# Effects of Dietary Fructooligosaccharide on Digestive Enzyme Activities, Intestinal Microflora and Morphology of Growing Pigs[A]

**Z. R. Xu, X. T. Zou, C. H. Hu*, M. S. Xia, X. A. Zhan and M. Q. Wang**

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

**ABSTRACT :** One hundred and twenty-eight growing barrows (Jiaxing Black×Duroc×Landrace) at an average BW of 20.8 kg were allocated to four treatments for 42 days, each of which was replicated four times with eight pigs per replicate and used to investigate the effects of fructooligosaccharide (FOS) on digestive enzyme activities, intestinal microflora and morphology of growing pigs. The pigs received the same basal corn-soybean meal diet and FOS was added to the basal diet at 0, 2, 4, 6 g/kg diet at the expense of corn, respectively. As compared to control, supplementation with 4 and 6 g/kg FOS significantly improved average daily gain and feed efficiency. Addition of FOS enhanced the growth of *Bifidobacterium* and *Lactobacillus*, but inhibited *Clostridium* and *Escherichia coli* in the small intestinal and proximal colonic contents. Supplementation with 4 and 6 g/kg FOS significantly improved the activities of total protease, trypsin and amylase in the small intestinal contents. However, FOS had no significant effect on the activity of lipase in the small intestinal contents as well as the digestive enzymes in pancreas. Morphological measurement of jejunal mucosa did show response to consumption of FOS. Villus height and the villus height to crypt depth ratio at the jejunal mucosa were significantly higher with 4 and 6 g/kg FOS supplementation as compared to control. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 12 : 1784-1789)***

**Key Words :** Fructooligosaccharide, Digestive Enzyme, Intestinal Microflora, Intestinal Morphology, Pig

## INTRODUCTION

In recent years, numerous investigations have been conducted to study effects of oligosaccharides on commensal bacteria and health of young animals and human infants (Gibson and Wang, 1994). Fructooligosaccharide (FOS) is β-linked fructose unit to the fructose moiety of sucrose. Because the β-linkages between fructose monomers cannot be hydrolyzed by enzymes of endogenous origin, FOS escapes enzymatic digestion in the small intestine and forms a substrate for the gastrointestinal microflora (Tokunaga et al., 1989). The FOS has been shown to enhance the growth of *Bifidobacterium* and *Lactobacillus*, but inhibit *Escherichia coli* in the large intestine (Hidaka et al., 1986b, 1991; Bunce et al., 1995; Roberfroid et al., 1998). There are large numbers of microorganisms in the small intestine of pigs, so FOS is fermented to some extent in the small intestine of pigs (Bolduan, et al., 1993). However, there is extremely limited information on the effect 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 pigs are lacking.

Therefore, an experiment was carried out to investigate the effects of dietary FOS on intestinal microflora, the digestive enzyme activities of pancreas and small intestinal contents, and intestinal morphology of growing pigs.

## MATERIALS AND METHODS

### Animals and experimental diets

A total of 128 growing barrows (Jiaxing Black×Duroc×Landrace) at an average BW of 20.8 Kg were allocated to 4 treatments for 42 days, each of which was replicated four times with eight pigs per replicate. The pigs received the same basal diet based on corn-soybean meal and FOS was added to the basal diet at 0, 2, 4, 6 g/kg diet at the expense of corn, respectively. The FOS (Meioligo-P) was provided by Meiji Seika kaisha, Ltd. (Tokyo, Japan) and the concentration of oligosaccharides was analyzed by HPLC. The main ingredients of this product are glucose, fructose, sucrose and FOS. The glucose, fructose and sucrose amount to 2.8% and the FOS amount to 97.2%. The FOS consist of three kinds of materials ($GF_n$: G refers to glucosyl moiety, F to the fructosyl moiety and n indicates the number of the fructosyl moieties in the molecules, n=2, 3, 4). $GF_2$, $GF_3$ and $GF_4$ amount to 46.8, 39.3 and 11.1%, respectively. Diets were formulated to meet or exceed nutrient requirements suggested by the NRC (1998) for 20 to 50 kg pigs. Antibiotic was excluded from all diets (Table 1). All pigs were given *ad libitum* access to feed and water. Growth performance results such as average daily gain (ADG), average daily feed intake (ADFI), and

** Supported by National Natural Science Foundation of China (Project 30100130) and Zhejiang Natural Science Foundation (Project ZD9902). The authours gratefully acknowledge to Junyi Shi, Weifen Li, Jianyi Sun for their skilled technical assistance. Special thanks are to Professor Jianxin Liu for experimental design and statistical analysis.

* Corresponding Author: C. H. Hu. Tel: +86-571-86985607, Fax: +86-571-86091820, E-mail: chhu@zju.edu.cn

Received April 1, 2002; Accepted August 1, 2002

**Table 1.** Formula and chemical composition of the basal diet

| Ingredients (%) | |
|---|---|
| Corn | 70.0 |
| Soybean meal (dehulled, solvent) | 25.0 |
| Animal fat | 2.0 |
| Limestone | 1.0 |
| Dicalcium phosphate | 1.2 |
| Sodium chloride | 0.3 |
| L-Lysine-HCI (78%) | 0.2 |
| Vitamin-mineral premix[1] | 0.3 |
| Analyzed chemical composition (% as feed) | |
| DE (MJ/kg) | 14.2 |
| Crude protein | 17.8 |
| Ether extraction | 4.8 |
| Crude fiber | 2.0 |
| Lysine | 0.94 |
| Met.+Cys. | 0.56 |
| Calcium | 0.71 |
| Phosphorus | 0.55 |

[1] The vitamin/mineral premix provided (per kg feed): 2,000 IU vitamin A, 200 IU vitamin $D_3$, 20 mg vitamin E, 1mg vitamin K, 1 mg thiamine, 3 mg riboflavin, 10 mg d-pantothenic acid, 0.5 mg folic acid, 1 mg pyridoxine, 20 mg niacin, 10 ug cobalamin, 500 mg choline chloride, 0.1 mg biotin, 0.2 mg Se, 0.2 mg I, 80 mg Fe, 5 mg Cu, 2 mg Mn and 80 mg Zn.

[2] DE was based on calculated values.

feed/gain (F/G) were collected.

At the 42th day of the feeding trial, eight pigs from each treatment (two pigs per pen) were slaughtered under general anaesthesia. The pigs were then immediately eviscerated in order to collect intestinal samples.

**The digestive enzyme activities in pancreatic tissue and small intestinal contents**

*Sampling procedure* : The contents taken from the small intestine were digesta from the distal end of the duodenum to the ileo-caecal junction. A homogenous intestinal digesta sample was collected by massaging the tract from both ends. The digesta sample were stored immediately at -20°C until used. Enzyme activity analyses of the samples obtained from the small intestine were performed on freeze-dried material, which was extracted with 1 mM HCl (50 mg lyophilized digesta in 1 ml 1 mM HCl ) for 1 h at 4°C followed by centrifugation (3,000×g) according to Jensen et al. (1998). The supernatants were then collected for analysis of protease, trypsin, chymotrypsin, amylase and lipase activities.

The pancreas from slaughtered pigs was homogenized in ice-cold 0.2 M Tris– HCl buffer, pH 8.0 containing 0.05 M NaCl in the ratio 1:4 (w/v). The homogenate was centrifuged at 3,000×g for 15 min at 4°C and the supernatant was saved. In the supernatant the activity of the enzymes: protease, trypsin,, chymotrypsin, amylase and lipase was determined.

*Digestive enzyme assay* : Protease activity was analyzed using the modified method of Lynn and Clevette-Radford (1984) using azocasein as the substrate. Trypsin (EC 3.4.21.4) activity was determined using benzoyl-DL-arginine-p-nitro-anilide (DL-BAPA) as substrate according to Erlanger et al. (1961). Chymotrypsin (EC 3.4.21.1) was determined according to Erlanger et al. (1966) using glutaryl-1-phenylalanine-p-nitroanilid (GPNA) as substrate.

Amylase (EC 3.2.1.1) activity was determined using a kit (No.700) from Sigma Chemical Company (Sigma Chemical Co., St. Louis, MO 63178-9916) and lipase (EC 3.1.1.3) by a pH-stat titration method using tributyrin as substrate according to Erlanson-Albertsson et al. (1987). The activity of protease, trypsin, chymotrypsin, amylase, and lipase are expressed as units (U) which is defined as the amount of enzyme that hydrolyses 1 μmole of substrate per minute.

**Intestinal microbial populations**

Samples of the contents from the small intestine (from the distal end of the duodenum to the ileo-caecal junction) and proximal colon were immediately collected into Qorpak glass containers under $CO_2$, sealed, and put on ice until they were transported to the lab for enumeration of microbial populations. Ten grams of mixed contents were blended under $CO_2$ in 90 mL of anaerobic dilution (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 (Oxoid; total anaerobes), MRS Agar (Oxoid; *Lactobacillus*), Reinforced Clostridial Agar plus supplements (Munoa and Pares, 1988; *Bifidobacterium*), Sulphite-Polymyxin Milk Agar (Mevissen-Cerhage et al., 1987; *Clostridium*), and MacConkey's No.2 (Oxoid; *Escherichia coli*). Single colonies were removed from selective media plates and grown in peptone yeast glucose (PYG) broth (Holdeman et al., 1977). Subsequently, the bacteria were characterized to genus level on the basis of colonial appearance, Gram reaction, spore production, cell morphology and fermentation end-product formation (Holdeman et al., 1977).

**Histomorphometry**

At slaughter, specimens (0.5 cm×0.5 cm) of intestinal tissue from the mid-jejunum were excised, rinsed in physiological saline. Samples were preserved in 10% formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Villus height and crypt depth were measured using image processing and analysis system

(Version 1, Leica Imaging Systems Ltd, Cambridge, England).

### Statistical analysis

One way analysis of variance was performed using the General Linear Model (GLM) Procedure of SAS (1989). Differences among means were tested using Duncan's multiple range test. A significant level of 0.05 was used.

## RESULTS

### Growth performance

Growth performance of pigs fed different levels of dietary FOS is presented in Table 2. As compared to control, supplementation with 4 and 6 g/kg FOS significantly improved ADG and feed conversion ratio. However, feed intake was unaffected by dietary treatments.

### Intestinal microflora

The total viable counts of anaerobes and the constitutions of microbes in the intestinal contents were shown in Table 3. As compared to control, supplementation with FOS increased the viable counts of *Bifidobacterium* and *Lactobacillus*, while reduced those of *Clostridium* and *Escherichia coli* in the small intestinal and proximal colonic contents.

### Digestive enzymes

The results on the effects of FOS on the digestive enzyme activities in the pancreas and the small intestinal contents of growing pigs are shown in Table 4 and Table 5, respectively. Supplementation with 4 and 6 g/kg FOS significantly improved the activities of total protease, trypsin and amylase in the small intestinal contents. However, FOS had no significant effect on the activities of chymotrypsin and lipase in the small intestinal contents as well as the digestive enzymes in pancreas.

### Morphological measurement of jejunal mucosa

Morphological measurement of jejunal mucosa did show response to consumption of FOS (Table 6). Villus height and the villus height to crypt depth ratio at the jejunal mucosa were significantly higher with 4 and 6 g/kg FOS supplementation as compared to control.

**Table 2.** Growth performance as affected by dietary FOS in growing pigs[1]

| | Dietary FOS level (g/kg) | | | | SEM[2] |
|---|---|---|---|---|---|
| | 0 | 2 | 4 | 6 | |
| Initial wt (kg) | 20.80 | 20.82 | 20.76 | 20.84 | 0.47 |
| Final wt (kg) | 48.11 | 49.35 | 50.38 | 50.08 | 0.61 |
| ADG (g) | $650^{b}$ | $679^{ab}$ | $705^{a}$ | $696^{a}$ | 15 |
| ADFI (kg) | 1 .73 | 1.72 | 1.75 | 1.71 | 0.02 |
| F/G | $2.66^{a}$ | $2.53^{ab}$ | $2.48^{b}$ | $2.46^{b}$ | 0.05 |

[1] Values are presented as means; n=32 for ADG, n=4 for ADFI and F/G per treatment. Means in a row with different letters differ significantly.
[2] Sand error of the mean.

**Table 3.** Viable cell counts of microflora in small intestinal and proximal colonic digesta of growing pigs[1,2]

| | Dietary FOS level (g/kg) | | | | SEM[3] |
|---|---|---|---|---|---|
| | 0 | 2 | 4 | 6 | |
| Small intestine | | | | | |
| Total anaerobes | 9.65 | 9.74 | 9.95 | 9.82 | 0.38 |
| Bifidobacterium | $6.95^{b}$ | $7.31^{ab}$ | $7.60^{a}$ | $7.53^{a}$ | 0.15 |
| *Lactobacillus* | $8.01^{b}$ | $8.62^{ab}$ | $8.86^{a}$ | $8.90^{a}$ | 0.26 |
| *Clostridium* | $6.48^{a}$ | $5.81^{ab}$ | $5.28^{b}$ | $5.04^{b}$ | 0.32 |
| *Escherichia coli* | 8.35 | 8.10 | 7.84 | 7.88 | 0.27 |
| Proximal colon | | | | | |
| Total anaerobes | 10.40 | 10.87 | 10.90 | 10.97 | 0.24 |
| Bifidobacterium | $7.79^{b}$ | $7.95^{ab}$ | $8.41^{a}$ | $8.42^{a}$ | 0.18 |
| *Lactobacillus* | $9.13^{b}$ | $9.50^{ab}$ | $10.16^{a}$ | $9.80^{ab}$ | 0.30 |
| *Clostridium* | $7.91^{a}$ | $6.94^{b}$ | $6.22^{b}$ | $6.65^{b}$ | 0.28 |
| *Escherichia coli* | $8.71^{a}$ | $8.43^{ab}$ | $7.99^{b}$ | $8.28^{ab}$ | 0.16 |

[1] Bacterial numbers are expressed as $\log_{10}$ cfu/g DM.
[2] Values are presented as means; n=8 per treatment. Means in a row with different letters differ significantly.
[3] Stand error of the mean.

**Table 4.** Effects of FOS on the digestive enzyme activities in the pancreas and the small intestinal contents of growing pigs[1,2]

| | Dietary FOS level (g/kg) | | | | SEM[3] |
|---|---|---|---|---|---|
| | 0 | 2 | 4 | 6 | |
| Pancreas | | | | | |
| Protease | 70.4 | 68.3 | 73.8 | 66.5 | 2.8 |
| Trypsin | 19.0 | 21.8 | 18.2 | 20.4 | 1.6 |
| Chymotrypsin | 0.35 | 0.22 | 0.38 | 0.47 | 0.1 |
| Amylase | 2,198 | 2,134 | 2,207 | 2,024 | 63.2 |
| Lipase | 56.6 | 52.8 | 58.1 | 56.2 | 2.7 |
| Small intestinal contents | | | | | |
| Protease | $32.6^{c}$ | $38.9^{bc}$ | $50.7^{a}$ | $45.8^{ab}$ | 2.4 |
| Trypsin | $15.7^{b}$ | $19.6^{ab}$ | $22.5^{a}$ | $23.5^{a}$ | 1.5 |
| Chymotrypsin | 0.14 | 0.17 | 0.23 | 0.26 | 0.07 |
| Amylase | $1,056^{b}$ | $1,181^{b}$ | $1,374^{a}$ | $1,478^{a}$ | 46.1 |
| Lipase | 88.5 | 90.1 | 83.8 | 86.4 | 3.2 |

[1] Digestive enzyme activities in the pancreas and the small intestinal contents are expressed as U/g pancreas and U/mg digesta DM, respectively.
[2] Values are presented as means; n=8 per treatment. Means in a row with different letters differ significantly:
[3] Stand error of the mean.

**Table 5.** Effects of FOS on the morphology of the jejunal mucosa[1]

| | Dietary FOS level (g/kg) | | | | SEM[2] |
|---|---|---|---|---|---|
| | 0 | 2 | 4 | 6 | |
| Villus height (μm) | $493^{b}$ | $529^{ab}$ | $577^{a}$ | $565^{a}$ | 20 |
| Crypt depth (μm) | 386 | 358 | 327 | 342 | 20 |
| Villus height:crypt depth | $1.29^{c}$ | $1.48^{bc}$ | $1.80^{a}$ | $1.67^{ab}$ | 0.07 |

[1] Values are presented as means; n=8 per treatment. Means in a row with different letters differ significantly.
[2] Stand error of the mean.

## DISCUSSION

### Effects of dietary FOS on growth performance and intestinal microflora of growing pigs

Numerous workers have reported increased growth and improved feed conversion ratio as a consequence of FOS inclusion in young pigs' diets (Hidaka et al., 1986a; Fukuyasu et al., 1987). Our study also verified this. Other authors, however, reported no or slightly negative effects of FOS on young pigs' growth performance (Kornegay et al., 1992; Farnworth et al., 1992).

It was reported that the biological effects of FOS on animals were mainly due to its preferential stimulatory effect on number of the health-promoting genus (*Bifidobacterium* and *Lactobacillus*), whilst maintaining populations of unprofitable or potential pathogens (*Escherichia coli* and *Clostridium*) at relatively low levels. The increases in numbers of *Bifidobacterium* and *Lactobacillus* and decreases in numbers of *Escherichia coli* and *Clostridium* in the colon of pigs supplemented with FOS in our study were in agreement with the work of most other research groups (Hidaka et al., 1986b, 1991; Fukuyasu et al., 1987; Bunce et al., 1995; Xu et al., 2002).

There are large numbers of microorganisms in the small intestine of pigs, so FOS is fermented to some extent in the small intestine of pigs (Bolduan et al., 1993). The results in the present study indicated that bacterial populations in the small intestinal digesta were affected by the supplementation of FOS. In an 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. To the author's knowledge, this is the only study that has investigated the effects of supplementing diets with FOS on the bacterial populations in the small intestine in the pigs. The reason of the lack of effects of FOS supplementation on the small intestinal bacterial populations in that study may be that the concentration of FOS (2 g/kg) was not adequate to alter microbial populations. In studies with poultry (Bailey et al., 1991), a 3.75 g/kg concentration of FOS was not sufficient to affect colonization of *Salmonella typhimurium*, whereas a 7.5 g/kg level affected concentrations of that species. Secondy, the overall intestine 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., 1986b; Howard et al., 1995). Addition of *Bifidobacterium* or oligosaccharedes to the diet of humans has been shown to have no effect when the natural level of *Bifidobacterium* is high (Hidaka et al., 1986b, 1991). For the animals in that study, the number of *Bifidobacterium* might have been enough that FOS did not increase *Bifidobacterium* numbers.

### Effects of dietary FOS on the digestive enzyme activities in the pancreas and the small intestinal contents

The results in the present study indicate that FOS has no significant effect on the digestive enzymes in pancreas. Ikegami et al. (1990) reported that when alginic acid and calcium alginate, insoluble polysaccharides that did not contribute to viscosity, were given to rats, they had no effect on the digestive enzymes in pancreas. However, administration of sodium alginate, the highly viscous polysaccharides, led to changes in the exocrine pancreatic-biliary function and may have increased the digestive enzymes in pancreas. The FOS used in this study might have little effect on the viscosity, so it did not affect the pancreatic enzyme activity.

The results on the effects of FOS on the digestive enzyme activity in the small intestinal contents indicated that supplementation with 4 and 6 g/kg FOS significantly improved the activities of total protease, trypsin and amylase. The intestinal microflora data showed that FOS exerted a preferential stimulatory effect on *Bifidobacterium* and *Lactobacillus*, whilst suppressed *Escherichia coli* and *Clostridium* in the small intestine. Such changes in microbial ecosystem in the presence of FOS might contribute to the observed effects on the digestive enzyme activity 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 *Escherichia coli* and *Clostridium* may damage the villus and microvillus of intestinal mucosa and inhibit the secretion of digestive enzymes (Gao, 1998). Moreover, the *Clostridium* could secrete the proteolytic enzymes, which may take the intestine digestive enzymes as selective nutrients, thus increasing the degradation of digestive enzymes (Conway, 1994).

However, in the present study, FOS has no significant effect on the activities of lipase in the small intestinal contents. Some investigators reported that addition of FOS to diets caused significantly greater fecal lipid excretion in rats (Delzenne, 1993; Kim et al., 1998). Addition of FOS enhanced the growth of *Bifidobacterium* and *Lactobacillus*, which had the action of precipitation and assimilation with bile salt (Zhan, 1998), thus increasing fecal bile acid excretion (Delzenne, 1993; Kim et al., 1998) and decreasing its intestinal concentration. Intestinal bile acid has a great impact on the lipid emulsification and the activities of lipase. Therefore, the increases in numbers of *Bifidobacterium* and *Lactobacillus* and decreases in numbers of *Clostridium* in the small intestine of pigs supplemented with FOS resulted in two-sided effects on the activities of lipase: On the one hand, this facilitated lipase secretion and inhibited its degradation; on the other hand,

this might lead to the decrease of intestinal bile acid, thus possibly reduced the activity of lipase. It is possible that such two-sided effects of microbial ecosystem might explain why the activity of lipase was not affected in the present study. However, this remains to be further investigated.

**Effects of dietary FOS on the 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 villus and deeper crypts have been associated with the presence of toxins (Yason et al., 1987; Anonymous, 1999). A shortening of the villus 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. 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 requirement for maintenance and will therefore lower the efficiency of the animal. Changes in intestinal morphology as descried above can lead to poor nutrient absorption, increased secretion in the gastrointestinal tract, diarrhoea, reduced disease resistance and lower overall performance.

In the present study, the increase in villus height and villus height: crypt depth ratio of the jejunal mucosa in FOS-fed pigs was found. It is likely that these changes are due to FOS's ability to improve the intestinal microflora and are not a direct action of FOS on the intestinal tissue. It is suggested that the energy conserved by the reduced turnover rate of the epithelial cells might be utilized for lean tissue mass synthesis and might explain some of the improvements seen in body weight gain and feed conversion with FOS.

## REFERENCES


Anonymous. 1999. How do mannanoligosaccharides work. Feeding Times (1):7-9.

Baily, J. S., L. C. Blankenship and N. A. Cox. 1991. Effect of fructooligosaccharide on Salmonella colonization of the chicken intestine. Poult. Sci. 70:2433-2438.

Bolduan, G., M. Beck and C. Schubert. 1993. Zur Wirkung von Oligosaccharіden beim Ferkel. Arch. Anim. Nutr. 44:21-27.

Bryant, M. P. and I. M. Allison. 196l. An improved non-selective culture medium for animal bacterial and its use in determining diurnal variation in numbers of bacteria in the rumen. J. Dairy Sci. 44:1446-1453.

Bryant, M. P. 1972. Commentary on the Hungate technique for culture for anaerobic bacteria. Am. J. Clin. Nutr. 25:1324-1330.

Bunce, T. J., M. D. Howard, G. L. Allee and L. W. Pace. 1995. Protective effects of fructooligosaccharide (FOS) in prevention of mortality and morbidity from infectious E.coli K: 88 challenge. J. Anim. Sci. 73(Suppl. 1):69(Abstr.).

Conway, P. L. 1994. The function of the gastrointestinal microflora and its regulation. In: Proceeding of the 6th international seminar on digestive physiology of pig. Sicuan: Sicuan science and technology press. pp. 233-242 (in Chinese).

Delzenne, N. M., N. Kok, M. F. Fiordaliso, D. M. Deboyser, F. M. Goethals and M. B. Roberfroid. 1993. Dietary fructooligosaccharides modify lipid metabolism in rats. Am. J. Clin. Nutr. 57(Suppl.):820S(Abstr.).

Erlanger, B. F., F. Edel and A. G. Cooper. 1966. The action of chymotrypsin on two new chromogenic substrates. Arch. Biochem. Biophys. 115:206–210.

Erlanger, B. F., N. Kokowsky and W. Cohen. 1961. The preparation and properties of two new chromogenic substrates of trypsin. Arch. Biochem. Biophys. 95:271–278.

Erlanson-Albertsson, C., A. Larsson and R. Duan. 1987. Secretion of pancreatic lipase and colipase from rat pancreas. Pancreas. 2:531-535.

Farnworth, E. R., H. W. Molder, J. D. Jones, N. Cave, H. Yamazaki and A. V. Rao. 1992. Feeding Jerusalem artichoke flour rich in fructo-oligosaccharides to weanling pigs. Can. J. Anim. Sci. 72:977-980.

Fukuyasu, T., T. Oshida and K. Ashida. 1987. Effects of oligosaccharides on growth of piglets and on bacteria flora, putrefactive substances and volatile fatty acids in their feces. Bulletin of Anim.Hygience 26:15-22.

Gao, L. S. (ed.). 1998. Digestive physiology and health protection, 1th edn. Beijing: China curatorial science and technology press. pp. 173-230 (in Chinese).

Gibson, G. R. and X. Wang. 1994. Bifidogenic properties of different types of fructooligosaccharides. Food Microbiol. 11:491-498.

Hidaka, H., T. Eida and T. Hamaya. 1986a. Livestock feed containing inulo- oligosaccharides and breeding of livestock by using the same. Eur. Pat. Appl. 0l7026A2:l0.

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

Hidaka, H., Y. Tashiro and T. Eida. 1991. Proliferation of bifidobacteria by oligosaccharides and their useful effect on human health. Bifidobact. Microflora l0:65-79.

Holdeman, L. V., E. P. Cato and W. E. C. Moore (ed.). 1977. Anaerobic laboratory mannual, 4th edn. Blacksburg: Virginia Polytechnic Institute and State University.

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 prolifertion in neonatal pigs. J. Gastroenterology Nutr. 21:297-303.

Ikegami, S., F. Tsuchihashi, H. Harada, N. Tsuchihashi, E. Nishidt and S. Innami. 1990. Effects of viscous indigestible polysaccharides on pancreatic-biliary secretion and digestive organs in rats. J. Nutr. 120:353-360.

Jensen, M. S., K. E. Bach Knudsen, J. Inborr and K. Jakobsen.

1998. Effect of β-glucanase supplementation on pancreatic enzyme activity and nutrient digestibility in piglets fed diets based on hulled and hulless barley varieties. Anim. Feed Sci. Technol., 72:329-345.

Kim, M. and H. K. Shin. 1998. The water soluble extract of chicory influences serum and liver lipid concentrations, cecal short-chain fatty acid concentrations and fecal lipid excretion in rats. J. Nutr. 128:1731-1736.

Kornegay, E. T., C. M. Wood and L. A. Eng. 1992. Effectiveness and safety of fructooligosaccharides for pigs. Anim. Sci. Res. Report Virginia Agr. Exp. Station 10:19-20.

Lynn, K. R. and N. A. Clevette-Radford. 1984. Purification and characterization of hevin, a serin protease from Hevea brazilliensis. Biochemistry 23:963-964.

Mevissen-Verhage, E .A .E., J. H. Marcelis, N. M. de Vos and J. Verhoef. 1987. Bifidobacterium, Bacteroides and Clostridium spp. in faecal samples from breast-fed and bottle-fed infants with and without iron supplement. J. Clin. Microbiol. 25:285-289.

Munoa, F. J. and R. Pares. 1988. Selective medium for the isolation and enumeration of Bifidobacterium spp. Appl. Environ. Microbiol. 54:1715-1718.

National Research Council. 1998. Nutrient requirements of swine. I0th Ed. National Academy Press. Washington, DC.

Roberfroid, M. B., J. A. E. Vanloo and G. R. Gibson. 1998. The bifidogenic nature of chicory inulin and its hydrolysis products. J. Nutr. 128:11-19.

SAS. Institute Inc. 1989. SAS/STAT User's Guide, Version 6. SAS Institute Inc., Cary, North Carolina.

Sissons, J. W. 1989. Potential of probiotic organisms to prevent diarrhea and promote digestion in farm animals: a review. J. Sci. Food Agric. 49:1-13.

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

Xu, Z. R., C. H. Hu and M. Q. Wang. 2002. Effects of fructooligosaccharide on conversion of L-tryptophan to skatole and indole by mixed populations of pig fecal bacteria. J. Gen. Appl. Microbial. 48:83-89.

Yason, C. V., B. A. Summers and K. A. Schat. 1987. Pathogenesis of rotavirus infection in various age groups of chickens and turkeys: pathology. Am. J. Vet. Res. 6:927-938

Zhan, C. 1998. The action of decreasing cholesterol of Bifidobacterium: precipitation and assimilation with biliary salt. Medicine in abroad: antibiotic. 19:146-149 (in Chinese).

.

3