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# Inhibition of the Growth of *Salmonella typhimurium* and *Escherichia coli* 0157:H7 on Chicken Feed Media by Bacteria Isolated from the Intestinal Microflora of Chickens

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**ABSTRACT**

Two lactic acid-producing bacteria, one volatile fatty acid-producing bacterium, and one starch-hydrolyzing bacterium were isolated from the cecal contents of adult chickens. The ability of these bacteria to produce lactic and volatile fatty acids in 5% chicken feed broth media and to inhibit the growth of *Salmonella typhimurium* and *Escherichia coli* 0157:H7 on a 5% chicken feed agar media was determined. Inhibition of the growth of the enteropathogens was due to bacteriostatic or bactericidal substances that the cecal isolates produced in the media. Depending on which isolates were used, the inhibitory substances were either high concentrations of lactic acid that created an inhibitory pH in the media or inhibitory concentrations of acetic and propionic acids that the isolates produced in the media.

Bacteria have been isolated from the cecal contents of adult chickens that can inhibit the growth of *Salmonella typhimurium* and *Escherichia coli* 0157:H7 in vitro (*9,10*). In one study, an *Enterococcus durans* strain was isolated that produced lactic acid from lactose in the media. A *Veillonella* isolate, grown with the *E. durans*, converted the lactic acid into acetic and propionic acids that inhibited the growth of both enteropathogens (*9*). In another study, a *Streptococcus* strain isolated from chickens produced large concentrations of lactic acid from lactose in the media. The high concentrations of lactic acid decreased the pH of the media below the range required for the growth of either enteropathogen (*10*).

The end products of lactose fermentation can also reduce cecal colonization of young chicks by *S. typhimurium* (*3,7,8*). In young chicks provided dietary lactose and cultures of cecal anaerobes from adult chickens, the concentrations of acetic, propionic, and lactic acids in the ceca increased. The increase in the cecal concentration of the total (*8*) or undissociated form (*3,7*) of these acids was correlated with a reduction in cecal colonization by *S. typhimurium*. Dietary lactose and cecal anaerobes also produced similar effects on *S. typhimurium* colonization when provided to adult chickens (*4*) and turkey poults (*5*).

However, the contents of the digestive tract of adult chickens (*13*) and anaerobic cultures of the cecal (*16*) and fecal (*14*) contents of adult chickens can also reduce salmonellae colonization of young chicks not provided dietary lactose. Different mechanisms have been proposed for how bacteria from adult chickens can reduce cecal colonization of young chickens by salmonellae (*14,18*). One of the proposed mechanisms of how the digestive tract of chicks and other animals resists colonization by enteropathogens is that bacteriostatic or bactericidal volatile fatty acids (VFA) produced in the ceca by the normal microflora inhibit the growth of the enteropathogens (*1,12,15*). Also, lactic acid is produced in the crops of chicks by lactobacilli that ferment carbohydrates consumed by the chicks (*6*). The lactic acid produced a low pH in the crop and was one of the factors that reduced colonization of the chicks by *E. coli*. The source of lactic acid in chicks not provided dietary lactose is carbohydrates in the chicken feed, and both lactic acid- and VFA-producing bacteria are among the bacteria in the normal flora from adult chickens (*9,10*).

The purpose of this research was to isolate bacteria from the cecal contents of adult chickens that can ferment the carbohydrates in a normal chick diet to produce lactic acid and bacteria that can convert the lactic acid into VFAs. The isolated bacteria were then tested for their ability to inhibit the growth of *S. typhimurium* and *E. coli* 0157:H7 on a medium that simulated a normal chick diet.

## MATERIALS AND METHODS

### *Growth of intestinal microflora*

Cecal contents were removed from adult broilers and stored at -70°C as previously described (*7*). Approximately 2 ml of the thawed cecal contents was added to 200 ml of modified Viande Levure (VL) broth. The composition of the broth was (g/L) tryptose (Difco Laboratories, Detroit, MI), 10; yeast extract (Difco), 5; sodium chloride (J. T. Baker, Phillipsburg, NJ), 5; beef extract (Difco), 2.4; L-cysteine hydrochloride (Sigma Chemical Co., St. Louis, MO), 0.4; dextrose (ICN Biochemicals, Cleveland, OH), 2.5; and Bacto agar (Difco), 0.6. Modified VL agar was made by increasing the agar concentration to 1.2%.

The inoculated broth was incubated in an anaerobic chamber (Coy Laboratory Products, Ann Arbor, MI) at 37°C for 18-24 h. Bacteria were isolated from the broth by plating serial dilutions of the broth onto VL agar plates that were incubated anaerobically at 37°C for 18-24 h. Tubes of reinforced clostridial medium (RCM) (Unipath Co., Oxoid Division, Ogdensburg, NY) were inoculated with the isolates and incubated anaerobically at 37°C for 18-24 h. Cultures were maintained by transferring to fresh RCM at 1 to 2-week intervals, incubating, and storing at ambient temperature in the anaerobic chamber.

*Selection of lactic acid and VFA producing bacteria*

Separate tubes containing the modified VL broth were inoculated with the isolates. The inoculated tubes were incubated anaerobically at 37°C for 18-24 h. After incubation, the growth medium was analyzed by gas chromatography with a Shimadzu Gas Chromatograph GC-9A (Shimadzu Corp., Columbia, MD) to determine the level of lactic acid produced, as previously described (*7*). The two isolates that produced the greatest concentrations of lactic acid were chosen for further study. Another lactic acid-producing bacterium that had been previously isolated (*10*) was also used in this study.

To select for bacteria that can convert lactic acid into VFAs, a lactic acid-based medium was prepared by substituting 0.25% L-lactic acid, lithium salt (Sigma) for dextrose in the modified VL media. Each isolate was added to separate tubes of this medium, and the tubes were incubated anaerobically at 37°C for 18-24 h. After incubation, the growth media of these isolates were also analyzed by gas chromatography. The isolate that produced the greatest amount of VFAs (acetic and propionic acids) was chosen for further study.

*Selection of a starch-hydrolyzing bacterium*

Serial dilutions of cecal contents incubated in modified VL broth were plated on a starch-based agar medium prepared by substituting 0.30% soluble starch (Sigma) for dextrose in the modified VL agar and adding 0.5% $K_2HPO_4$. Tubes of RCM and fresh plates of the starch medium were each inoculated with morphologically different isolates selected from the incubated plates. After incubation of the tubes and plates, the plates were flooded with Gram's iodine solution (Difco). The isolates that hydrolyzed the starch were selected by examining the flooded plates for zones of clearing around colonies on the agar. One starch-hydrolyzing bacterium was isolated. The bacterium was grown and stored in the anaerobic chamber in a starch medium containing (g/L) tryptone (Difco), 10; yeast extract (Difco), 10; $K_2HPO_4$ (Sigma), 5; and soluble starch (Difco), 3.

*Preparation and inoculation of chicken feed broth media*

A chicken feed medium was prepared using a corn-soybean based chicken feed obtained from the Poultry Science Department of Texas A & M University. The feed was composed of 53.32% yellow corn, 37.60% soybean meal (48% protein), 5.46% animal and vegetable fat blend, 1.64% calcium carbonate, 1.22% monocalcium diphosphate, 0.25% broiler vitamin premix, 0.25% sodium chloride, 0.21% methionine hydroxy analog (86% methionine), and 0.05% trace minerals. The chicken feed broth was prepared by blending a 5% suspension of chicken feed in distilled water (wt/vol) at high speed in a Waring blendor for 3 min. The blended suspension was filtered through cheese cloth, dispensed into test tubes, and autoclaved at 121°C for 15 min.

Ten-µl inoculating loops (Nunc Inter Med, Roskilde, Denmark) were used to inoculate test tubes containing 10 ml of the chicken feed broth with the appropriate lactic acid- and/or VFA-producing bacteria that had been grown in RCM broth for 24 h. Appropriate tubes of chicken feed broth were also inoculated with the starch-hydrolyzing bacteria that had been grown in the starch medium described above. The tubes were incubated anaerobically for 48 h at 37°C. After incubation, the pH of the media was measured with a pH meter. The concentrations of acetic and propionic acids in the incubated media were determined by gas chromatography, and the concentration of lactic acid was determined with a Gilford Impact 400E clinical chemical analyzer (Ciba Corning Diagnostics, Corp., Gilford Systems, Oberlin, OH).

*Inhibition studies*

Chicken feed agar medium was made by adding 1.2% Bacto agar (Difco) to the chicken feed broth medium. Ten-µl inoculating loops (Nunc Inter Med) were used to make a single streak of the appropriate bacterial cultures across the center of a petri dish containing the agar. These cultures had also been grown for 24 h in RCM broth or starch medium as stated above. The plates were incubated anaerobically for 48 h at 37°C. After incubation, the plates were removed from the anaerobic chamber and 3 ml of the melted feed agar tempered to 50°C was spread over the surface of the plate.

Cultures of *S. typhimurium* and *E. coli* 0157:H7 were grown in trypticase soy broth (BBL Microbiology Systems, Cockeysville, MD) at 37°C for 18 to 24 h. The cultures were centrifuged and washed twice in distilled water. The final suspension of each of these bacteria was used to produce an optical density equivalent to $10^9$ CFU/ml using a Spectronic 20D spectrophotometer (Milton Roy Co., Rochester, NY). Each bacterial suspension was diluted to $10^6$ CFU/ml, and a sterile cotton tipped applicator (Hardwood Products Co., Guilford, ME) was used to spread the bacterial suspension onto the surface of an incubated chicken feed agar plate that had been inoculated with the appropriate isolate(s). The plates were stored at 4°C overnight to allow the inhibitory substances produced by the isolate(s) to diffuse into the agar before the enteropathogens started to grow. Plates were then transferred to a 37°C incubator and incubated for 24 h. After incubation, the width of each enteropathogen's zone of inhibition adjacent to the length of the streak of the isolates was measured.

*Identification of the isolates and statistical analysis*

All isolates were identified using methods previously described (*10*).

All experiments were repeated three times. Data were analyzed with commercial statistical analysis software, PCSAS release 6.02 (SAS Institute, Cary, NC). Duncan's multiple range test was used for separation of significantly different treatment means (*11,17*). All statements of significance are based on the 0.05 level of probability.

## RESULTS

One of the lactic-acid producing isolates was a facultative gram-positive coccus that was identified as *E. durans* and labeled strain CA240. The other lactic acid-producing isolate was an anaerobic gram-positive rod that was identified as *Lactobacillus acidophilus* and was labeled strain CA255. The lactic acid-producing bacterium, *Streptococcus morbillorum* CA331, that was described in a previous study (*10*) was also used in this study. The starch-hydrolyzing isolate was an anaerobic gram-positive coccus that was identified as *Streptococcus intermedius* and was labeled strain CA502. The VFA-producing isolate was an anaerobic gram-negative coccus that was identified as *Veillonella parvula* and was labeled strain CA126. All isolates are part of the normal intestinal flora of animals (*2*).

Results of the inhibition of *S. typhimurium* and *E. coli* 0157:H7 on 5% chicken feed agar by the bacteria isolated

from chickens are listed in Table 1. No zones of inhibition of *S. typhimurium* were produced on the uninoculated control plates or on the plates inoculated with *S. intermedius*, *S. morbillorum*, *V. parvula*, or mixed cultures of *S. morbillorum* and *V. parvula*. Pure cultures of *E. durans* and mixed cultures containing *S. intermedius* and *V. parvula* did produce small zones of inhibition although zones were not significantly larger than on those plates where there was no inhibition. Zones of inhibition significantly larger than these were produced on plates inoculated with either pure cultures of *L. acidophilus*, or with mixed cultures of *E. durans* and *V. parvula*; *L. acidophilus*, *S. morbillorum*, *S. intermedius*, and *E. durans*; and *L. acidophilus* and *V. parvula*. The zones of inhibition produced when the plates were inoculated with the mixed culture containing all five isolates were significantly larger than any of the other zones produced except for those produced by the mixed culture of *L. acidophilus* and *V. parvula*.

TABLE 1. *Zones of inhibition of* S. typhimurium *and* E. coli *0157:H7 on 5% chicken feed agar by bacteria isolated from the intestinal contents of chicks.*

| Culture | Size of zones[1,2] (cm) *S. typhimurium* | *E. coli* 0157:H7 |
|---|---|---|
| None (control) | $0.00^{c} \pm 0.00$ | $0.00^{b} \pm 0.00$ |
| *L. acidophilus* | $1.23^{b} \pm 0.35$ | $1.63^{a} \pm 0.32$ |
| *S. morbillorum* | $0.00^{c} \pm 0.00$ | $0.00^{b} \pm 0.00$ |
| *S. intermedius* | $0.00^{c} \pm 0.00$ | $0.00^{b} \pm 0.00$ |
| *E. durans* | $0.13^{c} \pm 0.06$ | $0.47^{b} \pm 0.46$ |
| *V. parvula* | $0.00^{c} \pm 0.00$ | $0.00^{b} \pm 0.00$ |
| *L. acidophilus, S. morbillorum, S. intermedius*, and *E. durans* | $1.0^{b} \pm 0.17$ | $1.40^{a} \pm 0.10$ |
| *L. acidophilus* and *V. parvula* | $1.80^{a,b} \pm 0.10$ | $1.87^{a} \pm 1.03$ |
| *S. morbillorum* and *V. parvula* | $0.00^{c} \pm 0.00$ | $0.00^{b} \pm 0.00$ |
| *S. intermedius* and *V. parvula* | $0.13^{c} \pm 0.06$ | $0.30^{b} \pm 0.17$ |
| *E. durans* and *V. parvula* | $1.07^{b} \pm 0.70$ | $1.43^{a} \pm 0.31$ |
| *L. acidophilus, S. morbillorum S. intermedius, E. durans* and *V. parvula* | $2.13^{a} \pm 1.47$ | $1.57^{a} \pm 0.40$ |

[1] Mean ± standard deviation of widths of zones of inhibition.
[2] Values within a column followed by different superscripts show significant differences ($P<0.05$) in widths of zones of inhibition.

Bacteria that did not produce zones of inhibition on the *S. typhimurium* plates produced no zones of inhibition on the *E. coli* 0157:H7 plates either (Table 1). Again, pure cultures of *E. durans* and mixed cultures of *S. intermedius* and *V. parvula* produced small zones that were not significantly larger than on those plates where no inhibition had occurred. Significantly larger zones of inhibition were produced on plates inoculated with *L. acidophilus* alone; *L. acidophilus*, *S. morbillorum*, *S. intermedius*, and *E. durans* combined; *L. acidophilus* and *V. parvula* combined; *E. durans* and *V. parvula* combined; and the mixed culture containing all five of the isolates.

The amounts of VFAs and lactic acid that the isolates produced in the 5% chicken feed broth are listed in Table 2. The levels of acetic acid production by *L. acidophilus*, *S. morbillorum*, *S. intermedius*, and *E. durans* were not significantly greater than those found in the control samples. Pure cultures of *V. parvula* and mixed cultures of *S. morbillorum* and *V. parvula* produced significantly more acetic acid than was found in the controls, but significantly less than that produced by mixed cultures of *L. acidophilus* and *V. parvula*, *S. intermedius* and *V. parvula*, *E. durans* and *V. parvula*, and the mixed culture containing all five isolates.

The bacteria that produced significantly more propionic acid in the broth media than in the uninoculated media were mixed cultures of *L. acidophilus* and *V. parvula*, *S. intermedius* and *V. parvula*, *E. durans* and *V. parvula*, and the mixed culture containing all five isolates (Table 2).

The bacteria that did not produce significant increases in the lactic acid concentration of the feed broth were pure cultures of *V. parvula* and mixed cultures of *S. morbillorum* and *V. parvula*, *E. durans* and *V. parvula*, and *S. intermedius* and *V. parvula*. The isolates that did produce significantly more lactic acid than present in the uninoculated media, pure cultures of *S. morbillorum* and mixed cultures of *L. acidophilus* and *V. parvula*, and the culture containing all five isolates. The concentration of lactic acid produced by these isolates (5.26 to 5.84 μmol/ml) was not significantly different from the concentration produced by the mixed culture of *S. intermedius* and *V. parvula* (2.49 μmol/ml), but *S. intermedius* did produce significantly more lactic acid in the media when grown alone (10.81 μmol/ml). The 15.73 μmol/ml of lactic acid produced by the pure culture of *E. durans* was significantly greater than the 10.81 μmol/ml produced by *S. intermedius*, but the highest concentrations of lactic acid were produced in media inoculated with pure cultures of *L. acidophilus* or the mixed culture of *L. acidophilus*, *S. morbillorum*, *S. intermedius*, and *E. durans* (Table 2).

Increases in the lactic acid concentration of the incubated media were generally correlated with decreases in the pH of the media (Table 2). Media with a lactic acid concentration of 25.77 μmol/ml had a final pH of 4.18, and media with a lactic acid concentration of 0.49 μmol/ml had a final pH of 6.40.

## DISCUSSION

Previous in vitro inhibition studies using bacteria isolated from the cecal contents of chickens were done on media containing lactose (*9,10*). In those studies, the isolated bacteria inhibited the growth of *S. typhimurium* and *E. coli* 0157:H7 by either producing high concentrations of lactic acid from lactose in the media (*10*) or by one isolate producing acetic and propionic acids from lactic acid that another isolate produced from lactose in the media (*9*). In the present study, inhibition of the two enteropathogens

TABLE 2. *Concentrations of acetic, propionic, and lactic acids and pH of uninoculated 5% chicken feed broth and chicken feed broth inoculated with bacteria isolated from the cecal contents of adult chickens.*

| Culture | Acid[1] (μmol/ml) | | | pH[2] |
|---|---|---|---|---|
| | Acetic acid | Propionic acid | Lactic acid | |
| None (Control) | 0.40$^{e}$ ± 0.06 | 0.14$^{b}$ ± 0.01 | 0.57$^{e}$ ± 0.25 | 6.26 ± 0.01 |
| *L. acidophilus* | 0.50$^{e}$ ± 0.05 | 0.14$^{b}$ ± 0.01 | 24.60$^{a}$ ± 1.90 | 4.19 ± 0.04 |
| *S. morbillorum* | 0.45$^{e}$ ± 0.04 | 0.14$^{b}$ ± 0.01 | 5.26$^{d}$ ± 0.92 | 5.26 ± 0.10 |
| *S. intermedius* | 0.42$^{e}$ ± 0.05 | 0.14$^{b}$ ± 0.01 | 10.81$^{c}$ ± 1.52 | 4.93 ± 0.10 |
| *E. durans* | 0.49$^{e}$ ± 0.07 | 0.14$^{b}$ ± 0.01 | 15.73$^{b}$ ± 1.30 | 4.68 ± 0.08 |
| *V. parvula* | 3.84$^{b}$ ± 0.10 | 0.60$^{b}$ ± 0.08 | 0.52$^{e}$ ± 0.21 | 6.37 ± 0.10 |
| *L. acidophilus, S. morbillorum, S. intermedius,* and *E. durans* | 0.75$^{e}$ ± 0.01 | 0.14$^{b}$ ± 0.01 | 25.77$^{a}$ ± 1.31 | 4.18 ± 0.07 |
| *L. acidophilus* and *V. parvula* | 7.15$^{a}$ ± 2.05 | 8.21$^{a}$ ± 4.71 | 5.84$^{d}$ ± 3.58 | 4.69 ± 0.23 |
| *S. morbillorum* and *V. parvula* | 4.05$^{b}$ ± 0.03 | 0.75$^{b}$ ± 0.08 | 0.49$^{e}$ ± 0.22 | 6.40 ± 0.13 |
| *S. intermedius* and *V. parvula* | 6.62$^{a}$ ± 1.11 | 7.26$^{a}$ ± 1.83 | 2.49$^{d,e}$ ± 1.97 | 5.06 ± 0.12 |
| *E. durans* and *V. parvula* | 7.50$^{a}$ ± 1.08 | 7.40$^{a}$ ± 2.57 | 0.72$^{e}$ ± 0.23 | 5.17 ± 0.20 |
| *L. acidophilus, S. morbillorum, S. intermedius, E. durans,* and *V. parvula* | 8.11$^{a}$ ± 1.60 | 9.49$^{a}$ ± 2.38 | 5.62$^{d}$ ± 4.22 | 4.61 ± 0.32 |

[1] Mean ± standard deviation of μmol/ml acid. Within columns different lower case superscripts indicate significant differences ($P<0.05$) in acid concentration.

[2] Mean ± standard deviation of pH of media after incubation.

was due to the same factors, but lactic acid was produced from carbohydrates in the chicken feed media. Either the low pH created by high concentrations of lactic acid that the bacteria produced or the inhibitory concentrations of acetic and propionic acids that the bacteria produced prevented the growth of both enteropathogens on the chicken feed agar.

The ability of two of the pure cultures and one of the mixed cultures to inhibit the growth of the enteropathogens on the media was probably primarily due to the ability of these bacteria to produce high concentrations of lactic acid and a low pH in the media. *L. acidophilus* produced no significant increase in the concentration of VFAs in the broth media, but it produced a higher concentration of lactic acid than any of the other isolates. *E. durans* produced significantly less lactic acid in the media than *L. acidophilus*, and it also produced no significant increase in the VFA concentration of the broth media. The lower lactic acid production and the higher media pH were probably why *E. durans* produced significantly smaller zones of inhibition on the media than *L. acidophilus*. Inhibition of the enteropathogens by the mixed culture of *L. acidophilus*, *S. morbillorum*, *S. intermedius*, and *E. durans* was also due to the low pH that lactic acid produced in the media.

Four of the mixed cultures that contained *V. parvula* with one or more of the other isolates were also able to inhibit the growth of both enteropathogens on the agar plates. Inhibition of the enteropathogens by these cultures was probably due primarily to inhibitory concentrations of acetic and propionic acids produced in the media. Inhibitory concentrations of VFAs were produced when the lactic acid bacteria that could produce significantly more lactic acid from the media than pure cultures of *S. morbillorum* were grown with *V. parvula*. *V. parvula* converted the lactic acid produced by the other isolates into acetic and propionic acids. Lower concentrations of lactic acid produced by these cultures resulted in higher pHs in the media, but the isolates were still able to inhibit the growth of the enteropathogens on media with pHs as high as 5.17 because of the acetic and propionic acids that *V. parvula* produced.

When the plates were inoculated with the three isolates that produced low levels of VFAs or lactic acid, the growth of the enteropathogens was not inhibited. *Veillonella* species are asaccharolytic bacteria (*2*), and the significant increase in the concentration of acetic acid and the increase in propionic acid in media inoculated with *V. parvula* were not sufficient to inhibit the growth of the enteropathogens. The other two isolates, *S. morbillorum* and *S. intermedius*, produced significant increases in the concentration of lactic acid in the media, but no significant increases in the concentration of VFAs. The 5.26 μmol/ml of lactic acid that *S. morbillorum* produced and the 10.81 μmol/ml of

lactic acid that *S. intermedius* produced resulted in a pH of 5.26 and 4.93 in the media, respectively. These pHs do not inhibit the growth of either enteropathogen (*10*).

The only mixed culture that did not produce enough inhibitory substances to inhibit the growth of the enteropathogens consisted of *S. morbillorum* and *V. parvula*. Apparently, *S. morbillorum* could not provide sufficient lactic acid for *V. parvula* to convert into inhibitory concentrations of VFAs. Although *S. morbillorum* can apparently ferment only a small amount of the carbohydrates in the chicken feed media, it can produce large quantities of lactic acid from lactose (*10*). Perhaps *S. morbillorum* can also ferment other simple carbohydrates that starch-hydrolyzing bacteria, such as *S. intermedius*, produce from starch in the chicken feed.

Results of inhibition of the enteropathogens by pure cultures of *S. intermedius* and mixed cultures of *S. intermedius* and *V. parvula* may demonstrate the importance of isolates that can produce high concentrations of lactic acid in inhibiting the growth of enteropathogens even when a VFA-producing isolate is present. *S. intermedius* was the only bacterium that produced no zones of inhibition because of the low levels of lactic acid it produces when grown alone but did produce zones of inhibition when grown with *V. parvula*. The size of the zones of inhibition that *S. intermedius* and *V. parvula* produced when grown together was significantly smaller than the zones produced when *V. parvula* was grown with either *L. acidophilus*, *E. durans*, or a mixture of all of the other isolates. Generally, the higher the concentration of lactic acid that the lactic acid bacteria produced, the larger the zones of inhibition produced when the lactic acid bacteria were grown with *V. parvula*. This was true regardless of the final concentration VFAs produced in the media.

These results suggest that in young chicks provided microflora containing the proper lactic and VFA-producing bacteria may be able to resist colonization by enteropathogens. A large concentration of lactic acid produced in the crop of the chick by lactic acid bacteria may inhibit the growth of the enteropathogens in the chick's upper intestinal tract. VFA-producing bacteria are normally found in the lower intestinal tract of chickens (*1*), and lactic acid in the chick's lower intestinal tract may be converted to VFAs that may also inhibit the growth of the enteropathogens in the chick's lower intestinal tract.

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