ENVIRONMENT, WELL-BEING, AND BEHAVIOR

Effects of Galacto-Oligosaccharides and a Bifidobacteria lactis-Based Probiotic Strain on the Growth Performance and Fecal Microflora of Broiler Chickens

S. J. Jung,* R. Houde,† B. Baurhoo,‡ X. Zhao,‡ and B. H. Lee*§†

*Department of Food Science and Agricultural Chemistry, McGill University, Ste-Anne-de-Bellevue, Quebec, H9X 3V9, Canada; †Agro-Kimika Concepts, Dorval, Quebec, H9P 2A2, Canada; ‡Department of Animal Science, McGill University, Ste-Anne-de-Bellevue, Quebec, H9X 3V9, Canada; and §Food Research and Development Center, Agriculture and Agri-Food Canada, Ste-Hyacinthe, Quebec, J2S 8E3, Canada

ABSTRACT A galacto-oligosaccharide (GOS) prebiotic was prepared by reacting a high concentration of lactose (40% wt/vol) with a β-galactosidase enzyme for 24 h at 37°C. The enzyme was produced from recombinant Pichia pastoris X-33 cells. The study aimed at evaluating the effects of the prebiotic, a Bifidobacterium lactis-based probiotic, and the combination of these dietary additives on BW, feed intake, feed conversion ratio, and fecal counts of total anaerobic bacteria, lactobacilli, and bifidobacteria in broiler chickens. No significant differences in BW, feed intake and feed conversion ratio were found among the various groups. The study showed that GOS selectively stimulated the fecal microflora of broiler chickens. Total anaerobic bacteria and lactobacilli were increased by 3.4- and 3.56-fold, respectively, in chickens fed the diet containing GOS (3 kg per 25 kg) and B. lactis for 40 d compared with those fed the control diet. The bifidobacteria population in chickens fed the diet containing GOS (3 kg per 25 kg) and B. lactis significantly increased 21-fold in comparison to the control-fed birds. In particular, increasing the dietary concentration of GOS was accompanied by significant increases ($P < 0.05$) in bifidobacteria counts. The detectable population of bifidobacteria was also greater ($P < 0.05$) in chickens fed the diet containing GOS and bifidobacteria when compared with chickens fed a bifidobacteria-containing ration only. These results suggest that using GOS in combination with a B. lactis-based probiotic favored intestinal growth of bifidobacteria in broiler chickens.

Key words: galacto-oligosaccharide, prebiotic, recombinant lactase, bifidobacteria, probiotic

INTRODUCTION

Oligosaccharides are found as major components of several natural products such as plant extracts and mammalian milk, in either free or bound forms. In monogastric animals, many oligosaccharides are broken down in the upper intestine by hydrolytic enzymes. However, certain oligosaccharides such as galacto-oligosaccharides and mannan-oligosaccharides, due to their unique chemical structures, are resistant to digestive enzymes and transit unchanged into the large intestine. These nondigestible oligosaccharides (NDO) can reach the colonic area and are preferentially fermented and utilized by the Bifidobacterium genus as carbon and energy sources; thus, the definition of “bifidogenic factors” (Gomes and Malcata, 1999). Because of the selectivity of NDO as growth substrates along with the health-promoting properties of bifidobacteria, considerable attention is now focused on the use of these compounds in health and nutrition (O’Sullivan, 1996). Bifidobacteria and other probiotic lactic cultures are thought to contribute to human and animal health through mechanisms such as competitive exclusion of pathogenic and putrefactive bacteria, immune stimulation, increased production of short-chain fatty acids, control of intestinal function, prevention of cancer (Reddy and Rivenson, 1993; Sako et al., 1999), and improved digestion and nutrient absorption (Yaeshima, 1996). Moreover, these fermentable carbohydrates positively influence the composition and activity of indigenous microbiota of the gastrointestinal tract (Williams et al., 2001).

Oligosaccharide series such as lactulose-, malto-, fructo-, galacto-, and xylo-oligosaccharides are marketed as low-calorie, bioregulating dietary fiber and healthy foods in Japan (Farnworth et al., 1996). These oligosaccharides encompass beneficial health effects in...
humans as nutraceuticals (Smart, 1993) and in animals (Birlouez-Aragon, 1993). With a ban on dietary subtherapeutic antibiotics as growth promoters within the European Union and inevitably in North America, animal nutritionists are urgently seeking health-enhancement alternatives, particularly for young animals (Bauer et al., 2006). We hypothesize that galacto-oligosaccharides (GOS) represent a potential biological alternative to antibiotics in poultry production. However, one major challenge is the proper structural elucidation of GOS to determine which structure is most effective as a bifidogenic factor. Because the administration of subtherapeutic antibiotics to livestock involves a risk of antibiotic carry-over through milk and meat as well as the emergence of antibiotic-resistant pathogens, probiotic cultures have been used as alternatives for antibiotics to control pathogens. Because regulations concerning feed additives are becoming more stringent, pre- and probiotics with proven safety and efficacy that support growth and health of livestock animals might become widespread alternatives. To eventually achieve the industrial-scale production of GOS, we genetically overproduced the enzyme lactase (glycoside hydrolase; EC 3.2.1.23) with high galactosyltransferase (glycosyl transferase; EC 2.4.1.22) activity. Thus, lactose is used to form an active intermediate with the enzyme at a high concentration of substrate, and this intermediate reacts with any available sugar acceptor featuring a hydroxyl group (Birlouez-Aragon, 1993; Mahoney, 1998). Bifidogenic factors such as GOS could prove to be a valuable solution against cataract risk associated with increasing galactose absorption by humans (Smart, 1993).

The objective of this study was to investigate production responses and fecal microbiology of chicken fed diets containing GOS, Bifidobacterium lactis, or the combination of these dietary additives.

**MATERIALS AND METHODS**

**Preparation of GOS and Bifidobacteria Additives**

The GOS used in this study was prepared from a β-galactosidase-treated lactose solution. To prepare β-galactosidase from recombinant *Pichia pastoris* X-33, cells were incubated in buffered complex medium containing methanol (BMMY) broth with shaking at 200 rpm for 4 d at 30°C. Cells were centrifuged (5,900 × g for 10 min), washed twice with sodium phosphate buffer (50 mM, pH 7.0), and resuspended in the same buffer. Cells were then disrupted using a sonicator (550 Sonic Dismembrator, Fisher Scientific, Mississauga, Ontario, Canada) with the power level set at 6 for 1 h with 2-s pulsing and 10-s intervals under constant cooling. The disrupted cells were centrifuged (2,300 × g, 10 min, 4°C) and the supernatants (cell-free extracts) were used for GOS synthesis. The cell-free extracts were incubated with 40% (wt/vol) lactose (Saputo Inc., St-Hyacinthe, Quebec, Canada) aqueous solution for 24 h at 37°C with continuous stirring. The solution was then centrifuged (2,300 × g, 10 min, 4°C) and the supernatants used as GOS syrup. The pure commercial bifidobacterial strain for broiler feeding (*B. lactis* D 300; 300 × 10^9 cells/g) was provided by Abiassa Inc. (St-Hyacinthe, Quebec, Canada).

When the recombinant β-galactosidase was reacted with an aqueous 40% (wt/vol) lactose solution, the transgalactosylation ratio reached 25.2% at 83.1% conversion of initial lactose, and the maximum yield of GOS was 40.6%. Concentrations in the standardized GOS syrup supplement used in this study were 21% GOS, 10% lactose, 1.5% glucose, and 0.9% galactose.

**Bird Management**

Seven hundred twenty (n = 720) 1-d-old vaccinated (Marek’s disease and infectious bronchitis), male broiler chicks were obtained from a local commercial hatchery (Couvoir Ramsay, St-Félix-de-Valois, Quebec, Canada) and were grown over a 40-d experimental period. Birds were randomly allocated to 6 dietary treatments (3 pen replicates; 40 birds per pen). The birds were brooded following standard temperature regimens, which gradually decreased from 32 to 24°C and kept under a 20L:4D cycle throughout the study. All birds were housed and raised under a protocol approved by the McGill University Animal Care Committee. Birds were group-weighed by pen and feed consumption was determined at weekly intervals. Body weight, feed intake, feed conversion ratio, and mortality rate were recorded weekly and analyzed.

**Experimental Design and Diets**

Birds were fed a standard corn-soybean meal based diet (Moisson d’Or, AgriBrands Purina St-Clet, Quebec, Canada). A 2-phase feeding program was used with a starter diet from d 1 to 21 and a finisher diet from d 22 to 40. All diets were formulated to meet or exceed NRC (1994) requirements for macro- and micro-nutrients (Table 1). In this study, birds were randomly allotted to 1 of 6 treatments.

### Table 1. The calculated nutrient contents of basal diets

<table>
<thead>
<tr>
<th>Component</th>
<th>Starter diet</th>
<th>Finisher diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP (minimum), %</td>
<td>20.0</td>
<td>18.0</td>
</tr>
<tr>
<td>Crude fat (minimum), %</td>
<td>2.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Crude fiber (maximum), %</td>
<td>4.5</td>
<td>5.0</td>
</tr>
<tr>
<td>Sodium (actual), %</td>
<td>0.18</td>
<td>0.15</td>
</tr>
<tr>
<td>Calcium (actual), %</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Phosphorus (actual), %</td>
<td>0.50</td>
<td>0.70</td>
</tr>
<tr>
<td>Vitamin A (minimum), IU/kg</td>
<td>20,000</td>
<td>7,000</td>
</tr>
<tr>
<td>Vitamin D3 (minimum), IU/kg</td>
<td>2,000</td>
<td>1,500</td>
</tr>
<tr>
<td>Vitamin E (minimum), IU/kg</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>Added selenium, mg/kg</td>
<td>0.30</td>
<td>0.30</td>
</tr>
</tbody>
</table>

### Materials and Methods

**Preparation of GOS and Bifidobacteria Additives**

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For every 25-kg portion of the diet, treatments included 1) 0.15 kg of corn starch + 1.5 kg of water (T1, control); 2) diet T1 + 0.75 kg of GOS (T2); 3) 0.15 kg of corn starch + 3 kg of GOS (T3); 4) 1.5 kg of water + 0.15 kg of bifidobacteria (T4); 5) 0.75 kg of water + 0.15 kg of bifidobacteria + 0.75 kg of GOS (T5); and 6) 0.15 kg of bifidobacteria + 3 kg of GOS (T6). Preparation of the bifidobacteria probiotic included thorough mixing of 4 g of enterocoated B. lactis D300 pure culture (Abiasa Inc.) with 86 g of regular corn starch (Cargill Inc., Winnipeg, Manitoba, Canada) per 150 kg of feed. The mixture was then transferred into a Ziploc bag with 820 g of starch for mixing by inversion. Viability counts of the bacteria in the pure culture were 300 × 10^9 cfu/g and decreased only slightly after 40 d. Water and GOS syrup were spray-mixed onto the basal feed using a clean cement-type angled mixer, followed by addition of the dry bacterial premix. The 25-kg lots were allowed to dry to equilibrium overnight by spreading on clean tarps in a 20°C room. Diet composition and calculated nutrient contents of starter and finisher diets were similar to those of our previous study (Huang et al., 2004).

**Microbiological Analysis of Fecal Samples**

At 7 and 40 d of age, 10 birds from each treatment were randomly selected and weighed and fecal samples were collected. A portion (1 g) of feces was serially mixed with 9 mL of PBS containing 0.5 g/L of L-cystine. The samples were serially diluted to 10^−6, 10^−7, and 10^−8. For each dilution, 1 mL was used for inoculation. Bacterial populations examined were total culturable anaerobic bacteria, lactobacilli, and bifidobacteria. The counts (cfu) of total culturable anaerobic bacteria were enumerated on Reinforced Clostridial Medium agar (Fisher Scientific), lactobacilli were enumerated on Lactobacilli MRS agar (Fisher Scientific), and bifidobacteria on TOS Propionate agar (Yakult Co Ltd., Tokyo, Japan) after 3 d of incubation at 37°C in anaerobic jars, using the BD BBL GasPak Plus system (GasPak Jar Systems, Fisher Scientific).

**Statistical Analysis**

All data were analyzed using the SigmaStat software (Systat Software Inc., San Jose, CA); a 1-way ANOVA was used, with pen serving as the experimental unit for performance parameters and bird as the experimental unit for microbiology parameters. Treatment means were separated using the Bonferroni’s multiple comparison test, and statistical significance was declared at P < 0.05. All microbiological concentrations were subjected to log10 transformation before analysis.

**RESULTS**

**Bird Performance**

Performance responses of the broiler chickens are summarized in Table 2. When supplemented to the diet, neither GOS syrup nor B. lactis altered BW, feed intake, or feed conversion ratio of the chickens. Birds remained healthy and no significant mortality was observed during the experimental period.

**Enumeration of Total Anaerobic Bacteria, Lactobacilli, and Bifidobacteria**

Results of fecal microbial analysis are shown in Table 3. Feeding of GOS promoted greater numbers of total anaerobic bacteria, lactobacilli, and bifidobacteria than did the control diet (T1). Moreover, the increase in microbial counts was correlated with GOS dosage. The mean value of total anaerobic bacteria increased from 9.71 to 10.26 log cfu/g of feces in T6 birds after d 40. The average increase of 0.55 log units was significantly different according to the Bonferroni t-test (P <
Similarly, lactobacilli counts showed an increase of 0.53 log units, which was statistically significant \( P < 0.05 \). For bifidobacteria, the average count showed an increase of 1.32 log units (8.24 to 9.56 in log cfu/g) and was statistically significant \( P < 0.05 \). The bifidobacteria population was about 21- and 7.5-fold that in the negative control (T1) and positive control (T4), respectively. The bifidobacteria population in the T4 group (positive control) was 8.68 log cfu/g of feces at the end of d 40, and the bifidobacteria population of the T6 group was 0.88 log units greater than that of the T4 group. These results suggest that dietary intake of GOS has a potential prebiotic effect and the presence of symbiosis of the *B. lactis* and GOS combination on fecal microflora of broiler chicken.

### DISCUSSION

In the present study, neither GOS nor *B. lactis*, nor the combination of these additives had a positive or negative effect on BW, feed intake, or feed conversion of broiler chickens. Similar findings were reported when GOS was added to poultry (Biggs et al., 2007) and pig (Mountzouris et al., 2006) diets. Our results combined with the abovementioned studies show that feeding GOS has no benefit on production parameters. Galacto-oligosaccharide is a relatively less studied component in livestock but several studies report that GOS has bifidogenic effects in humans (Alander et al., 2001; Malinen et al., 2002; Gopal et al., 2003). Our in vitro studies with GOS (data not shown) indicated that the maximum growth rate of *Bifidobacterium breve* and *Lactobacillus acidophilus* in GOS syrup (5%, vol/ vol) media were 0.49 and 0.96 h\(^{-1}\) respectively; these are much higher growth rates than those of galactose- and lactose-containing media (Jung and Lee, 2008). The prebiotic GOS used in the study was found to preferentially stimulate the growth of bifidobacteria. We had previously demonstrated that disrupted lactobacillus and acidic fungus cells enhanced the performance of broiler chickens and in some cases enhanced the immune responses, but this effect was dependent on the strain: within bacteria or between bacteria and fungi (Huang et al., 2004). Moreover, in the present experiment the increase in bifidobacteria counts was correlated with GOS dosage compared with the control without GOS. This study was used to monitor microbial populations in broiler chicken feces during a feeding experiment with prebiotic (GOS) or probiotic (*B. lactis* strain), or both. Significantly greater counts of total anaerobes \( (P < 0.05) \), lactobacilli \( (P < 0.05) \), and bifidobacteria \( (P < 0.05) \) were found in feces of broiler chickens fed GOS compared with controls. In particular, significantly greater numbers of bifidobacteria counts were found in feces of chickens fed the mixture of *B. lactis* and GOS compared with the *B. lactis* group.

The potential of GOS to significantly modify intestinal microflora has previously been demonstrated in other animal species. For example, in studies conducted with pigs, dietary GOS significantly increased fecal populations of lactobacilli and bifidobacteria (Smiricky-Tjardes et al., 2003). Rowland and Tanaka (1993) studied the effects of a GOS-containing diet on gut flora metabolism in rats and reported significant increases in total anaerobes, bifidobacteria, and lactobacilli with significant decreases in enterobacteria. Similarly, Ito et al. (1990) observed significant increases in bifidobacteria and lactobacilli populations with significant decreases in *Bacteroides* and *Candida* populations on the human intestinal microflora. These studies showed that the intake of GOS significantly modified the fecal microbiota.

The current study suggests that the prebiotic effects of GOS may be dose dependent, host specific, and preferentially stimulate the growth of bifidobacteria among other beneficial microbes in the chicken intestines. The total number of bifidobacteria increased about 21-fold after feeding a diet containing GOS (3 kg/25 kg) and *B. lactis*. However, feeding birds with *B. lactis* alone did not significantly increase bifidobacteria in chicken.

### Table 3. Effects of galacto-oligosaccharides (GOS) and *Bifidobacterium lactis* on the population of total culturable anaerobic bacteria, lactobacilli, and bifidobacteria in fecal samples (log cfu/g) and d 7 and 40

<table>
<thead>
<tr>
<th>Item</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
<th>T6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total anaerobes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 7</td>
<td>8.98 ± 0.14(^{ab})</td>
<td>8.79 ± 0.05(^{b})</td>
<td>9.36 ± 0.22(^{ab})</td>
<td>9.56 ± 0.17(^{bc})</td>
<td>9.95 ± 0.14(^{a})</td>
<td>9.89 ± 0.06(^{a})</td>
</tr>
<tr>
<td>d 40</td>
<td>9.71 ± 0.04(^{bcd})</td>
<td>9.43 ± 0.14(^{c})</td>
<td>9.84 ± 0.05(^{cd})</td>
<td>9.61 ± 0.13(^{ce})</td>
<td>10.03 ± 0.01(^{cd})</td>
<td>10.26 ± 0.01(^{de})</td>
</tr>
<tr>
<td>Lactobacilli</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 7</td>
<td>8.79 ± 0.05(^{b})</td>
<td>8.74 ± 0.12(^{b})</td>
<td>9.35 ± 0.24(^{ab})</td>
<td>8.76 ± 0.06(^{b})</td>
<td>9.74 ± 0.20(^{b})</td>
<td>9.32 ± 0.18(^{ab})</td>
</tr>
<tr>
<td>d 40</td>
<td>9.66 ± 0.04(^{bc})</td>
<td>9.50 ± 0.22(^{bc})</td>
<td>9.82 ± 0.02(^{ab})</td>
<td>9.52 ± 0.10(^{b})</td>
<td>9.86 ± 0.02(^{bc})</td>
<td>10.19 ± 0.02(^{c})</td>
</tr>
<tr>
<td>Bifidobacteria</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 7</td>
<td>7.53 ± 0.04(^{ab})</td>
<td>7.95 ± 0.08(^{b})</td>
<td>8.00 ± 0.07(^{ab})</td>
<td>7.72 ± 0.07(^{ab})</td>
<td>8.38 ± 0.31(^{a})</td>
<td>8.35 ± 0.09(^{a})</td>
</tr>
<tr>
<td>d 40</td>
<td>8.24 ± 0.24(^{ab})</td>
<td>8.58 ± 0.06(^{b})</td>
<td>9.09 ± 0.21(^{a})</td>
<td>8.68 ± 0.11(^{b})</td>
<td>8.94 ± 0.14(^{bc})</td>
<td>9.56 ± 0.07(^{a})</td>
</tr>
</tbody>
</table>

\(^{a}\)Means with different superscripts within a row differ (Bonferroni t-test, \( P < 0.05 \)).

\(^{b}\)Mean ± standard error of triplicate.

\(^{c}\)T1 = 0.15 kg of corn starch + 1.5 kg of water; T2 = diet 1 + 0.75 kg of GOS; T3 = 0.15 kg of corn starch + 3 kg of GOS; T4 = 1.5 kg of water + 0.15 kg of bifidobacteria; T5 = 0.75 kg of water + 0.15 kg of bifidobacteria + 0.75 kg of GOS; T6 = 0.15 kg of bifidobacteria + 3 kg of GOS.
fects. The results of the present study suggest that the dietary intake of GOS has a potential prebiotic effect and the presence of some symbiotic effect of B. lactis and GOS combination on fecal microflora of broiler chicken. Alander et al. (2001) observed an increase in total bifidobacteria numbers in humans after 1 wk in a group consuming GOS-containing syrup and B. lactis Bb-12. Consumption of NDO in humans is associated with modification of the colonic microbiota with the aim of improving host health. Similarly, NDO are included in animal feed with the aim of improving growth performance and health status of farm animals. It was shown that GOS indeed modified numerous glycolytic activities, with an increase in β-galactosidase and α-glycosidic activities that can improve the fermentation of resistant starch and lactose, thus leading to improved short-chain fatty acid and lactic acid production (Macfarlane and Cummings, 1991). This in turn affected the association of Salmonella with Hep-2 cells (Durant et al., 1999). Some investigators found that oligosaccharides of the fructose and mannose series can effectively suppress enteric pathogens, enhance the immune response, and improve the integrity of the intestinal mucosa in broilers (Huang et al., 2004). Variations in the effectiveness of GOS may be related to differences among strains of Bifidobacterium used as co-additive, the type of animal, and diet components.

In conclusion, the present study indicates that the GOS produced in our lab has important prebiotic effects as demonstrated by increases in the beneficial bacteria population in broiler chickens. Additionally, the combination of GOS and B. lactis yielded greater prebiotic effects and represents an important dietary strategy that could potentially improve the intestinal microflora of chickens after the discontinued use of growth-promoting antibiotics.

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GALACTO-OLIGOSACCHARIDES AND BIFIDOBACTERIA LACTIS


