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Effects of fructooligosaccharide on conversion of L-tryptophan to skatole and indole by mixed populations of pig fecal bacteria

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An in vitro study was conducted to examine the effects of fructooligosaccharide (FOS) at levels of 0.5, 1.0, and 1.5% on conversion of L-tryptophan to skatole and indole by a mixed bacterial population from the large intestines of pigs. Microbial suspensions were anaerobically incubated at 38°C for 24 h. Samples were periodically removed for determination of pH and indole compounds. After 24 h incubation, microbial populations in each culture media were analyzed. Addition of 0.5, 1.0, and 1.5% FOS to the slurries with L-tryptophan significantly decreased the skatole concentration, the peak value of indole-3-acetic acid and the medium pH. The viable counts of *Bifidobacterium* were significantly higher as compared with the control. Addition of 1.0 and 1.5% FOS significantly decreased the rate of tryptophan degradation and the relative rate of skatole production. The relative rate of indole production was significantly increased. The viable counts of *Clostridium* and *Escherichia coli* were significantly reduced. The total viable counts of anaerobes were significantly increased. These results suggest that the reduced concentration of skatole observed in the presence of FOS may be caused by the decreased tryptophan degradation due to the increased need for amino acids in the synthesis of bacterial cellular protein, and by shifting microbial metabolism of tryptophan toward indole production at the expense of skatole, which might result from the changed microbial ecosystem and pH. Our observations open the possibility of inhibiting microbial production of skatole and decreasing the skatole concentration in backfat by feeding pigs diets containing FOS, but it remains to be demonstrated in vivo.

Key Words——fructooligosaccharide; indole; pig fecal bacteria; skatole; tryptophan

Introduction

The use of intact male pigs for meat production creates an economic opportunity because they generally have more lean meat per carcass and show better feed conversion ratios than castrates. Furthermore, animal welfare and the environment (due to improved nitrogen retention) benefit from entire male pig production. However, rearing intact male pigs is not used in a large number of countries because of the existence of boar taint, an objectionable odour affecting some boar meat (Bonneau, 1997). Moreover, boar taint is not a unique characteristic of boars only and may be detected, at low levels, in castrates and gilts as well (Baltic et al., 1997). Two compounds, androstenone (5a-androst-16-ene-3-one) and skatole, are held to be responsible for boar taint. Genetic factors are critical for the regulation of androstenone, whereas environ-

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mental factors, such as diet and feeding level, are the most important for skatole. The degree of microbial formation of skatole in the large intestine plays a very important role on the concentration of skatole in adipose tissue (Bonneau, 1997).

Skatole is produced in the large intestine of pigs by microbial degradation of L-tryptophan originating from dietary and endogenous protein (Yokoyama and Carlson, 1974). Anoxic metabolisis of L-tryptophan may give rise to two alternative volatile lipophilic compounds, indole and 3-methylindole (skatole). Many types of intestinal bacteria are capable of producing indole from L-tryptophan. The formation of skatole from tryptophan is a two-step process. Tryptophan is first converted to indole-3-acetic acid by *Escherichia coli* and *Clostridium* (Chung et al., 1975; Stowe, 1955). And then, the genera *Clostridium* and a *Lactobacillus* strain convert indole-3-acetic acid to skatole (Hengemnehle and Yokoyama, 1990; Jensen et al., 1995; Yokoyama et al., 1977).

The type and amount of carbohydrate entering the large intestine have a substantial effect on nitrogen metabolism and may therefore influence the synthesis of indoles (Hawe et al., 1992). Fructooligosaccharide (FOS) is a source of carbohydrate that is not digested by digestive enzymes in the upper gastrointestinal tract and reaches the large intestine in a largely intact form where it acts as selective nutrients for certain bacterial populations. The FOS has been shown to enhance the growth of *Bifidobacterium*, but inhibit *E. coli* and *Clostridium* (Roberfroid et al., 1998).

The objective of this study was to determine the effect of FOS on conversion of L-tryptophan to skatole and indole in vitro by a mixed bacterial population from the large intestines of pigs.

Materials and Methods

Materials. Fructooligosaccharide (FOS) was provided by Tian Yuan Health Food Co., Ltd. (YunNan, China) and the concentration of oligosaccharides was greater than 95% of total mixture. Bacteriological media were supplied by Oxoid (Hampshire, UK). All other chemicals used in the experiment were obtained from Sigma (St. Louis, MO, USA).

Incubation of pig fecal slurries. The in vitro technique was carried out according to the method described by Yokoyama and Carlson (1974) and Jensen et al. (1995). Fresh feces (total weight, 200 g) were collected from pigs fed a standard corn-soybean-based diet and were suspended in sterile anaerobic mineral salt medium which contained (per liter) 5.0 g $NaHCO_3$, 0.9 g NaCl, 0.9 g $(NH_4)_2SO_4$, 0.45 g KH_2PO_4, 0.45 g $K_2HPO_4 \cdot 3H_2O$, 0.03 g $CaCl_2 \cdot 2H_2O$, 0.02 g $MgCl_2$, 0.01 g $MnSO_4 \cdot 4H_2O$, 0.01 g $CoCl_2 \cdot 6H_2O$, 0.01 g $FeSO_4 \cdot 7H_2O$, and 1.0 g cysteine to give a 10% (wt/vol) fecal slurry. This suspension was transferred to a CO_2-flushed sterile plastic bag and stomached for 2 min under CO_2. The suspension was then pressed through cheesecloth to remove crude particulate material. Ninety-nine milliliters of this suspension, 5.1 mg of tryptophan in 1 ml of H_2O (final concentration of 250 μmol/L) and three levels of FOS (final concentration of 0.5, 1.0, and 1.5%) were mixed in triplicate in 125-ml sterile Erlenmeyer flasks fitted with rubber sleeves. Each flask was flushed for 30 min by inserting a syringe needle attached to a cylinder of CO_2 through the rubber sleeve and using another needle as an exhaust. After the flasks were flushed with CO_2, the exhaust needle was removed first, and positive pressure was attained by removing the gassing needle. Samples were incubated at 38°C in a shaking water bath for 24 h. The control containing only fecal slurries and tryptophan were incubated at the same time. One milliliter of each sample was periodically removed from the flasks at 0, 2, 4, 6, 8, 12, 18, and 24 h for determination of pH and indole compounds. After 24 h incubation, microbial populations in each culture medium were analyzed.

Analytical methods.

1. Analysis of indole compounds. Assays for skatole, indole, tryptophan and indole-3-acetic acid in culture media were carried out as described by Jensen et al. (1995). One milliliter of medium and 1.94 ml of HPLC grade methanol were transferred to a centrifuge tube with a conical bottom. Indole-2-carboxylic acid and 2-methylindole (60-μl portions of 1-mg/ml stock solutions in methanol) were added as internal standards. The mixture was vortex-mixed, placed in a freezer at −20°C for 15 min to accelerate precipitation of the particulate materials and centrifuged at 2,700×*g* for 10 min. One milliliter of the supernatant sample was transferred to an eppendorf tube and centrifuged at 15,000×*g* for 30 min. A portion of the supernatant (125 μl) was transferred to a fresh tube, and 4.875 ml of chlorhexidine (final concentration of 0.55%) as a preservative was added. The chromatographic conditions used were those described by Hansen-Moller

Table 1. In vitro skatole and indole production in pig fecal slurries with L-tryptophan and four levels of FOS[1)].

Item	FOS (%, wt/vol)			
	0	0.5	1.0	1.5
Skatole concentration (μmol/L)	104.3±9.6[a]	81.0±7.6[b] (↓22.3%)[2)]	56.5±16.8[c] (↓45.8%)	47.6±12.6[c] (↓54.4%)
Indole concentration (μmol/L)	134.3±13.3	128.5±10.1	117.5±17.0	120.8±9.5
Rate of tryptophan degradation (%)[3)]	83.9±8.9[a]	72.6±6.9[ab]	57.9±10.1[bc] (↓31.0%)	55.9±9.1[c] (↓33.4%)
Relative rate of skatole production (%)[4)]	44.7±4.5[a]	39.1±3.7[ab]	31.5±6.2[bc] (↓29.5%)	26.9±7.1[c] (↓39.8%)
Relative rate of indole production (%)[5)]	55.3±5.0[b]	60.9±8.1[ab]	68.5±4.9[a] (↑23.9%)	73.1±7.9[a] (↑32.2%)
Peak value of indole-3-acetic acid (μmol/L)	84.5±7.6[a]	68.3±5.5[b] (↓19.2%)	47.0±8.9[c] (↓44.4%)	32.1±7.6[d] (↓62.0%)

[1)] Results are the means and standard deviations of three independent experiments performed with fecal samples from three different pigs fed the same diet, each of which had triplicate incubations. Values within the same row with different superscripts are significantly different ($p<0.05$ or $p<0.01$).

[2)] The percent of decrease (↓) or increase (↑) as compared to the control.

[3)] Rate of tryptophan degradation=(skatole production+indole production)/added tryptophan.

[4)] Relative rate of skatole production=skatole production/(skatole production+indole production).

[5)] Relative rate of indole production=indole production/(skatole production+indole production).

(1992).

2. *pH determination.* The pH of the culture medium was measured with a precise pH meter (Model PHS-2C, Shanghai Rex Instruments Factory, P.R. China).

3. *Growth of mixed fecal bacteria.* Liquid samples (1 ml) were removed from each fermenter after 24 h and serially diluted in an anaerobic cabinet (10 : 10 : 80; $H_2 : CO_2 : N_2$ atmosphere) with half-strength Wilkins Chalgren Anaerobic Broth (Oxoid). Triplicate plates were then inoculated with 0.1 ml samples and incubated at 37°C aerobically or anaerobically as appropriate. 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-Verhage et al., 1987; *Clostridium*), and MacConkey's No. 2 (Oxoid; *E. 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).

4. *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.

Results

Effects of FOS on the metabolization of added L-tryptophan by pig fecal slurries

Addition of 0.5, 1.0, and 1.5% FOS to the bioreactor decreased the skatole concentration by 22.3% ($p<0.05$), 45.8% ($p<0.01$), and 54.4% ($p<0.01$), respectively (Table 1). The peak value of indole-3-acetic acid was reduced by 19.2% ($p<0.05$), 44.4% ($p<0.01$), and 62.0% ($p<0.01$), respectively. Addition of 1.0 and 1.5% FOS decreased the rate of tryptophan degradation by 31.0% ($p<0.01$) and 33.4% ($p<0.01$). The relative rate of skatole production was reduced by 29.5% ($p<0.05$) and 39.8% ($p<0.01$). The relative rate of indole production was increased by 23.9% ($p<0.05$) and 32.2% ($p<0.05$).

When fecal slurries were incubated with L-tryptophan, the L-tryptophan concentration decreased rapidly at a constant rate to zero within 6 h, while the indole-3-acetic acid concentration increased tran-

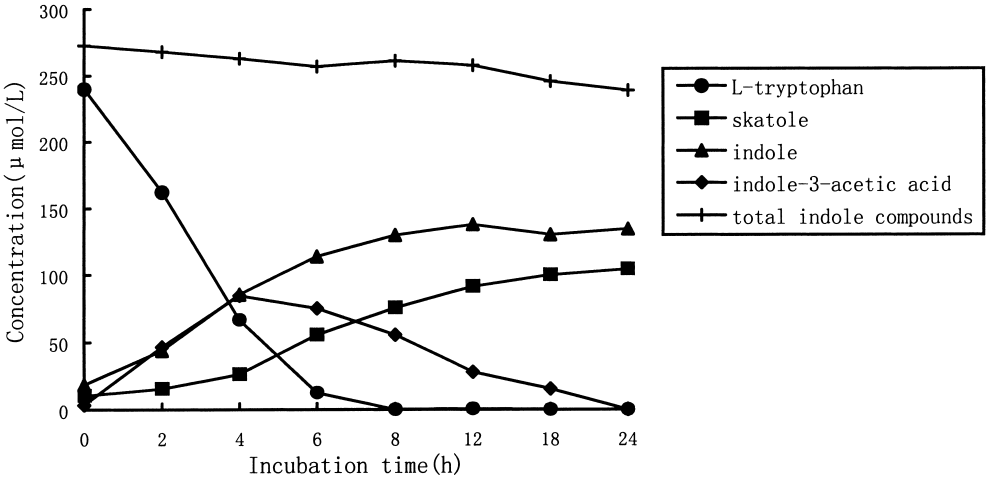

Fig. 1. Production of indole compounds from pig fecal slurries with addition of 250 μmol/L L-tryptophan.

Analyses were performed by HPLC. Each point is the mean of three independent experiments performed with fecal samples from three different pigs fed the same diet, each of which had triplicate incubations.

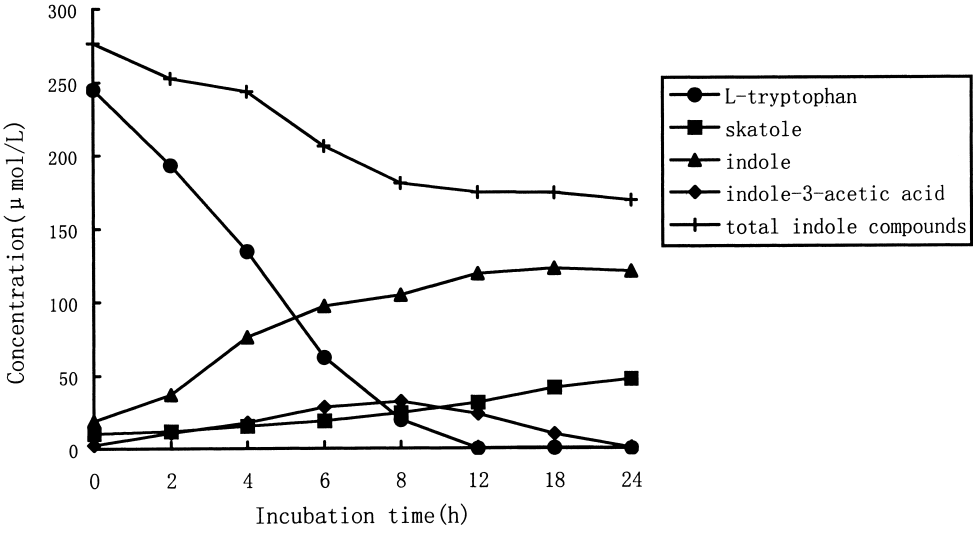

Fig. 2. Production of indole compounds from pig fecal slurries with addition of 1.5% FOS and 250 μmol/L L-tryptophan.

Analyses were performed by HPLC. Each point is the mean of three independent experiments performed with fecal samples from three different pigs fed the same diet, each of which had triplicate incubations.

siently to a peak value (84.5 μmol/L) at 4 h, which was followed by a slow decrease to zero (Fig. 1). The indole concentration increased rapidly at a constant rate to a maximun value at 8 h and then changed slightly. Production of skatole started with a lag phase followed by a rapid increase and then slower production. The total amounts of indole compounds detected decreased only slightly. When fecal slurries were incubated with L-tryptophan and 1.5% FOS, tryptophan catabolism was delayed and the L-tryptophan concentration decreased to zero only after 12 h (Fig. 2). The indole-3-acetic acid concentration increased to a peak value (32.1 μmol/L) at 8 h, followed by a slow decrease to zero. The FOS did not affect the production of indole. The skatole concentration increased slowly and remained low throughout the 24-h period. The total amounts of indole compounds detected decreased at a constant rate within 8 h and then leveled off.

Culture medium pH

The bacterial metabolism of FOS in pig fecal slurries caused a marked decrease in the culture medium pH (Fig. 3). Addition of 0.5, 1.0, and 1.5% FOS decreased the medium pH from 6.4 in control to 5.6 ($p<0.05$), 4.8 ($p<0.01$), and 4.4 ($p<0.01$), respectively.

Enumeration of selected bacterial genera utilizing FOS

The total viable counts of anaerobes and the constitutions of microbes in batch culture fermenters with different levels of FOS are shown in Table 2. Addition of 0.5, 1.0, and 1.5% FOS facilitated *Bifidobacterium* growth by 388.1 ($p<0.05$), 1,139.0 ($p<0.01$), and 1,440.7% ($p<0.01$), respectively. However, addition of 1.0 and 1.5% FOS reduced the viable counts of *Clostridium* by 67.9 ($p<0.05$) and 82.7% ($p<0.01$), and those of *E. coli* by 78.4 ($p<0.01$) and 75.4% ($p<0.01$), respectively. The total viable counts of anaerobes were increased by 332.5 ($p<0.01$) and 535.0% ($p<0.01$), respectively.

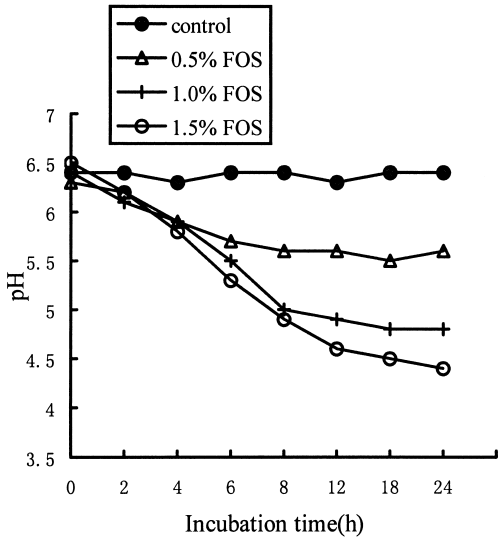

Fig. 3. Effect of FOS on the pH of pig fecal slurries with L-tryptophan.

Each point is the mean of three independent experiments performed with fecal samples from three different pigs fed the same diet, each of which had triplicate incubations.

Discussion

The present study indicated that addition of 1.0 and 1.5% FOS to the swine fecal slurries resulted in a significant reduction in the rate of tryptophan degradation (Table 1) and a delay in tryptophan catabolism (Figs. 1, 2). L-Tryptophan may be assimilated by microbial cells and incorporated into microbial biomass protein. It may also be degraded by the bacterial tryptophanase. Fordtran et al. (1964) observed a delay in tryptophan catabolism in the human large intestine in the presence of fermentable carbohydrate. Moreover, skatole-synthesizing bacteria isolated from the rumen exhibited a reduced production when readily fermentable carbohydrate, such as glucose, maltose, sucrose and mannose, were incorporated into the incubation medium (Yokoyama and Carlson, 1974). This may apply equally to skatole-producing bacteria in the

Table 2. Growth of selected populations of colonic bacteria in batch culture fermenters with different levels of FOS[1)].

	FOS (%, wt/vol)			
	0	0.5	1.0	1.5
Total anaerobes (10^{11}/L)	4.0 ± 2.8^{c}	11.5 ± 4.8^{bc}	17.3 ± 3.5^{ab} ($\uparrow$332.5%)[2)]	25.4 ± 6.3^{a} ($\uparrow$535.0%)
Bifidobacterium (10^{8}/L)	5.9 ± 5.9^{c}	28.8 ± 9.9^{b} ($\uparrow$388.1%)	73.1 ± 14.7^{a} ($\uparrow$1,139.0%)	90.9 ± 10.6^{a} ($\uparrow$1,440.7%)
Lactobacillus (10^{10}/L)	7.8 ± 5.8	9.9 ± 4.2	8.6 ± 6.5	10.1 ± 5.4
Clostridium (10^{7}/L)	32.4 ± 7.5^{a}	23.3 ± 12.2^{ab}	10.4 ± 6.1^{bc} ($\downarrow$67.9%)	5.6 ± 5.5^{c} ($\downarrow$82.7%)
Escherichia coli (10^{7}/L)	13.4 ± 4.1^{a}	8.0 ± 3.0^{ab}	2.9 ± 3.2^{b} ($\downarrow$78.4%)	3.3 ± 2.3^{b} ($\downarrow$75.4%)

[1)] Results are the means and standard deviations of three independent experiments performed with fecal samples from three different pigs fed the same diet, each of which had triplicate incubations. Values within the same row with different superscripts are significantly different ($p<0.05$ or $p<0.01$).

[2)] The percent of decreasing ($\downarrow$) or increasing ($\uparrow$) as compared to the control.

large intestine of pigs. Boyd and Lichstein (1955) found that bacteria harvested from carbohydrate-containing media exhibited markedly reduced tryptophanase activity. Microbial growth is stimulated by the presence of fermentable carbohydrate, such as FOS. Hence it is possible that growth in carbohydrate containing media favors the synthesis of tryptophan rather than its breakdown due to their increased need for amino acids in the synthesis of cellular protein.

Tryptophan can be either degraded directly to indole by many types of intestinal bacteria or converted to indole-3-acetic acid by *E. coli* and *Clostridium* and then to skatole by the genera *Clostridium* and a *Lactobacillus* strain (Chung et al., 1975; Jensen, et al., 1995; Stowe, 1955). In the present study, addition of FOS significantly decreased the skatole concentration and the peak value of indole-3-acetic acid (Table 1). Addition of 1.0 and 1.5% FOS significantly decreased the relative rate of skatole production but increased the relative rate of indole production. It can be concluded that at least a portion of the inhibition of skatole production due to FOS occurred at the phase of tryptophan conversion to indole-3-acetic acid. Bacterial growth data showed that FOS exerted a preferential stimulatory effect on *Bifidobacterium*, whilst significantly suppressing *E. coli* and *Clostridium* (Table 2). Such changes in the microbial ecosystem in the presence of FOS might contribute to the observed effects on the microbial conversion of tryptophan to skatole and indole. The effect of the microbial ecosystem on the microbial conversion of tryptophan to skatole and indole had previously been documented for pigs. By feeding pigs with fermented liquid feed, the microbial ecosystem in the intestinal tract of the pigs significantly changed in comparison with control. Such a change favored the microbial conversion of tryptophan in the hind gut toward indole at the expense of skatole, resulting in a lower skatole and higher indole concentration in blood and backfat (Jensen et al., 1997).

Clostridium and the genus *Lactobacillus* are responsible for converting indole-3-acetic acid to skatole. A *Lactobacillus* strain that produced skatole had been isolated and characterized from a bovine rumen (Yokoyama et al., 1977) and swine cecal contents (Hengemnehle and Yokoyama, 1990). The bacterium produced skatole by decarboxylating indole-3-acetic acid to skatole, but was not able to form skatole directly from tryptophan. In our study, although FOS reduced the viable counts of *Clostridium*, FOS had no significant effect on the viable counts of *Lactobacillus.* However, we could not provide conclusive evidence on the question of FOS inhibiting the conversion of indole-3-acetic acid to skatole. In a further study, indole-3-acetic acid may be used as substrate to study this.

The pH was lowered as a result of the production of short chain fatty acids and lactic acid from FOS fermentation (Fig. 3). Conversion of tryptophan to skatole appeared to be maximum at neutral pH, however the production was markedly reduced as the environment became more acidic (Hammond et al., 1984). It is possible that the reduced concentration of skatole in the presence of FOS might have been due to the effects of FOS on the acidity of the microbial environment.

Conclusion

It is desirable to minimize the amounts of skatole produced in pigs since accumulation of this compound in fat has negative effects on the quality of the meat. FOS reduced tryptophan degradation by the bacteria and shifted microbial metabolisis of tryptophan toward indole production at the expense of skatole, resulting in a lower skatole concentration. Our observations open the possibility of inhibiting microbial production of skatole and decreasing the skatole concentration in backfat by feeding pigs diets containing FOS, but it remains to be demonstrated in vivo.

Acknowledgments

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