**ENVIRONMENT AND HEALTH**

**The Influence of Complex Carbohydrates on *Salmonella typhimurium* Colonization, pH, and Density of Broiler Ceca**

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**ABSTRACT** *Salmonella typhimurium* colonization, pH, and density of ceca were measured in 3-5, and 6-wk-old broilers fed either a control ration or rations with added fructooligosaccharides (FOS) or lactose derivatives (LD). The purpose was to compare dietary crude FOS from Jerusalem artichokes with refined FOS and two LD for ability to reduce *Salmonella* colonization as determined by semi-quantitative procedures. Chicks were challenged commencing at 5 d, by exposure to chicks orally infected with *S. typhimurium*. With the exception of chicks fed crude FOS, the high prevalence of *Salmonella* infection at 3 wk declined as chicks aged. At 6 wk, broilers fed crude FOS had higher *Salmonella* counts than all other broiler groups, whereas broilers fed refined FOS had lower infections than control broilers. The decline of *Salmonella* infection of broilers fed either refined FOS or LD ceased after dietary additives were discontinued at 5 wk of age. At 6 wk, infection rates of the latter groups were at least as high as those of control broilers. Both FOS and LD reduced cecal pH and density. Broilers fed the control ration had higher pH at 5 and 6 wk and higher cecal densities at 3 and 5 wk than those of broilers fed rations containing 5% (wt/wt) carbohydrates. Treatment differences for cecal pH and density disappeared within 1 wk of withdrawal of carbohydrates from 5-wk-old broilers. No consistent effect of cecal pH and density on *Salmonella* infection was observed. Density was dependent on cecal volume, and at 6 wk of age, on broiler size.

(Key words: *Salmonella*, complex carbohydrates, cecal pH, cecal density, broiler)

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**INTRODUCTION**

The growth of desirable bacteria in the lower gastrointestinal (GI) tract of the chicken has been promoted as a mechanism for reducing undesirable pathogenic and putrefactive bacteria. Among other factors, diet has been reported to affect the composition of the enteric microflora of the chicken (Barrow, 1992).

There are several published studies testing the effects of either lactose (Corrier et al., 1990a,b; 1991; DeLoach et al., 1990; Hinton et al., 1990; Nisbet et al., 1993; Tellez et al., 1993) or fructooligosaccharides (FOS) (Bailey et al., 1990; Choi et al., 1994) on the diet for ability to control *Salmonella* in poultry. Modest reductions of cecal *Salmonella* counts or cecal *Salmonella* colonization associated with dietary lactose or lactose-containing products have been reported (DeLoach et al., 1990; Corrier et al., 1991; Hinton et al., 1990); however, Nisbet et al. (1994) observed little reduction of *Salmonella* colonization of 10-d-old broilers fed 2% dietary lactose unless lactose was used in conjunction with adult chicken cecal microflora. Corrier et al. (1990a) reported that dietary lactose increased cecal acidity and influenced intestinal flora involved in the control of enteropathogens in poultry. *Salmonella* control was believed to be achieved by stasis caused by low pH or elevated levels of volatile fatty acids. Dietary FOS did little to reduce *Salmonella* colonization of cecal contents of broiler chicks (Bailey et al., 1991; Conner and Moran, 1992) or contamination of broiler carcasses (Waldroup et al., 1993). However, in one study, dietary FOS reduced cecal colonization by *Salmonella typhimurium* as well as counts of *S. typhimurium* of colonized broiler chicks (Choi et al., 1994). In the above studies, only those of Corrier et al. (1990b) and Waldroup et al. (1993) involved chickens of market age.

Dietary lactose (Corrier et al., 1990a,b; DeLoach et al., 1990; Hinton et al., 1990) and FOS (Choi et al., 1994; Moran and Conner, 1992) have been associated with reductions in cecal pH. A trend for reduced cecal pH associated with elevated lactic acid levels and reduced *Salmonella* counts was apparent in some studies (Corrier et al., 1990a,b; Hinton et al., 1990).

Chicks consuming lactose had ceca that were “often distended as compared with the controls, and the cecal contents were frequently foamy in appearance” in...
broiler (Corrier et al., 1990a) and Leghorn chicks (Tellez et al., 1993). Instances of this phenomenon in chicks fed dietary FOS could not be found; however, Modler (1994) reported that lactulose and neosugars can be metabolized by gas-producing microorganisms.

In this study, dietary crude FOS was tested and compared with refined FOS and derivatives of lactose (LD) to determine effects on S. typhimurium colonization of broilers at 3, 5, and, especially, at 6 wk of age. In addition, the influence of these additives on cecal density and pH was evaluated to assess their possible roles in Salmonella control. Cecal volume and weight and broiler weight were measured and related to cecal density.

**MATERIALS AND METHODS**

**Diets and Complex Carbohydrates**

The chicks were fed corn-soybean broiler starter and finisher diets. Starter was fed for the first 3 wk. Finisher was fed thereafter for either 2 or 3 wk to broilers examined at 5 and 6 wk of age. The five dietary treatments, similar in energy and protein content, differed in added complex carbohydrate. The control ration (CONT) contained no added carbohydrates. The crude FOS diet consisted of the control ration with 8% Jerusalem artichoke flour (5% FOS); the refined FOS diet had 5% Raftiline\(^2\) added to the control ration. The LD diets consisted of the control ration with either 5% lactulose (LDL)\(^3\) or 5% lactosucrose (LDS)\(^4\). There were two replicate pens for each of the five dietary regimens to 5 wk of age. Broilers in one replicate of each treatment were fed control finisher during the 6th wk to study changes following withdrawal.

**Stocks**

Day-old Cornish broiler chicks hatched at the Centre for Food and Animal Research hatchery were delivered to the Animal Diseases Research Institute for rearing in a biocontained poultry house with 10 pens. Sixty-five chicks, 30 females and 35 males, were wing-banded (a unique number on each band) for each of the 10 pens. The above chicks were considered negative for Salmonella. Each cecum was weighed and immersed in water to determine its volume and the two measures used to calculate density according to Archimedes principle. Cecal pH was measured using a digital pH meter\(^6\) with an Orion Ross combination probe. The other half of the group had cecal Salmonella content determined on one cecum of each broiler as described by Blanchfield et al. (1984). A swab was used to smear cecal contents on a Brilliant Green Sulfa agar\(^6\) plate containing nalidixic acid. Agar plates were then incubated overnight at 37 C. Swab cultures were scored 0 for no colonies; 1 for 1 to 100 colonies; or 2 for more than 100 colonies of S. typhimurium. The sex of each broiler was verified at necropsy.

**Sulfa agar**

An additional 80 chicks, banded with green bands, were gavaged individually on the day of hatch with 10\(^7\) cfu of nalidixic acid resistant S. typhimurium. These chicks were reared in an isolated facility for 4 d and, on the 5th d, eight chicks (seeders) were added to each of the 10 pens of broilers being tested.

**Evaluation**

At 3, 5, and 6 wk of age, 18 to 20 broilers were removed from each pen and were killed for evaluation. Each broiler was weighed prior to examination. Half of each group of broilers was used to determine density and pH of each cecum of the broiler within 10 min after death. After removal, each cecum was weighed and immersed in water to determine its volume and the two measures used to calculate density according to Archimedes principle. Cecal pH was measured using a digital pH meter\(^6\) with an Orion Ross combination probe. The other half of the group had cecal Salmonella content determined on one cecum of each broiler as described by Blanchfield et al. (1984). A swab was used to smear cecal contents on a Brilliant Green Sulfa agar\(^6\) plate containing nalidixic acid. Agar plates were then incubated overnight at 37 C. Swab cultures were scored 0 for no colonies; 1 for 1 to 100 colonies; or 2 for more than 100 colonies of S. typhimurium. The sex of each broiler was verified at necropsy.

**Data Analysis**

Data were analyzed using the General Linear Models (GLM) procedure of the SAS Institute (1985). The model included cecal density, pH, and Salmonella score as the dependent variables and feed treatments (5), sexes (2), ages (3), replicates (2 up to 5 wk; 1 at 6 wk of age), and the two- and three-factor interactions as independent variables. Due to differences associated with age and the interaction of age with some of the other factors, analyses were repeated and presented within each age. Orthogonal contrasts of treatments were performed to compare chicks fed: CONT vs treated rations; FOS vs LD rations; crude FOS vs refined FOS rations; LDL vs LDS rations. Class and subclass least squares means for treatment groups of broilers were graphed to avoid confounding of factors. To determine the impact of the covariables cecal weight and volume and body wt, on cecal density, each was added to the above model for analyses of data at each age. Analyses of cecal density data revealed evidence of non-normality. Residual components of density data for each age were analyzed using the Univariate procedure of the SAS Institute (1985) testing for skewness, kurtosis, and normality.

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**RESULTS**

**Influence of Carbohydrates**

*Salmonella score.* Chicks were highly susceptible to cecal colonization by *Salmonella* at 3 wk of age, all but 4 of the 99 were culture positive (Table 1). *Salmonella* scores of 3-wk-old broilers fed diets with LD were lower (*P* < 0.05) than those fed diets with FOS. Otherwise, complex carbohydrates had little influence on the *Salmonella* score of 3-wk-old broilers. In comparison to broilers at 6 wk fed CONT, continuous feeding of rations with crude FOS to broilers gave rise to higher (*P* < 0.01) cecal colonization by *S. typhimurium*, whereas cecal colonization declined (*P* < 0.05) for broilers fed rations with refined FOS. *Salmonella* scores of 6-wk-old broilers fed rations with crude FOS were markedly higher (*P* < 0.001) than those of broilers fed refined FOS, LDS, and LDL. With the exception of broilers fed crude FOS, scores of the remaining groups of broilers declined with age, especially during the 6th wk of age. When CONT was fed during the 6th wk to broilers that were fed complex carbohydrates to 5 wk of age, the influence of the carbohydrates for the first 5 wk was lost and scores for these and the control groups of broilers did not differ (*P* > 0.05) at 6 wk.

*Cecal pH.* Mean cecal pH (Table 2) of broilers fed complex carbohydrates were lower than those of control broilers at 5 (*P* < 0.001) and 6 (*P* < 0.01) wk of age. Mean cecal pH of broilers fed a given ration tended to remain similar for all ages. Cecal pH of chicks fed FOS were higher at 3 (*P* < 0.001) and 5 (*P* < 0.05), but not at 6 wk of age, than those fed LD. Switching from diets containing carbohydrates to the control diet after 5 wk allowed pH means to rise to levels of broilers fed the control ration for 6 wk. The similarity of the cecal pH reduction in broilers fed crude FOS vs refined FOS is noteworthy because these broilers differed widely in cecal colonization by *S. typhimurium*. Correlation coefficients (r) between treatment means for *Salmonella* score and cecal pH were 0.88, 0.55, and 0.54 at 3, 5, and 6 wk of age, respectively; however, the positive trend suggested by these correlations was contradicted by treatment means for broilers fed crude FOS, which had the highest average pH and the highest average *Salmonella* scores.

*Cecal Density.* All rations with added complex carbohydrates reduced cecal density below that of the control group at 3 and 5 wk (*P* < 0.001) but not at 6 wk (*P* > 0.05) of age (Figure 1). Mean cecal densities for broilers of specific treatments were similar for all ages except those 6-wk-old broilers that had carbohydrates discontinued during the last week of the trial. Cecal densities of 3-wk-old broiler groups fed the various carbohydrates did not differ (0.46, 0.57, 0.58, and 0.61). Broilers fed crude FOS had the lowest cecal densities at all three ages (0.46, 0.49, and 0.48), and, by 6 wk of age, had lower densities (0.48 vs 0.65 to 0.73, *P* < 0.001) than all other groups of broilers. Cecal density increased more (*P* < 0.001) during the 6th wk for broilers fed crude FOS (from 0.49 to 1.03) than for those fed refined FOS to 5 wk of age (from 0.57 to 0.70). However, none of the groups differed from the CONT
TABLE 2. Cecal pH least squares means, ranges, and orthogonal contrasts of 3-, 5-, and 6-wk-old broilers fed various carbohydrates

<table>
<thead>
<tr>
<th>Treatment</th>
<th>3 wk</th>
<th>5 wk</th>
<th>6 wk</th>
<th>6-d wk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CONT</td>
<td>189</td>
<td>190</td>
<td>79</td>
<td>76</td>
</tr>
<tr>
<td>CFOS</td>
<td>5.38</td>
<td>5.80</td>
<td>5.85</td>
<td>5.86</td>
</tr>
<tr>
<td></td>
<td>(4.93 to 5.98)</td>
<td>(5.24 to 6.62)</td>
<td>(4.65 to 6.04)</td>
<td>(5.48 to 6.97)</td>
</tr>
<tr>
<td>RFOS</td>
<td>5.65</td>
<td>5.34</td>
<td>5.46</td>
<td>6.01</td>
</tr>
<tr>
<td></td>
<td>(4.89 to 6.69)</td>
<td>(4.43 to 6.10)</td>
<td>(4.85 to 5.93)</td>
<td>(5.46 to 6.56)</td>
</tr>
<tr>
<td>LDS</td>
<td>5.54</td>
<td>5.50</td>
<td>5.40</td>
<td>5.81</td>
</tr>
<tr>
<td></td>
<td>(4.94 to 6.47)</td>
<td>(4.22 to 6.26)</td>
<td>(4.73 to 6.34)</td>
<td>(5.15 to 6.56)</td>
</tr>
<tr>
<td>LDL</td>
<td>5.31</td>
<td>5.21</td>
<td>5.55</td>
<td>5.94</td>
</tr>
<tr>
<td></td>
<td>(4.31 to 6.19)</td>
<td>(4.03 to 6.33)</td>
<td>(4.87 to 6.00)</td>
<td>(5.26 to 6.42)</td>
</tr>
<tr>
<td>Orthogonal contrasts</td>
<td>NS</td>
<td>***</td>
<td>**</td>
<td>NS</td>
</tr>
<tr>
<td>CONT/Additives</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>FOS/LD</td>
<td>***</td>
<td>*</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>CFOS/RFOS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>LDL/LDS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

1Values in parentheses represent the range of pH values.
2CONT = control diet; CFOS = crude FOS; RFOS = refined FOS; LDL = lactulose; LDS = lactosucrose.
3Six-week-old broilers subjected to dietary treatments from hatch to 5 wk of age and fed the control ration only during the 6th wk.

*p < 0.05; **p < 0.01; ***p < 0.001.

Group at this age. Cecal volume was the only covariate consistently involved in accounting for variation (19 to 28%, data not shown) in cecal density. Like the cecal pH results, the broilers fed crude FOS had low cecal density values but high Salmonella scores, whereas other groups of broilers fed carbohydrates had low cecal densities and Salmonella scores.

General. In 6-wk-old broilers fed carbohydrates for the first 5 wk of age, variation accounted for by the model was 9% for Salmonella score, 8% for cecal pH, and 12% for cecal density. Corresponding values for the 3-, 5-, and 6-wk-old broiler groups fed carbohydrates continuously were higher, with ranges of 16 to 35%, 23 to 47%, and 26 to 40%, respectively. Feeding carbohydrates for 6 as opposed to 5 wk added to variation of cecal Salmonella score (0.628 vs 0.315).

Influence of Other Factors

For all three traits, there were no significant differences (Table 3) associated with effects of replicate (or pen) and replicate-sex interaction at 3 and 5 wk of age or sex at the three ages. Treatment-sex interactions were significant (P < 0.05) at 6 wk of age for all three traits.

In spite of the variables in the model, their predictive potential was low (< 50%) for the ages and traits studied. Internal factors appeared responsible for much of the unexplained variation of cecal pH and density. Analyses (not shown) of variation in these traits at the three ages revealed that variation between ceca from the same broiler was half and 90% as large, respectively, as that among ceca from different broilers subjected to the same treatment.
TABLE 3. ANOVA of complex carbohydrates and other factors influencing cecal Salmonella score, pH, and density of 3-, 5-, and 6-wk-old broilers

<table>
<thead>
<tr>
<th>Factors</th>
<th>3 wk</th>
<th>5 wk</th>
<th>6 wk</th>
<th>6-d wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treat</td>
<td>NS</td>
<td>NS</td>
<td>**</td>
<td>NS</td>
</tr>
<tr>
<td>Reps/Treat</td>
<td>NS</td>
<td>NS</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sex</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Treat × Sex</td>
<td>NS</td>
<td>NS</td>
<td>*</td>
<td>NS</td>
</tr>
<tr>
<td>Reps × Sex</td>
<td>NS</td>
<td>NS</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Salmonella score</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cecal pH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treat</td>
<td>***</td>
<td>***</td>
<td>*</td>
<td>NS</td>
</tr>
<tr>
<td>Reps/Treat</td>
<td>NS</td>
<td>NS</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sex</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Treat × Sex</td>
<td>NS</td>
<td>NS</td>
<td>*</td>
<td>NS</td>
</tr>
<tr>
<td>Reps × Sex</td>
<td>NS</td>
<td>NS</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cecal density</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treat</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>NS</td>
</tr>
<tr>
<td>Reps/Treat</td>
<td>NS</td>
<td>NS</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sex</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Treat × Sex</td>
<td>NS</td>
<td>NS</td>
<td>*</td>
<td>NS</td>
</tr>
<tr>
<td>Reps × Sex</td>
<td>NS</td>
<td>NS</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

1Salmonella scores, 0 = 0 cfu; 1 = 1 to 100 cfu; 2 = > 100 cfu.
2Treat = treatment; Reps = replicates.
3Six-week-old broilers subjected to dietary treatments from hatch to 5 wk of age, and fed the control ration only during the 6th wk.
*p < 0.05.
**p < 0.01.
***p < 0.001.
± = not applicable.

DISCUSSION

The existence of causal relations between either cecal pH or cecal density and Salmonella score were not supported by these results. Broilers fed the ration with crude FOS had the highest Salmonella scores with little reduction 1 wk after withdrawal of crude FOS from the diet. On the other hand, these broilers had reductions in cecal pH and cecal density equal to, or greater than, those of broilers fed refined FOS. Moreover, withdrawal of crude FOS and other complex carbohydrates for 1 wk allowed these traits to either resume or approach values for the CONT broilers. The positive coefficients for regression of treatment means for Salmonella score on pH is consistent with observations by Corrier et al. (1990a) suggesting that as pH rose, Salmonella score also rose. However, this trend is contradicted by the low pH for broilers that had high Salmonella scores.

The decline of Salmonella scores with age for the CONT and three of the four broiler groups fed complex carbohydrates is similar to observations by Sadler et al. (1969), and attests to the importance of this factor. It is not known why the broilers fed crude FOS or the broilers fed carbohydrates to 5 wk of age failed to exhibit the same response of Salmonella score to age between 5 and 6 wk of age. Age did not have a similar influence on cecal pH and density. As mentioned earlier, factors such as sex and replicate (or pen) were not important in determining levels of the three cecal traits measured. The biological significance of the treatment-sex interactions observed at 6 wk (P < 0.05) was not apparent. The dietary treatments imposed represented the only other important factor influencing the cecal traits measured. The involvement of cecal volume as a covariate supports the hypothesis that differences in cecal density arise due to gas production in the cecum and are independent of cecal size, and to 5 wk of age, body size.

With regard to other studies in the literature, results of this study are consistent in some instances and inconsistent in others. The lack of response of Salmonella score of cecal contents of 3-wk-old broilers fed FOS agrees with results of Bailey et al. (1991) and Conner and Moran (1992) but fails to support the more positive results of Choi et al. (1994). Reductions in cecal pH of 5- and 6-wk-old broilers are consistent with reports in other studies (Corrier et al., 1990a,b; Hinton et al., 1990; Moran and Conner, 1992; and Choi et al., 1994). The failure to detect a relationship between complex carbohydrates and cecal weight in this study differs from results of Tellez et al. (1993), who reported that feeding Leghorn chicks 10% dietary lactose to 14 or 19 d of age approximately doubled cecal weight. The Salmonella score and cecal pH increases following carbohydrate withdrawal are similar to those reported by Corrier et al. (1990b) following withdrawal of 7% lactose from 10-d-old chicks.

Variation in the three cecal traits accounted for by the models for 3-, 5-, and 6-wk-old broilers fed carbohy-
Salmonella obtain precise results. Hence, complex carbohydrates do influence these three cecal traits; however, the large amount of variation remaining unexplained by the model applied exceeded 50% for all three traits. Consequently, the error term for testing the significance of differences was relatively large, precluding the establishment of significance for small effects and reducing precision of estimates of these effects.

Cecal contents are the material of choice for detection of *Salmonellae* in chickens. In this study, large standard deviations are observed for the cecal traits consistent with the small amounts of variation accounted for by the model. This variation is attributable not only to measurement error and to factors not included in the model, but also to intrabroiler cecal differences for cecal pH and density. Large portions of residual variability in pH and density plus large ranges of values within treatment groups of broilers suggest that these are dynamic traits. The hypothesis that acidity and gas accumulate during fermentation in the cecum until near maximum levels are attained and then cecal contents are expelled is consistent with this phenomenon. Hence, intra-group variation should be expected due to differences in duration of fermentation of contents of the ceca being compared and may account for the observed deviation of residual cecal density measures from a normal distribution. Such variation could be reduced either by synchronization of cecal evacuation times or by the inclusion of fermentation duration of cecal contents as an independent variable in the model.

Information pertaining to factors influencing timing of cecal evacuation (Isshiki and Nakahiro, 1975; Isshiki, 1979; Hill, 1971) and to cecal motility (Roche 1973, 1974) is available in the literature. However, little information could be found relating to the timing or measurement of release of gas or contents from the cecum to clarify the above observations. Hence, it appears that large numbers of broilers for each treatment group are required to obtain precise results.

Some complex carbohydrates, as employed in this study, gave rise to minor reductions in broiler cecal *Salmonella* scores. Due to the ability of *Salmonella* to multiply in the environment, minor as opposed to major reductions of *Salmonella* are not adequate for food safety. In the current study, not inoculating broiler chicks with a cecal microflora may account for the delayed cecal *Salmonella* score reduction in broilers fed LD or FOS. Other researchers (Corrier et al., 1990a,b, 1991; Hinton et al., 1990; Bailey et al., 1991; Nisbet et al., 1994) have adopted competitive exclusion (introduced by Nurmi and Rantala, 1973 and reviewed by Pivnick and Nurmi, 1982) for use with dietary lactose and demonstrated marked reductions of *Salmonella* scores of cecal contents for broilers. Similarly, competitive exclusion seemed to enhance cecal pH reduction of broilers fed added carbohydrates (Corrier et al., 1990a,b; Hinton et al., 1990; Ziprin and DeLoach, 1993). In the present study, *Salmonella* colonization of the chicken was both increased and reduced by dietary complex carbohydrates. Due to the lack of knowledge concerning the microbial flora of these chickens, it is not known whether the microflora were optimal for the complex carbohydrates to reduce digestive tract colonization by *Salmonella*.

Further research is required to evaluate competitive exclusion cultures in conjunction with complex carbohydrates as a means of controlling *Salmonella* colonization in the chicken.

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