Research Notes

Effects of Chicken-Derived Cecal Microorganisms Maintained in Continuous Culture on Cecal Colonization by *Salmonella typhimurium* in Turkey Poults[1]

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ABSTRACT A characterized, chicken-derived, competitive exclusion culture of cecal bacteria was evaluated for effectiveness in the reduction of *Salmonella typhimurium* cecal colonization in growing turkey poults. The culture was administered by crop gavage on the day of hatch. All groups were challenged orally on Day 3 with 10^4 *S. typhimurium*. Compared with untreated controls, the percentage of poults that were *Salmonella* cecal-culture-positive at 10 d of age was significantly reduced ($P < 0.05$) in the poults provided culture. Additionally, the culture-treated poults had significantly ($P < 0.05$) fewer *Salmonella* per gram of cecal contents than the controls. The results indicated that treatment of turkey poults with the characterized chicken-derived culture effectively decreased *Salmonella* cecal colonization.

(*Key words*: *Salmonella*, turkey poults, competitive exclusion, PREEMPT™)

1999 Poultry Science 78:546–549

INTRODUCTION

Cecal cultures from adult turkeys have been tested for their ability to reduce *Salmonella* colonization in young poults on relatively few occasions. Lloyd *et al.* (1977) found that 1-d-old poults provided fresh cecal contents from an adult turkey and then challenged on Day 4 with *Salmonella typhimurium* were resistant to colonization when challenged with 10^3 cfu *S. typhimurium*. Similarly, Reid and Barnum (1983) found that a fresh culture (frozen for storage, then diluted in peptone water before administration) provided resistance to *Salmonella* colonization when poults were challenged at 2 d of age with 10^3 cfu. Additionally, these authors reported that treatment with culture at up to 6 h postchallenge also provided significant resistance to *Salmonella* colonization. Turkey-derived cecal cultures were tested alone or in combination with gentamicin to provide resistance to *Salmonella hadar* infection by Seuna *et al.* (1985). These researchers reported that the cecal culture was significantly more effective than the gentamicin for reducing the spread of the infection. When cecal culture and gentamicin were provided simultaneously, the effect was less than when the culture was provided alone.

Studies utilizing interspecies comparisons of the protective effects of cecal cultures are more numerous, but results have been variable. Weinack *et al.* (1982) found that a chicken-derived or a turkey-derived culture were both more effective in providing protection from *S. typhimurium* in chicks than the same cultures were in poults in three of four experiments. A fifth experiment, testing increasing challenge doses of *S. typhimurium*, indicated that chicken or turkey-derived cultures provided equal protection in chicks or poults at challenges up to 10^4 cfu per bird. Impey *et al.* (1984) reported that a defined chicken-derived culture protected chicks from *Salmonella kedougou* or *S. typhimurium* but did not protect poults, even after additional organisms were added to the culture. An undefined chicken-derived culture provided some protection in poults, but was not as effective as a turkey-derived culture (Impey *et al.*, 1984). A chicken-derived cecal culture was effective at protecting poults in one experiment when it was provided prior to challenge with *S. typhimurium* (Anderson *et al.*, 1984); however, the culture did not provide protection for poults given antibiotic injections at the hatchery (spectinomycin) or

Received for publication June 29, 1998.
Accepted for publication November 24, 1998.

[1]Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the USDA and does not imply its approval to the exclusion of other products that may be suitable.

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Abbreviation Key: BGA = brilliant green agar; CE = competitive exclusion; CF = continuous flow; NONA = novobiocin-nalidixic acid.

for poults infected with *S. bredeney* at the hatchery. Turkey poults provided chicken-derived cecal cultures intracloacally in the presence of dietary lactose were protected against *S. senftenberg* colonization (Corrier *et al*., 1991). Hollister *et al*. (1994) tested a chicken-derived continuous flow cecal culture that effectively controlled *Salmonella* colonization in broiler chicks provided dietary lactose and found that although turkey poults provided the same culture plus lactose had significantly fewer *Salmonella* per gram of cecal contents than control poults, the number was 100- to 1,000-fold higher than in chicks given the same treatment.

Continuous flow (CF) culture has been used to simulate the bacterial interactions of the mouse large intestine (Freter *et al*., 1983) and to study bacterial physiology and interactions in the rumen (Silley and Armstrong, 1984; Melville *et al*., 1988). A CF culture has also been used to maintain mixed chicken cecal microflora and to test the effectiveness of mixed chicken cecal microflora for reducing *Salmonella* colonization in broiler chicks (Nisbet *et al*., 1993) and poults (Hollister *et al*., 1994). The present series of experiments evaluated a defined, chicken-derived CF culture for effectiveness in decreasing *Salmonella* colonization in poults.

MATERIALS AND METHODS

Animals and Husbandry

Three identical experiments were conducted, each utilizing 40 female poults (Nicholas Large White) purchased from a commercial hatchery. The poults were placed in floor pens (550 cm^2 per bird) on pine shavings litter, under continuous fluorescent lighting and provided with water and an unmedicated corn-soybean meal mash ration that met or exceeded NRC requirements (1984) *ad libitum*. The paper liners from the poult transport containers and samples of the ration were cultured for *Salmonella* spp. as described previously (Andrews *et al*., 1992). *Salmonella* spp. were not detected. The poults were randomly assigned into two groups of 20 birds each and either not treated (controls) or were treated by crop gavage with 0.25 mL of CF culture on the day of hatch. On Day 3, all poults were challenged orally with 10^4 cfu *S. typhimurium*. This challenge dose was suggested by Mead *et al*. (1989) and agreed upon by laboratories in the U.K., France, The Netherlands, Canada, and the U.S. to be a standard challenge dose in order to facilitate inter-laboratory comparisons. On Day 10, 15 poults each from each treatment group were killed by cervical dislocation and ceca samples were collected aseptically to determine the presence of *Salmonella* and *Salmonella* colony-forming units per gram of cecal contents. Samples of cecal contents were also collected to measure cecal concentrations of acetic, propionic, butyric, and lactic acid.

Culture Preparation

Development of the chicken-derived CF culture and efficacy against experimental *Salmonella* challenge in broiler chicks was described previously (Corrier *et al*., 1995). Briefly, the mixed culture was selected during CF culture from a homogenate of cecal tissues and the contents were prepared from 10-wk-old broiler chickens. The culture was characterized to contain 29 bacterial strains composed of 15 facultative anerobes and 14 obligate anaerobes, representing 10 different genera. The culture was patented (Nisbet *et al*., 1995) and recently approved under the name Preempt® by the Food and Drug Administration for use in commercially reared poultry.

Salmonella Challenge

A primary poultry isolate of *S. typhimurium* from the National Veterinary Services Laboratory, Ames, IA 50010, was selected for resistance to novobiocin-nalidixic acid (NONA) in our laboratory and maintained on nutrient agar. Media used to culture the resistant isolate in experimental studies contained 25 μg/mL novobiocin and 20 μg/mL nalidixic acid to inhibit the growth of other bacteria. Inocula for challenge were prepared from overnight tryptic soy broth[3] cultures serially diluted in sterile PBS. The viable cell concentration of the inoculum was determined by colony counts on NONA brilliant green agar[4] (BGA) plates.

Cecal Colonization by Salmonella typhimurium

Cecal contents of one cecum from each poult were serially diluted in PBS to 1:100, 1:1,000, and 1:10,000, spread-plated on NONA BGA plates, and incubated for 24 h at 37 C. Total colony-forming units of *S. typhimurium* per gram of cecal contents was determined on an automatic colony counter.[5] Minced tissues and contents from the other ceca were aseptically placed in 30 mL of selenite cysteine broth,[3] agitated vigorously, and incubated overnight at 37 C. After incubation, the broth was streaked on NONA BGA plates, incubated overnight, and examined for typical *Salmonella* colonies. Cecal contents that were negative at the 1:100 dilution on BGA plates but positive after culturing in selenite-cysteine, and plating on BGA plates were arbitrarily assigned a value of 1.5 $\log_{10}$ *Salmonella* cfu/g of cecal contents. Cecal contents that were negative at the 1:100 dilution on BGA plates and negative after selenite-cysteine enrichment and BGA plating were scored as 0 cfu. *Salmonella* colonies were confirmed by biochemical tests on triple sugar iron agar and lysine iron agar[3] and confirmed as *S. typhimurium* by

[3]Difco Laboratories, Detroit, MI 48232.
[4]Oxoid Limited, Basingstoke, Hampshire, U.K.
[5]Biotran III, New Brunswick Scientific, Edison, NJ 08818.

serological tests with *Salmonella* O antiserum, poly A and Group B factors 1, 4, 5, and 12.[3]

Cecal Organic Acid Concentrations

Cecal contents (0.2 g) were collected from each poult and suspended in 0.8 mL sterile glass-distilled water. The concentrations of acetic, propionic, and butyric acids were measured with a gas chromatograph utilizing the method described by Hinton *et al.* (1990). Lactic acid concentration was measured by the enzymatic method of Hohorst (1965).

Statistical Analyses

Differences in cecal $\log_{10}$ *Salmonella* counts and organic acid concentrations among treatment groups, within experiments, were determined by analysis of variance using the General Linear Models procedures of SAS® software (Luginbuke and Schlotzhauer, 1987). Significant differences were separated using the Fisher's protected least significant difference procedure (Snedecor and Cochran, 1967). Chi-square analysis was used to determine significant differences between groups in percentage *Salmonella*-positive ceca samples. Differences among means were considered to be significant based on the 0.05 level of probability.

RESULTS AND DISCUSSION

Salmonella per Gram of Cecal Contents

The increase in *Salmonella* numbers in the cecal contents of the control poults on Day 10, above that present in the challenge dosage of Day 3 (10^4 cfu), clearly indicated *Salmonella* replication and amplification of the challenge dosage. Compared with the controls, the number of *Salmonella* in the cecal contents of the culture-treated poults decreased significantly by 4 to 5 $\log_{10}$ units during each of the three experiments (Table 1). The *Salmonella* challenge dosage of 10^4 cfu resulted in cecal colonization of 100% of the control poults on Day 10 in each of the three experiments (Table 2). Compared with the controls, the incidence of cecal colonization was significantly decreased in the treated poults during Experiments 1 and 2, and by analysis of the combined experiment results. The results agree with previous studies that reported a decrease in *Salmonella* cecal colonization in turkeys inoculated with cultures of intestinal microflora prepared from adult chickens (Loyd *et al.*, 1977; Snoeyenbos *et al.*, 1978; Weinack *et al.*, 1982; Reid and Barnum, 1983; Corrier *et al.*, 1991).

TABLE 1. Effect of a characterized competitive exclusion (CE) culture derived from chickens on *Salmonella typhimurium* concentration in cecal contents of poults

	$\log_{10}$ *Salmonella* per gram cecal contents[2]			
Treatment[1]	Experiment 1	Experiment 2	Experiment 3	Combined
Control	4.72 ± 1.49[a]	5.42 ± 1.09[a]	6.43 ± 0.65[a]	5.52 ± 1.08[a]
CE culture	0.70 ± 0.77[b]	0.00 ± 0.00[b]	1.30 ± 0.89[b]	0.67 ± 0.55[b]

[a,b]Means within columns with no common superscript differ significantly ($P < 0.05$).

[1]Treatments were: 1) no culture, (control); 2) chick-derived continuous flow culture. Poults were administered cecal cultures on Day 1, challenged orally with 10^4 *S. typhimurium* on Day 3, and killed on Day 10.

[2]$\log^{10}$ *S. typhimurium* per gram of cecal contents. Mean ± SD, n = 15.

TABLE 2. Effect of a characterized competitive exclusion (CE) culture derived from chickens on the percentage of *Salmonella typhimurium* cecal culture-positive poults

	Salmonella cecal culture-positive poults[2]			
Treatment[1]	Experiment 1	Experiment 2	Experiment 3	Combined
Control	15/15 (100)[a]	15/15 (100)[a]	15/15 (100)[a]	45/45 (100)[a]
CE culture	7/15 (47)[b]	0/15 (0)[b]	11/15 (73)[a]	18/45 (40)[b]

[a,b]Percentage within columns with no common superscript differ significantly ($P < 0.05$).

[1]Treatments were: 1) no culture, (control); 2) chick-derived continuous flow culture. Turkey poults were administered cecal cultures on Day 1, challenged orally with 10^4 *S. typhimurium* on Day 3 and killed on Day 10.

[2]Number and percentage of poults cecal-culture-positive for *S. typhimurium*, n = 15.

Compared with the control, the concentration of propionic acid in the cecal contents of the culture-treated poults was significantly higher on Day 3 of each experiment. Cecal propionic acid concentrations in the control poults were 6.1, 9.7, and 3.9 μmol/g compared to 19.5, 31.8, and 18.6 μmol/g in the culture-treated poults in Experiments 1, 2, and 3, respectively. These results agree with previous studies during which the concentration of propionic acid in the cecal contents of turkey poults treated with a chicken-derived CE culture increased significantly (Hollister *et al.*, 1994). The concentrations of acetic, butyric, and lactic acid did not differ consistently between the control and culture-treated poults (data not shown). The characterized CF culture contains several strains of anaerobic bacteria that produce propionic acid as a fermentation product. The significant increases in propionic acid in the ceca of the 3-d-old treated poults during this study may have been the result of the establishment of the propionic acid producing anaerobes that are present in the CF culture.

Previous studies have demonstrated that CE cultures prepared from the intestinal contents of chickens and composed of an undefined mixture of bacterial strains may effectively decrease *Salmonella* cecal colonization in turkeys. The results of the present study further demonstrated that the defined CF culture composed of 29 selected bacterial strains from adult chickens became established in newly hatched poults and significantly increased resistance to *S. typhimurium* cecal colonization.

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