Effect of dietary prebiotic supplementation on the performance, intestinal microflora, and immune response of broilers

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ABSTRACT This study investigated the effects of dietary supplementation with the prebiotics fructo-oligosaccharide (FOS) and mannan-oligosaccharide (MOS) on the performance, small intestinal microflora, and immune response of broilers. Two hundred forty 1-d-old Ross broiler chickens were randomly assigned to 6 dietary treatment groups: control, avilamycin (6 mg/kg), 0.25% FOS, 0.5% FOS, 0.025% MOS, and 0.05% MOS. Each treatment was fed to 4 replicates of 10 birds per diet for 4 wk. Except for the 0.5% FOS group, the overall BW gains of birds treated with avilamycin and prebiotics were significantly($P < 0.05$) higher than those of the control group. No significant differences were found between the control and supplemented groups in overall feed intake, feed conversion, and mortality. The 0.05% MOS group was significantly ($P < 0.05$) lower than the control and 0.5% FOS groups in heterophil:lymphocyte ratio and basophil level. Concentrations of plasma IgA and IgG were not significantly different among the treatment groups. Quantitative real-time PCR indicated that supplementation of the diet with avilamycin or prebiotics caused significant ($P < 0.05$) changes in the small intestinal microbial community, as determined in samples obtained at the ileocecal junction. The populations of *Clostridium perfringens* and *Escherichia coli* decreased with 0.25% FOS, 0.05% MOS, or avilamycin, and lactobacilli increased in the 0.25% FOS and 0.25% MOS treatment groups. Total bacteria increased in the 0.25% FOS and 0.05% MOS treatments and decreased in the avilamycin treatment. Feeding 0.25% FOS and 0.05% MOS resulted in an increase in lactobacillus community diversity in the ileum. Our results showed that 0.25% FOS and 0.05% MOS were comparable with avilamycin in improving productivity in broilers raised in wire floor cages up to 28 d of age. Plasma immunoglobulins were not affected by prebiotics, but the heterophil:lymphocyte ratio, basophil level, and microbial population in the ileum were significantly affected.

Key words: prebiotic, fructo-oligosaccharide, mannan-oligosaccharide, broiler, microflora, avilamycin

2011 Poultry Science 90:75–82
doi:10.3382/ps.2010-00732

INTRODUCTION

Dietary antibiotics are reported to have beneficial effects on animal and poultry growth and feed conversion efficiency and the inhibition of pathogen growth (Stutz and Lawton, 1984; Gaskins et al., 2002). The recent European Union ban on the prophylactic use of in-feed antibiotics has escalated the search for alternatives for use within the poultry industry (Janardhana et al., 2009). The use of compounds that may have prebiotic effects is a possible way to improve intestinal health and animal performance in the absence of antibiotic growth promoters. A prebiotic compound is defined as a nondigestible food ingredient that can be utilized by intestinal microflora, which beneficially affects the host (Gibson and Roberfroid, 1995).

Oligosaccharides are carbohydrates composed of short chains of monosaccharides. Some are thought to enhance the growth of beneficial organisms in the gut, and others are thought to function by competing with pathogenic bacteria for attachment sites in the lumen. In this way, prebiotic oligosaccharides may improve host health. Two of the most commonly studied prebiotic oligosaccharides are fructo-oligosaccharides (**FOS**) and mannan-oligosaccharides (**MOS**). Fructo-oligosaccharides are found naturally in some cereal crops and onions (Bailey et al., 1991), and MOS are obtained from the cell walls of yeast (*Saccharomyces cerevisiae*). Fructo-oligosaccharides can be fermented by bifidobacteria and lactobacilli (Hidaka et al., 1986; Bouhnik et al., 1994; Gibson and Roberfroid, 1995), which are generally classified as beneficial bacteria (Mizutani and Mitsuoka, 1980; Kawase, 1982; Gibson

Received February 24, 2010.
Accepted October 19, 2010.
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and Wang, 1994; Flickinger et al., 2003). Fructo-oligosaccharides may help to control or reduce the growth of harmful bacteria such as *Clostridium perfringens*—especially important to the poultry industry because it is a primary cause of necrotic enteritis—which has been estimated to cost the worldwide poultry industry $2 billion each year (Hofacre et al., 2005).

Mannose is the main component of MOS and is unique because it is bound by the type 1 fimbriae used by many enteric bacteria to attach to host cells. Therefore, mannose can result in the movement of undesirable bacteria through the intestine without colonization (Newman, 1994). Supplemental MOS has also been shown to increase the production of IgA in laying hens (Kim et al., 2009), rats (Kudoh et al., 1999), and dogs (Swanson et al., 2002a,b). Immunoglobulin A inhibits the attachment and penetration of bacteria in the lumen, increases the production of mucus (McKay and Perdue, 1993), and prevents inflammation that could cause epithelial tissue damage (Russell et al., 1989).

Traditionally, studies on the intestinal microbiota of chickens have used culture-based methods. Only 10 to 60% of total gastrointestinal tract bacteria are estimated to be culturable, so our understanding of intestinal microbiota may be inaccurate and is certainly incomplete (Gong et al., 2007). Culture-independent molecular approaches based on the 16S ribosomal RNA (**rRNA**) gene allow for faster and more detailed detection of changes in the composition of the microbial communities of various ecosystems than do cultivation methods (Janczyk et al., 2009). Polymerase chain reaction-denaturing gradient gel electrophoresis (**DGGE**) has been widely used to investigate changes in the intestinal microbial communities of humans and farm animals. Real-time PCR can quantify the number of 16S rRNA genes in a DNA sample extracted directly from an environmental sample. This technique has been successfully applied to the quantitative analyses of chicken intestinal microbial communities in feeding trials (Wise and Siragusa, 2007). The objective of this study was to compare the effects of supplementation of FOS and MOS on performance and on the small intestinal microflora and immune response of broiler chickens.

MATERIALS AND METHODS

Birds, Diets, and Nutrient Analysis

A total of 240 one-day-old Ross broiler chicks (120 birds of each sex) were randomly assigned to 1 of 6 dietary treatments. Each treatment had 4 replicates of 10 birds (5 birds of each sex). Birds were raised in wire-floored batteries (width: 76 cm; length: 76 cm; height: 50 cm) and diets were fed ad libitum for 28 d. Body weight gain and feed intake were recorded on d 14 for the starter period (0 to 2 wk) and d 28 for the grower period (3 to 4 wk; Table 1). Dietary treatments were a control, avilamycin (6 mg/kg), **FOS 0.25** (0.25% Sun-Oligo), **FOS 0.5** (0.5% Sun-Oligo), **MOS 0.025** (0.1% Bio-Mos), and **MOS 0.05** (0.2% Bio-Mos). Supplements were added at the expense of wheat shorts (2,070 kcal of ME, 17.60% CP), which are fine particles of bran, germ, flour, and tailings (Kellems and Church, 2002). Avilamycin (3% avilamycin and 97% carrier as wheat shorts; Elanco Co. Ltd., Greenfield, IN) was used as the positive control because it is still used commercially as an antibiotic growth promoter. It belongs to the orthosomycin group, which is not absorbed in the intestine and is effective on harmful intestinal bacteria (Boll et al., 2006) . The FOS was supplied as Sun-Oligo (95% fructo-oligosaccharide powder; 2,860 kcal of ME, 0% CP; Samyang Genex Co., Seoul, Korea), composed of 1-kestose, nystose, and 1^{f}-β-fructofuranosyl nystose. The MOS was supplied as Bio-Mos (25% mannan-oligosaccharides; 2,053 kcal of ME, 30% CP; Alltech, Nicholasville, KY). Nutrients were analyzed based on AOAC (1990) official methods: CP, method 990.02; Ca, method 935.13; available P, method 965.17; Lys, method 975.44, and Met + Cys; method 985.28. Minerals were determined by inductively coupled plasma spectrometry (Optima 5300 DV, Perkin Elmer Inc., Shelton, CT) following the Hardware Guide of the Optima 5000 Series instrument, and amino acids were determined with an Amino Acid Analyzer (S433D, Sykam GmbH, Eresing, Germany) following the Sykam Manual for Amino Acid Analyzer S433.

Preparation of Biological Samples

At the end of wk 4, 8 birds (equal numbers of each sex) from each treatment group were killed by cervical dislocation, a method approved by the Animal Care Committee of Chung-Ang University. Immediately after cervical dislocation, blood samples (5 mL each) were collected by heart puncture using EDTA-treated Vacutainer tubes and a One-Use Holder (Becton Dickinson, Franklin Lakes, NJ). The whole blood samples were kept on ice and provided for immediate analysis of hematology. The gastrointestinal tract was removed from the carcasses, 10-cm segments of the upper part of the ileocecal junction were dissected, and approximately 2 g of ileal content was aseptically collected into a 2-mL Eppendorf tube. The ileocecal contents were immediately frozen at −40°C until use. The frozen samples were kept at 4°C for 12 h before isolation of genomic DNA.

Analyses of Blood Parameters and Serum Immunoglobulin

Leucocytes (white blood cells, heterophils, lymphocytes, monocytes, eosinophils, basophils) of blood samples were analyzed using Hemavet Multispecies Hematology Systems (Drew Scientific Inc., Oxford, CT). Immediately after blood analysis, plasma samples were obtained by centrifuging for 20 min at 25,000 × *g* at

Table 1. Composition of the experimental broiler diets

Item	Control	Avilamycin	FOS 0.25	FOS 0.5	MOS 0.025	MOS 0.05
Ingredient (%)						
Corn, US No. 3	54.05	54.05	54.05	54.05	54.05	54.05
Soybean meal	25.71	25.71	25.71	25.71	25.71	25.71
Wheat shorts	4.00	3.98	3.75	3.50	3.975	3.95
Feather meal	1.50	1.50	1.50	1.50	1.50	1.50
Corn gluten meal	6.74	6.74	6.74	6.74	6.74	6.74
Dicalcium phosphate	2.08	2.08	2.08	2.08	2.08	2.08
Tallow	4.00	4.00	4.00	4.00	4.00	4.00
Limestone	1.08	1.08	1.08	1.08	1.08	1.08
Sodium chloride	0.20	0.20	0.20	0.20	0.20	0.20
L-Lys-HCl	0.39	0.39	0.39	0.39	0.39	0.39
Premix[1]	0.10	0.10	0.10	0.10	0.10	0.10
Choline chloride	0.15	0.15	0.15	0.15	0.15	0.15
Avilamycin[2]	—	0.02	—	—	—	—
Fructo-oligosaccharide[3] (FOS)	—	—	0.25	0.50	—	—
Mannan-oligosaccharide[4] (MOS)	—	—	—	—	0.025	0.05
Total	100.00	100.00	100.00	100.00	100.00	100.00
Nutrient content[5]						
ME (kcal/kg)	3,100	3,100	3,102	3,104	3,100	3,101
CP (%)	21.90	21.90	21.86	21.81	21.90	21.91
Ca (%)	0.91	0.91	0.91	0.91	0.91	0.91
Available P (%)	0.49	0.49	0.49	0.49	0.49	0.49
Lys (%)	1.30	1.30	1.30	1.30	1.30	1.30
Met + Cys (%)	0.78	0.78	0.78	0.78	0.78	0.78

[1]Provided per kilogram of diet: vitamin A, 12,500 IU; vitamin D_3, 2,500 IU; vitamin E, 20 IU; vitamin K_3, 2 mg; vitamin B_1, 2 mg; vitamin B_2, 5 mg; vitamin B_6, 3 mg; vitamin B_{12}, 18 μg; calcium pantothenate, 8 mg; folic acid, 1 mg; biotin, 50 μg; niacin, 24 mg; Zn, 60 mg; Mn, 50 mg; Fe, 50 mg; Cu, 6 mg; Co, 250 μg; I, 1 mg; Se, 150 μg.

[2]Avilamycin (3% avilamycin, Elanco Co. Ltd., Greenfield, IN).

[3]Sun-Oligo (95% fructo-oligosaccharides, Samyang Genex Co. Ltd., Seoul, Korea).

[4]Bio-Mos (25% mannan-oligosaccharides, Alltech, Nicholasville, KY).

[5]Nutrient contents of the control diet were analyzed, except ME, which was calculated based on NRC (1994). Nutrient contents of diets with supplements were calculated.

room temperature and were stored at −15°C until measurement of IgG and IgA. The plasma IgG and IgA were measured using ELISA Quantitation Kits (Bethyl Laboratories, Montgomery, TX) of chicken IgG (Cat. No. K0231089) and IgA (Cat. No. K0231034).

Analysis of Intestinal Microflora

Real-Time PCR Analysis. Genomic DNA was isolated from 250 mg of ileal content using an UltraClean Fecal DNA Kit (Mobio, Carlsbad, CA) and was stored at −20°C until use. The colonizations of total bacteria, *C. perfringens*, *Escherichia coli*, and lactobacilli were analyzed by real-time PCR. Sample genomic DNA was used as a template for PCR amplification using SYBR Green PCR technology (Applied Biosystems, Foster City, CA) and an ABI 7500 real-time PCR instrument (Applied Biosystems). Species-specific 16S rRNA primers were used for the *C. perfringens* group [F: 5′-ATGCAAGTCGAGCGA(G/T)G-3′; R: 5′-TATGCGGTA TTAATCT(C/T)CCTT T-3′, where F is forward and R is reverse], the *E. coli* subgroup (F: 5′-GTTAATACCTTTGCTCATTGA-3′, R: 5′-ACCAGGGTATCTAATCC TGT-3′), and the *Lactobacillus* spp. (F: 5′-AGCAGTAG GGAATCTTCCA-3′, R: 5′-CACCGCTACACATGGAG-3′). Univ-518F (5′-CCAGCAGC- CCGCGGTAATACG-3′) and Univ-800R (5′-TACCAG GGTATCTA ATCC-3′) were used as universal primers (Malinen et al., 2005). A quantitative real-time PCR-based method was used to measure the total concentrations of bacteria, lactobacilli, *C. perfringens*, and *E. coli* in the contents of the upper ileocecal junction segments. Species-specific primers amplified 318 bp for total bacteria-specific amplicons, 341 bp for *Lactobacillus* spp. amplicons, 120 bp for *C. perfringens* amplicons, and 340 bp for *E. coli* amplicons. Amplification was performed in 20 μL containing 10 μL of 2× SYBR Green PCR Master Mix (Applied Biosystems), 2 μL of primer (1 μL of forward and 1 μL of reverse in each), 1 μL of template, and 7 μL of PCR-grade water. Standard curves were constructed using the PCR product of the 16S rRNA gene from the target species genomic DNA preparations at 1, 10, 100, and 1,000 pg/μL. Absolute quantification was achieved using standard curves constructed by amplification of known amounts of target DNA, following the mathematical model of Rutledge and Cote (2003).

DGGE Analysis of Lactobacilli. *Lactobacillus*-specific regions of the 16S rRNA gene were amplified using the primers Lac1 (5′-AGCAGTAGGGAATCTTCCA-3′) and Lac2-GC (5′-CGCCCGGGGCGCGCCCGGGCGGCCCGGGGGCACCGGGGGATTTCACCGCTACACATG-3′; Walter et al., 2001). After visual confirmation of PCR products by agarose gel electrophoresis, DGGE was performed using the Bio-Rad D-code system (Bio-Rad Laboratories Inc., Hercules, CA) as described by the manufacturer. A 6% acrylamide gel with 30 to 50% denaturant was used, where 100% denaturant was

Table 2. Body weight gain, feed intake, feed efficiency, and mortality of broiler chickens fed experimental diets for 4 wk[1]

	Treatment[2]						
Item	Control	Avilamycin	FOS 0.25	FOS 0.5	MOS 0.025	MOS 0.05	SEM
BW gain (g/bird)							
0 to 2 wk	404.6	396.4	410.5	386.6	393.9	404.6	10.10
3 to 4 wk	944.0^{b}	986.4^{a}	974.2^{ab}	956.6^{ab}	967.5^{ab}	975.1^{ab}	9.93
0 to 4 wk	1,348.2^{c}	1,382.8^{a}	1,384.8^{a}	1,343.3^{c}	1,361.4^{b}	1,379.7^{a}	3.66
Feed intake (g/bird)							
0 to 2 wk	655.7	633.1	655.8	665.8	621.5	647.3	22.39
3 to 4 wk	1,536.2^{ab}	1,596.1^{a}	1,575.0^{ab}	1,525.5^{b}	1,591.4^{a}	1,577.9^{ab}	19.35
0 to 4 wk	2,191.8	2,229.3	2,230.7	2,191.4	2,212.9	2,225.3	20.51
Feed conversion (feed:gain)							
0 to 2 wk	1.62	1.60	1.60	1.72	1.57	1.60	0.063
3 to 4 wk	1.62	1.62	1.61	1.59	1.64	1.62	0.030
0 to 4 wk	1.62	1.61	1.61	1.62	1.62	1.61	0.012
Mortality (%)							
0 to 2 wk	2.50	0.00	0.00	2.50	0.00	2.50	1.767
3 to 4 wk	0.00	0.00	2.50	0.00	0.00	0.00	1.020
0 to 4 wk	2.50	0.00	2.50	2.50	0.00	2.50	2.040

[a–c]Values with different superscripts in the same row are significantly different ($P < 0.05$).

[1]Values are the means of 4 pens of 10 birds per pen.

[2]Control = control diet; avilamycin = control diet + 6 mg/kg of avilamycin (Elanco Co. Ltd., Greenfield, IN); FOS 0.25 = control diet + 0.25% Sun-Oligo (Samyang Genex Co. Ltd., Seoul, Korea); FOS 0.5 = control diet + 0.5% Sun-Oligo; MOS 0.025 = control diet + 0.1% Bio-Mos (Alltech, Nicholasville, KY); MOS 0.05 = control diet + 0.2% Bio-Mos.

7 *M* urea and 40% deionized formamide. Electrophoresis was performed at 60°C and 70 V for 16 h. Gels were stained with ethidium bromide, destained in distilled water, and viewed using a Gel Doc 2000 image analysis system (Bio-Rad Laboratories Inc.). The DGGE bands were removed and placed into a 1.5-mL vial with 20 μL of sterilized distilled water and kept at 4°C overnight to allow for passive diffusion into the water, before 1 μL of the eluted ribosomal DNA was amplified with the *Lactobacillus*-specific primers Lac1F (5′-AGCAGTAGGGAATCTTCCA-3′) and Lac2R (5′-ATTTCACCGCTACACATG-3′; Walter et al., 2001). The PCR products were cloned in *E. coli* JM109 using a pGEM-T Easy Vector System (Promega, Madison, WI). Plasmid DNA from selected transformants was isolated using a QIAprep Spin Miniprep Kit (Qiagen, Valencia, CA). Sequence analysis was performed using purified plasmid DNA and the universal primers T7 and SP6 (SolGent Co. Ltd., Korea). Analysis of sequence data and sequence similarity was performed using the Basic Local Alignment Search Tool of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/).

Statistical Analysis

Data were subjected to ANOVA using a GLM procedure (SAS Institute, 2000). Significant differences among treatment means were measured by Duncan's multiple range test at $P < 0.05$ (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

No significant differences were seen among treatments for feed intake, feed:gain, or mortality during the overall period (0 to 4 wk; Table 2). Body weight gain in the starter period (0 to 2 wk) was not significantly different, but BW gain for the grower (3 to 4 wk) and overall periods differed by treatment. The avilamycin, FOS 0.25, and MOS 0.05 groups showed significantly greater overall BW gains than did birds in the MOS 0.025 treatment group, and BW gain in this group was greater than BW in the FOS 0.5 and control groups. The avilamycin and MOS 0.025 groups had significantly higher feed intake than did the FOS 0.5 group during the grower period (3 to 4 wk). No significant differences were seen in feed conversion and mortality among treatments. It was previously reported that BW gain and feed conversion ratio were improved in broilers fed diets supplemented with 0.2 to 0.4% FOS (Xu et al., 2003). In the present experiment, birds in the FOS 0.5 treatment group gained less BW than did birds in the other prebiotic treatment groups over 0 to 4 wk. Mikkelsen et al. (2004) reported that a high level of FOS increases gas production by intestinal microbiota, which causes diarrhea. Dietary mannan oligosaccharides improved broiler and turkey performance at a level of 0.0125 to 0.1% (Hooge, 2003; Sims et al., 2004) and improved the egg production of layers at a level of 0.025 to 0.05% (Woo et al., 2007; Kim et al., 2009).

The heterophil:lymphocyte ratio and basophil leukocytes were higher in the control and FOS 0.5 groups compared with those of the other treatments (Table 3). Because few reports are available regarding this topic, more studies may be needed to verify the effects of prebiotics on leukocytes in poultry.

Plasma IgA and IgG concentrations were not significantly different between groups (Table 3). In other studies, however, IgA was increased in laying hens (Kim et al., 2009) and dogs (Swanson et al., 2002a,b) by supplementation of FOS and MOS. Cetin et al. (2005) report-

Table 3. Effects of prebiotics and avilamycin on the parameters of leucocytes and immunoglobulins in the blood of broilers[1]

	Treatment[2]						
Item	Control	Avilamycin	FOS 0.25	FOS 0.5	MOS 0.025	MOS 0.05	SEM
Leukocyte[3]							
WBC (thousand/μL)	23.72	22.95	22.94	23.05	23.52	21.97	0.998
HE (thousand/μL)	8.90	8.44	8.63	8.86	8.60	7.96	0.381
LY (thousand/μL)	10.23	9.73	9.92	9.57	10.61	9.72	0.482
H:L	0.92[a]	0.83[ab]	0.87[ab]	0.92[a]	0.82[ab]	0.81[b]	0.031
MO (thousand/μL)	2.64	2.62	2.44	2.53	2.61	2.47	0.121
EO (thousand/μL)	1.48	1.19	1.43	1.45	1.26	1.31	0.104
BA (thousand/μL)	0.67[a]	0.49[ab]	0.52[ab]	0.62[ab]	0.50[ab]	0.41[b]	0.062
Immunoglobulin							
IgG (mg/mL)	6.63	6.73	6.83	6.92	6.74	6.95	0.339
IgA (mg/mL)	5.86	5.98	5.86	5.75	5.91	5.99	0.451

[a,b]Values with different superscripts in the same row are significantly different ($P < 0.05$).

[1]Values are the means of 8 birds per diet.

[2]Control = control diet; avilamycin = control diet + 6 mg/kg of avilamycin (Elanco Co. Ltd., Greenfield, IN); FOS 0.25 = control diet + 0.25% Sun-Oligo (Samyang Genex Co. Ltd., Seoul, Korea); FOS 0.5 = control diet + 0.5% Sun-Oligo; MOS 0.025 = control diet + 0.1% Bio-Mos (Alltech, Nicholasville, KY); MOS 0.05 = control diet + 0.2% Bio-Mos.

[3]Leukocytes: WBC = white blood cells; HE = heterophils; LY = lymphocytes; MO = monocytes; EO = eosinophils; BA = basophils; H:L = heterophil:lymphocyte ratio.

ed that IgG levels were increased by MOS supplementation in turkeys, and Woo et al. (2007) reported that IgG was increased by MOS supplementation in layers. Yin et al. (2008) reported that supplementation with a prebiotic oligosaccharide galacto-mannan increased the serum levels of IgA, IgG, and IgM compared with the level of lincomycin in early-weaned pigs.

Avilamycin treatment significantly decreased the total microbial population and the populations of *E. coli* and *C. perfringens* compared with those of the control group (Figure 1). Except for the FOS 0.5 group, the prebiotic treatment groups showed significantly higher lactobacillus levels than did the avilamycin and control treatment groups. The populations of *E. coli* and *C. perfringens* were significantly lower in the FOS 0.25, MOS 0.05, and avilamycin treatment groups compared with birds in the FOS 0.5 and control groups. Overall, FOS 0.25 and MOS 0.05 decreased populations of *C. perfringens* and *E. coli* and increased populations of lactobacilli and total bacteria. Choi et al. (1993) reported that FOS supplementation at the level of 0.22% increased bifidobacteria and lactobacilli and decreased *C. perfringens* and *E. coli* populations in the ileal content of broilers. Sims et al. (2004) showed that 6-wk-old turkeys in a MOS treatment group had significantly less *C. perfringens* in their large intestines than did the control birds. Fructo-oligosaccharide may help to control or reduce the growth of harmful bacteria such as *C. perfringens* (Hofacre et al., 2005). Bailey et al. (1991) reported that chicken diets with 0.375% FOS had little effect on the colonization of *Salmonella enterica* serovar Typhimurium, whereas diets containing 0.75% FOS led to a 12% lower level of *Salmonella* Typhimurium than in the control group. Choi et al. (1994) reported that FOS supplementation of broiler diets at a level of 0.22% suppressed cecal *Salmonella* Typhimurium colonization and alleviated the depression of broiler performance induced by *Salmonella* Typhimurium invasion. Most previous studies have focused on the effect of dietary prebiotics on the composition of intestinal microflora, and many have assumed that improvements in host resistance to intestinal infections were likely. In the present experiment, birds in the FOS 0.5 treatment group gained less BW and had less intestinal lactobacilli and more *E. coli* and *C. perfringens* than those in the FOS 0.25 treatment group. This result indicates that supplementation of FOS at the level of 0.5% was excessive. Ten Bruggencate et al. (2005) reported that rapid fermentation of FOS by endogenous microflora impairs the gut mucosal barrier, as indicated by increased intestinal permeability before infection. Lactobacilli have been detected in the chicken small intestine in other studies. In this present study, lactobacilli were detected by PCR-DGGE as amplicons of 341 bp (Figure 2). The results clearly demonstrated the variations in bacterial constituents between the supplement groups. Elution of DNA from the bands in Figure 2 (1 to 10) was performed, and the DNA was cloned and sequenced for Basic Local Alignment Search Tool analysis. Sequence similarities for each band are shown in Table 4. Polymerase chain reaction-DGGE showed significant changes in the lactobacillus community of the ileum. Antibiotic treatment resulted in a decrease in diversity, whereas probiotics increased the diversity of the lactobacilli. Bands 2 and 7 in Figure 2 were present in most samples from birds treated with prebiotics. These bands were closely related to *Lactobacillus crispatus* and *Lactobacillus salivarius.* Band 2 was present at a very high intensity in all antibiotic-treated birds, and sequence analysis revealed that it was closely related to *L. crispatus.* This result implies that *L. crispatus* may have high antibiotic resistance, consistent with the results of a previous report (Cauwerts et al., 2006).

In this study, we showed the effects of prebiotics on the intestinal microbiota of broilers. Feeding FOS 0.25 and MOS 0.05 to broilers resulted in an increase in di-

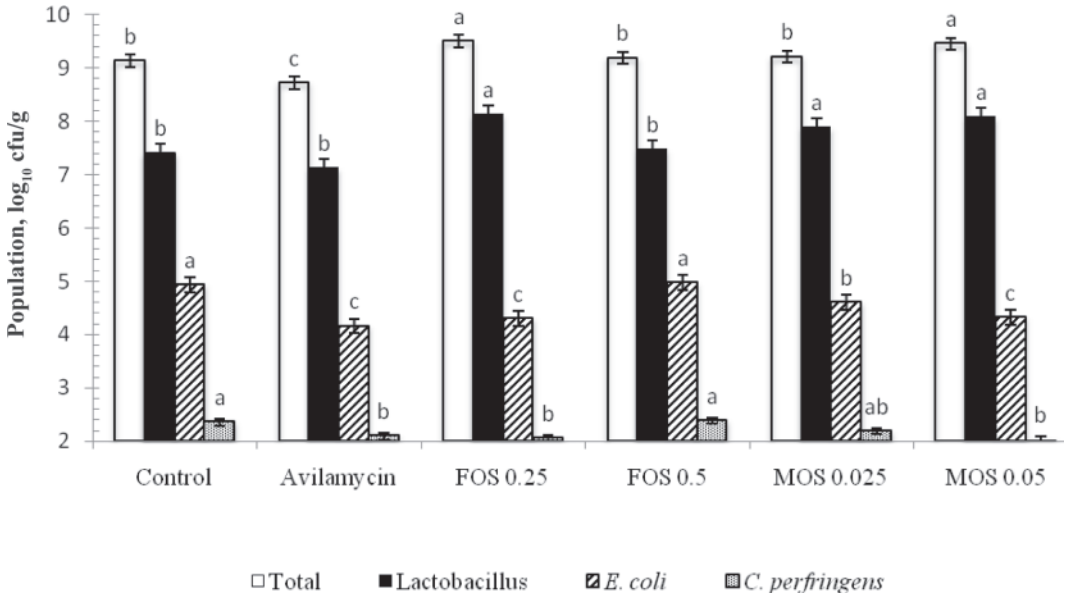

Figure 1. Microbial populations in the small intestinal content of broiler chickens at 4 wk of age. Total bacteria, *Clostridium perfringens*, *Escherichia coli*, and lactobacilli in ileocecal junction samples were analyzed by real-time PCR. Within a bacterial species (or total), bars with different letters (a–c) are different ($P < 0.05$, n = 8). Control = control diet; avilamycin = control diet + 6 mg/kg of avilamycin (Elanco Co. Ltd., Greenfield, IN); FOS 0.25 = control diet + 0.25% Sun-Oligo (Samyang Genex Co. Ltd., Seoul, Korea); FOS 0.5 = control diet + 0.5% Sun-Oligo; MOS 0.025 = control diet + 0.1% Bio-Mos (Alltech, Nicholasville, KY); MOS 0.05: control diet + 0.2% Bio-Mos.

versity in lactobacillus DGGE fingerprints in the ileum. Because greater diversity of the intestinal tract microbiota community is believed to have a positive effect on the welfare and productivity of the host bird (Janczyk et al., 2009), the changes observed in this study suggest the possibility of long-term feeding (e.g., >28 d) with FOS and MOS as an alternative to antibiotic growth promoters. Because the birds were housed in raised wire-floor cages in the present study, it is acknowledged that performance results could be different if birds were grown on litter in floor pens. Our results showed that supplementation with 0.25% FOS and 0.05% MOS performed better than supplementation with 0.5% FOS and 0.025% MOS in broiler production. Plasma immunoglobulins were not affected but the heterophil:lymphocyte ratio, and basophil levels were significantly affected by prebiotics. Feeding FOS 0.25 and MOS 0.05 increased the diversity and populations

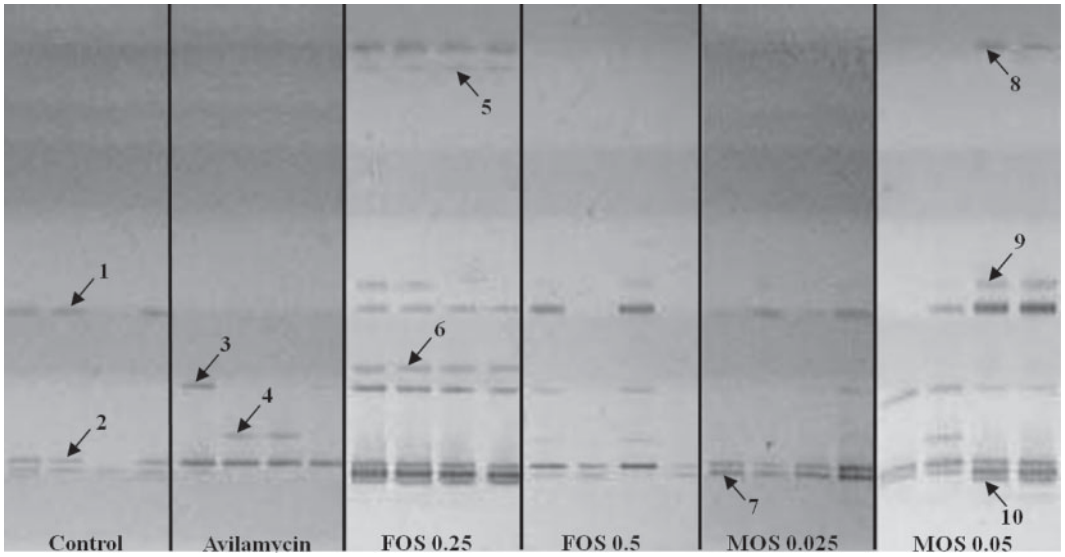

Figure 2. Polymerase chain reaction-denaturing gradient gel electrophoresis of *Lactobacillus*-specific PCR products (341 bp) from ileocecal junction samples of broiler chickens at 4 wk of age. Bands 1 to 10 refer to the corresponding clones in Table 4 (n = 4). Control = control diet; avilamycin = control diet + 6 mg/kg of avilamycin (Elanco Co. Ltd., Greenfield, IN); FOS 0.25 = control diet + 0.25% Sun-Oligo (Samyang Genex Co. Ltd., Seoul, Korea); FOS 0.5 = control diet + 0.5% Sun-Oligo; MOS 0.025 = control diet + 0.1% Bio-Mos (Alltech, Nicholasville, KY); MOS 0.05 = control diet + 0.2% Bio-Mos.

Table 4. Sequence analysis of *Lactobacillus*-specific PCR-denaturing gradient gel electrophoresis bands[1]

Band no.	NCBI BLAST match[2]	NCBI accession no.	Sequence similarity (%)
1	*Lactobacillus aviarius* ssp. *aviarius*	AB326355	100
2	*Lactobacillus crispatus*	AY335500	100
3	*Lactobacillus johnsonii*	AB425921	99
4	*Lactobacillus helveticus*	AB446394	99
5	*Weissella cibaria*	GU223368	99
6	*Lactobacillus agilis*	AB425919	100
7	*Lactobacillus salivarius*	AB425928	100
8	*Lactobacillus plantarum*	GU138613	99
9	*L. aviarius*	AB175728	100
10	*L. agilis*	AB425914	100

[1]Amplicon sizes of the PCR products were 341 bp. NCBI = National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/); BLAST = Basic Local Alignment Search Tool.

[2]Only the highest matches are presented.

of lactobacilli, and decreased the populations of *E. coli* and *C. perfringens* in the ileum of broilers.

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