Effects of Dietary Fructooligosaccharide on Digestive Enzyme Activities, Intestinal Microflora and Morphology of Male Broilers

Z. R. Xu, C. H. Hu,[1] M. S. Xia, X. A. Zhan, and M. Q. Wang

Zhejiang University, Animal Science College; Key Laboratory of Molecular Animal Nutrition, Ministry of Education, HangZhou, 310029, P. R. China

ABSTRACT Two hundred forty male Avian Farms broiler chicks, 1 d of age, were randomly allocated to four treatments, each of which had five pens of 12 chicks per pen. The chicks were used to investigate the effects of fructooligosaccharide (FOS) on digestive enzyme activities and intestinal microflora and morphology. The chicks received the same basal diet based on corn-soybean meal, and FOS was added to the basal diet at 0, 2.0, 4.0, and 8.0 g/kg diet at the expense of corn. Addition of 4.0 g/kg FOS to the basal diet significantly increased average daily gain of broilers. The feed-to-gain ratios were significantly decreased for the birds fed diets with 2.0 and 4.0 g/kg FOS versus the control. Addition of 4.0 g/kg FOS enhanced the growth of *Bifidobacterium* and *Lactobacillus*, but inhibited *Escherichia coli* in the small intestinal and cecal digesta. Supplementation of 2.0 or 4.0 g/kg FOS to chicks significantly improved the activities of amylase compared to the control (12.80 or 14.75 vs. 8.42 Somogyi units). A significant increase in the activities of total protease was observed in 4.0 g/kg FOS-treated birds versus controls (83.91 vs. 65.97 units). Morphology data for the duodenum, jejunum, and ileum showed no significant differences for villus height, crypt depth, or microvillus height at the duodenum. By contrast, addition of 4.0 g/kg FOS significantly increased ileal villus height, jejunal and ileal microvillus height, and villus-height-to-crypt-depth ratios at the jejunum and ileum and decreased crypt depth at the jejunum and ileum. However, addition of 8.0 g/kg FOS had no significant effect on growth performance, digestive enzyme activities, intestinal microflora, or morphology.

(*Key words*: broiler, digestive enzyme, fructooligosaccharide, intestinal microflora, intestinal morphology)

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INTRODUCTION

Fructooligosaccharides (FOS) reportedly can be substituted for antibiotics to enhance the growth and production efficiency of broilers (Ammerman et al., 1988a,b, 1989; Wu et al., 1999). They can be classified as nondigestible oligosaccharides because the β-linkages between fructose monomers cannot be hydrolyzed by enzymes of endogenous origin. As a consequence, FOS are quantitatively available as substrates for gastrointestinal microflora (Roberfroid et al., 1998). The FOS have been shown to enhance the growth of *Bifidobacterium* and *Lactobacillus* but inhibit *Escherichia coli* and *Salmonella* in the large intestine (Hidaka et al., 1986; Choi et al., 1994; Bunce et al., 1995; Roberfroid et al., 1998; Fukata, 1999; Xu, et al., 2002). There are several microorganisms in the small intestine of chicks, and so FOS may be fermented to some extent in the small intestine of chicks. However, there is extremely limited information on the effects of FOS on activity of the microflora in the small intestine. Moreover, data on the effects of FOS on the digestive enzyme activities and intestinal morphology in broilers are lacking.

Therefore, an experiment was carried out to investigate the effects of dietary FOS on intestinal microflora, the digestive enzyme activities of the small intestinal digesta, and the intestinal morphology in male broilers.

MATERIALS AND METHODS

Birds and Diets

All procedures were approved by the University of Zhejing Institutional Animal Care and Use Committee. Two hundred forty male Avian Farms broiler chicks, 1 d of age, were randomly allocated to four treatments, each of which had five pens of 12 chicks per pen. The chicks received the same soybean meal, and FOS was added to the basal diet at 0, 2.0, 4.0, 8.0 g/kg diet at the expense of corn. The concentration of oligosaccharides

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[1]To whom correspondence should be addressed: chhu@zju.edu.cn.
[2]Meiji Seika Kaisha Ltd., Tokyo, Japan.

Abbreviation Key: ADG = average daily gain; ADS = anaerobic dilution solution; FOS = fructooligosaccharide.

of the FOS (Meioligo-P)[2] were greater than 95% of the total mixture.

Diets were fed from 1 to 49 d including starter (1 to 21 d), grower (21 to 42 d), and finisher (42 to 49 d). Nutrient levels of the diets (Table 1) were based on NRC (1994) recommendations. Feeds were analyzed for CP, calcium, and total phosphorous according to the methods of AOAC (1990). Antibiotic was excluded from all diets. All chicks were given ad libitum access to feed and water. The chicks were raised in wooden cages (120 × 90 × 50 cm, length × width × height), equipped with nipple waterers and tube feeders. Temperature was maintained at 32°C for the first 5 d and then gradually reduced according to normal management practices until a temperature of 22°C was achieved. Continuous lighting was maintained. Chicks were weighed individually at 1 and 49 d of age to determination average daily gain (ADG). Feed consumed per cage basis was recorded daily; the uneaten feed was discarded, and the feeders were replenished with fresh feed. Average daily feed intake, and feed/gain were calculated.

At the 49th day of the feeding trial, 15 chickens per treatment (3 per cage) were slaughtered by severing the jugular vein. The birds were then immediately eviscerated for collection of gastrointestinal tract digesta and intestinal samples for morphological changes.

Intestinal Microbial Populations

Samples of the contents from the small intestine (from the distal end of the duodenum to the ileocecal junction) and cecum were immediately collected into glass containers under CO_2, sealed, and put on ice until they were transported to the laboratory for enumeration of microbial populations. Ten grams of mixed contents were blended under CO_2 in 90 mL of anaerobic dilution solution (ADS) (Bryant and Allison, 1961). Further serial dilutions were made in ADS for anaerobic bacterial enumeration (Bryant, 1972). The initial dilution in ADS was also used as a source for serial dilutions in PBS for enumeration of aerobic bacterial populations. Triplicate plates were then inoculated with 0.1-mL samples and incubated at 37°C aerobically or anaerobically as appropriate. Three dilutions were plated for each medium. Bacteria were enumerated on Wilkins Chalgren agar[3] (total anaerobes), MRS agar[3] (*Lactobacillus*), reinforced clostridial agar plus supplements (Munoa and Pares, 1988; *Bifidobacterium*), and MacConkey's No. 2[3] (*Escherichia coli*). Single colonies were removed from selective media plates and grown in peptone yeast glucose broth (Holdeman et al., 1977). Subsequently, the bacteria were characterized to genus on the basis of colonial appearance, Gram reaction, spore production, cell morphology, and fermentation end-product formation (Holdeman et al., 1977).

Digestive Enzyme Activities in the Small Intestinal Contents

Sampling Procedure. The following procedures were according to the method described by Jin et al. (2000). The contents taken from the small intestine were digesta from the distal end of the duodenum to the ileocecal junction. A homogenous intestinal digesta sample was collected by massaging the tract from both ends. The digesta samples were stored immediately at –70°C until used. The small intestinal digesta samples were diluted 10×, based on the sample weight, with ice-cold PBS (pH 7.0), homogenized for 60 s, and sonicated for 1 min with three cycles at 30-s intervals. The sample was then centrifuged at 18,000 × *g* for 20 min at 4°C. The supernatants were divided into small portions and stored at –70°C for enzyme assays.

Digestive Enzyme Assay. Assays for digestive enzymes were carried out as described by Jin et al. (2000). Amylase (a-1,4-glucan 4-glucanohydrolase, EC 3.2.1.1) activity was determined using the method of Somogyi (1960). Amylase activity unit (1 Somogyi unit) was defined as the amount of amylase that will cause formation of reducing power equivalent to 1 mg of glucose in 30 min at 40°C/mg of intestinal digesta protein. The substrate used in the analysis was cornstarch. All chemicals were purchased in a kit (No. 700).[4] The analytical procedure was in accordance with supplier instructions.[4]

Lipase (triacylglycerol lipase. EC 3.1.1.3.) activity was assayed using the method described by Tietz and Fiereck (l966). Lipase activity unit (Sigma-Tietz units) was equal to the volume (mL) of 0.05 *M* NaOH required to neutralize the fatty acid liberated during 6 h of incubation with 3 mL of lipase substrate at 37°C/mg of intestinal digesta protein. Olive oil was used as the substrate in this assay. All chemicals were purchased in a kit (No. 800).[4]

Protease activity was analyzed using the modified method of Lynn and Clevette-Radford (1984). The protease activity unit was defined as milligrams of azocasein degraded during 2 h of incubation at 38°C/mg of intestinal digesta protein. Azocasein was used as the substrate.

The intestinal digesta protein concentrations were determined by the method of Lowry et al. (1951). Ovine serum albumin was used as a standard. All chemicals were purchased in a kit (No. 690).[4]

Light Mcroscopy

Segments were removed from the duodenum, jejunum, and ileum as follows: 1) intestine from the gizzard to pancreatic and bile ducts was referred to as the duodenum, the middle section of which was taken for microscopy; 2) midway between the point of entry of the bile ducts and Meckel's diverticulum (jejunum), and 3) 10 cm proximal to the ileocecal junction (ileum). The samples were flushed with physiological saline and fixed in 10% formalin. Three cross-sections for each in-

[3]Oxoid, Ltd., www.oxoid.com.
[4]Sigma Chemical Co., St. Louis, MO.
[5]Leica Imaging Systems Ltd., Cambridge, UK.

TABLE 1. Ingredients and nutrient composition of diets

Item	Starter 0 to 21 d	Grower 21 to 42 d	Finisher 42 to 49 d
Ingredient (%)			
Corn	56.00	62.00	64.04
Soybean meal (dehulled, solvent)	30.30	27.40	28.40
Fishmeal	6.00	3.00	0.00
Rapeseed oil	4.00	4.00	4.00
NaCl	0.30	0.30	0.30
Calcium phosphate	1.50	1.50	1.50
Limestone	1.20	1.20	1.20
DL-Methionine	0.20	0.10	0.06
Vitamin-mineral premix[1]	0.50	0.50	0.50
Nutrition composition			
Calculated[2]			
ME (Mcal/kg)	3.06	3.12	3.12
Crude protein (%)	22.51	19.61	18.20
Lysine (%)	1.28	1.07	0.96
Methionine + cysteine (%)	0.90	0.70	0.62
Calcium (%)	1.01	0.88	0.76
Total phosphorus (%)	0.82	0.72	0.64
Available phosphorus (%)	0.46	0.37	0.33
Analyzed composition			
Crude protein (%)	22.84	19.72	18.16
Calcium (%)	0.94	0.90	0.81
Total phosphorus (%)	0.85	0.70	0.67

[1]Supplied per kilogram of diet: vitamin A, 1,500 IU; cholecalciferol, 200 IU; vitamin E, 10 IU; riboflavin, 3.5 mg; pantothenic acid, 10 mg; niacin, 30 mg; cobalamin, 10 μg; choline chloride, 1,000 mg; biotin, 0.15 mg; folic acid, 0.5 mg; thiamine 1.5 mg; pyridoxine 3.0 mg; iron, 80 mg; zinc, 40 mg; manganese, 60 mg; iodine, 0.18 mg; copper, 8 mg; selenium, 0.15 mg.

[2]Based on NRC (1994) feed composition tables.

testinal sample (total of 15 samples for each of the three intestinal segments per dietary treatment) were then prepared after staining with hematoxylin and eosin using standard paraffin embedding procedures (Uni et al., 1998).

A total of 10 intact, well-oriented crypt-villus units were selected in triplicate for each intestinal cross-section (30 measurements for each sample, total of 450 measurements for each of the three intestinal segments per dietary treatment). Villus height was measured from the tip of the villi to the villus crypt junction, and crypt depth was defined as the depth of the invagination between adjacent villi. Morphological indices were determined using image processing and analysis system (Version 1).[5]

Transmission Electron Microscopy

Specimens of the duodenum, jejunum, and ileum were collected from the same sites as those used for tissues examined with light microscopy. Samples were fixed in 2.5% glutaraldehyde in 0.1 *M* phosphate buffer (pH 7.0). The fixed samples were washed in the buffer, dehydrated with graded ethanol, and embedded in Spurr's resin. Three ultrathin sections for each intestinal sample (total of 15 samples for each of the 3 intestinal segments per dietary treatment) were cut and stained with 2% uranyl acetate, poststained with 0.2% lead citrate, and examined in a Jeol JEM-1200 transmission electron microscope at 60 kV. Midvillus regions were identified under low magnification. After midvillus regions were located, micrographs of the microvillus border were taken at 20,000× magnification. A total of 10 well-oriented longitudinal microvilli were assessed for each intestinal section (30 measurements for each sample, total of 450 measurements for each of the 3 intestinal segments per dietary treatment).

Statistical Analysis

One-way analysis of variance was performed using the general linear model procedure of SAS software (1989). Differences among means were tested using Duncan's multiple-range tests. A significance level of 0.05 was used.

RESULTS

Growth Performance

Addition of 4.0 g/kg FOS to the basal diet significantly increased ADG of broilers (Table 2). Addition of 2.0 or 8.0 g/kg FOS had no effect on ADG. The feed-to-gain ratios were decreased by 0.12 ($P < 0.05$) and 0.20 units ($P < 0.05$) for the birds fed diets with 2.0 and 4.0 g/kg FOS versus the control, respectively. However, feed intake was unaffected by dietary treatments.

Intestinal Microflora

In the small intestinal digesta, the viable counts of *Bifidobacterium* and *Lactobacillus* were significantly in-

TABLE 2. Effect of FOS on growth performance of male broilers[1]

Item	Dietary FOS[2] level (g/kg) 0	2.0	4.0	8.0	SEM
ADG (g)	47.16[b]	50.22[ab]	52.53[a]	49.41[ab]	1.264
ADFI (g)	104.75[a]	105.46[a]	106.11[a]	104.76[a]	0.725
F/G	2.22[a]	2.10[b]	2.02[b]	2.12[ab]	0.035

[a,b]Means within a row with different letters differ significantly ($P < 0.05$) when tested with Duncan's new multiple-range test following analysis of variance.

[1]Data represent mean values of five replicate cages of 12 chicks each.

[2]FOS = fructooligosaccharide; ADG = average daily gain; ADFI = average daily feed intake; F/G = feed per gain.

creased, whereas those of *Escherichia coli* were significantly reduced for the broilers fed diets with 4.0 g/kg FOS versus control (Table 3). Similarly, in cecal digesta, the viable counts of total anaerobes and *Bifidobacterium* were significantly increased for the birds fed diets with 4.0 g/kg FOS, and those of *Lactobacillus* were significantly increased, whereas those of *E. coli* were significantly reduced for the birds fed with 2.0 and 4.0 g/kg FOS versus control.

Digestive Enzymes

Supplementation of 2.0 or 4.0 g/kg FOS to chick diets significantly improved the activities of amylase after 49 d of feeding versus control (12.80 or 14.75 vs. 8.42 Somogyi units; Table 4). Supplementation of 4.0 g/kg FOS to chick diets significantly improved the activities of total protease versus control (83.91 vs. 65.97 Units). However, supplementation of 8.0 g/kg FOS had no effect on digestive enzymes. The intestinal lipolytic activities were unaffected by dietary treatments.

Morphological Measurement of the Small Intestinal Mucosa

No dietary effect was apparent for villus height and crypt depth at the duodenum (Table 5). By contrast, villus height at the ileum was significantly higher and crypt depths at the jejunum and ileum were significantly lower for the birds fed diets with 4.0 g/kg FOS versus control. Addition of 4.0 g/kg FOS to the basal diet significantly increased the ratios of the villus height to crypt depth at the jejunum and ileum versus control.

Microvillus Height at Different Sites of the Small Intestine

No dietary effect was apparent for microvillus height at the duodenum (Table 6). By contrast, the jejunal microvillus was significantly longer for the birds fed diets with 4.0 g/kg FOS versus control. The ileal microvillus was significantly longer for the birds fed diets with 2.0 and 4.0 g/kg FOS versus control.

DISCUSSION

Effects of Dietary FOS on Growth Performance

Some workers have reported increased growth and improved feed conversion ratio as a consequence of FOS inclusion in broiler diets (Ammerman et al., 1988 a,b, 1989; Bailey et al., 1991; Fukata, 1999; Wu et al., 1999). However, the recommended concentration of FOS in

TABLE 3. Viable cell counts of microflora in small intestinal and cecal digesta of male broilers[1,2]

Site and microflora	Dietary FOS[3] level (g/kg) 0	2.0	4.0	8.0	SEM
Small intestine					
Total anaerobes	8.47[a]	8.69[a]	8.75[a]	8.81[a]	0.36
Bifidobacterium	7.22[b]	7.82[ab]	8.11[a]	7.64[ab]	0.25
Lactobacillus	7.46[b]	8.11[ab]	8.47[a]	8.20[ab]	0.31
Escherichia coli	7.03[b]	6.46[ab]	6.18[a]	6.71[ab]	0.22
Cecum					
Total anaerobes	9.55[b]	9.73[ab]	9.85[a]	9.73[ab]	0.09
Bifidobacterium	8.36[b]	8.81[ab]	8.94[a]	8.68[ab]	0.18
Lactobacillus	8.42[b]	9.02[a]	9.08[a]	8.80[ab]	0.15
Escherichia coli	7.72[a]	7.11[b]	7.17[b]	7.73[a]	0.17

[a,b]Means within a row with different letters differ significantly ($P < 0.05$).

[1]Bacterial numbers are expressed as $\log_{10}$ colony-forming units per gram of DM.

[2]Means represent 15 chicks at 49 d of age (three chicks from each of five cages) per treatment for a total of five experimental units per treatment.

[3]Fructooligosaccharide.

TABLE 4. Effects of FOS on the digestive enzyme activities in the small intestinal contents of male broilers[1,2]

	Dietary FOS[3] level (g/kg)				
Enzyme	0	2.0	4.0	8.0	SEM
Protease (unit)	65.97[b]	75.53[ab]	83.91[a]	77.35[ab]	5.104
Amylase (Somogyi unit)	8.42[c]	12.80[ab]	14.75[a]	10.68[bc]	0.956
Lipase (Sigma-Tietz unit)	22.68[a]	24.37[a]	20.91[a]	25.86[a]	2.754

[a–c]Means within a row with different letters differ significantly ($P < 0.05$).

[1]Amylase activity unit (1 Somogyi unit) was defined as the amount of amylase that would cause formation of reducing power equivalent to 1 mg of glucose in 30 min at 40°C/mg of intestinal digesta protein. Lipase activity unit (Sigma-Tietz units) was equal to the volume (mL) of 0.05 *M* NaOH required to neutralize the fatty acid liberated during 6 h incubation with 3 mL of lipase substrate at 37°C/mg of intestinal digesta protein. The protease activity unit was defined as mg of azocasein degraded during 2 h incubation at 38°C/mg of intestinal digesta protein.

[2]Means represent 15 chicks at 49 d of age (three chicks from each of five cages) per treatment for a total of five experimental units per treatment.

[3]Fructooligosaccharide.

broiler diets is not consisten among authors. Ammerman et al. (1988b) found that addition of 2.5 and 5.0 g/kg FOS significantly improved feed efficiency over the entire feeding period of 46 d. They also noted that treatments of 3.75 g/kg FOS produced heavier birds at 47 d than the control or 7.5 g/kg FOS (Ammerman et al., 1989). Fukata (1999) reported that 1 g/kg FOS significantly decreased the mean numbers of cecal *Salmonella enteritidis* in the chicks. Bailey et al. (1991) reported that 3.75 g/kg FOS was not sufficient to affect colonization of *Salmonella typhimurium*, whereas 7.5 g/kg affected levels of that species. Wu et al. (1999) observed that the optimal FOS level was 2.5 to 5.0 g/kg, which had a beneficial effect on BW gain and feed efficiency, whereas too much FOS (10 g/kg) caused diarrhea, thus decreasing production performance. In the present study, addition of 4.0 g/kg FOS to the basal diet improved growth performance. Moreover, treatment with 8.0 g/kg FOS resulted in poorer performance than 2.0 and 4.0 g/kg FOS. Other authors, however, reported no effects of FOS on growth of broilers (Waldroup et al., 1993).

Effects of Dietary FOS on Intestinal Microflora in the Small Intestinal and Cecal Digesta

The results from our experiment showed that FOS exerted a preferential stimulatory effect on several bacteria of the health-promoting genus (*Bifidobacterium* and *Lactobacillus*), while maintaining populations of unprofitable or potential pathogens (*E. coli*) at relatively low levels in the small intestinal and cecal digesta. The increases in numbers of *Bifidobacterium* and *Lactobacillus* and decreases in numbers of *E. coli* in the large intestine supplemented with FOS were consistent with the results of most other research groups (Hidaka et al., 1986; Choi et al., 1994; Bunce et al., 1995; Roberfroid et al., 1998; Fukata, 1999; Xu et al., 2002).

TABLE 5. Effects of fructooligosacchqride (FOS) on the morphology of the intestinal mucosa at different sites in the small intestine[1]

	Dietary FOS level (g/kg)				
Site	0	2.0	4.0	8.0	SEM
Villus height (μm)					
Duodenum	674.13[a]	652.71[a]	688.16[a]	670.33[a]	20.26
Jejunum	780.45[a]	803.61[a]	814.73[a]	803.04[a]	28.27
Ileum	541.18[b]	598.04[ab]	625.40[a]	570.13[ab]	24.15
Crypt depth (μm)					
Duodenum	481.52[a]	487.09[a]	498.67[a]	482.25[a]	22.14
Jejunum	530.92[b]	495.51[ab]	441.16[a]	499.27[ab]	26.75
Ileum	436.44[b]	367.81[ab]	325.33[a]	389.35[ab]	30.81
Villus height: crypt depth					
Duodenum	1.40[a]	1.34[a]	1.38[a]	1.39[a]	0.06
Jejunum	1.47[b]	1.62[ab]	1.85[a]	1.61[ab]	0.09
Ileum	1.24[c]	1.63[b]	1.92[a]	1.46[bc]	0.08

[a–c]Means within a row with different letters differ significantly ($P < 0.05$).

[1]Means represent 15 chicks at 49 d of age (three chicks from each of five cages) per treatment for a total of five experimental units per treatment. There was one sample for each of the three intestinal segments per chick, three cross-sections per sample, 45 cross-sections for each of the three intestinal segments per treatment, and 10 measurements per cross-section for a total of 450 measurements for each of the three intestinal segments per treatment).

TABLE 6. Effects of fructooligosaccharide (FOS) on the microvillus height at different sites in the small intestine[1]

	Dietary FOS level (g/kg)				
Site	0	2.0	4.0	8.0	SEM
Duodenum (μm)	1.96[a]	2.04[a]	2.12[a]	2.06[a]	0.09
Jejunum (μm)	2.15[b]	2.42[ab]	2.61[a]	2.43[ab]	0.11
Ileum (μm)	1.40[c]	1.75[ab]	1.94[a]	1.54[bc]	0.08

[a–c]Means in a row with different letters differ significantly ($P < 0.05$).

[1]Means represent 15 chicks at 49 d of age (three chicks from each of five cages) per treatment for a total of five experimental units per treatment. There was one sample for each of the three intestinal segments per chick, three ultrathin sections per sample, 45 sections for each of the three intestinal segments per treatment, and 10 measurements per section for a total of 450 measurements for each of the three intestinal segments per treatment).

There are several microorganisms in the small intestine of broilers, and so FOS may be fermented to some extent in the small intestine. The results in the present study indicated that bacterial populations in the small intestinal digesta were affected by supplementation with FOS. In another study, Bolduan et al. (1993) reported that supplementation with 2 g/kg FOS for weaned pigs did not affect bacterial populations in the small intestinal digesta. The reason of the lack of effect of FOS supplementation on the small intestinal bacterial populations in their study may be because the concentration of FOS (2 g/kg) was not adequate to alter microbial populations. In studies with poultry (Bailey et al., 1991), 3.75 g/kg of FOS was not sufficient to affect colonization of *Salmonella typhimurium*, whereas 7.5 g/kg decreased concentrations of that species. Secondly, the overall intestinal health of those piglets may have been a factor in the lack of response to the dietary treatments. Oligosaccharides may not selectively enrich for *Bifidobacterium* when the indigenous population is high before treatment (Hidaka et al., 1986). Addition of *Bifidobacterium* or oligosaccharides to the diet of humans has been shown to have no effect when the natural level of *Bifidobacterium* is high (Hidaka et al., 1986). For the species in that study, the number of *Bifidobacterium* might have been enough that FOS did not increase *Bifidobacterium* numbers.

Effects of Dietary FOS on Digestive Enzyme Activities in the Small Intestinal Digesta

The results of the effects of FOS on the small intestinal digestive enzyme activities indicated that supplementation with FOS improved the activities of total protease and amylase. Data from the intestinal microflora showed that FOS exerted a preferential stimulatory effect on *Bifidobacterium* and *Lactobacillus*, while it suppressed *E. coli* in the small intestine. Such changes in microbial ecosystem in the presence of FOS might contribute to the observed effects on the digestive enzyme activities in the small intestinal contents. The *Bifidobacterium* and *Lactobacillus* colonizing the intestine have been reported to deliver enzymes, thus increasing the intestine digestive enzyme activity (Sissons, 1989). However, the *E. coli* may damage the villus and microvillus of intestinal mucosa and inhibit the secretion of digestive enzymes (Gao, 1998). Moreover, *E. coli* could secrete the proteolytic enzyme, which may use the intestinal digestive enzymes as selective nutrients, thus increasing the degradation of digestive enzyme (Conway, 1994).

Effects of Dietary FOS on Change in Intestinal Morphology

The structure of the intestinal mucosa can reveal some information on gut health. Stressors that are present in the digesta can lead relatively quickly to changes in the intestinal mucosa due to the close proximity of the mucosal surface and the intestinal content. Changes in intestinal morphology such as shorter villi and deeper crypts have been associated with the presence of toxins (Yason et al., 1987; Anonymous, 1999). A shortening of the villi decreases the surface area for nutrient absorption. The crypt can be regarded as the villus factory, and a large crypt indicates fast tissue turnover and a high demand for new tissue (Yason et al., 1987; Anonymous, 1999). Demand for energy and protein for gut maintenance is higher compared to other organs. A fast-growing broiler devotes about 12% of the newly synthesized protein to the digestive tract (Anonymous, 1999). Any additional tissue turnover will increase nutrient requirements for maintenance and will therefore lower the efficiency of the animal. A shortening of the villus and a large crypt can lead to poor nutrient absorption, increased secretion in the gastrointestinal tract, diarrhea, reduced disease resistance, and lower overall performance. In the present study, increases in ileal villus height and villus height (crypt depth ratio at the jejunum and ileum) in FOS-fed chicks were found. Similarly, Sonmez et al. (1999) observed that addition of 1 g/kg mannanoligosaccharide to the basal diet increased ileal and jejunal villus heights. It is likely that these changes are due to the ability of FOS to create a more favorable intestinal microbial environment and are not a direct action of FOS on the intestinal tissue.

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