

Effects of Mushroom and Herb Polysaccharides, as Alternatives for an Antibiotic, on the Cecal Microbial Ecosystem in Broiler Chickens

F. C. Guo,*†¹ B. A. Williams,* R. P. Kwakkel,† H. S. Li,‡ X. P. Li,‡ J. Y. Luo,‡
W. K. Li,§ and M. W. A. Verstegen*

*Animal Nutrition Group, and †Animal Production Systems Group, Wageningen University,
PO Box 338, 6700 AH Wageningen, The Netherlands; ‡Laboratory of Veterinary Clinic,
Institute of Animal Science and Veterinary Pharmaceuticals, CAAS, 730050 Lanzhou, China;
and §Department of Parasitological Diseases, Gansu Animal Health Service, 730046, Lanzhou, China

ABSTRACT An in vivo experiment was conducted to study the potential prebiotic effects of mushroom and herb polysaccharide extracts, *Lentinus edodes* extract (*LenE*), *Tremella fuciformis* extract, and *Astragalus membranaceus Radix* extract, on chicken growth and the cecal microbial ecosystem, as compared with the antibiotic Apramycin (APR). This investigation was carried out in terms of a dose-response study. The chickens were naturally infected with avian *Mycoplasma gallisepticum* prior to the experiment. The BW gain, cecal pH, viscosity, and predominant microbial populations were measured 1 wk after the extract and APR treatments. The extracts and APR significantly stimulated growth of the chickens infected with avian *Mycoplasma gallisepticum*. The average BW gain of the groups fed with the extracts was significantly lower than that of the antibiotic group. The extracts

had no significant effect on cecal pH. However, cecal viscosity and microbial populations were significantly affected by feeding extracts and antibiotic. In contrast to APR, the extracts stimulated the number of the potentially beneficial bacteria (bifidobacteria and lactobacilli), while reducing the number of the potentially harmful bacteria (*Bacteroides* spp. and *Escherichia coli*). Of the 3 extracts, *LenE* was associated with the most cecal bifidobacteria and lactobacilli. With each increase in the *LenE* dose, birds tended to have higher BW gain and total aerobe and anaerobe counts. Numbers of predominant cecal bacteria, in particular, *E. coli*, bifidobacteria, and lactobacilli, were significantly increased with increases in the *LenE* dose. It would seem that these specific mushroom and herb polysaccharide extracts hold some promise as potential modifiers of intestinal microbiota in diseased chickens.

(Key words: mushroom and herb polysaccharide extracts, chicken, growth, cecal microbial ecosystem)

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INTRODUCTION

The cecum can be described as the location for a very complex microbial ecosystem, although other parts of the digestive tract of chickens might also be important sites for microbial colonization. The cecum is one of the areas of greatest microbial activities in the gastrointestinal tract of chickens. Relative to other parts of the gastrointestinal tract, the cecum provides a stable environment for microorganisms, resulting in a large microbial population due to the slower transit time. According to Barnes et al. (1972), the total number of bacteria is around 10^{11} cells/g (wet weight) in the ceca of chickens, with anaerobic bacteria at 10^8 to 10^9 cells/g. Intestinal microflora play an important role in the health status of host animals. In general, intestinal bacteria may be divided into species

that exert either harmful (pathogenic) or beneficial effects on host health (Macfarlane and Cummings, 1991). Therefore, a common approach to maintain host health is to increase the number of desirable bacteria in order to inhibit colonization of invading pathogens (Rolfe, 1991). The composition and activity of intestinal microbiota can be altered by diet composition and dietary manipulations such as the use of feed additives and antibiotics (Coates et al., 1981; Jensen, 1993). Antibiotic therapies have been reported as major factors in the etiology of gut health disorders (Gardiner et al., 1993; Solomons, 1993). Increasing insight into the potentially beneficial activities of the gastrointestinal microbiota and increasing public concern about antibiotic resistance and residues in animal products have resulted in the search for alternatives, such as prebiotics, probiotics, and other feed additives.

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¹To whom correspondence should be addressed: fucun.guo@wur.nl.

Abbreviation Key: AMG = avian *Mycoplasma gallisepticum*; APR = Apramycin; *AstE* = *Astragalus membranaceus* polysaccharide extract; *LenE* = *Lentinus edodes* polysaccharide extract; *TreE* = *Tremella fuciformis* polysaccharide extract.

Certain plant polysaccharides are now recognized as having a prebiotic effect (Verstegen and Schaafsma, 1999; Cummings and Macfarlane, 2002). Prebiotics are defined as nondigestible food ingredients that beneficially affect the host by selective stimulation of growth or activity of one or a limited number of bacterial species in the colon, thus benefiting host health (Gibson and Roberfroid, 1995). Carbohydrates, especially oligo- and polysaccharides, have been used as prebiotics to influence the composition of the bacterial populations in the large intestine of a number of animal species (Baily et al., 1991; Newman, 1995; Grizard and Barthomeuf, 1999; Jensen, 1999; Hughes et al., 2000; Breves et al., 2001; Rycroft et al., 2001; Zimmermann et al., 2001; Korakli et al., 2002).

Natural medicinal products originating from fungi and herbs have been used as feed additives for farm animals in China for centuries, and show many bioactivities such as antimicrobial activities, immune enhancement, and stress reduction (Wang et al., 1998). The bioactive components of these products are quite complex (Yang and Feng, 1998), but of these, polysaccharides are considered to be the most important immunoactive components (Xie and Niu, 1996; Xue and Meng, 1996). It is well documented that polysaccharides derived from *Astragalus membranaceus Radix* (Huang Qi), *Lentinus edodes* (Shiitake), and *Tremella fuciformis* (White Jelly), which have been used as immune enhancers, also show antibacterial (Yuan et al., 1993), antiviral (Wei et al., 1997; Cheng et al., 1998; Liu et al., 1999b; Yu and Zhu, 2000) and antiparasitic activities (Hu et al., 1998; Pang et al., 2000) in chickens. It has been hypothesized that some of these effects are actually the result of a prebiotic effect by which the polysaccharides stimulate growth of the beneficial bacterial populations in the large intestine and thus increase resistance to pathogens. However, the effects of mushroom and herb polysaccharides on the microbial ecosystem in the large intestine of animals have not been well investigated as such. Also, it is not known what an optimal inclusion level of these mushroom and herb polysaccharides might be, when used as modifiers of the intestinal microbiota.

The present in vivo experiment was conducted to study the prebiotic effects of the mushroom and herb polysaccharide extracts, *L. edodes* extract (*LenE*), *T. fuciformis* extract (*TreE*), and *A. membranaceus* extract (*AstE*), as alternatives for the antibiotic Apramycin (APR), on the growth and cecal microbial ecosystem of chickens and to investigate the optimum supplemental level of the potential prebiotic for enhanced growth and beneficial intestinal microbiota.

MATERIALS AND METHODS

Animal Husbandry and Diets

A total of 200 1-d-old female Huangyu broiler chicks² were reared in horizontal battery brooders using wood

²A local slow-growing breed from Gansu Poultry Breeding Company, Lanzhou, China.

³Gansu Province Animal Health Service, Lanzhou, China.

⁴Gansu Huanghe Pharmacy Market, Lanzhou, China.

TABLE 1. Composition of the diets (g/kg)

Ingredient	Starter diet	Grower diet
Maize	570	635.3
Soybeans (500 g/kg of CP)	320	270
Fish meal	40	30
Animal fat	20	20
Limestone	14	12
NaCl	3.5	3.5
Dicalcium phosphate	17	16
DL-Methionine	2.6	2.1
Lysine	2.9	1.1
Premix ¹	10	10
Calculated analysis (g/kg)		
Crude protein	210	190
Metabolizable energy (kcal/kg)	3,000	3,050
Calcium	10	9
Available phosphate	4.5	4.3
Lysine	11.7	10.5
Methionine	6.0	5.1

¹Supplied for per kilogram of diet: vitamin A (retinal acetate), 8,500 IU; cholecalciferol, 1,800 IU; DL- α -tocopherol acetate, 13.0 IU; vitamin K₃, 3.4 mg; thiamin, 1.8 mg; riboflavin, 5.2 mg; D-pantothenic acid, 9.8 mg; niacinamide, 38 mg; folic acid, 0.90 mg; pyridoxine, 3.60 mg; vitamin B₁₂, 18 μ g; d-biotin, 80 μ g; choline chloride, 200 mg; Fe, 80 mg; Zn, 75 mg; Mn, 100 mg; Se, 0.15 mg; I, 0.3 mg; carrier was maize.

sawdust as litter, with a density of 10 birds/m². The brooder temperature was set at 30 \pm 3°C during the first week and gradually decreased by 2°C per week until 28°C was reached by the third week. Relative humidity was between 65 and 70%. The lighting program was 24 h light throughout the experiments. All birds were fed ad libitum. The diet was based on maize and soybean meal and was used throughout the experiment. The diet composition is shown in Table 1.

Avian *Mycoplasma gallisepticum* Infection

Birds proved to be naturally infected with avian *Mycoplasma gallisepticum* (AMG) prior to the experiment. A few birds showed respiratory symptoms at 3 d of age, and the disease then spread to the whole flock 1 wk later. Mortality reached 8% at 13 d of age. Presumptive diagnosis was based on the occurrence of typical signs (mucous discharge from the mouth and nostrils, increased respiratory rate, and swollen head at the time of death) and lesions (airsacculitis, sinusitis, and synovitis) together with differential diagnosis (isolation and identification of the causative organisms), and final diagnosis was based on serological tests.³

Polysaccharide Preparation

Intact mushroom and herb materials were purchased from a local source.⁴ The intact mushroom and herb materials were dried overnight at 45°C and ground through a 1-mm sieve for polysaccharide extraction according to the general procedure of water-soluble polysaccharide extraction (Liu et al., 1999a). The yields of the polysaccharide fractions and their total sugar contents are shown in Table 2.

TABLE 2. Yields and total sugar contents of the mushroom and herb water-soluble polysaccharide extracts¹

Composition	<i>LenE</i>	<i>TreE</i>	<i>AstE</i>
Yield of polysaccharides ² (%)	6.8	16.0	10.1
Dry matter ³ (g/kg)	958	965	967
Total sugar content ⁴ (g/kg DM)	655	613	687

¹*LenE* = *Lentinus edodes* polysaccharide extract; *TreE* = *Tremella fuciformis* polysaccharide extract; *AstE* = *Astragalus membranaceus* polysaccharide extract.

²Yields of polysaccharides were based on air-dry matter of the intact materials.

³The dry matter contents of the extracts were determined by freeze-drying.

⁴Total sugar content was determined using the phenol-sulfuric method (Dubois et al., 1956).

Experimental Design

At 14 d of age, 135 broilers, after exclusion of extreme weights, were randomly assigned to 9 dietary treatments: addition of *LenE* at 1, 2, 3, 5, and 10 g/kg of the diet; *TreE* and *AstE* both at 2 g/kg; antibiotic; and control. The antibiotic used was APR sulfate soluble powder (C₂₁H₄₁N₅O₁₁) at 20 mg/kg of BW.⁵ Polysaccharide extracts and APR were administered orally twice daily from 15 to 21 d of age. Appropriate proportions were prepared so that each chicken received the intended dose directly into the crop by means of oral gavage. Each treatment consisted of 3 pens with 5 birds per pen. A pen was considered as the replicate experimental unit.

Data Collection

Body weight and mortality per pen were recorded at 14 and 21 d of age. At the end of the experiment, all birds were killed by cervical dislocation in a germ-free isolation chamber sterilized by ultraviolet radiation. The cecum was then removed from each bird, and the fresh excreta of the cecum were gently squeezed and carefully collected in sterilized 25-mL tubes, each tube contained pooled excreta for 5 birds (per pen).

Three grams of fresh cecal samples was diluted with 10 mL distilled water and vortexed before pH⁶ and viscosity⁷ were measured.

One gram of wet sample was diluted with 10 mL of sterilized distilled water, of which 1 mL was transferred into 9 mL of sterilized distilled water. Samples were serially diluted from 10⁻¹ to 10⁻⁷. One-tenth milliliter of each diluted sample was plated on the appropriate medium for enumeration of microbial populations. Bacterial counts were performed using the appropriate dilution and plate culture techniques under aerobic or anaerobic conditions

according to Barnes and Impey (1970), and the results were expressed as colony forming units log₁₀ per gram of fresh sample.

The bacterial groups and species determined included total anaerobes (reinforced clostridial agar), total aerobes (nutrient blood agar), lactobacilli (citromalic acid-enriched MRS agar), *Bacteroides* spp. (EMB agar), enterococci (esculin-crystal violet-aesculine azide agar), *Escherichia coli* (MacConkey agar), and bifidobacteria (bifidobacterium agar composed of tomato juice, 400 mL; dissoluble amylum, 0.5 g; peptone, 15 g; yeast extract, 2 g; glucose, 20 g; sodium chloride, 5 g; Tween-80, 1 mL, 5% cysteine, 0.5 mL; liver extract, 80 mL; agar powder, 20 g; and distilled water, 520 mL; pH = 7.0, at 37°C for 72 h).⁸

Statistical Analysis

The data were subjected to statistical analysis by ANOVA using SPSS 8.0 (SPSS, 1997). Orthogonal contrasts were used to assess 1) the effect of antibiotic, examining the contrast between the antibiotic and control groups; 2) the effect of polysaccharide extracts, examining the contrast between the overall extracts (*LenE*, *TreE*, and *AstE* at 0.2%) and the antibiotic group; 3) the difference between the 3 extracts, examining the contrasts, *LenE* vs. *TreE*, and *AstE* vs. *LenE* and *TreE*.

Differences of BW gain, cecal pH, viscosity, and microbial counts among the different dietary levels of *LenE* (1, 2, 3, 5, and 10 g/kg) were tested by Tukey's multiple range test.

The dose response of *LenE* was also fitted to both linear [1] and quadratic [2] regression functions:

$$Y = b_0 + b_1 X \quad [1]$$

$$Y = b_0 + b_1 X + b_2 X^2 \quad [2]$$

where Y = predicted response, X = the dose of the extract, b₀ = intercept (i.e., BW gain, cecal pH, viscosity, or bacterial populations on the basal diet), and b₁ and b₂ = a linear or quadratic regression coefficient.

The significance of the linear and quadratic models was assessed by an *F*-test.

RESULTS

Effects of *LenE*, *TreE*, and *AstE*

The BW gain of the birds fed with APR was significantly higher compared with that of the nonsupplemented birds (Table 3). The overall mean of the BW gain of the groups fed with the extracts was significantly lower than the mean of the antibiotic group. There was no significant difference in BW gain among the groups fed the extracts. The pH value of the different treatments was not significantly different. The birds fed with APR showed significantly higher cecal viscosity than the nonsupplemented birds. The overall mean of the cecal viscosity of the groups

⁵Antibiotic Laboratory, CAAS, Lanzhou, China.

⁶pH meter, pHB-5A, Lida, Shanghai, China.

⁷NZ-6 type conical viscosity meter, with shearing rate 50-1, Shanghai, China.

⁸Institute of Biological Products, China Hygiene Ministry, Lanzhou, China.

TABLE 3. The BW gain, cecal pH, viscosity, and microbial counts of chickens fed with the mushroom and herb water-soluble polysaccharide extracts¹

Treatment ²	BW gain (g/bird/d)	pH	Viscosity (cpm) ⁵	Aerobes	Anaerobes	<i>Bacteroides</i>	<i>Escherichia</i>	Enterococci	Bifidobacteria	Lactobacilli
						spp.	<i>coli</i>			
Control	5.7	7.0	0.88	5.93	9.52	6.70	6.93	7.36	8.55	7.54
APR	25.6	7.2	1.29	9.08	9.39	8.72	9.69	7.25	8.12	6.58
<i>LenE</i> , 2 g/kg	17.6	6.8	1.17	6.19	9.53	6.38	6.58	7.29	9.09	8.33
<i>TreE</i> , 2 g/kg	18.9	7.0	1.22	5.99	9.09	5.74	6.14	7.16	8.69	8.16
<i>AstE</i> , 2 g/kg	21.6	7.0	1.17	6.18	9.26	6.45	6.73	7.23	9.03	8.21
SEM	1.84	0.13	0.03	0.32	0.05	0.27	0.03	0.02	0.10	0.096
Contrast statements ³						Probability levels of contrasts				
Control vs. APR	<0.000	0.725	0.008	0.001	0.071	<0.000	<0.000	0.029	0.015	0.005
Extracts ⁴ vs. APR	0.001	0.346	0.081	<0.000	0.062	<0.000	<0.000	0.496	<0.000	<0.000
<i>LenE</i> vs. <i>TreE</i>	0.558	0.706	0.039	0.109	0.002	0.002	0.029	0.226	0.001	0.008
<i>AstE</i> vs. <i>LenE</i> and <i>TreE</i>	0.066	0.359	0.141	0.220	0.413	0.003	0.005	0.960	0.032	0.203

¹Results are given as means (n = 3).

²APR = Apramycin; *LenE* = *Lentinus edodes* polysaccharide extract; *TreE* = *Tremella fuciformis* polysaccharide extract; *AstE* = *Astragalus membranaceus* polysaccharide extract.

³Orthogonal contrasts.

⁴Extracts = all extracts (n = 9).

⁵cpm = cycles per minute.

⁶Colony-forming units are expressed as log₁₀ colonies per gram of cecal contents.

fed with the extracts was not significantly different from the antibiotic group. Of the 3 extracts, the *TreE* group showed the highest cecal viscosity.

The total aerobic counts of the antibiotic group were significantly higher than those of the control group and of the groups fed with the extracts (Table 3). Total anaerobic counts were not significantly different between the antibiotic and control groups or among the extracts and the antibiotic group. Of the 3 extracts, the *LenE* group had the significantly highest anaerobe counts.

The number of *Bacteroides* spp. and *E. coli* for the birds fed with APR was significantly higher compared with that of the nonsupplemented birds and the overall mean of the birds fed with the extracts (Table 3). Of the 3 extracts, the *TreE* group showed the lowest *Bacteroides* spp. and *E. coli* counts. The antibiotic group had significantly lower enterococci counts than the control group was not significantly different from the extract groups. The antibiotic group showed significantly lower bifidobacteria and lactobacilli counts compared with the control group. The overall mean of bifidobacteria and lactobacilli counts of the groups fed with the extracts was significantly higher compared with the antibiotic group. Of the 3 extracts, the *LenE* group showed highest bifidobacteria and lactobacilli counts, whereas the *TreE* group showed the lowest counts.

Dietary Level Effect of *LenE*

BW gain increased with increasing *LenE* dose in the diet (Table 4). The 5 g/kg *LenE* showed the highest BW gain. However, cecal pH, and viscosity among the different levels of *LenE* were not different.

Total aerobic and anaerobe counts increased with increasing *LenE* dose, and *LenE* at a dose of 1 g/kg at the diet showed significantly the lowest total aerobic and anaerobe counts (Table 4). Both 1 and 5 g/kg levels of

LenE had significantly lower *Bacteroides* spp. counts than 3 and 10 g/kg levels of *LenE*, and 3 g/kg *LenE* had significantly highest number of *Bacteroides* spp. The number of enterococci decreased with increasing *LenE* dose (from 1 to 5 g/kg), and the highest and lowest doses (1 and 10 g/kg) of *LenE* showed the significantly highest enterococci counts. The number of *E. coli* increased with increase of the *LenE* dose, and 5 and 10 g/kg *LenE* levels showed the significantly highest counts. The number of bifidobacteria and lactobacilli increased with increases of the *LenE* dose. A level of 2 g/kg *LenE* showed the highest bifidobacteria and lactobacilli counts.

The estimated parameters for regression functions of the *LenE* dose response are shown in Table 5.

Compared with the linear model, the quadratic model significantly improved the regression relationship for the observed traits (Table 5). There was ($P < 0.001$) an increased quadratic regression relationship between the *LenE* supplemental levels and the BW gain and enterococci and *E. coli* counts. The data of total aerobes and anaerobes fitted well to the quadratic model ($P < 0.01$). The cecal viscosity also showed a ($P < 0.05$) quadratic function. However, the pH value, *Bacteroides* spp., bifidobacteria and lactobacilli counts all fitted poorly to both the linear and quadratic functions ($P > 0.05$).

DISCUSSION

BW Gain, Cecal pH, and Viscosity

In this study, the broiler chicks became naturally infected with AMG prior to the experiment. Therefore, feeding both APR and the extracts might have significantly improved the health status of the infected birds, as indicated by the growth values. The BW gain of the birds given either APR or the extracts was significantly improved as compared with the untreated birds which

TABLE 4. The BW gain, cecal pH, viscosity and microbial counts of chickens fed with different levels of *LenE*¹

<i>LenE</i> ²	BW gain	pH	Viscosity ³	Aerobes	Anaerobes	<i>Bacteroides</i> spp.	Enterococci	<i>Escherichia coli</i>	Bifidobacteria	Lactobacilli
(g/kg)	(g/bird/d)		(cpm) ³	(cfu) ⁴						
1	16.4 ^b	7.2	1.10	5.57 ^b	8.80 ^b	6.25 ^c	7.58 ^a	6.32 ^c	8.53 ^d	7.91 ^d
2	17.6 ^b	6.8	1.17	6.09 ^a	9.10 ^{ab}	6.38 ^{bc}	7.45 ^b	6.63 ^b	9.08 ^a	8.49 ^a
3	21.0 ^{ab}	6.3	1.16	6.14 ^a	9.17 ^a	6.87 ^a	7.20 ^c	6.78 ^{ab}	8.69 ^{cd}	8.19 ^c
5	26.5 ^a	6.4	1.18	6.21 ^a	9.20 ^a	6.35 ^c	7.13 ^c	6.92 ^a	8.81 ^{bc}	8.30 ^b
10	22.5 ^{ab}	6.7	1.15	6.00 ^{ab}	9.13 ^a	6.58 ^b	7.57 ^a	6.96 ^a	8.93 ^{ab}	8.37 ^b
SEM	1.09	0.13	0.01	0.07	0.05	0.06	0.05	0.07	0.05	0.05
Dietary effects	0.001	0.246	0.075	0.013	0.012	<0.001	<0.001	<0.001	<0.001	<0.001

^{a-d}Means with different superscripts within the column are significantly different ($P < 0.05$).

¹Results are given as means ($n = 3$) \pm SE.

²*LenE* = *Lentinus edodes* polysaccharide extract.

³cpm = cycles per minutes.

⁴The cfu is expressed as \log_{10} colonies per g cecal contents.

showed very poor growth. Furthermore, BW gain increased linearly ($P < 0.001$) with increasing *LenE* dose.

The advantages of using antibiotics as feed supplements in terms of growth stimulation and improvement of host health and feed efficiency have been well documented (Miles et al., 1984; Ensminger, et al., 1990; Peterson et al., 1991). The efficacy of antibiotics is more obvious in diseased birds than in normal birds. For example, a study of Eyssen and De Somer (1963) demonstrated that birds had a better response to antibiotic growth promoters under dirty housing conditions than under clean housing conditions.

There are few reports in the literature comparing mushroom and herb polysaccharides with antibiotic growth promoters in poultry. However, it has been well documented that the polysaccharides derived from *A. membranaceus*, *L. edodes*, and *T. fuciformis* decreased mortality and

incidence of bacterial (Yuan et al., 1993), viral (Wei et al., 1997; Liu et al., 1999b; Yu and Zhu, 2000), and parasitic diseases (Hu et al., 1998; Pang et al., 2000) in chickens. Yuan et al. (1993) reported that *TreP_m*, a polysaccharide extract isolated from mycelia of *T. fuciformis*, can be a good adjuvant for vaccines. A dose-response trial of Yuan et al. (1993) showed that the protection rate of pasteurellosis vaccine increased 67 to 160% with increases of the *TreP_m* level in chickens postinfection with pasteurellosis (MLD C48⁻¹). This finding is similar to the current study in which BW gain was significantly increased with increases in *TreP_m* dose.

Although the pH value numerically decreased with increasing *LenE* dose, the expected significantly lower cecal pH by feeding the mushroom and herb polysaccharide extracts was not observed in this study. This result is not consistent with the in vitro fermentation test (Guo

TABLE 5. Dose response of *LenE*¹, parameter estimates, and asymptotic SE (in parentheses), described by linear and quadratic functions in broilers

Type of model ²	Observed trait	b0	b1	b2	SE	<i>P</i> -value ³	R ²	F (1, 12) ⁴
Linear	BW gain	17.92 (1.57)	0.69 (0.30)		3.68	0.037	0.29	
	PH	6.79 (0.23)	-0.03 (0.04)		0.53	0.478	0.04	
	Viscosity	1.14 (0.02)	0.002 (0.003)		0.04	0.433	0.05	
	Aerobes	5.90 (0.12)	0.02 (0.02)		0.28	0.318	0.08	
	Anaerobes	8.99 (0.07)	0.02 (0.01)		0.17	0.120	0.18	
	<i>Bacteroides</i> spp.	6.41 (0.10)	0.02 (0.02)		0.24	0.348	0.07	
	Enterococci	7.36 (0.09)	0.06 (0.03)		0.20	0.717	0.01	
	<i>Escherichia coli</i>	6.47 (0.07)	0.06 (0.01)		0.17	0.001	0.59	
	Bifidobacteria	8.71 (0.09)	0.02 (0.02)		0.20	0.187	0.13	
	Lactobacilli	8.14 (0.08)	0.03 (0.02)		0.19	0.113	0.18	
	Quadratic	BW gain	10.98 (1.85)	4.65 (0.91)	-0.35 (0.08)	2.35	<0.001	0.73
PH		7.46 (0.37)	-0.42 (0.18)	0.034 (0.016)	0.47	0.107	0.31	4.74
Viscosity		1.09 (0.02)	0.03 (0.01)	-0.003 (0.001)	0.03	0.048	0.40	7.64
Aerobes		5.42 (0.16)	0.30 (0.08)	-0.002 (0.007)	0.21	0.009	0.54	12.14
Anaerobes		8.71 (0.10)	0.18 (0.05)	-0.014 (0.004)	0.13	0.006	0.57	10.98
<i>Bacteroides</i> spp.		6.26 (0.19)	0.10 (0.09)	-0.007 (0.008)	0.24	0.450	0.12	0.78
Enterococci		7.83 (0.04)	-0.26 (0.02)	0.024 (0.002)	0.05	<0.001	0.94	200.80
<i>Escherichia coli</i>		6.13 (0.07)	0.25 (0.03)	-0.017 (0.003)	0.09	<0.001	0.89	32.56
Bifidobacteria		8.65 (0.16)	0.06 (0.08)	-0.003 (0.007)	0.21	0.388	0.15	0.23
Lactobacilli		7.98 (0.15)	0.12 (0.07)	-0.008 (0.006)	0.19	0.135	0.28	1.70

¹*LenE* = *Lentinus edodes* polysaccharide extract.

²Models: linear: $Y = b_0 + b_1 X$; quadratic: $Y = b_0 + b_1 X + b_2 X^2$.

³ $P < 0.05$ are printed in bold.

⁴F (1, 12) represents the significance of adding a quadratic component to the linear model. Critical F (1, 12) values: 4.75 ($P < 0.05$) and 9.33 ($P < 0.01$).

et al., 2003). To some extent, it was surprising given that fermentation of carbohydrates leads to the production of straight-chain acids, and the fermentation of protein results in production of branched-chain acids (e.g., from amino acids such as valine, leucine, and isoleucine), both of which can lower intestinal pH (Cummings, 1981; Macfarlane et al., 1992). Unlike in vitro fermentation, acid concentrations in ceca were probably relatively low and, after polysaccharides were fermented by microbes, these fermentation end products may have already been absorbed from the lumen.

The viscosity of cecal contents of the birds fed with the extracts was significantly higher compared with the control group, which is consistent with previous studies (Hughes et al., 2000; Iji et al., 2001) which showed that inclusion of nonstarch polysaccharides in broiler diets raised intestinal viscosity and excreta moisture. According to these reports, viscosity is dependent on several factors including the size of the polysaccharide molecule, whether it is branched or linear, the presence of charged groups, the surrounding structures, and the concentration. In this experiment *TreE* had more influence on viscosity of cecal contents than *LenE* or *AstE*, which might have been due mainly to difference in physicochemical properties of the extracts, although other unidentified substances may also influence the viscosity of the cecal contents. It has been reported that sugar composition, molecular weights, and structures of these polysaccharides are all different (Xia and Cheng, 1988; Pang et al., 1995; Yang et al., 1999, 2001).

Predominant Intestinal Microbiota

Nondigestible carbohydrates (oligo- and polysaccharides) are potential prebiotics which could selectively enrich for beneficial bacterial species. Due to their chemical structure, these compounds are not hydrolyzed by digestive enzymes or absorbed in the upper part of the gastrointestinal tract. Such ingredients therefore enter the large intestine and may serve as substrates for the endogenous colonic bacteria (Salyers, 1979; Eastwood, 1992; Gibson and Roberfroid, 1995). Intestinal bacteria may be grouped into those species that may have harmful or pathogenic influences on host health such as *Proteus* spp., staphylococci, clostridia, and veillonellae; those that may have beneficial effects such as lactobacilli and bifidobacteria; and those that may have both effects such as enterococci, *E. coli*, streptococci and *Bacteroides* spp. (Macfarlane and Cummings, 1991).

There were significant changes in microbial counts for animals fed the APR and the polysaccharide extract diets, with an increase in the total number of aerobes and anaerobes. Hesselman and Aman (1986) reported that viscosity was increased in the presence of water-soluble nonstarch polysaccharides, which also decreased digesta passage rate. Thus, the highest viscosity that resulted from feeding APR and the polysaccharide extracts in the present study may have slowed down the digesta transit time in the lower part of the small intestine (ileum) and in the large

intestine (cecum and rectum) of chickens. This could mean that intestinal bacteria had sufficient time to multiply, resulting in an increase in the microbial population. Surprisingly, *TreE* showed the highest viscosity and yet the cecal microbial counts of the *TreE* group were significantly lower compared with the *LenE* and *AstE* groups. This may be due to confounding factors because the bacterial populations and activities can fluctuate in response to substrate availability and pH, as well as the viscosity of digesta in the intestines (Cummings and Macfarlane, 1991).

The mushroom and herb extract diets were largely associated with reduced *Bacteroides* spp., enterococci and *E. coli* numbers, but increased numbers of bifidobacteria, and lactobacilli, relative to the control and antibiotic groups. As demonstrated by several studies (Macy and Probst, 1979; Bailey et al., 1991; Gibson and Roberfroid, 1995; Newman, 1995; Sunvold et al., 1995; Langhout, 1999), some bacteria in the large intestine are more specialized in the hydrolysis of large molecular carbohydrates such as oligo- and polysaccharides, producing small molecular weight carbohydrates from large polymers and then fermenting them, which can lead to greater bacterial numbers. Fermentation endproducts such as short-chain fatty acids lower intestinal pH, which can depress harmful bacteria and stimulate beneficial bacteria.

There were large variations in numbers of *Bacteroides* spp. among the *LenE* levels and, as a result, these did not fit either linear or quadratic regression functions. Although the bifidobacteria and lactobacilli counts increased with increasing *LenE* dose, both fitted poorly to the linear or quadratic function. It may be associated with the exceptionally high bifidobacteria and lactobacilli counts of the 2 g/kg *LenE* group.

Of the 3 extracts, the *LenE* and *AstE* groups were associated with the highest cecal microbial populations. The different response of birds to the 3 extracts may be related to the physicochemical properties of these polysaccharide fractions and to their fermentation characteristics in the large intestine of chickens. According to an in vitro study, *AstE* and *LenE* were rapidly degraded and highly fermentable, whereas *TreE* was less readily fermented (Guo et al., 2003). Fermentation of these polysaccharides resulted in a great shift of the cecal microbial community of chickens (Guo et al., unpublished).

Unlike the extracts, APR treatment significantly increased the number of *Bacteroides* spp. and *E. coli*, but inhibited growth of enterococci, bifidobacteria and lactobacilli in ceca. It was reported that growth-promoting antibiotics lower the number and activity of intestinal microbes (Rosen, 1995). Cecal enterococci of chickens were largely reduced by feeding APR. It has been shown that microorganisms responsible for subclinical infections may be reduced or eliminated by antibiotic treatments (Jensen, 1993).

In summary, the mushroom and herb polysaccharide extracts and APR stimulated growth of the chickens naturally infected with AMG after 1-wk treatments. The aver-

age BW gain of the groups fed with the extracts was significantly lower compared with the BW gain of those fed APR-containing diets. However, cecal viscosity and microbial population counts were significantly higher in chickens fed the extracts and APR. Unlike APR, presence of the extracts seems to have stimulated the number of potential beneficial bacteria such as bifidobacteria and lactobacilli, while reducing the number of harmful bacteria such as *Bacteroides* spp. and *E. coli*. Birds had different responses to the 3 extracts, with *LenE* showing the greatest potential as a prebiotic. Birds tended to have higher BW gain and increased cecal microbial counts when increased doses of *LenE* were administered. Thus, the mushroom and herb polysaccharide extracts, *LenE*, *TreE*, and *AstE*, may have some prebiotic effect leading to shifts in the intestinal microbial populations of chickens. One of the limitations of using traditional culture-based microbiology techniques is that only easily cultivable organisms are counted, which does not represent the total number of bacterial species present in the gastrointestinal tract of the chickens in terms of describing the species present within a complex microflora. This experiment was conducted using diseased birds, and the results suggest that, in infected birds, these extracts had a positive effect. However, it is not yet known whether a similar effect might be found for noninfected birds. Further work is required to investigate this.

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