Cecal Populations of Lactobacilli and Bifidobacteria and *Escherichia coli* Populations After In Vivo *Escherichia coli* Challenge in Birds Fed Diets with Purified Lignin or Mannanoligosaccharides

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ABSTRACT Two experiments were conducted to evaluate lignin and mannanoligosaccharides as alternatives to antibiotic growth promoters in broilers. Dietary treatments for the 2 studies were 1) negative control (CTL–, antibiotic free); 2) positive control (CTL+, diet 1 + 11 mg of virginiamycin/kg); 3) mannanoligosaccharide (MOS; diet 1 + BioMos: 0.2% to 21 d and 0.1% thereafter); 4) LL (diet 1 + 1.25% Alcell lignin); and 5) HL (diet 1 + 2.5% Alcell lignin). In experiment 1, each treatment was assigned to 4 pen replicates (52 birds each). Body weight and feed intake were recorded weekly for 38 d. At 28 and 38 d, cecal contents were assayed for lactobacilli and bifidobacteria. Body weight and feed intake did not differ among dietary treatments. At d 38, the lactobacilli population was greatest ($P < 0.05$) in birds fed MOS, whereas LL-fed birds had greater ($P < 0.05$) lactobacilli load than those fed CTL+. Bifidobacteria load was greater ($P < 0.05$) in birds fed MOS or LL compared with those fed CTL+ at both d 28 and 38. However, at d 28 and 38, lactobacilli and bifidobacteria loads were lowest ($P < 0.05$) in CTL+ or HL-fed birds. In experiment 2, 21-d-old birds from the initial flock were transferred to cages for oral *Escherichia coli* (O2 and O88 serotypes) challenge (12 birds/treatment). After 3, 6, and 9 d, cecal loads of *E. coli* were determined. Birds fed HL had a lower *E. coli* load ($P < 0.05$) than birds fed CTL– or CTL+ at d 3, and lower than birds fed CTL– at d 6. At d 9, the *E. coli* load was lower ($P < 0.05$) in birds fed MOS or HL than in those fed the CTL– or CTL+ diets; LL-fed birds had lower *E. coli* load than those fed CTL–. Birds fed MOS or LL had a comparative advantage over CTL+ birds in increasing populations of lactobacilli and bifidobacteria and lowering *E. coli* loads after challenge.

Key words: antibiotic, mannanoligosaccharides, lignin, food safety, poultry

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INTRODUCTION

The subtherapeutic usage of antibiotic growth promoters (**AGP**) is under intense scientific and public scrutiny because their use has been linked to the development of antibiotic-resistant pathogenic bacteria, which pose a threat to human health (Smith et al., 2003). At the same time, food safety remains a major public health concern worldwide. The principal pathogenic bacteria causing foodborne illnesses are *Campylobacter*, *Salmonella*, and *Escherichia coli* (Mead et al., 1999). The critical point of bacterial contamination of poultry products occurs at the slaughter house when pathogens in the intestinal contents make contact with chicken carcasses (Heyndrickx et al., 2002). Different strains of antibiotic-resistant *E. coli* have been isolated from poultry and poultry meat products in several countries (Sackey et al., 2001; Zhao et al., 2001; Mayrhofer et al., 2004). Human infections with antibiotic-resistant pathogenic bacteria are more difficult to treat and therefore increase hospitalization costs (Mead et al., 1999; Lees and Aliabadi, 2002).

The threat of antibiotic-resistant bacteria and global demands for safe poultry products have prompted the need for effective biological modulators of enteric microflora in the poultry industry, and in this context, there is increased interest in the use of prebiotics. Mannanoligosaccharides and purified lignin have the potential to eliminate or kill intestinal pathogenic bacteria (Nelson et al., 1994; Newman, 1994), but very little research on these additives has been carried out with poultry. Spring et al. (2000) and Fernandez et al. (2002) observed that when broilers were fed diets containing a commercial mannanoligosaccharide (BioMos, Alltech Inc., Nicholasville, KY) and challenged with pathogenic strains of *Salmonella*, the cecal populations of these specific strains of *Salmonella* were significantly reduced. However, in turkeys challenged with pathogenic strains of *E. coli*, the mannanoli-

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gosaccharide did not affect the total intestinal population of *E. coli* (Fairchild et al., 2001). There are no research reports on broilers fed mannanoligosaccharide and challenged with *E. coli*.

The polyphenolic fragments of Alcell lignin (Alcell Technologies Inc., Montreal, Québec, Canada) inhibited the growth of aerobic bacteria in the cecum of rats, and inhibited the in vitro growth of *E. coli*, *Pseudomonas*, and *Staphylococcus aureus* (Nelson et al., 1994). There are no published studies on the effects of lignin on in vivo growth of *E. coli*. Nevertheless, research findings suggest that lignin could have a role in reducing the intestinal load of pathogenic bacteria in poultry, thereby improving the safety of poultry products. Given the need for biological additives as alternatives to antibiotics in poultry production, research into lignin and mannanoligosaccharides is very valuable.

The objectives of this study were to evaluate the effects of dietary addition of purified lignin (Alcell lignin) and a mannanoligosaccharide (BioMos) to broiler diets free of antibiotics on 1) cecal populations of lactobacilli and bifidobacteria in birds grown under normal conditions and 2) cecal populations of total *E. coli* after an in vivo challenge with known pathogenic strains of *E. coli*; and also to compare these effects with a diet containing an antibiotic growth promoter (virginiamycin).

MATERIALS AND METHODS

Bird Management

One thousand and forty 1-d-old male Cobb 500 broilers were obtained from a commercial local hatchery (Couvoir Simetin, Mirabel, Québec, Canada) and grown over a 38-d experimental period. Birds were randomly assigned to 1 of 5 dietary treatments (4 pen replicates; 52 birds per pen). Each pen was covered with 8 cm of clean pine wood shavings and was equipped with 1 tube feeder and 1 automatic waterer. The birds were brooded following a standard temperature regimen that gradually decreased from 32 to 24°C, and under a 20L:4D lighting cycle throughout the studies. Bird management and care were conducted following the animal care protocol approved by the McGill University Animal Care Committee. Birds were group weighed by pen and feed consumption was determined at weekly intervals.

Experimental Diets

The birds were fed a corn-soybean meal based diet. All the diets were formulated to be isonitrogenous and isoenergetic and to meet or exceed NRC (1994) requirements for macro- and micronutrients. A 2-phase feeding program was used, with a starter diet from d 1 to 21 and a grower diet from d 22 to 38. The 5 dietary treatments included 1) negative control diet (**CTL**–, AGP-free); 2) positive control diet (**CTL+**, diet 1 + 11 mg of virginiamycin/kg); 3) **MOS** (diet 1 + BioMos): 0.2% of starter diet and 0.1% of grower diet); 4) **LL** (diet 1 + 1.25% Alcell lignin); and 5) **HL** (diet 1 + 2.5% Alcell lignin).

Enumeration of Lactobacilli and Bifidobacteria

At d 28 and 38, 1 bird from each pen was euthanized by electrical stunning and bleeding of the carotid artery; the ceca were aseptically collected into sterile plastic bags. Bird sampling was performed as described previously (Fernandez et al., 2002; Denev et al., 2005). Samples of the fresh cecal contents were diluted 10-fold by weight in buffered peptone water (Fisher Scientific, Ottawa, Ontario, Canada) and mechanically homogenized using a stomacher (model 400 Lab Blender, Seward Medical, London, UK) for 30 s. The samples were then serially diluted in 0.85% sterile saline solution for enumeration of lactobacilli and bifidobacteria. All microbiological analyses were performed in duplicate and the average values were used for statistical analysis. Lactobacilli were anaerobically assayed using Lactobacilli MRS agar (Fisher Scientific) and incubated at 37°C for 48 h. Bifidobacteria were anaerobically assayed using Wilkins-Chalgren agar (Oxoid, Nepean, Ontario, Canada) supplemented with glacial acetic acid (1 mL/L) and mupirocin (100 mg/L) extracted from antimicrobial discs (Oxoid) (Rada et al., 1999). Petri dishes were incubated at 37°C for 3 d. After the incubation periods, colonies of lactobacilli and bifidobacteria were counted.

E. coli Challenge

Bird Transfer. At d 21, 6 birds from each pen replicate of the initial flock were randomly removed, separated into 2 groups, and transferred to wire cages equipped with individual feeders and nipple drinkers for *E. coli* challenge. For each group, birds (3 birds/cage, 12 birds/dietary treatment) were housed in 2 separate rooms with environmentally controlled conditions following the same temperature and light conditions as above.

E. coli Challenge and Enumeration. Two *E. coli* serotypes (O2 and O88) were used based on pathogenicity to poultry (Menao et al., 2002) and agglutination to MOS (Mirelman et al., 1980). The O2 and O88 serotypes were isolated from chicken carcasses and obtained from the Meat Safety Laboratory, Faculty of Veterinary Medicine (University of Montreal, St Hyacinthe, Québec, Canada). A growth curve was constructed to determine the point at which the cultures reached and maintained a concentration of 10^7 cfu/mL, corresponding to an exponential phase of growth, which was the gavage target dose desired in this study. The *E. coli* concentrations were verified by serial dilutions and plated on sheep blood agar (Oxoid) at 37°C for 24 h.

Before the challenge study, litter samples were screened for *E. coli* to confirm that birds were free from the administered O2 and O88 serotypes of *E. coli*. At d 29, caged birds in the first room were orally challenged with a mixed culture of *E. coli* (O2 and O88 serotypes) at a concentration of 1×10^7 cfu/mL of sterile PBS (pH = 7.2), whereas birds in the second room were orally gavaged with 1 mL of sterile PBS, serving as control.

Table 1. Effects of antibiotics, mannanoligosaccharide, and lignin on BW, feed intake, and feed conversion of broiler chickens[1]

	Treatment[2]					
Age	CTL–	CTL+	MOS	LL	HL	SEM
BW (g)						
d 7	145.00	145.87	142.16	139.16	138.42	2.13
d 21	856.43	839.90	849.54	829.72	831.15	6.77
d 35	2,135.64	2,082.01	2,144.57	2,116.48	2,070.01	18.27
Feed intake (g)						
d 1 to 7	124.33	113.85	115.64	114.01	109.83	4.21
d 1 to 21	966.26	1,013.03	984.26	976.80	922.35	21.77
d 1 to 35	3,968.89	4,088.27	4,014.10	4,041.57	3,964.76	44.65
Feed conversion						
d 1 to 7	0.86	0.78	0.82	0.82	0.79	0.03
d 1 to 21	1.13	1.21	1.16	1.18	1.11	0.03
d 1 to 35	1.86[b]	1.97[a]	1.87[ab]	1.91[ab]	1.92[ab]	0.02

[a,b]Values with different superscripts within the same row are different (Bonferroni *t*-test, $P < 0.05$).

[1]Mean of 4 replicates.

[2]CTL– = antibiotic-free diet; CTL+ = antibiotic-free diet supplemented with 11 mg of virginiamycin/kg; MOS = antibiotic-free diet supplemented with 0.2% and 0.1% BioMos (Alltech Inc., Nicholasville, KY) in the starter (1 to 21 d) and grower feed (22 to 38 d), respectively; LL and HL = antibiotic-free diet supplemented with 1.25 or 2.5% Alcell lignin (Alcell Technologies Inc., Montreal, Québec, Canada), respectively.

At 3, 6, and 9 d postinoculation, 4 birds from each dietary treatment (1 bird per cage) of the 2 groups were euthanized, and the ceca were aseptically removed for enumeration of total *E. coli*. Samples of the fresh cecal contents were serially diluted and plated on Rapid *E. coli* 2 agar (BioRad Laboratories, Mississauga, Ontario, Canada), modified using *E. coli* supplement (BioRad) to be selective for *E. coli* for identification and quantification of *E. coli*. Microbiological analyses of the cecal samples were performed in duplicate and the average values were used for statistical analysis. At each time interval (d 3, 6, and 9), 10 *E. coli* isolates from each sample replicate were subcultured on sheep blood agar and then O-serotyped by agglutination technique (EcL Laboratories, Faculty of Veterinary Medicine, University of Montreal) to verify that the serotypes recovered in the ceca matched those administered. Any isolate of the O2 or O88 serotypes of *E. coli* recovered in the ceca of PBS-gavaged birds and samples of isolates of the same serotypes from *E. coli*-challenged birds were genotyped using the pulsed field gel electrophoresis (**PFGE**) method (Caya et al., 1999) to verify if these were identical to the serotypes used to challenge the birds.

Statistical Analysis

Data were analyzed by 1-way ANOVA using the GLM procedure of SAS (SAS Institute, 2003) with pen as the experimental unit for performance parameters and bird as the experimental unit for microbiological parameters. The ANOVA power of the test was determined using the MIXED procedure of SAS and it was consistently above $1 - \beta = 0.85$ with an $\alpha = 0.05$. Treatment means were separated using the least squares means option of SAS. Differences between treatment means were tested using Bonferroni's multiple comparison test, and statistical significance was declared at a probability of $P < 0.05$. All microbiological concentrations were subject to $\log_{10}$ transformation before analysis.

RESULTS

Bird Performance and Enumeration of Lactobacilli and Bifidobacteria

Dietary treatments did not alter growth performance or feed intake (Table 1). Feed conversion ratio was not different among dietary treatments up to d 28. However, at d 35, feed conversion ratio was greater in birds fed the CTL+ diet than in those fed the CTL– diet; there were no other treatment effects on feed conversion ratio.

At d 28, the cecal population of lactobacilli was greatest in birds fed MOS or CTL–, but there was no difference between these 2 treatments (Figure 1). At d 38, the lactobacilli population was significantly increased in birds fed the MOS diet compared with the other treatments. Birds fed the CTL+ or HL diet had lower lactobacilli loads than those fed the CTL– diet at both d 28 and 38. However, the magnitude of reduction with HL was much more pronounced at d 28 than at d 38. Birds fed the LL diet showed increased cecal populations of lactobacilli compared with those fed the CTL+ or HL diet at d 28 and 38. However, the lactobacilli load was not different between birds fed LL or CTL– at d 38, but the lactobacilli load was lower in LL-fed birds at d 28.

Neither MOS nor LL altered the cecal population of bifidobacteria compared with the CTL– diet at d 28 (Figure 2). However, at d 38, birds fed the LL diet had smaller populations of bifidobacteria than those fed the MOS or CTL– diet. When compared with the CTL–, MOS, or LL diet, the CTL+ and HL diets significantly reduced the populations of bifidobacteria at d 28 and 38, but there was no difference between these 2 diets (CTL+ and HL).

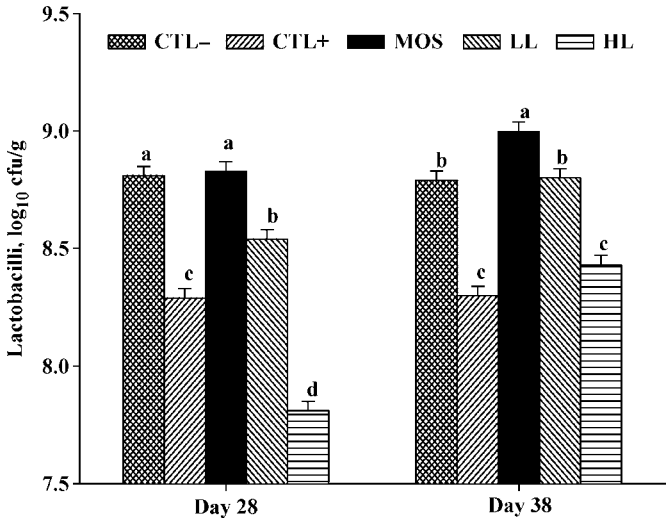

Figure 1. Concentrations ($\log_{10}$ cfu/g) of lactobacilli in the ceca of broiler chickens fed antibiotic-free diets (CTL–); antibiotic-free diets supplemented with 11 mg of virginiamycin/kg (CTL+); antibiotic-free diets supplemented with BioMos (MOS; Alltech Inc., Nicholasville, KY) at 0.2% and 0.1% in the starter (1 to 21 d) and in the grower feed (22 to 38 d), respectively; and antibiotic-free diets supplemented with low (LL, 1.25%) or high (HL, 2.5%) Alcell lignin (Alcell Technologies Inc., Montreal, Québec, Canada). [a–d]Values with different letters within a group are different (Bonferroni *t*-test, $P < 0.05$).

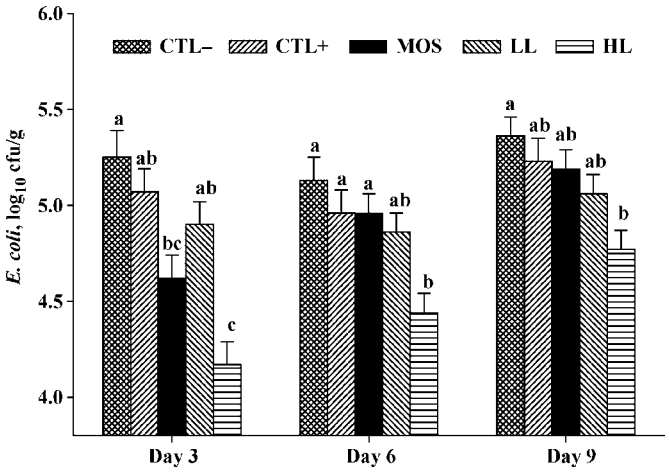

Figure 3. Concentrations ($\log_{10}$ cfu/g) of *Escherichia coli* in the ceca of PBS-gavaged broiler chickens fed antibiotic-free diets (CTL–); antibiotic-free diets supplemented with 11 mg of virginiamycin/kg (CTL+); antibiotic-free diets supplemented with BioMos (MOS; Alltech Inc., Nicholasville, KY) at 0.2% and 0.1% in the starter (1 to 21 d) and in the grower feed (22 to 38 d), respectively; and antibiotic-free diets supplemented with low (LL, 1.25%) or high (HL, 2.5%) Alcell lignin (Alcell Technologies Inc., Montreal, Québec, Canada). [a–c]Values with different letters within a group are different (Bonferroni *t*-test, $P < 0.05$).

E. coli Challenge and Enumeration

PBS Gavage. Serotyping results of *E. coli* isolates indicated that the O88 serotype was absent in the ceca of the birds at all intervals (d 3, 6, and 9) after PBS gavage. However, about 10% of all the *E. coli* isolates belonged to the O2 serotype ("O2-PBS"); results from PFGE revealed that these colonies were genetically different from the O2 serotype used to challenge the birds ("O2-challenge").

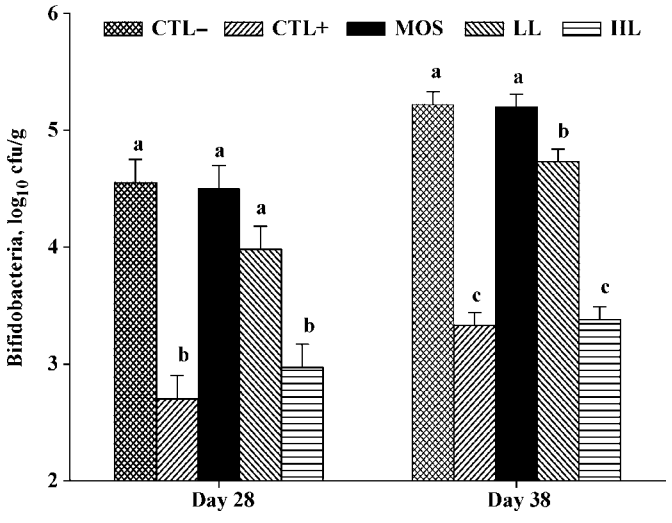

Figure 2. Concentrations ($\log_{10}$ cfu/g) of bifidobacteria in the ceca of broiler chickens fed antibiotic-free diets (CTL–); antibiotic-free diets supplemented with 11 mg of virginiamycin/kg (CTL+); antibiotic-free diets supplemented with BioMos (MOS; Alltech Inc., Nicholasville, KY) at 0.2% and 0.1% in the starter (1 to 21 d) and in the grower feed (22 to 38 d), respectively; and antibiotic-free diets supplemented with low (LL, 1.25%) or high (HL, 2.5%) Alcell lignin (Alcell Technologies Inc., Montreal, Québec, Canada). [a–c]Values with different letters within a group are different (Bonferroni *t*-test, $P < 0.05$).

At 3 and 6 d, PBS-gavaged birds fed the HL diet had lower populations of *E. coli* compared with those fed the CTL– or CTL+ diet; at d 9, the *E. coli* population was lower in HL-fed birds than those fed the CTL– diet but not in birds fed the CTL+ diet (Figure 3). Moreover, at d 3, birds fed the HL diet had reduced *E. coli* populations compared with those fed the LL diet and, at d 6, *E. coli* load was lower than in MOS-fed birds. Birds fed MOS had reduced *E. coli* load compared with those fed the CTL– diet at d 3. There were no differences in the populations of *E. coli* among birds fed the CTL–, CTL+, or LL diets at any interval after the gavage.

E. coli Challenge. Results from serotyping indicated that the 2 serotypes of *E. coli* (O2 and O88) used in the challenge were recovered in the cecal digesta of birds at all intervals (d 3, 6, and 9) after the challenge. However, irrespective of time intervals after the challenge and dietary treatments, the O2 serotype was recovered more frequently (11 times or 84%) than the O88 serotype. Results from PFGE revealed that the O2 serotype used to challenge the birds (O2-challenge) was recovered in the cecal digesta. Moreover, the O2 serotype identified in the ceca of PBS-gavaged birds (O2-PBS) was also recovered in the ceca of *E. coli*-challenged birds. Several other serotypes of *E. coli* were also recovered in the ceca of the birds but these were not relevant to the study.

At all intervals after the *E. coli* challenge, the effects of CTL– and CTL+ on the populations of *E. coli* were similar (Figure 4). At d 3 and 9, the HL diet significantly reduced the cecal populations of total *E. coli* compared with the CTL– or CTL+ diet but at d 6, *E. coli* load was lower in

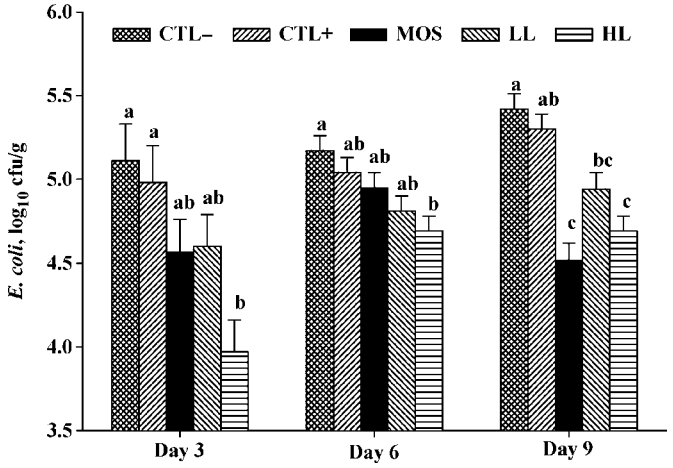

Figure 4. Concentrations ($\log_{10}$ cfu/g) of *Escherichia coli* in the ceca of *E. coli*-challenged broiler chickens fed antibiotic-free diets (CTL–); antibiotic-free diets supplemented with 11 mg of virginiamycin/kg (CTL+); antibiotic-free diets supplemented with BioMos (MOS; Alltech Inc., Nicholasville, KY) at 0.2% and 0.1% in the starter (1 to 21 d) and in the grower feed (22 to 38 d), respectively; and antibiotic-free diets supplemented with low (LL, 1.25%) or high (HL, 2.5%) Alcell lignin (Alcell Technologies Inc., Montreal, Québec, Canada). [a–c]Values with different letters within a group are different (Bonferroni *t*-test, $P < 0.05$).

the HL diet only compared with the CTL– diet. At d 9, birds fed MOS had a lower load of *E. coli* than those fed the CTL– or CTL+ diet; whereas LL-fed birds had a lower *E. coli* load compared with those fed the CTL– diet. At all intervals after the *E. coli* challenge, the cecal populations of *E. coli* were not different among birds fed the MOS, LL, or HL diet.

DISCUSSION

Our results show that, under the conditions of this study, the addition of the antibiotic virginiamycin to an antibiotic-free diet did not alter growth performance or feed intake in broilers. Feed conversion ratio, however, was increased with the addition of virginiamycin at d 35. This was an unexpected finding; virginiamycin has been reported to improve BW and feed efficiency when added to broiler or turkey diets (Parks et al., 2005; Miles et al., 2006). Although a decrease in feed efficiency was observed despite the lack of change in growth performance and feed intake, similar observations have been made previously between diets containing bacitracin and enramycin (Pedroso et al., 2006).

The observation that MOS and lignin failed to alter production responses are consistent with our previous study (Baurhoo et al., 2007). However, Hooge (2004) reported that growth performance and feed efficiency were improved in birds fed MOS compared with those fed AGP-free diets; but performance was similar between MOS and AGP-fed birds. Indulin, a purified lignin byproduct from the paper industry (Ross et al., 1986), has been shown to improve weight gain and feed efficiency in broilers (Ricke et al., 1982). Based on the current study and published research, it appears that MOS, lignin, and AGP have variable effects on broiler performance. These may be attributed to differences in the type of lignin product, experimental conditions, diet formulation, and health status of the birds. It is reported that AGP (Sims et al., 2004) and the most beneficial additives (Hooge, 2004) are most effective under stress and disease conditions.

Our findings that birds fed the CTL+ diet had significantly lower cecal loads of lactobacilli and bifidobacteria than CTL– birds at d 28 and 38 were expected because AGP are known to inhibit the growth and colonization of these intestinal gram-positive bacteria (Engberg et al., 2000). In contrast, MOS increased the cecal populations of lactobacilli and bifidobacteria and, in the challenge study, reduced the cecal population of *E. coli*. Therefore, the results clearly demonstrate that MOS was effective in suppressing the growth of *E. coli* in broilers. Reports indicate that competitive exclusion is a mechanism involving the establishment of an intestinal population of beneficial bacteria, such as lactobacilli, that prevents the colonization of pathogenic bacteria (Van der Wielen et al., 2002). It is quite possible that the increase in both lactobacilli and bifidobacteria may be based on the same principle. Mannanoligosaccharides competitively exclude gram-negative pathogenic bacteria from the intestine. The mannose-specific type-1 fimbriae of *E. coli* adsorb to MOS and are ultimately excreted without colonizing the chicken gut (Newman, 1994). Research to date has revealed equivocal responses in intestinal populations of lactobacilli and bifidobacteria in broilers (Spring et al., 2000; Fernandez et al., 2002; Denev et al., 2005) and turkeys (Fairchild et al., 2001; Sims et al., 2004). In the present study and our previous study (Baurhoo et al., 2007), MOS consistently increased the cecal populations of lactobacilli and bifidobacteria, in support of the evidence of the positive effects of MOS on the beneficial bacteria in the intestine of broilers.

The LL diet significantly increased the cecal populations of lactobacilli and bifidobacteria compared with the AGP diet, in agreement with our previous findings (Baurhoo et al., 2007). These effects are similar to those observed with MOS. But when the comparison was made with the AGP-free diet, lignin did not show any beneficial effects. Mannanoligosaccharides are classified as prebiotics (Ferket, 2004). Prebiotics have the effect of selectively stimulating the growth or metabolic activity of a limited number of intestinal microorganisms (Gibson and Roberfroid, 1995). Given the similarity in the effects of MOS and LL on lactobacilli and bifidobacteria, lignin at low levels has the potential to be classified as a prebiotic. Maintenance of a good symbiotic relationship between the host and its intestinal microflora is recognized as being critical for optimal performance and health of broilers (Ferket, 2000). The intestinal populations of lactobacilli and bifidobacteria compete against potential pathogens for nutrients and binding sites, thereby reducing the intestinal population of pathogens (Rolfe, 2000). Furthermore, lactobacilli secrete bacteriocins (Jin et al., 1996ab) and bifidobacteria produce organic acids and other bactericidal substances

(Gibson and Wang, 1994); all of these substances can suppress the colonization of the intestines by pathogenic bacteria. Therefore, under the conditions of this study, diets containing MOS and LL offered a significant advantage over virginiamycin by improving the intestinal microbial ecology of broilers.

In contrast to the LL diet, HL inhibited the growth of lactobacilli and bifidobacteria. These findings demonstrate that lignin at a high level possesses antibacterial effects against the intestinal beneficial bacteria and would, therefore, preclude the use of lignin at dietary levels that exceed 1.25%. Previous studies, both in vivo and in vitro, have demonstrated that Alcell lignin (10%) inhibited bacterial growth (Nelson et al., 1994).

Results indicate that, in birds subjected to the *E. coli* challenge, 11 times (84% more) as many O2 serotypes were recovered in the ceca as the O88 serotype, suggesting that the O2 serotype can colonize the gut more efficiently than the O88 serotype. According to Menao et al. (2002), the O2 and O88 serotypes of *E. coli* are pathogenic to poultry, but the O2 serotype is most commonly isolated on large-scale broiler farms and in chicken carcasses. The O2 serotype (O2-PBS) was isolated from the ceca of PBS gavage and *E. coli*-challenged birds; this finding indicates that this O2 serotype was present in the gut of the birds before the experiment. Before the initiation of the challenge study, the litter was screened for the presence of O2 and O88 serotypes but neither was detected. The O2-PBS serotype may, therefore, have been present at a low concentration and not detectable at the time the litter was screened.

In birds gavaged with PBS or challenged with *E. coli*, the cecal populations of total *E. coli* were not different whether birds were fed the CTL+ or CTL− diet. *Escherichia coli* is resistant to most of the AGP used in poultry production because of its complex cell wall structure (Ferket, 2000), explaining our findings. Moreover, by inhibiting the intestinal growth of lactobacilli and bifidobacteria, AGP limit the opportunity for competitive exclusion of *E. coli* from the gut.

The cecal population of total *E. coli* after challenge in birds fed MOS was reduced, but only at d 9. It seems likely, therefore, that there was a time delay for MOS to act on the cecal population of *E. coli*. Fernandez et al. (2002) reported similar findings when MOS-fed broilers were orally challenged with *Salmonella enteritidis* (PT4). Results of this study also indicate that the effects of MOS in reducing the cecal concentration of total *E. coli* were more pronounced in *E. coli*-challenged birds than in PBS-gavaged birds. These findings agree with reports of Hooge (2004) that MOS is most effective under disease and stress conditions.

In broilers fed MOS and challenged with pathogenic strains of *Salmonella*, the cecal populations of the specific strains of *Salmonella* were significantly lowered compared with those fed AGP-free diets (Spring et al., 2000; Fernandez et al., 2002). However, in an *E. coli* (O2, O19, O88, and O159 serotypes) challenge study conducted with turkeys, Fairchild et al. (2001) observed that the intestinal concentration of coliforms did not differ when turkeys were fed an AGP-free diet or one containing MOS or AGP. Sims et al. (2004) also reported that the concentrations of *E. coli* and coliforms in the large intestine of MOS-fed turkeys did not differ from those fed an AGP or AGP-free diet. Therefore, a positive response of MOS in reducing the intestinal population of pathogenic bacteria may occur mainly in broilers rather than turkeys.

The challenge study indicates that both LL and HL reduced the cecal population of total *E. coli* compared with the CTL− diet. However, the effects were more pronounced with the HL diet, suggesting that the inhibition of *E. coli* by lignin may be dose related. In studies conducted in vitro, Phillip et al. (2000) reported a greater inhibition of *E. coli* growth in culture medium containing 10% (wt/vol) compared with 5% (wt/vol) Alcell lignin. Although the exact mechanism of lignin action is not clear, Jung and Fahey (1983) proposed that the polyphenolic compounds of lignin cause cell membrane damage and lysis of bacteria. Other phenolic compounds such as carvacrol, thymol, and cinnamaldehyde have been shown to exert antimicrobial effects against lactobacilli, bifidobacteria, and *E. coli* (Lee et al., 2004; Bozin et al., 2006). Carvacrol and thymol are reported to cause cell membrane disintegration and release of the bacterial cell contents, whereas cinnamaldehyde penetrates the bacterial cell membrane to impair the enzyme system, reduce the intracellular pH, and cause depletion of adenosine triphosphate (Helander et al., 1998; Oussalah et al., 2006).

That MOS and LL significantly reduced the cecal population of total *E. coli* in birds challenged with pathogenic strains of *E. coli* is an important finding from this study. Intestinal *E. coli* contaminates poultry carcasses during processing at the slaughter house (Heyndrickx et al., 2002), representing an important cause of foodborne illnesses in humans (Mead et al., 1999). Therefore, the addition of MOS or a low level of lignin to poultry diets could be a useful dietary strategy to improve the safety of poultry products. The cecal population of total *E. coli* was also significantly reduced with the HL diet but this treatment also caused a significant reduction in the cecal population of lactobacilli and bifidobacteria. Such an outcome is not desirable so it is not advisable to use high levels of lignin in poultry diets.

In conclusion, the dietary addition of MOS increased the cecal populations of lactobacilli and bifidobacteria; in *E. coli*-challenged birds, MOS and LL reduced the cecal populations of total *E. coli*. A greater level of lignin (2.5% of DM) caused a major reduction in the cecal population of *E. coli*, but lactobacilli and bifidobacteria loads were also reduced. Under the conditions of this study, virginiamycin failed to improve production performance when compared with an AGP-free diet or one containing MOS or lignin. It seems that MOS and a low level of Alcell lignin could replace AGP in poultry production; these natural feed additives have the potential to improve the safety of poultry products without risking the spread of antibiotic resistance in bacteria.

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