

Effect of Dietary Oligochitosan Supplementation on Ileal Digestibility of Nutrients and Performance in Broilers

R.-L. Huang,[†] Y.-L. Yin,^{†‡}¹ G.-Y. Wu,^{†§} Y.-G. Zhang,^{†#} T.-J. Li,[†] L.-L. Li,[†] M.-X. Li,^{†#} Z.-R. Tang,^{†#} J. Zhang,^{†#} B. Wang,^{†#} J.-H. He,^{||} and X.-Z. Nie^{||}

[†]Key Laboratory of Subtropical Agro-ecology, Institute of Subtropical Agriculture, The Chinese Academy of Sciences, Hunan 410125, The People's Republic of China; [‡]Department of Food Science and Engineering, Jiangxi 330047, The People's Republic of China; [§]Department of Animal Science, Texas A&M University, College Station 77843; ^{||}College of Animal Science, Hunan Agricultural University, Changsha, Hunan 410128; [#]Graduate School of The Chinese Academy of Sciences, Beijing, 100864, The People's Republic of China

ABSTRACT The effect of dietary chitosan oligosaccharides (COS) supplementation on ileal digestibilities of nutrients and performance in broilers was assessed by feeding graded levels (0, 50, 100, 150 mg/kg) of COS. Two thousand four hundred male commercial Avian broilers (1-d-old) were assigned randomly to 5 dietary treatment groups (60 birds per pen with 8 pens per treatment). Diet A was a typical corn- and soybean meal-based diet supplemented with 6 mg/kg of an antibiotic flavomycin (positive control). Diet B was the basal diet without any supplement. Diets C, D, and E were formulated by adding 50, 100, and 150 mg/kg of COS to the basal diet, respectively. On the morning of d 21 and 42, 64 birds (8 per pen with 8 pens per treatment) from the growth trial for

each age group were killed by cervical dislocation for determination of the ileal digestibilities of nutrients. Dietary supplementation with COS and antibiotic enhanced ($P < 0.05$) the ileal digestibilities of DM, Ca, P, CP, and all amino acids (except for alanine in the 21-d-old birds or phenylalanine, glutamate, and glycine for the 42-d-old birds). Feed efficiency was improved ($P < 0.05$) in response to dietary supplementation of an antibiotic or COS (150 mg/kg for d 1 to 21, and 100 and 150 mg/kg for d 21 to 42). The results demonstrate for the first time to our knowledge that dietary COS supplementation was effective in increasing the ileal digestibilities of nutrients and feed efficiency in broilers. Our findings may explain a beneficial effect of COS on chicken growth performance.

(Key words: chitosan, ileal digestibilities, performance, broilers)

2005 Poultry Science 84:1383–1388

INTRODUCTION

A decrease in the therapeutic effectiveness of antibiotics in treating a wide array of bacterial infections in humans has prompted several European countries to ban the use of dietary antibiotics (Simon et al., 2003). With increased concerns about the use of antibiotics in animal production, alternative means to enhance gut health need to be explored (Ricke et al., 2005). Birds use a variety of mechanisms against pathogens, including gastric acidification of ingested feed, rapid transit through sections of the intestinal tract, inhibition of pathogen colonization by the indigenous microbiota, the physical and enzymatic barrier of the epithelial lining, and the mucosa immune system (Guo, 1997). The intestinal microbiota, epithelium, and immune system are effective barriers against patho-

gen colonization. However, when pathogens are successful in colonizing the intestinal tract, the immune system reacts with an inflammatory and/or an antibody response (Guo, 1997). The primary alternatives to enhance gut function studied to date include acidification of feed, feeding probiotic organisms, and prebiotic compounds (Patterson and Burkholder, 2003).

Prebiotics are defined as “nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of the bacteria in the colon” (Gibson and Roberfroid, 1995). As regulators of nutrient utilization, oligosaccharides, mainly fructooligosaccharides and mannanoligosaccharide, are commonly fed to weaning pigs and poultry in Japan and are increasingly being used in Europe (Patterson and Burkholder, 2003). Fructooligosaccharides and mannanoligosaccharide have been shown to have prebiotic effects (Newman, 1994; Gao et al., 2001).

©2005 Poultry Science Association, Inc.
Received for publication March 10, 2005.
Accepted for publication June 9, 2005.

¹To whom correspondence should be addressed: yyulong2003@yahoo.com.cn.

Abbreviation Key: COS = chitosan oligosaccharides, ADG = average daily gain.

Chitosan oligosaccharides (COS) may have similar properties. Previous research has shown that dietary supplementation with COS inhibits the growth of oral (Tarsi et al., 1998) and intestinal (Xiao and Wu, 1996; Wang et al., 2003) pathogenic bacteria, while increasing the density of small-intestinal microvilli (Wang et al., 2003). The feeding of COS to pigs also favorably alters whole-body lipid metabolism (Jabbal et al., 1998; Wang, 1998; Tang et al., 2005) and increases serum levels of total protein, growth hormone, and insulin-like growth factor-I as well as hepatic and i.m. mRNA levels for insulin-like growth factor-I (Tang et al., 2005). Additionally, dietary COS supplementation to mice reduces the levels of early preneoplastic markers for colon carcinogenesis, while stimulating mucosal and systemic antibody responses against *Bordetella pertussis* filamentous hemagglutinin and recombinant pertussis (Torzsas et al., 1996; Jabbal et al., 1998). However, the mechanisms responsible for the observed effects of COS are not known, but could be due to improved digestibilities of dietary nutrients. Therefore, the objective of the study was to determine the effects of dietary COS supplementation on the ileal digestibilities of nutrients and performance in broilers.

MATERIALS AND METHODS

Experimental Design and Diets

Two thousand four hundred male commercial Avian broilers (1-d-old) were obtained from Zheng Da Thailand, Inc.² and assigned randomly to 5 dietary treatment groups (60 birds per pen with 8 pens per treatment). The experiment was carried out in accordance with the Chinese guidelines for animal welfare and approved by the animal welfare committee of Institute of Subtropical Agriculture, The Chinese Academy of Sciences.

Diets, Housing, and Management

Five diets were prepared for the study. Diet A was a typical corn- and soybean meal-based diet meeting NRC (1994) nutrient requirements for broilers and supplemented with 6 mg/kg of an antibiotic flavomycin³, as a positive control diet. Diet B was the basal diet without any supplement (Table 1). Diets C, D and E were formulated by the addition of 50, 100, and 150 mg/kg of COS to the basal diet, respectively. The diets were supplemented with 0.3% of Cr₂O₃ as an indigestible marker for determining the ileal digestibilities of nutrients. The chitosan used in this trial was provided by Dalian Chemical and Physical Institute⁴ and is a 6 sugar units of *N*-acetyl glucosamine with 1 to 4 β -linkages. The COS used in this study was a mixture of oligosaccharides with molecular weights

TABLE 1. Composition of the basal diets (% , as-fed basis) for starter and grower broilers

Ingredients	Starter (d 1 to 21)	Grower (d 22 to 42)
Corn	58.4	66.4
Soybean meal (45% CP)	35.5	27.3
Canola oil	1.3	1.6
Ground limestone	1.2	1.2
Calcium phosphate (20% P)	2.1	2.0
Methionine	0.08	0.08
Salt	0.25	0.25
Mineral and vitamin mix ¹	1.17	1.17
Chemical composition (analyzed contents; DM basis)		
Crude protein, %	21.00	18.20
Metabolizable energy, MJ/kg	12.34	12.74
Calcium, %	0.99	0.95
Available phosphorus, %	0.44	0.41
Sodium, %	0.20	0.17
Essential amino acid, %		
Arginine	0.67	0.60
Cystine	0.34	0.30
Histidine	1.13	1.00
Isoleucine	1.10	0.98
Leucine	1.73	1.54
Lysine	1.05	0.92
Methionine	0.46	0.40
Phenylalanine	2.07	1.84
Threonine	0.85	0.76
Valine	1.66	1.48
Nonessential amino acid, %		
Alanine	0.69	0.61
Aspartic acid	0.59	0.52
Glutamic acid	1.30	1.16
Glycine	2.21	1.96
Proline	0.59	0.52
Serine	1.12	1.00
Tyrosine	1.04	0.93

¹Supplying per kilogram of diet: vitamin A, 8,000 IU; vitamin D₃, 3,500 IU; vitamin E, 35.0 IU; vitamin K, 2.0 mg; thiamin, 4 mg; riboflavin, 6.0 mg; calcium pantothenate, 10 mg; vitamin B₁₂, 12 μ g; niacin, 76.4 mg; folic acid, 4.9 mg; choline chloride, 862.4 mg (=700 mg of choline); biotin, 200 μ g; niacin, 40 mg; pyridoxine, 4.0 mg; folic acid, 1.0 mg; manganic sulfate, 215.1 mg; zinc sulfate, 92.7 mg; copper sulfate, 39.3 mg; ferric citrate, 500 mg; Na₂SeO₃, 0.22 mg; potassium iodide 0.52 mg (=0.4 mg iodine).

between 10³ and 10⁴ Da. The diets were fed in a pellet form. All groups of broilers had free access to feed and drinking water.

The broilers were raised on a battery brooder house during d 1 to 21 and then transferred into floor pens during d 22 to 42. The size of each pen was 2 m² for the starter (d 1 to 21) and 4.6 m² for the grower (d 22 to 42). Birds were maintained on a 24-h constant-light schedule. The initial room temperature was set at approximately 32°C and reduced by 2 to 3°C weekly until reaching 22°C at wk 4, which was maintained for the remaining 6 wk. At the end of 1- and 4-wk periods of the experiment, all groups of chicks received i.m. administration of 0.5 mL of a commercial inactivated Newcastle disease vaccines. Throughout the study, broilers were checked daily for signs of disease and mortality. The birds were individually weighed, whereas feed intake and feed efficiency were determined for each pen, on a weekly basis.

²Yueyang, People's Republic of China.

³Hoechst AG, Frankfurt, Germany.

⁴The Chinese Academy of Sciences, Dalian, People's Republic of China.

Ileal Digestibility Trial

On the morning of d 21 and 42, 64 chicks (8 per pen with 8 pens per treatment) from the growth trial for each age group were killed by cervical dislocation. All of the ileal digesta between the yolk sac and the terminal ileum (2 cm above the ileal-cecal junction) were obtained immediately and carefully, as described by Gong et al. (2003). The digesta from each of the 8 birds per pen was pooled as one sample into a plastic bag and immediately stored at -20°C . Digesta were freeze-dried, ground through a 1.00-mm mesh screen, and mixed thoroughly before analyses.

Dry matter, gross energy, CP ($\text{N} \times 6.25$, macro-Kjeldahl), Ca, and P contents were determined according to AOAC (1990) methods. Samples also were analyzed for chromic oxide (Fenton and Fenton, 1979). Amino acid contents were determined by ion-exchange chromatography following hydrolysis in 6 M HCl at 110°C under N_2 for 24 h, as we described (Yin et al., 2002). Methionine and cysteine were determined as methionine sulfone and cysteic acid after oxidation with performic acid (AOAC, 1990). The values for glutamic acid and aspartic acid include glutamate plus glutamine and aspartate plus asparagine, respectively.

The apparent digestibility values for dietary nutrients were calculated as follows:

$$\text{DD} = 1 - [(\text{ID} \times \text{AF}) / (\text{IF} \times \text{AD})]$$

where DD is the apparent digestibility of a nutrient in diet; ID is the concentration of an indigestible marker in diet; AF is the nutrient concentration in ileal digesta; IF is the indigestible-marker concentration in ileal digesta; and AD is the nutrient concentration in diet.

Statistical Analysis

The data on the growth performance and ileal digestibilities of nutrients were analyzed as a completely randomized design (Steel et al., 1997). This was further split by periods using the GLM procedure of the SAS User's Guide.⁵ Differences between means were compared using the Student-Neuman-Keuls multiple comparison test. Probabilities less than 0.05 were taken to indicate statistical significance.

RESULTS

Average daily gain (ADG), feed intake, and feed efficiency data were computed and analyzed for d 1 to 21 and d 22 to 42 (Table 2). Broilers fed the 100 mg/kg of COS and 6 mg/kg of flavomycin diets had greater ADG ($P < 0.01$), compared with other diets, during the first 3 wk. There was no difference ($P > 0.05$) in ADG among the broilers fed the basal, 50, and 150 mg/kg COS diets.

Similar results were obtained for d-22 to 42 birds. Particularly, ADG (47.8 g) was the same for broilers fed the 100 mg/kg of COS and 6 mg/kg of flavomycin diets, which was greater ($P < 0.05$) than that for other treatment groups. Feed intake over d 1 to 21 did not differ ($P > 0.05$) among chicks fed the 6 mg/kg flavomycin diet and the 0, 50 and 100 mg/kg of COS diets. However, chicks fed the 150 mg/kg of COS diet exhibited lower feed intake when compared with those fed the 50 and 100 mg/kg of COS diets (Table 2). Feed intake over d 22 to 42 was not different among the 5 treatment groups ($P > 0.05$). The feed efficiency, however, was improved ($P < 0.05$) by dietary supplementation of 6 mg/kg of flavomycin, as well as 100 and 150 mg/kg of COS during the entire feeding period (d 1 to 42).

The effects of COS on ileal digestibilities (coefficients) of DM, energy, Ca, P, CP, and amino acids in 21-d-old broilers are summarized in Table 3. The ileal digestibilities of DM and energy in chicks fed the 100 mg/kg of COS diet were similar to those for birds fed the 160 mg/kg of COS diet ($P > 0.05$), but were greater ($P < 0.05$) than the values for birds fed the 6 mg/kg of COS diet, as well as the 0 and 50 mg/kg of COS diets. The ileal digestibilities of calcium and phosphorus increased ($P < 0.05$) with increasing the dietary supplemental level of COS from 0 to 100 mg/kg; the values did not differ ($P > 0.05$) between the 2 groups of chicks fed the 6 mg/kg of flavomycin diet and the 100 mg/kg of COS diet. Dietary supplementation with 150 mg/kg of COS increased and decreased ($P < 0.05$) the ileal digestibilities of phosphorus and calcium, respectively, in comparison with feeding of the 0 mg/kg of COS diet. The ileal digestibilities of CP and all amino acids (except for alanine) in chicks fed the 100 mg/kg of COS diet were similar to those fed the 150 mg/kg of COS and the 6 mg/kg of flavomycin diets ($P > 0.05$), but were greater ($P < 0.05$) than the values for birds fed the 0 and 50 mg/kg of COS diets. Increasing dietary supplemental levels of COS from 0 to 100 mg/kg increased ($P < 0.05$) the ileal digestibilities of phenylalanine in a concentration-dependent manner. The ileal digestibilities of measured nutrients (except for energy) did not differ ($P > 0.05$) between the 2 groups of chicks fed the 0 and 50 mg/kg diets. Results obtained for 42-d-old broilers (Table 4) were in general agreement with those for the 21-d-old chicks, except for the following observations for the older birds. First, the ileal digestibilities of DM, energy, calcium, CP, and histidine were greater ($P < 0.05$) for chicks fed the 50 mg/kg of COS diet, compared with birds fed the 0 mg/kg of COS diet. Second, the ileal digestibilities of alanine in chicks fed the 6 mg/kg diet and the 100 and 150 mg/kg of COS diets were similar ($P > 0.05$), but were greater ($P < 0.05$) than the values for birds fed the 0 and 50 mg/kg of COS diets. Third, the ileal digestibilities of phenylalanine and tyrosine did not differ ($P > 0.05$) among the 5 treatment groups.

DISCUSSION

Dietary supplementation with prebiotics, such as COS, has been shown to improve the health status of the gastro-

⁵SAS Institute Inc., Cary, NC.

TABLE 2. Effects of chitosan oligosaccharides (COS) on the growth performance of broilers

	6 mg/kg of flavomycin	0 mg/kg of COS	50 mg/kg of COS	100 mg/kg of COS	150 mg/kg of COS	SEM ¹	P-value
Treatment	A	B	C	D	E		
BW, g							
1 d	44.1 ^a	44.6 ^a	43.3 ^a	44.8 ^a	44.9 ^a	0.39	0.0642
21 d	812.6 ^{ab}	786.0 ^b	824.1 ^a	825.8 ^a	796.9 ^b	7.25	0.0221
42 d	2036.4 ^a	1976.2 ^b	1955.3 ^b	2053.9 ^a	1970.2 ^b	19.19	0.0006
Average daily gain, g							
1 to 21 d	37.2 ^a	35.3 ^b	36.6 ^b	37.2 ^a	35.8 ^b	0.34	0.0183
22 to 42 d	58.3 ^a	55.8 ^{ab}	53.9 ^b	58.5 ^a	55.9 ^{ab}	0.83	0.0004
1 to 42 d	47.8 ^a	45.5 ^b	45.2 ^b	47.8 ^a	45.8 ^b	0.46	0.0007
Average daily feed intake, g							
1 to 21 d	51.5 ^{ab}	51.0 ^{ab}	52.7 ^a	52.1 ^a	49.5 ^b	0.66	0.0411
22 to 42 d	144.9	144.4	141.5	145.0	140.3	2.99	0.7682
1 to 42 d	98.2	97.7	97.1	98.6	94.9	1.61	0.4201
Feed:gain							
1 to 21 d	1.384 ^b	1.440 ^a	1.439 ^a	1.401 ^{ab}	1.382 ^b	0.014	0.0052
22 to 42 d	2.516 ^b	2.587 ^a	2.627 ^a	2.479 ^b	2.511 ^b	0.028	0.0002
1 to 42 d	2.050 ^b	2.147 ^a	2.148 ^a	2.060 ^b	2.070 ^b	0.020	0.0264

¹Pooled standard error of the mean for 60 chicks per pen with 8 pens per treatment.

^{a,b,c}Values in the same row with a common letter are significantly different at $P < 0.05$.

intestinal tract; therefore, these substances are being actively investigated as indirect growth promoters (Newman, 1994; Patterson and Burkholder, 2003). The use of such compounds in diets for food animals has been assumed to minimize the use of antibiotics and thus reduce the possible occurrence of drug resistance in bacteria (Simon et al., 2003). To our knowledge, little is known about the effect of COS on nutrient digestion in poultry or other livestock species, and this is the first study to determine the effect of dietary COS supplementation on the ileal digestibilities of DM, energy, Ca, P, CP, and amino acids

in growing broilers. For comparison, we used an antibiotic (flavomycin) as a positive control to evaluate the efficacy of COS supplementation. Our results indicate that the efficiency of the utilization of dietary nutrients in broilers fed the 100 and 150 mg/kg of COS diets was higher than birds fed the 0 and 50 mg/kg of COS diets, and was similar to that for chicks fed the flavomycin-supplemented diet.

The enhanced ileal digestibilities of nutrients in the broilers fed the COS-containing diets might be explained by the following findings. First, COS supplementation

TABLE 3. Effects of chitosan oligosaccharides (COS) on the ileal digestibilities (coefficients) of DM, energy, Ca, P, CP, and amino acids in 21-d-old broilers

	6 mg/kg of flavomycin	0 mg/kg of COS	50 mg/kg of COS	100 mg/kg of COS	150 mg/kg of COS	SEM ¹	P-value
Treatment	A	B	C	D	E		
DM	0.80 ^{bc}	0.76 ^d	0.78 ^{cd}	0.85 ^a	0.83 ^{ab}	0.01	0.0001
Energy	0.82 ^b	0.77 ^c	0.82 ^b	0.88 ^a	0.86 ^a	0.01	0.0001
Ca	0.79 ^a	0.75 ^b	0.73 ^{bc}	0.78 ^a	0.71 ^c	0.01	0.0001
P	0.83 ^{ab}	0.80 ^b	0.81 ^{ab}	0.87 ^a	0.85 ^a	0.02	0.0099
CP	0.88 ^a	0.70 ^b	0.72 ^b	0.87 ^a	0.83 ^a	0.01	0.0001
Essential amino acid							
Arginine	0.85 ^a	0.77 ^b	0.78 ^b	0.84 ^a	0.82 ^a	0.01	0.0001
Cystine	0.84 ^a	0.79 ^b	0.79 ^b	0.84 ^a	0.83 ^a	0.01	0.0001
Histidine	0.90 ^a	0.78 ^b	0.80 ^b	0.90 ^a	0.86 ^{ab}	0.02	0.0001
Isoleucine	0.81 ^a	0.70 ^b	0.72 ^b	0.81 ^a	0.84 ^a	0.01	0.0001
Leucine	0.86 ^a	0.75 ^b	0.72 ^b	0.86 ^a	0.87 ^a	0.02	0.0001
Lysine	0.85 ^a	0.73 ^b	0.74 ^b	0.87 ^a	0.84 ^{ab}	0.02	0.0001
Methionine	0.82 ^a	0.75 ^b	0.74 ^b	0.84 ^a	0.83 ^a	0.00	0.0001
Phenylalanine	0.83 ^a	0.70 ^c	0.77 ^b	0.85 ^a	0.84 ^a	0.01	0.0001
Threonine	0.84 ^a	0.78 ^b	0.74 ^b	0.82 ^a	0.81 ^{ab}	0.01	0.0005
Valine	0.91 ^a	0.79 ^b	0.81 ^b	0.92 ^a	0.82 ^b	0.01	0.0001
Nonessential amino acid							
Alanine	0.83	0.82	0.81	0.82	0.79	0.01	0.06523
Aspartic acid	0.83 ^a	0.71 ^b	0.72 ^b	0.85 ^a	0.83 ^a	0.01	0.00001
Glutamic acid	0.83 ^a	0.78 ^b	0.77 ^b	0.84 ^a	0.82 ^a	0.01	0.00001
Glycine	0.86 ^a	0.77 ^b	0.80 ^b	0.88 ^a	0.88 ^a	0.02	0.0001
Proline	0.84 ^a	0.80 ^b	0.79 ^b	0.84 ^a	0.84 ^a	0.01	0.0037
Serine	0.86 ^a	0.72 ^b	0.76 ^b	0.83 ^a	0.82 ^a	0.01	0.0001
Tyrosine	0.89 ^a	0.80 ^b	0.78 ^b	0.88 ^a	0.86 ^a	0.01	0.0001

¹Pooled standard error of the mean for pooled samples of 8 chicks per pen with 8 pens per treatment.

^{a,b,c}Values in the same row with a common letter are significantly different at $P < 0.05$.

TABLE 4. Effects of chitosan oligosaccharides (COS) on the ileal digestibilities (coefficients) of DM, energy, Ca, P, CP, and amino acids in 42-d-old broilers

	6 mg/kg of flavomycin	0 mg/kg of COS	50 mg/kg of COS	100 mg/kg of COS	150 mg/kg of COS	SEM ¹	P-value
Treatment	A	B	C	D	E		
DM	0.85 ^a	0.82 ^b	0.86 ^a	0.86 ^a	0.86 ^a	0.01	0.0001
Energy	0.86 ^a	0.83 ^b	0.86 ^a	0.86 ^a	0.87 ^a	0.01	0.0003
Ca	0.66 ^b	0.60 ^c	0.75 ^a	0.69 ^a	0.68 ^b	0.01	0.0001
P	0.82 ^a	0.82 ^a	0.85 ^a	0.87 ^a	0.85 ^a	0.01	0.0938
CP	0.81 ^a	0.74 ^b	0.79 ^a	0.83 ^a	0.81 ^a	0.01	0.0001
Essential amino acid							
Arginine	0.78 ^a	0.73 ^b	0.74 ^{ab}	0.75 ^{ab}	0.81 ^a	0.02	0.0001
Cystine	0.87 ^a	0.81 ^b	0.79 ^b	0.84 ^a	0.84 ^a	0.02	0.0197
Histidine	0.85 ^a	0.77 ^b	0.81 ^a	0.82 ^a	0.81 ^a	0.02	0.0058
Isoleucine	0.80 ^a	0.77 ^b	0.77 ^b	0.83 ^a	0.80 ^a	0.02	0.0327
Leucine	0.86 ^a	0.79 ^b	0.79 ^b	0.84 ^a	0.81 ^{ab}	0.02	0.0034
Lysine	0.86 ^a	0.76 ^b	0.82 ^{ab}	0.86 ^a	0.87 ^a	0.01	0.0001
Methionine	0.86 ^a	0.80 ^b	0.80 ^b	0.84 ^a	0.82 ^a	0.01	0.0091
Phenylalanine	0.88	0.86	0.84	0.84	0.85	0.01	0.1025
Threonine	0.79 ^a	0.73 ^b	0.75 ^b	0.80 ^a	0.81 ^a	0.01	0.0007
Valine	0.78 ^b	0.77 ^b	0.79 ^b	0.79 ^b	0.83 ^a	0.01	0.0132
Nonessential amino acid							
Alanine	0.75 ^a	0.70 ^b	0.69 ^b	0.75 ^a	0.77 ^a	0.02	0.0003
Aspartic acid	0.85 ^a	0.81 ^b	0.78 ^b	0.86 ^a	0.85 ^a	0.01	0.0001
Glutamic acid	0.85 ^a	0.88 ^a	0.88 ^a	0.85 ^a	0.86 ^a	0.01	0.3391
Glycine	0.96	0.95	0.95	0.94	0.95	0.00	0.0761
Proline	0.78 ^a	0.73 ^b	0.75 ^b	0.79 ^a	0.79 ^a	0.02	0.0410
Serine	0.80 ^a	0.73 ^b	0.70 ^b	0.80 ^a	0.78 ^a	0.02	0.0024
Tyrosine	0.89	0.86	0.86	0.87	0.87	0.01	0.3197

¹Pooled standard error of the mean for pooled samples of 8 chicks per pen with 8 pens per treatment.

^{a,b,c}Values in the same row with a common letter are significantly different at $P < 0.05$.

reduced the number of pathogenic bacteria (e.g., *Escherichia coli*, *Salmonella typhimurium*) (Choi et al., 1994; LeMieux et al., 2003; Wang et al., 2003) and increased the beneficial bacteria (e.g., *Lactobacilli*) numbers (Oli et al., 1998) in the intestine. Such changes in the intestinal bacterial population resulted in a decrease in the incidence of diarrhea (Oli et al., 1998) and an increase in immune function (Gibson and Roberfroid, 1995; Patterson and Burkholder, 2003). Second, COS may stimulate the secretion of digestive enzymes from the stomach, pancreas, and intestinal mucosa (Hou and Gao, 2001). This effect is expected to reduce local inflammation in the intestinal mucosa, facilitate the breakdown of complex molecules into simpler ones, and enhance the integrity of enterocytes, thereby promoting the digestion and absorption of nutrients (Wu, 1998). Through an increase in the digestion and absorption of nutrients, dietary supplementation with COS reduces the excretion of fecal nitrogen and phosphorus from animals, thereby minimizing the major sources of environmental pollution. Dietary supplementation of 100 mg/kg of COS to broilers was as effective as a well-documented antibiotics (6 mg/kg of flavomycin) in enhancing the ileal digestibilities of nutrients and average daily gain, compared with the broilers fed the basal diet. Thus, compared with feeding an antibiotic, dietary COS supplementation to poultry and other livestock species offers 3 unique advantages: 1) preventing drug resistance in animals and humans; 2) improving the health of the small intestine; and 3) increasing the ileal digestibility of dietary phosphorus.

According to the limited published reports to date, the effects of COS on the growth performance of poultry or

other livestock species are not consistent. Some studies indicate that COS does not affect weight gain or feed efficiency in chickens (Hill et al. 1998; Wang, 1998; Zhang et al., 1998). However, Ma et al. (2001) and Wang et al. (2003) reported that COS increased the ADG of 0- to 42-d broilers. These discrepancies may result in part from the different molecular weights and different doses of the COS used in the experiments. The COS can serve only as adhesives and carrying agents when its molecular weight is greater than 10^5 Da. In contrast, COS can modulate immune responses and reduce the establishment of pathogens in the intestine when its molecular weights range between 10^3 to 10^4 , as was the case with the COS used in the present study. At a molecular weight $<10^3$, COS can stimulate the growth of bifid bacteria that have a number of health-promoting properties (Shigehiro et al., 1990; Hou and Gao, 2001). Importantly, results of the present study demonstrate that the growth performance and nutrient digestibilities in 1- to 42-d-old chicks exhibited a dose-dependent response to dietary COS supplementation (Tables 2 to 4). Compared with the 0 mg/kg COS diet, dietary supplementation with 100 mg/kg COS to broilers resulted in higher ileal digestibilities of almost all measured nutrients and higher average daily gain, but dietary supplementation with a lower (50 mg/kg) or a higher (150 mg/kg) dose of COS neither increased the ileal digestibilities of some essential amino acids (in both 21- and 42-d-old chicks), Ca (in 21-d-old chicks), or P (in 42-d-old chicks) (Tables 3 and 4), nor affected ADG (Table 2). Therefore, the enhanced ileal digestibilities of dietary nutrients contributed to the improved feed efficiency in COS-supplemented broilers.

In conclusion, dietary supplementation of 100 mg/kg of COS to broilers is as effective as a well-documented antibiotic in enhancing the ileal digestibilities of nutrients and ADG. Our results establish an optimal dose of COS for improving the efficiency of feed utilization in poultry. We suggest that an increase in the digestion and absorption of nutrients is a major mechanism responsible for the enhanced growth performance of broilers in response to dietary COS supplementation.

ACKNOWLEDGMENTS

This research was jointly supported by grants from the National Basic Research Program of China (contract/grant No. 2004CB117502), NSFC (contract/grant No. 30371038), the Outstanding Overseas Chinese Scholar Fund of the Chinese Academy of Sciences (No. 2005-1-4), and the Chinese Academy of Science Knowledge Innovation Project (contract No. KSCX2-SW-323).

REFERENCES

- AOAC. 1990. Official Methods of Analysis. 16th ed. Assoc. Off. Anal. Chem., Washington, DC.
- Choi, K. H., H. Namkung, and I. K. Paik. 1994. Effects of dietary fructooligosaccharides on the suppression of intestinal colonization of *Salmonella typhimurium* in broiler chickens. Korean J. Anim. Sci. 36:271-284.
- Fenton, T. W., and M. Fenton. 1979. An improved procedure for the determination of chromic oxide in feed and feces. Can. J. Anim. Sci. 59:631-634.
- Gao, F., G. H. Zhou, and Z. K. Han. 2001. Effect of fructooligosaccharides (FOS) on growth performance, immune function and endocrine secretion in chicks. Acta Zoonutr. Sin. 13:51-55.
- Gibson, G. R., and M. B. Roberfroid. 1995. Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. J. Nutr. 125:1401-1412.
- Gong, L. M., D. F. Li, F. L. Wang, and D. S. Huang. 2003. The effect of the ratio of threonine to lysine on performance, carcass quality, nutrient digestibility and plasma biochemistry indexes in broilers. Pages 52-63 in Proc. 2003 Studies on Animal Nutrition and Metabolism. Beijing Agricultural Press, Beijing, China.
- Guo, Y. M. 1997. Poultry Nutrition and Feedstuff. China Agricultural Univ. Press, Beijing, China.
- Hill, D. J., J. Petrik, and E. Arany. 1998. Growth factors and the regulation of fetal growth. Diabetes Care 21:B60-B69.
- Hou, Q. L., and Q. S. Gao. 2001. Chitosan and Medicine. Shanghai Science Technology Press, Shanghai, China.
- Jabbal, G. I., A. N. Fisher, R. Rappouli, S. S. Davis, and L. Illun. 1998. Stimulation of mucosal and systemic antibody responses against *Bordetella pertussis* filamentous haemagglutinin and recombinant pertussis toxin after nasal administration with chitosan in mice. Vaccine 16:2039-2046.
- LeMieux, F. M., L. L. Southern, and T. D. Bidner. 2003. Effects of mannan oligosaccharides on growth performance of weanling pigs. J. Anim. Sci. 81:2462-2487.
- Ma, X. Z., Y. Yang, X. D. Xie, and Y. L. Feng. 2001. Effects of chitosan on growth performance and lipometabolism of male broiler chickens. Fujian J. Agric. Sci. 16:30-34.
- Newman, K. E. 1994. Mannan-oligosaccharides: natural polymers with significant impact on the gastro-intestinal microflora and the immune system. Pages 167-174 in Proc. 10th Annu. Symp. Biotechnol. Feed Ind. Nottingham Univ. Press, Loughborough, UK.
- NRC. 1994. Nutrient Requirements of Poultry. 9th rev. ed. Natl. Acad. Press, Washington, D.C.
- Patterson, J. A., and K. M. Burkholder. 2003. Prebiotic feed additives: Rational and use in pigs. Pages 319-332 in Proc. 9th Int. Symp. Digest. Physiol. Pigs. Banff, Alberta, Canada.
- Oli, M. W., B. W. Petschow, and R. K. Buddington. 1998. Evaluation of fructooligosaccharide supplementation of oral electrolyte solution for treatment of diarrhea. Digest. Dis. Sci. 43:1380-147.
- Ricke, S. C., M. M. Kunderinger, D. R. Miller, and J. T. Keeton. 2005. Alternatives to antibiotics: Chemical and physical antimicrobial interventions and foodborne pathogen response. Poult. Sci. 84:667-675.
- Shigehiro, H., I. Chitoshi, and S. Haruyoshi. 1990. Chitosan as an ingredient for domestic animal feeds. J. Agric. Food Chem. 87:1214-1217.
- Simon, O., W. Vahjen, and L. Scharek. 2003. Micro-organisms as feed additives-probiotics. Pages 295-318 in Proc. 9th Int. Symp. Digest. Physiol. Pigs. Banff, Alberta, Canada.
- Steel, R. G. D., J. H. Torrie, and D. A. Dickey. 1997. Principles and Procedures of Statistics. McGraw-Hill, New York.
- Tang, Z. R., Y. L. Yin, C. M. Nyachoti, R. L. Huang, T. J. Li, C. B. Yang, X. J. Yang, J. Peng, D. S. Qi, J. J. Xing, Z. H. Sun, and M. Z. Fan. 2005. Effect of dietary supplementation of chitosan and galacto-mannan-oligosaccharide on serum parameters and the insulin-like growth factor-I mRNA expression in early-weaned piglets. Domest. Anim. Endocrinol. 28:430-441.
- Tarsi, R., B. Corbin, C. Pruzzo, and R. A. Muzzarelli. 1998. Effect of low molecular-weight chitosans on the adhesive properties of oral streptococci. Oral Microbiol. Immunol. 34:217-224.
- Torzsas, T. L., C. W. C. Kendall, M. Sugano, Y. Iwamoto, and A. V. Rao. 1996. The influence of high and low molecular weight chitosan on colonic cell proliferation and aberrant crypt foci development in CF1 Mice. Food Chem. Toxicol. 34:73-77.
- Wang, X. B. 1998. Research of effect of chitosan on cholesterol content of meat of broiler. Feedstuff Res. 5:9-10.
- Wang, X. W., Y. G. Du, X. F. Bai, and S. G. Li. 2003. The effect of oligochitosan on broiler gut flora, microvilli density, immune function and growth performance. Acta Zoonutr. Sin. 15:32-45.
- Wu, G. 1998. Intestinal mucosal amino acid catabolism. J. Nutr. 128:1249-1252.
- Xiao, W. X., and K. L. Wu. 1996. The Function and Properties of chitosan. J. Wuxi Light Industry Univ. 15:297-320.
- Yin, Y. L., R. L. Huang, H. Y. Zhong, T. J. Li, W. B. Souffrant, and C. F. M. de Lange. 2002. Evaluation of mobile nylon bag technique for determining apparent ileal digestibilities of protein and amino acids in growing pigs. J. Anim. Sci. 80:409-420.
- Zhang, L. Y., B. W. Wang, and G. Y. Ying. 1998. Effect of chitosan on cholesterol content of serum and yolk in egg-chicken. Feedstuff Res. 10:31-32.