Research Note

Isomaltooligosaccharide Increases Cecal *Bifidobacterium* Population in Young Broiler Chickens[1]

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ABSTRACT A newly developed compound derived by fermentation, isomaltooligosaccharide (IMO), was hypothesized to enrich cecal bifidobacterial populations and reduce colonization levels of *Salmonella* in the ceca of broiler chickens. Broiler starter diets were prepared with final IMO concentrations of 1% (wt/wt), 2% (wt/wt), and 4% (wt/wt) and a control diet without IMO supplementation. Chickens were divided into 4 groups and challenged with 10^8 cell of *Salmonella enterica* ser. Typhimurium with 200 μg/mL nalidixic acid resistant (*S.* Typhimurium Nalr) after 7 d of placement. The experiment was done in 3 replications. IMO-supplemented diets resulted in significantly higher cecal bifidobacteria compared with the control diet ($P < 0.05$). However, there was no significant difference in bifidobacteria counts among the treatment groups. Chickens fed diets with 1% IMO had a significant 2-log reduction in the level of inoculated *S*. Typhimurium Nalr ($P < 0.05$) present in the ceca compared with the control group, but no differences were found between the control group and the groups fed 2 or 4% IMO for *S.* Typhimurium Nalr. No differences in feed consumption, feed conversion, or feed efficiency compared with the control group were observed; however, the result showed a significant reduction in weight for birds fed 1% IMO diet compared with those fed the control diet.

(*Key words*: isomaltooligosaccharide, *Bifidobacterium*, broiler chicken, colonization, competitive exclusion)

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INTRODUCTION

Antibiotics have been used in animal agriculture since shortly after their discovery in the 1950s (Fuller, 1989). They are used for treatment and prevention of bacterial infections. In food-producing animals such as poultry, some antibiotics are used for growth promotion and improving feed efficiency (Turnidge et al., 1999; Mathews, 2001). However antibiotic growth promotants have received increasing attention as a contributory factor in the international emergence of antibiotic-resistant bacteria in human health and agriculture (Fuller, 1989; World Health Organization, 1997; Wegener et al., 1998; Aarestrup, 1999; Turnidge et al., 1999; Wegener et al., 1999; Aarestrup et al., 2000; Wray and Davies, 2000; Turnidge, 2004). Most classes of antibiotics used in animals have human analogs, and bacteria are capable of selecting for resistance to human antibiotics.

The concept of competitive exclusion (Nurmi and Rantala, 1973) and the use of complex dietary carbohydrates opened a new, promising approach to the control of *Salmonella* in poultry and have been confirmed by a number of studies in various countries (Oyofo et al., 1989; Baba et al., 1991; Bailey et al., 1991; Nisbet et al., 1993; Terada et al., 1994; Orban et al., 1997; Patterson et al., 1997; Fernandez et al., 2000, 2002; Kleessen et al., 2003). Many oligosaccharides such as fructooligosaccharides, when fed to animals or humans can reach the colon undegraded and provide a carbohydrate substrate for the growth of beneficial microorganisms, such as bifidobacteria (Tomomatsu, 1994; Playne and Crittenden, 1996) and some lactic acid bacteria (Flickinger et al., 2000), which are thought to create conditions unfavorable to growth of pathogens, such as *Salmonella* (Isolauri et al., 2001).

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[1]Mention of trade names of commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

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Abbreviation Key: BGS = brilliant green sulfa agar; IMO = isomaltooligosaccharide; MLIA = modified lysine iron agar; Nal = nalidixic acid; Nalr = nalidixic acid-resistant; RV = Rappaport-Vassiliadis R10 broth; TOS-A = transoligosaccharide propionate agar medium supplemented with glacial acetic acid; VFA = volatile fatty acids.

In a previous in vitro study by Chung and Day (2004), isomaltooligosaccharide (IMO) was used preferentially by strains of *Bifidobacterium bifidum*, *Bifidobacterium longum*, and *Lactobacillus johnsonii*. The use of oligosaccharides as prebiotics should lead to production of intestinal lactic acid, an increase in short-chain fatty acid production, and lowering of pH in the large intestine (Chung and Day, 2004), thereby being a potential feed additive to help modify poultry intestinal microflora. Ultimately it is hoped that addition of IMO to poultry feed could lead to intestinal microflora balance, allowing for more consistent production responses in the absence of prophylactic antibiotics. The objectives of this study were to test the hypothesis that addition of IMO in the diet would increase the *Bifidobacterium* spp. populations in the chicken cecum, which, in turn, would reduce the colonization by *Salmonella enterica* ser. Typhimurium and improve growth performance in the 3-wk-old broiler chickens.

MATERIALS AND METHODS

Experimental Birds

In each of 3 replications, 40 broilers (*Gallus domesticus*, Ross × Ross) that were 1 d old were obtained from a local commercial hatchery. The chicks were randomly allocated to 4 isolator units[3] containing 10 birds per units, and each unit was randomly assigned to 1 of 4 dietary treatments; control (0% IMO), 1% IMO, 2% IMO, and 4% IMO of the diet (wt/wt). All chicks were placed at room temperature maintained at approximately 35°C for the first week and at approximately 32°C thereafter. Water and assigned feed were provided ad libitum for the duration of the experiment. Feed consisted of a standard unmedicated diet[4] based on corn and soybean meal (22.5% crude protein, 5.28% crude fat, 2.53% crude fiber, 0.95% calcium, 0.45% available phosphorus, and 3,080 kcal of estimated energy/kg) that exceeded levels of critical nutrients as recommended by the National Research Council (1994). All chicks were inoculated orally, through a gavage needle (Animal Feeding Needles,[5] 1 mL syringe,[6] size 0.4 × 13 mm) inserted approximately 2.5 cm into the esophagus with 0.25 mL (containing 10^8 total cells) of a culture of *Salmonella enterica* ser. Typhimurium nalidixic acid strain after 7 d of placement. Twenty-one days after placement, 3 chicks were randomly selected from each treatment group and euthanized by cervical dislocation. All animal care procedures were in compliance with the local animal care and use committee.

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[6]Becton Dickinson and Co., Franklin Lakes, NY.
[7]Difco Laboratories, Detroit, MI.
[8]Yakult Pharmaceutical, Tokyo, Japan.
[9]Oxoid, Hampshire, UK.

Determination of Bird Body Weight, Feed Conversion, and Feed Efficiency

Body weights of chickens in all groups were measured on d 1 and 21. The mean body weight, feed conversion, and feed efficiency per treatment group were calculated from 30 pooled samples per treatment group.

Salmonella enterica Ser. Typhimurium Nalidixic Acid Resistant

A culture of *S.* Typhimurium resistant to 200 μg of nalidixic acid (Nal) per milliliter was obtained from the culture collection of the Poultry Microbiological Safety Research Unit of the Agricultural Research Service, Russell Research Center; Athens, GA. Inocula for infectious challenge exposure was prepared from brilliant green sulfa agar (BGS)[7] supplemented with 200 μg/mL of Nal. Cultures were incubated at 37°C for 24 h. The culture was diluted in sterile isotonic saline solution to obtain challenge inoculate containing 1×10^8 *S.* Typhimurium Nal resistant (Nalr)/0.25 mL.

Microbiological Media

The transoligosaccharide propionate agar medium[8] supplemented with glacial acetic acid (TOS-A); 1% (vol/vol) TOS-A was used as the enumerating media for *Bifidobacterium*. Samples for bifidobacteria analysis were first diluted in prereduced Wilkins-Chalgren broth.[9] Trypticase phytone yeast extract broth (Scardovi 1986) was used to culture presumptive *Bifidobacterium* subsequent to isolation from TOS-A, for fructose-6-phosphate phosphoketolase assay, and for maintaining the *Bifidobacterium* cultures. BGS supplemented with 200 μg/mL of Nal (BGS+Nal) was used to enumerate *S.* Typhimurium Nalr. Modified lysine iron agar (MLIA),[9] buffered peptone water (BPW),[7] Rappaport-Vassiliadis R10 broth (RV),[7] tetrathionate broth,[7] triple sugar iron agar,[7] and lysine iron agar[7] slants were used as a confirmational media for *Salmonella*. Reinforced clostridial agar[9] and deMan Rogosa Sharpe agar[9] were used to enumerate total anaerobic bacteria and total lactic acid bacteria, respectively. All media were autoclaved at 121°C for 15 min prior to use and were used within 2 wk.

Cecal Bacterial Population Determination

In each of 3 replications, 3 birds were randomly selected from each isolator unit and euthanized by cervical dislocation. The ceca from individual birds were removed, weighed, and kept under anaerobic condition in an anaerobic jar[9] equipped with AnaeroGen sachets[9] until analyses. Immediately, the contents of each cecum were blended in Wilkins-Chalgren broth (1:9, wt/vol), and further serial dilutions were made in the same media for enumeration. A 100-μL aliquot of appropriate dilutions was spread plated on TOS-A, BGS+Nal, reinforced clostridial, and deMan Rogosa Sharp agars. All steps were

performed under anaerobic condition (except for *Salmonella*) in an anaerobic chamber[10] (5% hydrogen, 5% CO_2, balanced nitrogen). All media (except for BGS+Nal) were incubated anaerobically in anaerobic jars equipped with AnaeroGen sachets at 37°C for 72, 48, and 24 h for *Bifidobacterium*, total lactic acid bacteria, and total anaerobic bacteria, respectively. The BGS+Nal plates were incubated aerobically at 37°C for 24 h for *Salmonella*. All bacterial population counts were calculated as colony-forming units per gram of wet weight of cecal content. Bacterial populations were enumerated in duplicate at 2 different dilutions.

Confirmation of Bifidobacterium

For each sample, colonies were identified as members of the genus *Bifidobacterium* by the following criteria: (1) they were gram positive, pleomorphic rods with characteristic bifurcated *Bifidobacterium* cell morphology; (2) they were unable to grow under aerobic conditions; (3) they were catalase negative; and (4) they showed fructose-6-phosphate phosphoketolase (EC 4.1.2.22) activity as described by Scardovi (1986)

Confirmation of S. Typhimurium NalR

Negative samples from BGS+Nal media (no viable count on BGS+Nal) were preenriched, from the original dilution, overnight aerobically at 37°C. Then 0.5 mL of preenriched samples was transferred to 10 mL of tetrathionate broth and 0.1 mL to 10 mL RV, followed by aerobic incubation at 37°C for 24 h. Next, one loopful each of tetrathionate broth and RV was streaked on MLIA, BGS, and BGS+Nal and then incubated aerobically at 37°C for 24 h. After incubation, a well-isolated colony with typical *Salmonella* spp. morphology (purple colony with or without black center) was picked from MLIA agar. A well-isolated opaque colony with a smooth appearance and entire edge surrounded by a red medium was picked from BGS+Nal, and triple sugar iron and lysine iron agar slants were inoculated with the isolate by stabbing the agar butts and streaking the slants. After aerobic incubation at 37°C overnight, reactions were determined.

Statistical Analysis

The data obtained from this study were analyzed as a randomized complete block design. Treatment means were analyzed using the GLM with least squares means procedure of SAS software (SAS Institute, 2001). Bacterial populations were analyzed after $\log_{10}$ transformation. Differences in the mean were compared by least significant difference (LSD). All statements of statistical significance were based on $P < 0.05$.

[10]Sheldon Manufacturing Inc., Cornelius, OR.

RESULTS AND DISCUSSION

Feed Conversion, Feed Efficiency, and Body Weight Gain

Feed consumption, feed conversion, and feed efficiency were similar for birds fed a treated IMO diet compared with those on the control diet (Table 1). However, results showed a significant reduction in weights of birds fed the 1% IMO diet compared with the control diet. The diets containing 2 and 4% IMO showed similar results for bird body weight when compared with control diet. These results were similar to those of Oyofo et al. (1989), Patterson et al. (1997), and Iji et al. (2001). Oyofo et al. (1989) found no significant difference on weight gain between chickens fed several types of carbohydrates (dextrose, lactose, sucrose, mannose, and maltose; all at 2.5% wt/wt) when compared with control group in a 10-d trial period. Patterson et al. (1997) found that weight gain, feed consumption, feed efficiency, and feed digestibility were similar for 4-wk-old birds fed thermally produced kestoses (at 2%) compared with the control group and birds fed other sugars (8% sucrose and 8% glucose). Iji et al. (2001) found no significant effects of mannanoligosaccharide (at 0, 1.0, 3.0, and 5.0 g/kg diet) on feed consumption and weight gain when fed to broiler chickens during a 28-d trial. Waldroup et al. (1993) reported body weight gains for birds fed fructooligosaccharides (FOS), but the differences were not significant. However they found significant body weight gains for birds fed FOS with bacitracin methylene disalicylate. The chickens in this study were raised at 32°C, whereas chickens in the study by Waldroup et al. (1993) were raised at a lower temperature (32.2°C for the first week and reduced by 2.8°C/wk to 21.1°C). It may be suggested that undetermined factors such as stress, temperature, animal health, and others may influence the efficacy of IMO on broiler chicken performance. High environmental temperature causes reduction of feed consumption and body weights of broilers (Orban and Roland, 1992). The precise mechanisms of how chicks fed 1% IMO had a significant reduction in weight are not known.

Total Anaerobic Bacteria Population

There were no significant changes in the number of total anaerobic bacteria for all treatment groups compared with the control group (Table 2).

Lactic Acid Bacteria and Bifidobacterium Population

All of the IMO treatment diets significantly increased the number of bifidobacteria in birds compared with the control diet (Table 2). However, there was not a significant difference among the treatment groups (1, 2, and 4% IMO diets). IMO had little effect on lactic acid bacteria numbers when compared with the control diet (Table 2). Others have reported that bifidobacteria increased in poultry ce-

TABLE 1. Performance of 3-wk-old broilers fed IMO supplemented diets[1]

Parameter	Treatment[2]			
	Control	1% IMO	2% IMO	4% IMO
Body weight gain (kg)	6.85 ± 0.36[a]	6.17 ± 0.26[b]	6.51 ± 0.26[ab]	6.91 ± 0.27[a]
Feed consumption (kg)	10.95 ± 1.58[a]	9.32 ± 0.96[a]	9.98 ± 0.53[a]	11.36 ± 1.90[a]
Feed conversion (kg:kg)	1.60 ± 0.22[a]	1.51 ± 0.09[a]	1.53 ± 0.12[a]	1.65 ± 0.22[a]
Feed efficiency (kg:kg)	0.63 ± 0.08[a]	0.67 ± 0.04[a]	0.65 ± 0.05[a]	0.62 ± 0.08[a]

[a,b]Means within the same row followed by different superscript letters are significantly different ($P < 0.05$).

[1]Isomaltooligosaccharide (IMO) was added to the diet at 1% (wt/wt), 2% (wt/wt), or 4% (wt/wt); the control was not supplemented with IMO.

[2]Values within parameters are means ± SD (n = 30 for each treatment).

cal content when birds were fed specific prebiotics or oligosaccharides. For example, Patterson et al. (1997) studied the effects of thermally produced kestoses on broiler performance and selective enrichment of bifidobacteria in the intestinal tract of 4-wk-old broilers. They found that dietary administration of thermally produced kestoses at 2% of the diet significantly increased the number of bifidobacteria and lactobacilli in the chicken cecum. They found a smaller increase in number of *Lactobacillus acidophilus* and *Lactobacillus salivarius* when compared with bifidobacteria numbers. Orban et al. (1997) found a significant increase in the number of bifidobacteria in the cecas of broilers fed a diet supplemented with 7.5% sucrose thermal oligosaccharide caramel (STOC) in a 4-wk trial. Rada et al. (2001) found a highly significant 1-log increase in bifidobacteria ($P < 0.01$) when an inulin-supplemented diet (at 5%) was fed to 1-wk-old laying hens.

Cecal Colonization by S. Typhimurium Nalr

Chickens treated with the 1% IMO diet had a significant 2-log reduction in the level of S. Typhimurium Nalr present in the ceca compared with the control group (Table 2). This reduction was not observed in samples from chickens fed a greater IMO concentration (2 or 4%).

A reduction in *Salmonella* colonization of birds provided the IMO diet was associated with more bifidobacteria and lactic acid bacteria present in the ceca. Bailey et al. (1991) found that FOS had minimal effect on *Salmonella* colonization in 2-wk-old broiler chicks fed a supplemented FOS diet at 0.375 and 0.75%; they did not, however, report the bifidobacterial levels. Fernandez et al. (2002) studied the effects of a mash diet or mash supplemented with 2.5% mannose oligosaccharide or palm kernel meal on the microflora of the hen cecal contents over a 4-wk trial. They found the diet supplemented with mannose oligosaccharide and palm kernel meal affected the bird intestinal microflora by increasing the number of *Bifidobacterium* spp. and *Lactobacillus* spp. while decreasing the *Enterobacteriaceae* groups. The diets were also found to reduce the susceptibility to colonization of 4-wk-old hens by *Salmonella enterica* ser. Enteritidis.

Oligosaccharides and related carbohydrates are neither degraded nor hydrolyzed in the upper intestinal tract of animals and, hence, reach the cecum intact (Hidaka et al., 1986; Oku, 1986). Because of its low digestion and absorption, IMO passes into the lower portions of the intestine and cecum and is subsequently available for bifidobacteria and lactic acid bacteria to use as a growth substrate. The importance of normal intestinal flora in the gastrointestinal tract in reducing pathogen colonization in animals has been well documented (Mulder et al., 1997; Bezkorovainy, 2001; Ishibashi and Yamazaki, 2001; Isolauri et al., 2001; Marteau et al., 2001; Gong et al., 2002) and by the Nurmi concept of competitive exclusion in poultry (Oyofo et al., 1989; Baba et al., 1991; Bailey et al., 1991; Nisbet et al., 1993; Terada et al., 1994; Orban et al., 1997; Patterson et al., 1997; Fernandez et al., 2000, 2002; Henriksson and Conway, 2001; Kleessen et al., 2003). However, the mechanisms by which probiotic strains prevent colonization by enteropathogens are not known

TABLE 2. Cecal bacterial populations of 3-wk-old broilers fed isomaltooligosaccharide (IMO)-supplemented diets

Treatment[2]	Bacteria (log cfu/g of cecum wet weight)[1]			
	S. Typhimurium Nalr[3]	Bifidobacteria	Total anaerobic bacteria	Total lactic acid bacteria
Control diet	7.91 ± 2.27[a]	9.07 ± 0.69[a]	10.40 ± 0.86[a]	9.88 ± 1.43[ab]
1% IMO	5.42 ± 1.93[b]	10.09 ± 1.54[b]	10.66 ± 0.73[a]	10.28 ± 1.31[a]
2% IMO	7.51 ± 2.28[a]	10.04 ± 1.46[b]	10.58 ± 1.02[a]	10.05 ± 1.50[ab]
4% IMO	6.74 ± 1.47[a]	9.98 ± 1.21[b]	10.81 ± 0.71[a]	9.67 ± 1.67[b]

[a,b]Means within the same column followed by different superscript letters are significantly different ($P < 0.05$).

[1]Values within columns are mean ± SD (n = 9 for each treatment).

[2]Isomaltooligosaccharide (IMO) was added to the diet at 1% (wt/wt), 2% (wt/wt), or 4% (wt/wt); the control diet was not supplemented.

[3]*Salmonella enterica* ser. Typhimurium, nalixidic acid resistant strain (200 μg/mL).

(Chung, 2002). In the normal intestinal microflora, Mead (2000) described that volatile fatty acids (VFA) are produced mainly as a result of the metabolism of sporulating and nonsporulating anaerobic bacteria. The VFA can be inhibitory to other organisms present, especially in the undissociated state below pH 6.0. VFA that are inhibitory to salmonellas include acetic and propionic to suppress the salmonellas. The precise mechanism of the protective effect is unknown and may never be determined because of the complexity of the gut as a habitat for microorganisms and the variety of host-microbe and microbe-microbe interactions that can occur (Rolfe, 1991).

This study suggests that a 1% IMO diet showed a reduction in the number of S. Typhimurium Nalr in the ceca of 3-wk-old chickens compared with a diet without IMO added or a diet with 2 or 4% IMO. Feeding the optimum level of IMO (1%) in diets of chickens may reduce Salmonella colonization of young chickens.

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