon.

The results of the use of the mixtures of organic acids (A-1 and A-2) demonstrated that although the amounts of accumulated undissociated acids of each acid in A-1 or A-2 are less than in individual use of acids at the same low pH levels, particular at pH 4.5, the mixtures gave a higher reduction rate. Minor and Marth (1972) reported that there was no synergetic activity on *Staphylococcus aureus* when acetic acid and HCl were combined. Our results strongly demonstrate that the combination of organic acids gives a remarkably high bactericidal effect on *Campylobacter jejuni/coli* viability in low pH aqueous solutions. The reason for this synergistic effect is, as yet, unknown.

There is a lack of knowledge of *Campylobacter* disinfectants under field conditions, especially on poultry farms. Our present experiment also demonstrates the different bactericidal effects of commercial products on *Campylobacter* that are routinely used on Dutch broiler farms. Although Blaser and colleagues (1986) showed that standard chlorinated water could inhibit growth of *Campylobacter* spp. within a few minutes, our results of the use of product A, based on chlorine compounds, demonstrated no influence on *Campylobacter jejuni/coli* populations living in water feed mixtures within 2 h. The effect of chlorinated water on *Campylobacter* might be reduced by feed. In contrast, product B based on organic substances accomplished *Campylobacter jejuni/coli* death within 1 h under similar conditions.

In general, rearing water on broiler farms is intensively contaminated with dirty organic matter such as feed, feces, soil, or bedding. This water is a good survival place for pathogenic and nonpathogenic agents. Therefore, re-infection with any bacteria, particularly *Campylobacter* spp., can certainly occur. This experiment is the first to demonstrate the ability of organic acids to kill *Campylobacter jejuni/coli* without causing cell membrane changes.

The use of organic acids in rearing water systems for broilers could reduce cross-infection of *Campylobacter* spp. via rearing water on farm level. Further research, especially animal infection experiments, is necessary to specify the exact effect of acidified water on transmission of *Campylobacter* bacteria among chickens at farm level.

ACKNOWLEDGMENTS

The authors thank E. R. Spek for providing transmission electron microscopic pictures and Nancy Bleumink and J. van Putten for valuable comments on TEM pictures.

REFERENCES

- Baronosky, J. J., W. J. A. Shreurs, and E. R. Kashket. 1984. Uncoupling by acetic acid limits growth of and acetogenesis by *Clostridium thermoaceticum*. Appl. Environ. Microbiol. 48:1134-1139.
- Blaser, M. J., H. L. Hardesty, B. Power, and W.-L. Wang. 1980. Survival of *Campylobacter fetus* susp. *jejuni* in Biological Milieus. Clin. Microbiol. 11:309-313.
- Blaser, M. J., P. F. Smith, W.-L. Wang, and J. C. Hoff. 1986. Inactivation of *Campylobacter jejuni* by chlorine and monochloramine. Appl. Environ. Microbiol. 51:307-311.
- Cherrington, C. A., M. Hinton, G. R. Pearson, and I. Chopa. 1991. Short-chain organic acids at pH 5. 0 kill *Escherichia coli* and *Salmonella* spp. without causing membrane perturbation. Appl. Bacteriol. 70:161-165.
- Cudjoe, K. S., and G. Kapperud. 1991. The effect of lactic acid spray on *Campylobacter jejuni* inoculated onto poultry carcasses. Acta Vet. Scand. 32:491-498.
- Doyle, M. P., and D. J. Roman. 1981. Growth and survival *Campylobacter* subsp. *jejuni* as a function of temperature and pH. Food Prot. 44:596-601.
- Eklund, T. 1983. The antimicrobial effect of dissociated and undissociated sorbic acid at different pH levels. Appl. Bacteriol. 54:383-389.
- Epling, L. K., J. A. Carpenter, and L. C. Blankenship. 1993. Prevalence of *Campylobacter* spp. and *Salmonella* spp. on pork carcasses and the reduction effected by spraying with lactic acid. Food Prot. 56:536-537.
- Fletcher, R. D., A. C. Albers, A. K. Chen, and J. N. Alberton, Jr. 1983. Ascorbic acid inhibition of *Campylobacter jejuni* growth. Appl. Environ. Microbiol. 45:792-795.
- Hinton, H., and A. H. Linton. 1988. Control of *Salmonella* infections in broiler chicken by the acid treatment of their feed. Vet. Rec. 15:416-421.
- Jacobs-Reitsma, W. F., A. W. Van de Giessen, N. M. Bolder, and R. W. A. W. Mulder. 1995. Epidemiology of *Campylobacter* spp. at two Dutch broiler farms. Epidemiol. Infect. 114:413-421.
- Koenraad, P. M. F. J., R. Ayling, W. C. Hazeleger, F. M. Rombouts, and D. G. Newell. 1995. The speciation and subtyping of *Campylobacter* isolate from sewage plants and waste water from a connected poultry abattoir using molecular techniques. Epidemiol. Infect. 115:485-494.
- Kroll, R. G., and R. A. Patchett. 1991. Biocide-induce perturbations of aspects of cell homeostasis: Intercellular pH, membrane potential and solute transport. Pages 189-202 in Mechanisms of Action of Chemical Biocide. S. P. Denyer and W. B. Hugo, ed. Blackwell, Oxford, England.
- Minor, T. E., and E. H. Marth. 1972. Loss of viability by *Staphylococcus aureus* in acidified media. Milk Food Technol. 35:191-196.
- Pearson, A. D., M. H. Greenwood, R. K. A. Feltham, T. D. Healing, J. Donaldson, D. M. Jones, and R. R. Colwell. 1993. Microbial Ecology of *Campylobacter jejuni* in a United Kingdom chicken supply chain Intermittent common source, vertical transmission, and amplification by flock propagation. Appl. Environ. Microbiol. 62:4614-4620.
- Rotimi, V. O., L. Egwari, and B. Akande. 1990. Acidity and intestinal bacteria: An in-vitro assessment of the bactericidal activity of hydrochloric acid on intestinal pathogens. Afr. J. Med. Sci. 19:275-280.

- Salmond, C. V., R. G. Kroll, and I. R. Booth. 1984. The effect of food preservative on pH homeostasis in *Escherichia coli*. Gen. Microbiol. 130:2845–2850.
- Shanker, S., J. A. Rosenfield, G. R. Davey, and T. C. Sorrell. 1982. *Campylobacter jejuni*: Incidence in processed broiler and biotype distribution in human and broiler isolates. Appl. Environ. Microbiol. 43:1219–1220.
- SPSS. 1997. SPSS Advance Statistics. Version 7.5.2. SPSS, Inc., Chicago, IL.
- Stern, N. J., P. J. Rothenberg, and J. M. Stone. 1985. Enumeration and reduction of *Campylobacter jejuni* in poultry and red meat. Food Prot. 48:606–610.
- Thomson, J. L., and M. Hinton. 1997. Antibacterial activity of formic and propionic acids in the diet of hens on *Salmonellas* in the crop. Br. Poult. Sci. 38:59–65.
- Van Netten, P., J. H. J. Huis in't veld, and D. A. A. Mossel. 1994. The immediate bactericidal effect of lactic acid on meat-borne pathogen. Appl. Bacteriol. 77:490–496.
- Wang, W.-L., B. W. Powers, N. W. Luechtefeled, and M. L. Blaser. 1983. Effects of disinfectants on *Campylobacter jejuni*. Appl. Environ. Microbiol. 45:1202–1205.
- Waterman, S. R., and P. L. C. Small. 1998. Acid-sensitive enteric pathogens are protected from killing under extremely acidic conditions of pH 2.5 when they are inoculated onto certain solid food sources. Appl. Environ. Microbiol. 64:3882–3886.