The Effects of Dietary Mannanoligosaccharides on Cecal Parameters and the Concentrations of Enteric Bacteria in the Ceca of Salmonella-Challenged Broiler Chicks[1]

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ABSTRACT The ability of different enteric pathogens and coliforms to trigger agglutination of yeast cells (*Saccharomyces cerevisiae*, NCYC 1026) and a yeast cell wall preparation (MOS) was examined. Five of seven strains of *Escherichia coli* and 7 of 10 strains of *Salmonella typhimurium* and *Salmonella enteritidis* agglutinated MOS and *Sac. cerevisiae* cells. Strains of *Salmonella choleraesuis*, *Salmonella pullorum*, and *Campylobacter* did not lead to agglutination. Two strains that agglutinated MOS (*S. typhimurium* 29E and *Salmonella dublin*) and one nonagglutinating strain (*S. typhimurium* 27A) were selected as challenge organisms for *in vivo* studies in chicks under controlled conditions.

In a series of three trials in which 3-d-old chicks were orally challenged with 10^4 cfu of *S. typhimurium* 29E, birds receiving 4,000 ppm of dietary MOS had reduced cecal *S. typhimurium* 29E concentrations (5.40 *vs* 4.01 log cfu/g; $P < 0.05$) at Day 10. In a second series of three trials with *S. dublin* as challenge organism, the number of birds that tested salmonella positive in the ceca at Day 10 was less when MOS was part of the diet (90 *vs* 56%; $P < 0.05$). To test the effect of MOS on concentrations of bacteria that do not express Type 1 fimbriae, a challenge trial was conducted with *S. typhimurium* 27A. However, strain 27A did not colonize the birds sufficiently to evaluate whether MOS affected its cecal concentration. Mannanoligosaccharide did not significantly reduce the concentrations of cecal coliforms ($P < 0.10$) although they were numerically lower. It had no effect on cecal concentrations of lactobacilli, enterococci, anaerobic bacteria, lactate, volatile fatty acid, or cecal pH.

(*Key words*: *Salmonella*, chicken, mannans, yeast, ceca)

2000 Poultry Science 79:205–211

INTRODUCTION

Newly hatched birds lack complex gastrointestinal microflora and are especially prone to colonization of enteric pathogens (Milner and Shaffer, 1952). However, the application of cultures of cecal microflora obtained from healthy adult birds (competitive exclusion cultures) to the newly hatched chick has been shown to increase the complexity of their gastrointestinal microflora and give protection against enteric pathogens (Nurmi and Rantala, 1973; Stavric *et al.*, 1985; Corrier *et al.*, 1995). Many enteric pathogens must attach to the mucosal surface of the gut wall to establish themselves in the gastrointestinal tract (Costerton *et al.*, 1978, Fuller *et al.*, 1981). One mode of action of competitive exclusion (CE) cultures to exclude pathogens is related to their ability to increase the competition for attachment sites (Schneitz *et al.*, 1993). Because attachment is often mediated through binding of bacterial lectins to receptors containing D-mannose (Eshdat *et al.*, 1978), it may be possible to block the lectins with mannose or similar sugars and to inhibit bacterial attachment.

Oyofo *et al.* (1989a) tested the effect of different sugars on the *in vitro* adherence of *Salmonella typhimurium* to epithelial cells from 1-d-old chicks. They reported inhibition of adherence by methyl-a-D-mannoside and mannose by more than 90%. The inhibition of the adherence of *S. typhimurium* by mannose (Oyofo *et al.*, 1989a) is reflected in decreased colonization of *S. typhimurium* when mannose is added to the diet of young chicks (Oyofo *et al.*, 1989b,c). Because relatively high concentrations of mannose are required to control colonization of pathogenic bacteria, the cost of using pure mannose in commercial production is prohibitive even for short periods. However, mannose-based carbohydrates occur naturally

Received for publication April 5, 1999.
Accepted for publication September 30, 1999.
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Abbreviation Key: BGA = brilliant green agar; CE = competitive exclusion; MOS = mannanoligosaccharide; OD = optical density; VFA = volatile fatty acids.

in many products, such as yeast cell walls or different gums, which are available at reasonable prices. The hypothesis tested in this study was that mannanoligosaccharides (MOS) from yeast cell walls are able to decrease the concentrations of enteric pathogens that express Type 1 fimbriae in poultry.

The objectives of these studies were to screen different bacterial strains for their ability to agglutinate MOS in yeast cell preparations and to determine the effect of MOS on cecal fermentation parameters, cecal microflora, and enteric pathogen and coliform colonization in chicks under controlled conditions.

MATERIALS AND METHODS

Mannanoligosaccharides

The MOS preparation used in these studies was the commercial product Bio-Mos.[3] The product contains yeast cell wall fragments derived from *Saccharomyces cerevisiae*. The cell wall fragments are obtained by centrifugation from a lysed yeast culture. The pellet containing the yeast cell wall fragments is then washed and spray dried.

Agglutination of Yeast Products

The ability of the bacteria to adhere to the mannan associated with the yeast cell wall was measured with an agglutination test. Agglutination of MOS (yeast cells) by different enteric pathogens and coliforms in the presence and absence of specific sugars was qualitatively examined (Table 1). These tests were conducted using a modification of the method described by Mirelmann *et al.* (1980). To standardize the concentration of bacteria, bacterial cells were grown for 24 h on the following media: 10 g/L Bacto-Peptone[4]; 5 g/L yeast extract; and 5 g/L NaCl (Firon *et al.*, 1983). Cells were harvested by centrifugation and suspended in PBS (0.05 *M*, pH 7.2) to reach an optical density at 660 nm (OD_{660}) of 1.0. Mannanoligosaccharide was suspended at 1 g/L in PBS (OD_{660} = 1.60). A 24-h culture of *Sac. cerevisiae* NCYC 1026, grown in Tryptic Soy Broth[4], was harvested by centrifugation, and cells were suspended in PBS to reach an OD similar to the one of the MOS suspension (OD_{660} = 1.60). The yeast and MOS suspensions were blended for 1 min in a kitchen blender to separate clumping cells and MOS particles. Agglutination tests were then performed with 1.0 mL of the bacterial suspension and 1.0 mL of the MOS or yeast suspension. Agglutination was determined microscopically. To determine the inhibitory effect of carbohydrates on agglutination, each agglutinating strain was first incubated for 5 min in PBS containing 50 m*M* glucose, mannose, galactose, or fructose.

Animal Trials

Experimental Design. All animal trials were conducted following the animal care protocol IACUC 94-0004A approved by the University of Kentucky Institutional Animal Care and Use Committee. The ability of salmonella to colonize the intestinal tracts of chickens was evaluated in six separate trials. In each of the first two trials, the birds were divided into two control and two treatment groups containing 10 birds each. Trial 3 was conducted with 26 birds per group to study salmonella colonization over time. The fourth and fifth trials also used four groups of 10 birds, but because of low hatchability only 36 birds were assigned to treatment groups (9 birds per group). Birds were hatched and housed in bacterial isolation chambers. All diets met NRC requirements for young birds (NRC, 1994). Gastrointestinal microflora of birds were standardized at the beginning of each experiment by inoculating each bird with 1×10^4 cfu of one of the challenge organisms on Day 3. Cecal parameters were determined on Day 10. Dietary treatments in all trials included an unsupplemented diet (control) and a diet supplemented with 4,000 ppm of dietary Bio-Mos.

Bacterial Isolation Chambers. Five bacterial isolation chambers[5] were used for these studies. One chamber containing the incubator was outfitted with a bacterial trap filled with disinfecting solution; the other four contained plastic housing boxes (3,840 $cm^2 \times 320$ cm^2 per bird) and were equipped with regular inlet ports. The chambers were sanitized as described by Makin and Tzipori (1980). Feed, water, and 500 g wood shavings were placed in autoclave bags and were placed in the chambers prior to fumigation.

Eggs. Eggs (broiler type, line 24[6]) were sanitized by spraying with a disinfectant solution (2.0% H_2O_2, 0.8% quaternary ammonium, and 0.25% acetic acid) and were misted daily during incubation. On Day 19, eggs were dipped for 30 s in disinfectant solution (37 C) and were transferred into the sterile incubator, which was placed in one bacterial isolator.

Standard Inoculum. Newly hatched chicks were inoculated with a standard inoculum to provide similar basic microflora for each bird at the beginning of each experiment. The standard inoculum was derived from the hatching debris from a commercial hatchery. The debris were mixed and stored in 100-g aliquots at −80 C. The inoculum was tested to be salmonella-free according to the approved standard method for isolation of salmonellae (Wallace *et al.*, 1992). Microbial counts on the debris revealed the following bacterial concentrations: total bacteria, 2.5×10^7 cfu/g (Viand Levure Agar at pH 6.7; Barnes *et al.*, 1979); coliforms, 8.03×10^6 cfu/g (MacConkey Agar); enterococci, 7.50×10^6 cfu/g (KF Streptococcal Agar); and lactobacilli, <1,000 cfu/g (Rogosa Agar). The defrosted debris was mixed 1:5 with 0.1% peptone water, blended in a sterile kitchen blender, and each bird was given 0.25 mL from the tip of a pipette (1.25×10^6 cfu/bird). Six h after standard inoculation, chicks were randomly as-

[3]Alltech Inc., Nicholasville, KY 40356.
[4]Difco Laboratories, Detroit, MI 48232.
[5]Standard Safety Equipment Co., Palatine, IL 60067.
[6]Avian Farms, Sommerset, KY 42633.

TABLE 1. Inhibitory effects of fructose, galactose, glucose and mannose on bacterial agglutination of mannanoligosaccharide preparations (MOS)

Strain	Source	Agglutination of MOS	Inhibition of agglutination by			
			Fructose	Galactose	Glucose	Mannose
Escherichia						
E. coli K99	A[1] K99	+[2]	Yes	No	No	Yes
E. coli K99	ATCC[3]31619	+	Yes	No	No	Yes
E. coli 4157	A 4157	–[4]				
E. coli 15R	UKAS[5] 15R	+	Yes	No	No	Yes
E. coli	A 39639 B2	+	Yes	No	No	Yes
E. coli O157:H7	A 0157H7	–				
E. coli	A 39639 B2	+	Yes	No	No	Yes
Salmonella						
S. enteritidis	A 13A	+	Yes	No	No	Yes
S. enteritidis	A 371	+	Yes	No	No	Yes
S. enteritidis	A 3	+	Yes	No	No	Yes
S. enteritidis	A 52	+	Yes	No	No	Yes
S. enteritidis	ATCC 13076	–				
S. typhimurium	UKAS 29E	+	Yes	No	No	Yes
S. typhimurium	ATCC 14028	+	Yes	No	No	Yes
S. typhimurium	ATCC 13311	+	Yes	No	No	Yes
S. typhimurium	ATCC 29630	–				
S. typhimurium	UKAS 27A	–				
S. montevideo	A 95111010 J	+	Yes	No	No	Yes
S. give	A 95111010 F	+	Yes	No	No	Yes
S. kedougou	NCTC[6] 12173	+	Yes	No	No	Yes
S. dublin	ATCC 15480	+	Yes	No	No	Yes
S. pullorum	ATCC 9120	–				
S. pullorum	ATCC 19945	–				
S. choleraesuis	ATCC 13317	–				
S. choleraesuis	ATCC 9150	–				
S. choleraesuis	ATCC 13312	–				
Campylobacter						
C. jejuni	ATCC 29428	–				
C. jejuni	ATCC 25217	–				
C. jejuni	UKAS 240	weak[7]	No	No	No	No
C. jejuni	UKAS 215	–				
C. jejuni	UKAS W91	–				
C. coli	ATCC 43481	–				
C. coli	ATCC 33559	–				
C. coli	UKAS 237	–				
C. coli	UKAS 216	–				
C. coli	UKAS 218	weak	No	No	No	No
C. lari	UKAS 234	–				

[1]Culture Collection of Alltech Inc., Nicholasville, KY.

[2]The MOS particles were clumping together.

[3]American Type Culture Collection, Rockville, MD.

[4]No MOS particles were clumping together.

[5]Culture Collection of the University of Kentucky, Department of Animal Science, Lexington, KY.

[6]National Collection of Type Cultures, Central Public Health Laboratory, London, U.K.

[7]A few particles were clumped, but the majority were free.

signed to four groups of 10 chicks each and were transferred into the bacterial isolation chambers.

Husbandry. Room temperature was adjusted to 34 C for the first day and was then gradually lowered to 28 C at Day 10. Light was on continuously for the first 24 h after the chicks were transferred into the chamber and for 20 h each of the following days. Feed and water were consumed *ad libitum*. The feed was a corn-soybean meal diet that was formulated to meet NRC recommendations and contained no antimicrobial supplement (NRC, 1994).

Challenge Cultures and Their Application. Challenge cultures of *S. typhimurium* (strains 29E and 27A) and *S. dublin* for *in vivo* studies were grown in the basal peptone-yeast extract medium described earlier. The cultures were then diluted in sterile 0.1% peptone solution to a final concentration of 4×10^4 cfu/mL. Concentrations of salmonellae were determined on brilliant green agar (BGA) using spread-plate technique. One quarter of a milliliter of the diluted culture (1×10^4 cfu/bird) was given to each bird from the tip of a pipette.

Sampling and Sample Analysis. After 10 d, the chicks from each group were sacrificed by asphyxiation with CO_2, and both ceca were aseptically removed. The content of one cecum was placed in a sterile test tube, weighed, and diluted 1:10 with sterile 0.1% peptone solution. The empty cecum was cut longitudinally and placed in lactose broth. Decimal dilutions of each sample were prepared in 0.1% peptone solution. Salmonellae were enumerated on BGA containing 30 mg naladixic acid/L after 24 h incubation at 37 C. Naladixic acid was added to the media

to facilitate the selection of the antimicrobial-resistant challenge organisms. The tube containing the empty cecum in lactose broth was incubated at 37 C for 24 h for enrichment of salmonellae (Wallace *et al.*, 1992). Cecal samples that were salmonella-negative on BGA plates, but positive in the enrichment test, were assigned as 1.50 log cfu/g (Nisbet *et al.*, 1993). Samples that were confirmed negative in the enrichment test were assigned a concentration of 1 cfu/g (0 log cfu/g) (Nisbet *et al.*, 1993). Four birds were randomly selected from each group for analysis of the concentrations of different groups of intestinal bacteria. Lactobacilli, enterococci, coliforms, and anaerobic bacteria were enumerated in duplicate using the pour-plate technique on Rogosa SL agar, KF streptococcal agar, McConkey agar, and reinforced clostridial agar,[7] respectively.

Determination of pH, Volatile Fatty Acid, and Lactate Concentrations. The content of the second cecum was diluted 1:10 in distilled water, and pH was determined with a combined electrode.[8] Samples were stored at –20 C until analyses of volatile fatty acids and lactate. Volatile fatty acid analysis was conducted on a Hewett-Packard model 5890 gas chromatograph,[9] fitted with a 180-cm × 4-mm glass column, containing 10% SP 1000 and 1% H_3PO_4 on 100/120 Chromosorb WAW[10] with a modification of the method described by Erwin *et al.* (1961). D- and L-lactate were determined spectrophotometrically with D- and L-lactate dehydrogenase, respectively, in the presence of nicotinamide adenine dinucleotide (Brandt *et al.*, 1980).

Statistical Analysis. Data were analyzed by ANOVA appropriate for the randomized complete block design; chambers (10 birds) were considered to be the experimental unit. Differences between treatment means were assessed by least significant difference test (Kuehl, 1994). Bacterial concentrations were subject to log 10 transformation prior to analysis. To evaluate the overall effects of MOS supplementation, the data was initially blocked by trial. No significant trial by treatment interactions were observed, and the data were pooled across the trials. Statistical analyses were conducted using the general linear models procedure of SAS® (SAS Institute, 1985).

RESULTS

Agglutination

Five of seven strains of *Escherichia coli* and 7 of 10 strains of *S. typhimurium* and *Salmonella enteritidis* agglutinated MOS (Table 1). *Salmonella montevideo*, *Salmonella give*, *Salmonella kedougou*, and *S. dublin* also triggered agglutination. Addition of *Salmonella choleraesuis* and *Salmonella pullorum* strains did not lead to agglutination of the MOS particles. Agglutination associated with these Gram-negative bacterial strains could be inhibited with mannose or fructose but not with glucose or galactose. However, it generally took 9 to 16 times more fructose to bring about the inhibitory responses observed with mannose (data not shown). This result suggests that fructose was less effective as a blocking agent. Agglutination patterns of MOS were similar to patterns of *S. cerevisiae* (data not shown). Different strains of *Campylobacter* were also tested for their abilities to agglutinate MOS (Table 1). Only two strains showed some agglutination; however, these agglutinations were very weak and were not affected by addition of carbohydrates.

Challenge Trials

The concentrations of salmonellae and coliforms in chicks challenged with *S. typhimurium* 29E are shown in Table 2. The concentrations of salmonella were consistently lower in birds fed MOS than in birds fed the unsupplemented diet. Salmonellae concentrations were between 4.97 and 5.73 cfu/g for the control and ranged from 3.79 to 4.29 log cfu/g for the MOS treatment. Over the three trials, MOS-supplemented chicks had lower *S. typhimurium* 29E concentrations by about 25-fold (4.01 *vs*. 5.40 log cfu/g; $P < 0.05$). Concentrations of coliforms also tended to be lower ($P < 0.1$) when MOS was added to the feed (8.47 *vs*. 8.71 log cfu/g). Concentrations of lactobacilli, enterococci, and anaerobic bacteria were not affected by treatment. Volatile fatty acid concentrations, lactic acid concentrations, and pH were not affected by dietary treatment. Average values were as follows: pH 5.61, acetate 61.7 m*M*, propionate 5.9 m*M*, butyrate 12.2 m*M*, and lactate 9.0 m*M*. In Trial 3, the cecal *S. typhimurium* 29E concentrations in the unsupplemented group rose from 3.61 log cfu/g at 2 d to 4.97 log cfu/g at 7 d after challenge (Figure 1). *Salmonella typhimurium* 29E concentrations in the MOS treatment group were lower ($P < 0.05$) than those of the unsupplemented control group 7 d after challenge. Birds receiving MOS had average cecal salmonella concentrations of 2.98 log cfu/g at 2 d and 3.79 log cfu/g at 7 d after challenge.

The percentage of birds from which *S. dublin* could be recovered was lower in the MOS-treated groups than in the control group in all three trials (Table 3). In Trial 6, 90% of the birds in one of the groups receiving MOS were colonized with *S. dublin*. As a result, the mean reduction in the percentage of colonized birds with MOS addition appears lower in Trial 6 (24%) than in Trial 4 (47%) or Trial 5 (44%). Over the three trials, MOS decreased the number of salmonella-positive birds from 89.8 to 55.7% ($P < 0.05$). Concentrations of coliforms, lactobacilli, enterococci, and anaerobic bacteria were not different in the two treatment groups. Cecal pH, VFA, and lactate concentrations were in a similar range as in the trials with *S. typhimurium* and were not affected by treatment (data not shown).

Salmonella typhimurium 27A, a nonaggultinating strain of salmonella, colonized the chicks at a very low rate.

[7]All media were purchased from Difco Laboratories.
[8]Orion Research Inc., Boston, MA 02129.
[9]Hewlett-Packard, Palo Alto, CA 94304.
[10]Supelco Inc., Bellofonte, PA 16823.

TABLE 2. Effect of dietary mannanoligosaccharide preparations (MOS) on concentrations of different bacterial populations in the ceca of chicks maintained in microbiological isolators and challenged with *Salmonella typhimurium* 29E[1]

Bacterial group	Trial 1		Trial 2		Trail 3		Overall		
	Control	MOS	Control	MOS	Control	MOS	Control	MOS	SE
	(log cfu/g)								
Salmonella	5.73	4.29	5.50	3.95	4.97	3.79	5.40[a]	4.01[b]	0.08
Coliforms	8.78	8.40	8.87	8.73	8.48	8.29	8.71	8.47	0.05
Lactobacilli	<6.00	<6.00	<6.00	<6.00	7.41	7.61			
Enterococci	8.12	8.35	8.26	8.25	8.03	8.24	8.13	8.28	0.05
Anaerobes	9.37	9.14	9.20	9.11	9.21	8.91	9.26	9.05	0.04

[a,b]Values in same row within trial with no common superscript differ significantly ($P < 0.05$).
[1]n = 6.

This organism could only be recovered from the ceca of 3 of 40 chicks that were challenged with doses of up to 1×10^4 cfu/bird. Two control chicks and one bird receiving MOS were salmonella positive at 10 d, which made it impossible, using the bacterial isolation chambers, to evaluate the effects of MOS on the colonization of birds by this strain.

DISCUSSION

The colonization of bacteria on mucosal tissues is recognized as an important step in the infectious process. To colonize the mucosal surfaces, bacteria must first bind to the epithelial cells of these tissues. One way of binding to epithelial cells is through attachment of Type-1 fimbriae (Ofek *et al.*, 1977). A variety of strains of *E. coli*, *Salmonella*, and *Campylobacter* were tested for the presence of Type-1 fimbriae by means of yeast and MOS agglutination. No differences were observed between agglutination pattern of the two yeast products. The majority of the strains of *E. coli* and *S. typhimurium* and *S. entritidis* tested agglutinated both the yeast culture and MOS. All agglutination observed in these enteric bacteria was mannose sensitive. This result is in agreement with findings by Duguid (1964) and Duguid *et al.* (1966) who reported high frequency of Type-1 fimbriae expression in *S. typhimurium*, *Salmonella typhi*, and *S. enteritidis*. However, Mirelmann *et al.* (1980) only found 4 of 13 strains of *S. typhimurium* to express Type-1 fimbriae. The poultry-specific salmonella types, *Salmonella pullorum* and *Salmonella gallinarum*, did not trigger agglutination in the work of Duguid (1964) or in the study presented here. Four of seven tested strains of *E. coli* agglutinated MOS. Mirelmann *et al.* (1980) reported that about half of the tested strains of *E. coli* expressed Type-1 fimbriae. None of the tested *Campylobacter* strains caused a mannose-sensitive agglutination of MOS. However, mannose was reported to inhibit adherence of *C. jejuni* to epithelial cells *in vitro* (McSweegan and Walker, 1980), and protective effects with dietary mannose were also reported against *Campylobacter jejuni* colonization in chickens (Schoeni and Wong, 1994). These authors did not determine whether the *Campylobacter* strains used in their study expressed mannose-sensitive fimbriae. Therefore, it is not possible to say whether inhibition of attachment or other mechanisms were involved in their studies. Fucose is a constituent of the mucus the primary site of *Campylobacter* colonization, and inhibition of *Campylobacter* colonization has also been reported with addition of fucose to the diet (Cinco *et al.*, 1984).

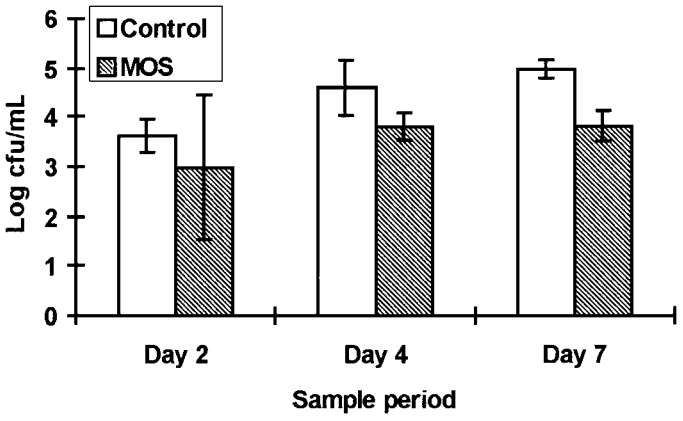

FIGURE 1. Effect of dietary mannanoligoscharrides on the concentration of salmonellae in the chicks maintained in microbial isolators for 2, 4, and 7 d after challenge with *Salmonella typhimurium* 29E. Bars represent standard deviations of the mean values.

Standardization of experimental conditions is important to achieve reproducible results. Initial results obtained from challenge studies with birds from a commercial hatchery were not reproducible. It was hypothesized that the birds might have been exposed to different bacterial flora at the hatchery, which could explain differences between trials. Birds that would have been exposed to a more complex microflora at the hatchery would exert a stronger CE effect in the gastrointestinal tract and would, consequently, be colonized with the challenge organisms at lower rates. Therefore, the experimental conditions were modified to standardize microflora of each bird at the beginning of the experiment by removing the native microflora of the egg through sanitation and then exposing the newly hatched bird to a constant level of microorganisms derived from hatching debris in bacterial isolators. This process allowed for the standardization of the microflora at the beginning of the experiment and allowed the composition of the cecal gastrointestinal microflora, cecal pH, and cecal acid concentrations of the

TABLE 3. Effect of dietary mannanoligosaccharide preparations (MOS) on concentrations of different bacterial populations in the ceca of chicks maintained in microbiological isolators and challenged with *Salmonella dublin*[1]

	Trial 4		Trial 5		Trail 6		Overall		
Bacterial group	Control	MOS	Control	MOS	Control	MOS	Control	MOS	SE
Prevalence of salmonella (% colonized)	95.0	50.0	80.0	45.0	94.4	72.2	89.8[a]	55.7[b]	8.15
	(log cfu/g)								
Coliforms	9.50	8.97	9.16	8.69	8.45	8.62	9.03	8.76	0.28
Lactobacilli	7.40	7.57	7.38	7.31	7.25	6.09	7.34	6.99	0.50
Enterococci	7.91	9.01	8.71	9.29	9.19	9.13	8.60	9.14	0.41
Anaerobes	9.70	9.68	9.79	9.78	9.58	9.71	9.69	9.72	0.06

[a,b]Values in same row within trial with no common superscript differ significantly ($P < 0.05$).

[1]n = 6.

birds to be similar in each experiment and each treatment group.

The main goal of these trials was to evaluate the effects of MOS on salmonella colonization in chicks. The focus was on *Salmonella* strains expressing Type-1 fimbriae. We hypothesized that MOS might affect colonization of such strains through blocking bacterial attachment to the gut mucosa. In a first series of three trials, dietary addition of MOS decreased the cecal concentration of *S. typhimurium* 29E. To further investigate whether MOS could decrease the concentration of a strain of *Salmonella* that colonizes birds with a cecal concentration less than 10^4 cfu/g, another series of trials was conducted using *S. dublin* as a challenge organism. Mannanoligosaccharide treatment also reduced the number of birds from which *S. dublin* could be isolated in these trials. The challenge trials with *S. dublin* and *S. typhimurium* 29E suggest that MOS can lower cecal salmonella colonization of strains that colonize at high or low concentrations. The study conducted over time showed that MOS reduced cecal *S. typhimurium* 29E concentrations 7 d after challenge. To observe cecal *S. typhimurium* 29E concentration over a longer period of time, with and without withdrawing MOS, might help to further investigate what effect MOS exerts on Type-1 fimbriated enteric pathogens. An inhibitory effect on salmonella colonization in chicks has also been shown with live yeast culture. Line *et al.* (1995) reported decreased colonization of *S. typhimurium* with dietary *Saccharomyces boulardii*. However, different modes of action might be involved with live yeast cells. Live yeast cells excrete metabolites, which are known to affect the composition of the gastrointestinal microflora (Girard, 1996) and could, therefore, alter the CE effect of the indigenous microflora. In addition, the strain of *S. typhimurium* used by Line *et al.* (1995) has not been screened for presence of Type-1 fimbriae. Therefore, it can not be determined whether the observed reduction was due to yeast cell wall mannan or other mechanisms.

The decrease in concentrations of *Salmonella* expressing Type-1 fimbriae was not accompanied by changes in cecal parameters such as decreases in pH or increase in propionic acid concentrations, which are known to reduce salmonella concentrations, which suggests that other modes of action are responsible for the decrease salmonella concentrations with dietary MOS. *In vitro* agglutination results suggest that adsorption of salmonellae by MOS, which would keep them from adhering to the intestinal wall, could be a possible mode of action. Oyofo *et al.* (1989a) tested the effect of different sugars on the adherence of *S. typhimurium* to epithelial cells of 1-d-old chicks *in vitro*. They reported inhibition of adherence by methyl-a-D-mannoside and mannose by more than 90%. In different *in vivo* trials, mannose has also been shown to decrease cecal colonization of *S. typhimurium* in young chicks (Oyofo *et al.*, 1989b,c). The fact that MOS had no effect on the concentrations of lactobacilli and enterococci, two bacterial species that are known to not express Type-1 fimbriae, further suggests that MOS affects bacterial concentrations in the gastrointestinal tract by adsorbing bacteria and keeping them from adhering to the gut wall.

Dietary MOS has been shown to decrease the prevalence of strains of *Salmonella* expressing Type-1 fimbriae in young chicks under laboratory-scale conditions. No changes in cecal parameters such as a major shift in bacterial populations or changes in pH or VFA concentrations, which are known to affect salmonella, were observed with MOS addition. Field trials will be required to show how the reduction noticed under small-scale conditions will transfer into commercial production systems. Because MOS can be considered animal, environmental, and consumer friendly, it might offer an additional and interesting tool that can be included in a hazard analysis critical control point program of poultry production.

REFERENCES

Barnes, E. M., C. S. Impey, and B.J.H. Stevens, 1979. Factors affecting the incidence and anti-salmonella activity of the anaerobic caecal flora of the young chick. J. Hyg. Camb. 82:263–283.

Brandt, R. B., S. A. Seige, M. G. Walters, and M. H. Bloch, 1980. Spectrophotometric assay for D-lactate in plasma. Anal. Biochem. 102:39–46.

Cinco, M., E. Banfi, E. Ruaro, D. Crevatin, and D. Crotti, 1984. Evidence for L-fucose-(6-deoxy-L-galactopyranose) mediated adherence of *Campylobacter* ssp. to epithelial cells. FEMS Microbiol. Lett. 21:347–351.

Corrier, D. E., D. J. Nisbet, C. M. Scanlan, A. G. Hollister, D. J. Caldwell, L. A. Thomas, B. M. Hargis, T. Tomkins, and J. R. DeLoach, 1995. Treatment of commercial broiler chickens with a characterized culture of cecal bacteria to reduce salmonella colonization. Poultry Sci. 74:1093–1101.

Costerton, J. W., G. G. Geesey, and K. J. Cheng, 1978. How bacteria stick. Sci. Am. 238:86–95.

Duguid, J. P., 1964. Functional anatomy of *Escherichia coli* with special reference to enteropathogenic *E. coli*. Rev. Latinoam. Microbiol. 7(Suppl. 13-4):1–16.

Duguid, J. P., E. S. Anderson, and I. Campbell, 1966. Fimbriae and adhesive properties in salmonellae. J. Pathol. Bacteriol. 92:107–138.

Erwin, E. S., G. J. Marco, and E. M. Emery, 1961. Volatile fatty acid analysis of blood and rumen fluid by gas chromatography. J. Dairy Sci. 44:1768–1771.

Eshdat, Y., I. Ofek, Y. Yehuidit-Gan, Y. Yashouv-Gan, N. Sharon, and D. Mirelmann, 1978. Isolation of a mannose-specific lectin from *E. coli* and its role in the adherence of the bacteria to epithelial cells. Biochem. Biophy. Res. Com. 85:1551–1559.

Firon, N., I. Ofek, and N. Sharon, 1983. Carbohydrate specificity of the surface lectins of *Escherichia coli*, *Klebsiella pneumoniae* and *Salmonella typhimurium*. Carbohydr. Res. 120:235–249.

Fuller, R., S. B. Houghton, and B. E. Brooker, 1981. Attachment of *Streptococcus faecium* to the duodenal epithelium of the chicken and its importance in colonization of the small intestine. Appl. Environ. Microbiol. 41:1433–1441.

Girard, I. D., 1996. Characterization of stimulatory activities from *Saccharomyces cerevisiae* on the growth and activities of ruminal bacteria. Ph.D. Diss., University of Kentucky, Lexington, KY.

Kuehl, R. O., 1994. Statistical Principles of Research Design and Analysis. Duxbury Press, Belmont, CA.

Line, E. J., N. J. Stern, N. A. Cox, and J. S. Bailey, 1995. *Saccharomyces* treatment to diminish *Campylobacter* and *Salmonella* in chicks. Poultry Sci. 74(Suppl. 1):201. (Abstr.).

Makin, T. J., and S. Tzipori, 1980. Inexpensive techniques for the production and maintenance of gnotobiotic piglets, calves and lambs. Aust. Vet. J. 56:353–358.

McSweegan, E., and R. I. Walker, 1980. Identification and characterization of two *Campylobacter jejuni* adhesins for cellular and mucus substrates. Infect. Immun. 53:141–148.

Milner, K. C., and M. F. Schaffer, 1952. Bacteriologic studies of experimental salmonella infection in chicks. J. Infect. Dis. 90:81–96.

Mirelmann, D., G. Altman, and Y. Eshdat, 1980. Screening of bacterial isolates for mannose-specific lectin activity by agglutination of yeast. J. Clin. Microbiol. 11:328–331.

NRC, 1994. Nutrient requirement of poultry. National Academy Press, Washington, DC.

Nisbet, D. J., D. E. Corrier, and J. DeLoach, 1993. Effect of mixed cecal microflora maintained in continuous culture and of dietary lactose on *Salmonella typhimurium* colonization in broiler chicks. Avian Dis. 37:528–535.

Nurmi, E., and M. W. Rantala, 1973. New aspect of salmonella infection in broiler production. Nature 241:210–211.

Ofek, I., D. Mirelmann, and N. Sharon, 1977. Adherence of *E. coli* to human mucosal cells mediated by mannose receptors. Nature 265:623–625.

Oyofo, B. A., J. R. DeLoach, D. E. Corrier, J. O. Norman, R. L. Ziprin, and H. H. Mollenhauer, 1989a. Effect of carbohydrates on *Salmonella typhimurium* colonization in broiler chickens. Avian Dis. 33:531–534.

Oyofo, B. A., J. R. DeLoach, D. E. Corrier, J. O. Norman, R. L. Ziprin, and H. H. Mollenhauer, 1989b. Prevention of *Salmonella typhimurium* colonization of broilers with D-mannose. Poultry Sci. 68:1357–1360.

Oyofo, B. A., R. E. Droleskey, J. O. Norman, H. M. Mollenhauer, R. L. Ziprin, D. E. Corrier, and J. R. DeLoach, 1989c. Inhibition by mannose of *in vitro* colonization of chicken small intestine by *Salmonella typhimurium*. Poultry Sci. 68:1351–1356.

SAS Institute, 1985. SAS® User's Guide. SAS Institute Inc., Cary, NC.

Schneitz, C., L. Nuotio, and K. Lounatma, 1993. Adhesion of *Lactobacillus acidophilus* to avian intestinal epithelial cells mediated by the crystalline bacterial cell surface layer (S-layer). J. Appl. Bacteriol. 74:290–294.

Schoeni, J.C.L., and A.C.L. Wong, 1994. Inhibition of *Campylobacter jejuni* colonization in chicks by defined competitive exclusion cultures. Appl. Environ. Microbiol. 60:1191–1197.

Stavric, S., T. M. Gleeson, B. Blanchfield, and H. Pivnick, 1985. Competitive exclusion of salmonella from newly hatched chicks by mixtures of pure bacterial cultures isolated from fecal and cecal contents of adult birds. J. Food. Prot. 48:778–782.

Wallace, H. A., V. R. Bruce, G. June, F. Satchell, and P. Sherrod, 1992. Salmonella. Pages 57–58 *in*: Bacterial Analytical Manual. 7th ed. AOAC International, Arlington, VA.