Study of the Application of Fructooligosaccharides in Piglets

Chuanlai Xu*, Xudong Chen[1], Cheng Ji[1], Qiugang Ma[1] and Kai Hao
School of Food Science and Technology, Southern Yangtze University, Wuxi, JiangSu, 214036, P. R. China

ABSTRACT : In this study, 90 crossbred weaned pigs (Duroc×Landrace×Large White) weighing ~ 7.86±0.06 kg each were randomly allotted to one of three dietary treatments. Control pigs were a fed corn-soybean meal diet with no additives. The two treatment groups were fed the basal diet supplemented either with 75 mg/kg Aureomycin or 0.4% fructooligosaccharides (FOS) in order to study the effects on performance, serological indices, and enteric morphology in addition to examining the content of volatile fatty acids in intestinal digesta. The results indicate that the diets containing FOS and antibiotics had a significant effect on feed conversion ratios (FCR) and diarrhea incidence, as well as increasing the concentrations of isobutyric and butyric acid and total VFAs in the caecum, and acetic acid, isovaleric acid, and total VFAs in feces. Supplementation with FOS also resulted in significantly longer mucosal villi height and a higher percentage of goblet cells compared with the control. No difference was found in crypt depth among the three treatments. While serum glucose levels were significantly higher following FOS supplement, differences in serum total protein, albumin, globulin, and urea nitrogen levels were not significant. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 7 : 1011-1016)***

Key Words : FOS, Growing Performance, Enteric Morphology, Serum, VFAs, Piglet

INTRODUCTION

The period around weaning is a critical time in the life of a young piglet. The change from milk to solid food, the absence of maternal immunoglobulins, and the stress of a change of environment and littermates can endanger the intestinal health of the animal (Mathew et al., 1998). The immune system of the young pig is also not yet fully developed, allowing increased susceptibility to pathogen activity. Sub-therapeutic levels of feed grade antibiotics are typically used to reduce stress and to decrease possible negative effects during the transition (Corpet, 1996). However, the use of antibiotics in animal feed is a cause for concern in view of the risk of selection of resistant strains of microorganisms (Turner et al., 2001). It is possible that a total ban on several of the feed grade antibiotics may be implemented in the near future (Muirhead, 1998; White et al., 2002). To maintain animal health and productivity in such a scenario, alternatives need to be evaluated.

One possible alternative to the use of growth promoting antibiotics might be found in prebiotics. Prebiotics are food ingredients that selectively stimulate the activity and/or growth of beneficial bacteria such as Bifidobacteria and Lactobacilli (Gibson et al., 1995). They are short-chain carbohydrates (SCCs) that are non-digestible by animal enzymes (Quigly et al., 1999). Current findings concerning the use of fructooligosaccharides (FOS) suggests they are resistant to digestion by gastric acid and pancreatic enzymes both *in vivo* and *in vitro* (Cummings et al., 2001). FOS produced from sucrose by the action of fungal β-fructofuranosidase have been found to be non-digestible in humans and other animals. The selective utilization of FOS by intestinal bacteria led to a remarkable increase in bifidobacteria in the gut according to Hidaka et al. (1986). On the other hand, Houdijk (1998) concluded that FOS was fermented before it reached the colon, and therefore could not serve as a substrate for bifidobacteria. VFA production resulting from fermentation capacity, might help protect the animal against pathogenic activity. Howard (1995) suggested that the dietary consumption of FOS tended to enhance bifidobacteria populations and prevent colonic epithelial mucosa atrophy in neonatal pigs fed an elemental diet. Among the metabolites of FOS, such as short-chain fatty acids (SCFA), lactic acids and gases, butyrate is the major substrate for energy metabolism in colonic mucosa, stimulating epithelial cell growth (Roediger, 1980). The other SCFA and lactic acids are rapidly absorbed, presenting potential substrates for the liver and various tissues (Bergman, 1990).

The objective of this study was to investigate the effect of FOS addition to piglet diets on performance, intestinal VFA patterns, serum indices, and enteric morphology. In addition, the study also sought to determine whether FOS could be used as an alternative to feed grade antibiotics.

MATERIALS AND METHODS

Animals and diets

Ninety cross-bred weaned pigs(Duroc×Landrace×Large White) weighing ~7.86±0.06 kg each (33±1 d of age) were randomly divided on the basis of initial weight and gender into one of fifteen pens of three treatment groups (six pigs per pen, three barrows and three gilts, five pens per

* Corresponding Author: Chuanlai Xu. Tel: +86-510-5881769, Fax: +86-510-5881769, E-mail: xcl@sytu.edu.cn
[1] College of Animal Science and Technology, China Agricultural University Beijing, 100094, P. R. China.
Received September 22, 2004; Accepted January 27, 2005

Table 1. Composition and nutritive value of experimental diet (as fed basis)

Ingredient	Composition (%)	Nutrient level	Content (MJ/kg, %)
Corn, ground	60.8	Digestible energy	13.90
Soybean meal	15.0	Crude protein	18.0
Soybean, extruded	10.0	Lysine	1.24
Fish meal	5.0	Methionine	0.32
Whey, roller-dried	6.0	Methionine+cysteine	0.68
Dicalcium phosphate	1.2	Calcium	0.81
Limestone, ground	0.6	Total phosphorus	0.70
Salt, iodized	0.3		
L-lysine HCl	0.1		
Premix	1.0		
Total	100		

Values are from the Tables of Feed Composition and Nutritive Value in China (2000), while the values for CP, Ca and Total P were calculated.

Premix provided the following per kg feed: Vitamin A, 16,000 IU; D_3, 3,200 IU; E, 32 IU; K_3, 4.0 mg; B_1, 3.2 mg; B_2, 10.9 mg; B_6, 1.6 mg; B_{12}, 0.01 mg; Niacin, 32.0 mg; Pantothenic acid, 20 mg; Folic acid, 1.35 mg; Biotin, 0.05 mg; Choline, 0.5 g; Cu, 145 mg; Fe, 115 mg; Mn, 65 mg; Zn, 100 mg; I, 1.0 mg; Co, 1.0 mg; Se, 0.3 mg.

Table 2. Effect of diet treatments on the performance of weaning piglets

Indices \ Treatments	Control	Aureomycin	FOS	SEM	P
Average daily feed intake (g/d)	475±27[a]	532±7[b]	474±14[a]	25.73	0.068
Average daily gain (g/d)	253±22[a]	345±17[b]	333±18[b]	27.04	0.011
F distribution/gravity	1.90±0.08[b]	1.56±0.07[a]	1.45±0.11[a]	0.1246	0.009
Diarrhea rate (%)	3.80±0.97[b]	1.43±0.30[a]	1.11±0.60[a]	1.0546	0.05
Mortality (%)	10.00±4.08	3.33±3.33	3.33±3.33	5.0928	0.351

Values are mean±std. Error.

Different superscripts within a row indicates significant differences ($p<0.05$); No superscripts or same superscripts within a row indicates no significant difference ($p>0.05$). Similar to the next table.

treatment) and fed one of three dietary treatments. No significant differences were present in the initial bodyweight of the piglets ($p>0.05$) (Table 2). Each pen (1.00×1.80 m) contained a self-feeder and a nipple waterer to provide *ad libitum* access to feed and water. Pigs were housed in an environmentally regulated nursery in pens with woven wire flooring, allowing 0.30 m^2 per pig. The pigs were fed five times daily (08:00, 10:00, 12:30, 15:30 and 18:30 h) with equal portions allotted at each meal. Animal handling procedures were in accordance with guidelines approved by China Agricultural University's Animal Care Committee.

The diets consisted of a control diet (Table 1), the control diet with the addition of an antibiotic (Aureomycin, 75 mg/kg), and the control diet with the addition of 0.4% FOS (FOS, 0.4%). Previous research by the Animal Nutrition Lab, Animal Sci-Technology College, China Agricultural University found that the addition of 0.4% FOS to diets effectively increased performance in piglets. All diets were typical corn-soybean diets, formulated to meet or exceed the nutrient requirements (NRC, 1998) of 5 to 10 kg pigs and contained 18% CP and not less than 13.90 MJ/kg of digestible energy (on a as fed basis). The pigs were fed their diets for 21 days prior to one pig per pen being selected and killed. Feed intake was measured daily and body weight measured at 08:00 at the end of 21days.

Aureomycin (15% mean efficiency) was manufactured by Fu Kang (Fu zhou, Fujian Province, P. R. China) and bought from the market. FOS was provided by the School of Food Science and Technology, Southern Yangtze University. Based on high performance liquid chromatography (HPLC) analysis, the FOS contained 1.83% monosaccharides, 1.13% sucrose, 39.81% kestose, 49.78% nystose, 7.06% furanosyl nystose, for a total FOS of 96.65%.

Chemical analysis

Samples of the contents from the cecum were immediately collected upon the death of the animals and frozen in a jar of liquid nitrogen until subsequent analysis. Cecal contents and feces samples were also measured for VFA concentration by gas chromatography (Varian Model 3400; Varian Instrument Group, Walnut Creek, CA, USA). The digestion products were weighed, diluted with 2 g of deionized redistilled water, and centrifuged at 10,000 g for 10 min. Supernatant (1 ml) was decanted and mixed with 200 μl of 25% (w/v) metaphosphoric acid containing 2 g/L of 2-ethyl butyric acid (internal standard). This mixture was placed in an ice bath for 30 min and centrifuged at 10,000 g for 10 min, and the supernatant was decanted for injection in the gas chromatograph. Chemical analyses were conducted in duplicate. Representative feed samples were collected and analyzed for crude protein, Calcium and total Phosphorus content according to AOAC (1990, method 988.05, method 927.02 and method 965.05) procedures. Crude protein was calculated from Kjeldahl N (N×6.25,

Table 3. Effect of diet treatments on serum indices of weaning piglets

Indices \ Treatments	Control	Aureomycin	FOS	SEM	P
Glucose (mmol/L)	2.11±0.22[a]	7.64±0.47[c]	5.13±0.16[b]	0.4447	0.000
Total phosphorus (g/L)	36.96±1.73[a]	69.50±3.80[b]	41.01±0.74[a]	3.4604	0.000
Albumin (g/L)	18.16±1.50[a]	15.85±2.27[a]	20.15±0.88[a]	2.3365	0.238
Globulin (g/L)	18.79±2.25[a]	53.65±2.91[b]	20.86±1.58[a]	3.2665	0.000
Albumin/globulin	1.04±0.21[b]	0.30±0.05[a]	1.00±0.12[b]	0.2056	0.009
Serum urea nitrogen (mg/L)	78.57±2.38[a]	152.78±26.97[b]	116.27±8.96[ab]	23.2867	0.033

Table 4. Effect of diet treatments on the VFA of cecum content in weaning piglets (mmol/g fresh content)

Indices \ Treatments	Control	Aureomycin	FOS	SEM	P
Acetic acid	51.03±7.87	45.92±1.80	61.03±4.57	7.5691	0.273
Propionic acid	18.95±2.68[a]	16.23±2.01[a]	29.56±0.37[b]	2.7487	0.033
Isobutyric acid	0.18±0.18	0	0	0.1429	0.465
Butyric acid	8.99±0.25[a]	8.59±0.37[a]	12.94±1.27[b]	1.0946	0.049
Isovaleric acid	0.83±0.06	0.94±0.10	3.09±1.78	1.4522	0.347
Valeric acid	2.58±1.10	1.40±0.40	1.78±1.05	1.2773	0.677
Total VFAs	82.54±4.57[a]	73.06±0.14[a]	108.38±4.39[b]	5.1724	0.013
Acetic/total VFA	61.82	62.85	56.31		
Propionic/total VFA	22.96	22.21	27.27		

AOAC 1990).

Morphological measurements were made for crypt depth, villi height and percentage of goblet cells of the jejunum, ileum and cecum. Samples of tissue were cut from the intestin immediately after excising the digestive tract. Tissues were placed in 10% formalin. Fixed specimens were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The supportive tissue was thinly sliced (five slices) and mounted on microscope slides. Slides were viewed by light microscopy for morphological determination.

Serum glucose, total protein, albumin, globulin, and urea nitrogen concentrations were measured in blood samples using commercial enzyme kits (Zhongsheng Beikong Bio-Technology and Science Inc. 100083, Beijing, China). Blood samples were collected via jugular venipuncture before the morning feeding (0800 h for free access phase).

Statistical analysis

The results are expressed as mean values with standard errors. Data were analyzed using analysis of variance for a completely randomized design, and differences between means were determined using one-way ANOVA procedures. Multi-comparison was used to test significance and probability values of $p<0.05$ were taken as significant.

RESULTS

Performance

There were no significant differences in initial weight and mortality among the treatment groups (Table 2) ($p>0.05$).

The final weight ($p = 0.997$) of Control treatment is improved 70.8% than initial weight ($p = 0.007$), and the Aureomucin treatment improved 96.6%, while the FOS treatment improved 93.2%.

Feed conversion efficiency was significantly meaning improver for the Aureomycin and FOS treatments than for the control treatment ($p<0.05$), while the diarrhea rate was significantly lower. No significant differences were found between the two treatment groups on average daily gain (ADG), final weight (Table 2), but significant differences were found between the two treatments and the control.

Serological indices

Table 3 provides the results for the serological indices. Compared with the control, both treatments significantly increased serum glucose concentration ($p<0.05$), especially in Aureomycin treatment. The serum urea nitrogen (SUN) concentration was significantly affected by the Aureomycin treatment ($p<0.05$), although the FOS treatment difference was not significant ($p<0.05$).

The aureomycin treatment also significantly increased serum total protein and globulin concentrations and decreased the ratio of albumin to globulin (A/G), but did not affect serum albumin contents. Compared with the control, FOS did not affect serum total protein, albumin, globulin or A/G ($p>0.05$).

VFAs in cecal contents

The results presented in Table 4 indicate that FOS significantly increased the amount of propionic acid, butyric acid and total VFAs in the cecal contents, while aureomycin tended to decrease the contents of acetic acid, propionic acid, butyric acid and total VFAs.

Table 5. Effect of diet treatments on VFA content of feces in weaning piglets (mmol/g fresh content)

Indices \ Treatments	Control	Aureomycin	FOS	SEM	P
Acetic acid	57.44±4.59[a]	56.46±3.46[a]	81.97±2.90[b]	5.2538	0.027
Propionic acid	22.46±1.05	34.66±2.26	31.37±6.32	5.5418	0.222
Isobutyric acid	2.84±0.39[b]	1.68±0.11[a]	3.67±0.06[b]	0.3345	0.048
Butyric acid	19.62±0.24	18.22±0.93	23.92±2.63	2.2848	0.170
Isovaleric acid	4.69±0.58[a]	3.50±0.19[a]	6.34±0.11[b]	0.5064	0.025
Valeric acid	2.79±0.21	3.21±0.65	5.02±0.77	0.8404	0.1436
Total VFAs	109.84±4.96[a]	117.72±1.41[a]	151.69±11.13[b]	10.0134	0.048

Table 6. Morphological measurements of different dietary treatments from weanling pigs

Indices \ Treatments	Control	Aureomycin	FOS	SEM	P
Villous height (μm)					
Jejunum	350±35[a]	433±11[b]	507±6[c]	30.65	0.001
Ileum	502±21[ab]	445±20[a]	510±20[b]	28.92	0.081
Cecum	0±0[a]	132±16[b]	90±41[b]	35.68	0.007
Crypt depth (μm)					
Jejunum	372±14[a]	320±18[a]	370±19[a]	24.08	0.082
Ileum	310±20[a]	305±28[a]	367±23[a]	33.89	0.164
Cecum	397±8[a]	360±25[a]	405±7[a]	22.05	0.129
Percent of goblet cell (%)					
Jejunum	4.00±1.00[a]	4.50±1.12[a]	8.00±0.86[b]	1.41	0.025
Ileum	3.83±0.48[a]	11.17±2.41[b]	11.17±0.83[b]	2.12	0.004
Cecum	0±0[a]	21.83±2.10[c]	10.50±4.75[b]	4.24	0.000

The VFA pattern was also found to differ with diet. The proportion of propionic acid increased from 22.96 to 27.27% with FOS consumption, while the proportion of acetic acid was decreased from 61.82 to 56.31%.

VFAs in fecal contents

FOS had a positive influence on the amount of VFA, acetic acid and isovaleric acid in the feces of weaned piglets ($p<0.05$), while the Aureomycin treatment had a weakly negative influence on those indices (Table 5).

Morphological measurement

Morphological measurements of jejunal, ileal and cecal tissue showed responses to the consumption of FOS and Aureomycin, with the most pronounced differences occurring in the villous height and percentage of goblet cells in mucosa. FOS was beneficial in increasing the villi height of mucosa from jejunum to cecum in piglets compared with both the Aureomycin treatment and the control (Table 6). Table 6 also shows that the crypt depth of the mucosa for the different treatments was not affected ($p>0.05$), although FOS did significantly improve the percentage of goblet cells in the GI tract ($p<0.05$).

DISCUSSION

Weaning is stressful to young pigs because it takes several days for bifidobacterial populations to increase through selective enrichment (Howard et al., 1995). In the present study, the affects of FOS on growth performance were not as remarkable as reported in other FOS studies on poultry and swine (Fukuyasu et al., 1987; Ammerman et al., 1988; Bailey et al., 1991; Waldroup et al., 1993; Orban et al., 1993, 1995a, b). Dietary levels of FOS did not limit performance response in the present study and higher levels would not be practical. When pigs consumed a 0.75% FOS diet in a preliminary study (Orban et al., 1994) pigs wasted more feed because the wet feed stuck to the waterer, feed trough, and pen. Further, pigs fed the 0.75% FOS diet tended to paw the feed out of the feeder adding to the waste problem. The lack of effect of either antibiotics or FOS may be related to the overall intestinal health of these pigs. Feeding FOS to young piglets, however, may have other effects. A number of complex sugars have been shown to alter the morphology of the intestinal lining, presumably through increased production of short chain fatty acids (Tellez et al., 1993; Yoshioka et al., 1994; Howard et al., 1995). Feeding FOS has also been shown to increase magnesium absorption in rats (Ohta et al., 1994). There are also research findings suggesting that bifidobacteria stimulate components of the immune system in rats (Lee et al., 1993). We have seen that broilers fed FOS diets supplemented with NRC-recommended levels of trace minerals and vitamins perform as well as birds fed control diets supplemented with twice the level of trace minerals and vitamins (Orban et al., 1995b). Thus, depending on

dietary conditions, FOS could potentially have beneficial effects not observed using standard performance indices or without selectively enriching bifidobacteria. Of interest in the present study was the fact that FOS did not significantly alter crypt depth as indicated by Tsukahara et al. (2003).

Either the addition of antibiotic or FOS significantly increased pig performance or changed the intestinal microbial populations examined in this study. The lack of response to antibiotic feeding may suggest that there were dietary, pig, or environmental conditions that limited detection of a response. It may be important to determine conditions under which one can and cannot obtain responses to dietary manipulations, such as feeding live microorganisms or selective enrichment compounds in pigs.

Cecal concentrations of VFA indicate more fermentation occurs in this region than in the ileum or jejunum. The ileal, jejunal, and cecal concentrations of VFA found in this study agree with those from Mathew et al. (1998), although the cecal and jejunal VFA levels were only obtained by killing animals at the end of that experiment. As such, a direct comparison of these studies over the course of the weaning was not possible. Changes in fermentation patterns postweaning are also indicated by shifts in VFA production (Mathew et al., 1998), as was observed in this study. VFA declines may have significant affects on the energy available to the intestinal mass. Large intestinal VFA production in pigs has been estimated to contribute between 5 and 28% of the total maintenance energy requirement (Friend et al., 1964; Imoto and Namioka, 1978). In other species, VFA has been shown to be the primary energy source for the intestinal mass, contributing up to 70% of the required maintenance needs (Imoto and Namioka, 1978).

IMPLICATIONS

The efficacy of FOS as a growth-promoting agent in swine diets was evaluated in this study. There were no significant differences in growth performance between pigs fed FOS diets or diets supplemented with aureomycin, although there were some in consistent trends for improved performance. Further studies will be necessary to determine if FOS is truly effective in swine, or under what dietary conditions the FOS may be beneficial in swine.

REFERENCES

Ammerman, E., C. Quarles and P. V. Twining, Jr. 1988. Broiler response to the addition of dietary fructooligosaccharides. Poult. Sci. 67(Suppl. 1):67(Abstr.).

Bailey, J. S., L. C. Blackenship and N. A. Cox. 1991. Effect of fructooligosaccharides on salmonella colonization of the chicken intestine. Poult. Sci. 70:2433-2438.

Bergman, E. N. 1990. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. Physiological Reviews.70:567-590.

Cummings, J. H., G. T. Macfarlane and H. N. Englyst. 2001. Prebiotic digestion and fermentation. J. Clin Nutr. Am. 73:415-420.

Friend, D. W., J. W. G. Nicholson and H. M. Cunningham. 1964. Volatile fatty acid and lactic acid content of pig blood. Can. J. Anim. Sci. 44:303-309.

Fukuyasu, T., T. Oshida and K. Ashida. 1987. Effect of oligosaccharides on growth of piglets and on bacterial flora, putrefactive substances and volatile fatty acids in their feces. Bull. Anim. Hyg. 26:15-22.

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.

Hidaka, H., T. Eida, T. Takizawa, T. Tokunaga and Y. Tashiro. 1986. Effects of fructooligosaccharides on intestinal flora and human health. Bifidobact. Microflora. 5:37-50.

Houdijk, G. M., M. W. Bosch and H. J. Verstegen. 1998. Effects of dietary oligosaccharides on the peformance and faecal characteristics of young growing pigs. Anim. Feed Sci. Technol. 71:35-48.

Howard, M. D., D. T. Gordon, L. W. Pace, K. A. Garleb and M. S. Kerley. 1995. Effects of dietary supplementation with fructooligosaccharides on colonic microbiota populations and epithelial cell proliferation in neonatal pigs. J. Ped. Gastroenter. Nutr. 21:297-303.

Imoto, S. and S. Namioka. 1978. VFA production in the pig large intestine. J. Anim. Sci. 47:467-478.

Lee, J., A. Ametani, A. Enomoto, Y. Sato, H. Motoshima, F. Ike and S. Kaminogawa. 1993. Screening for the immunopotentiating activity of food microorganisms and enhancement of the immune response by Bifidobacterium adolescentis M101-4. Biosci. Biotechnol. Biochem. 57:2127-2132.

Mathew, A. G., S. E. Chattin, C. M. Robbins and D. A. Golden. 1998. Effects of a direct-fed yeast culture on enteric microbial populations, fermentation acids, and performance of weanling pigs. J. Anim. Sci. 76:2138-2145.

NRC, National Research Council 1998. Research Priorities for Airborne Particulate Matter, National Academy Press, Washington, DC.

Ohta, A., S. Baba, T. Takizawa and T. Adachi. 1994. Effects of fructooligosaccharides on the absorption of magnesium in the magnesium-deficient rat model. J. Nutr. Sci. Vitaminol. 40:171-180.

Orban, J. I., J. A. Patterson, A. L. Sutton and G. N. Richards. 1993. Effect of sucrose thermal oligosaccharide caramel on growth and intestinal microflora of broiler chickens. Poult. Sci. 72(Suppl. 1):132(Abstr.).

Orban, J. I., J. A. Patterson, A. L. Sutton and G. N. Richards. 1994. Effect of sucrose thermal oligosaccharide caramel (STOC) on growth performance and intestinal microbial populations in growing pigs. In: Proc. VIth Int. Symp. on Digestive Physiology in Pigs. EAAP Publ. 80:280. Bad Doberan, Germany.

Orban, J. I., J. A. Patterson, A. L. Sutton, O. Adeola and G. N. Richards. 1995a. Growth performance and intestinal microbial populations in White Pekin ducks fed diets containing sucrose thermal oligosaccharide caramel. Poult. Sci. 74(Suppl. 1):

209(Abstr.).

Orban, J. I., J. A. Patterson, A. L. Sutton and G. N. Richards. 1995b. Influence of sucrose thermal oligosaccharide caramel and vitamin-mineral level on growth and changes in intestinal microbial populations in broilers from day-old to four weeks of age. Poult. Sci. 74 (Suppl. 1):209(Abstr.).

Patterson, J. A., A. L. Sutton, J. I. Orban and G. N. Richards. 1993. Effect of kestoses from thermolysis of sucrose on growth of intestinal bacteria, performance and intestinal microflora of broiler chickens. Poult. Sci. 72(Suppl. 1):132(Abstr.).

Quigly, M. E., G. J. Hudson, H. N. Englyst. 1999. Determination of resistant short-chain carbohydrates (non-digestible oligosaccharides) using gas-liquid chromatography. Food Chem. 65:381-390.

Roediger, W. E. W. 1980. Role of anaerobic bacteria in the metabolic welfare of the colonic mucosa in man. Gut. 21:793-798.

SAS. 1987. SAS User's Guide: Statistics (Version 6.01 Ed.). SAS Inst. Inc., Cary, NC.

Tellez, G., C. E. Dean, D. E. Corrier, J. R. Deloach, L. Jaeger and B. M. Hargis. 1993. Effect of dietary lactose on cecal morphology, pH, organic acids and Salmonella enteritidis organ invasion in leghorn chicks. Poult. Sci. 72:636-642.

Tokunaga, T., T. Oku and N. Hosoya. 1989. Utilization and excretion of a new sweetener, fructooligosaccharide (Neosugar), in rats. J. Nutr. 119:553-559.

Tsukahara, T., Y. Iwasaki, K. Nakayama and K. Ushida. 2003. Stimulation of butyrate production in the large intestine of weaning piglets by dietary fructooligosaccharides and its influence on the histological variables of the large intestinal mucosa. J. Nutr. Sci. Vitaminol (Tokyo). 49(6):414-421.

Waldroup, A. L., J. T. Skinner, R. E. Hierholzer and P. W. Waldroup. 1993. An evaluation of fructooligosaccharide in diets for broiler chickens and effects on Salmonella contamination of carcasses. Poult. Sci. 72:643-650.

Yoshioka, M., Y. Shimomura and M. Suzuki. 1994. Dietary polydextrose affects the large intestine in rats. J. Nutr. 124:539-547.