Influence of Dietary Oligosaccharides on Growth Performance and Intestinal Microbial Populations of Piglets*

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ABSTRACT : An experiment was conducted to determine the effects of dietary oligosaccharides on performance and intestinal microbial populations of piglets. Ten litters of piglets were assigned to five groups randomly, with two litters per group. The control group was fed with corn-soybean basal diet. Oligosaccharides was added to the basal diet at the level of 0.05%, 0.1%, 0.2% and 0.35% respectively to form four experimental diets. The experiment was conducted with two periods. The first period (suckling period) was from 7 to 28 days of age and the second period (weanling period) was from 28 to 56 days of age. Fresh fecal samples were collected at 21 days of age and assayed for Escherichia coli concentration, pH and moisture content. Three pigs per group were slaughtered at 42 days of age and cecum, colon, and rectum content samples were collected and assayed immediately for *Escherichia coli* and Bifidobacterium concentration, pH and moisture content. The results showed that dietary oligosacchrides decreased fecal *Escherichia coli* population and pH significantly ($p<0.05$), but did not affect performance and fecal moisture content during suckling period. 0.1% oligosaccharides for weanling pigs increased growth and improved feed conversion ratio together with a reduction of diarrhea ($p<0.05$), but 0.35% oligosaccharides did not affect growth performance. 0.1% and 0.2% oligosaccharides for weanling pigs had a suppression to *Escherichia coli* colonization in rectum and an enrichment to Bifidobacterium in colon ($p<0.05$). Oligosaccharides decreased significantly ($p<0.05$) rectum moisture content, but did not affect cecum, colon and rectum pH. ***(Asian-Aust. J. Anim. Sci. 2001. Vol 14, No. 12 : 1747-1751)***

Key Words : Piglets, Oligosaccharides, Performance, Intestinal Microbial Populations

INTRODUCTION

Oligosaccharides are water-soluble carbohydrates consisting of 2 to 10 monomeric units. They can be classified as non-digestible oligosaccharides (NDOs) because they cannot be hydrolyzed by endogenous enzymes (Burvall et al., 1979; Oku et al., 1984), but may be delivered to the lower intestinal tract where they act as selective nutrients for beneficial members of the normal gastrointestinal microflora, including lactobacilli and bifidobacteria (Tokunaga et al., 1989). Recently, oligosaccharides have been used to enrich the beneficial bacterial populations in domestic livestock and humans (Monsan and Paul, 1995). Research efforts into the commercial manufacture of oligosaccharides were stimulated by the realization that many of the compounds possessed beneficial effects when present in diets (Perry, 1995; Monsan and Paul, 1995). The synthetic route varies and may be dependent on the direct polymerization of disaccharides or the fractionation of microbial cells to obtain the material from the cell wall (Fishbein et al., 1998; Altech Inc., 1994; Manley and Richards, 1994). Polysaccharides, when fermented, also yield oligosaccharides (Pusztai et al., 1995). The objectives of this study were to evaluate the growth response and changes in the intestinal microbial populations of piglets fed diets containing synthetic (industrial) oligosaccharides.

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MATERIALS AND METHODS

Animals and experimental design

Ten litters of 7-d-old crossbred (Yorkshire×Yorkshire-Landrace-Beijing Black Pig) piglets (100 piglets, kept 10 piglets per litter with similar sex ratio and live weight) were assigned to five groups randomly, with two litters per group. Ten sows (second parity sows) had similar backfat thickness and live weight, and their initial litter size was from 11-13 respectively. They were housed individually in an environmentally regulated nursery in pens (4.5 m^2) with woven wire flooring. The mean temperature was 23°C, humidity was between 60-80% in the farrowing pen. The experiment was conducted with two periods. The first period (suckling period) was from 7 to 28 days of age and the second period (weanling period) was from 28 to 56 days of age. At the 28 days of age, the piglets were weaned, moved to growing pens, and 15 weanling piglets from 2 litters per group were selected and allotted by weight and sex in three replications (pens). Each pen contained a self-feeder and a nipple waterer to provide *ad libitum* access to feed and water. Animal handling procedures were approved by the traditional feeding.

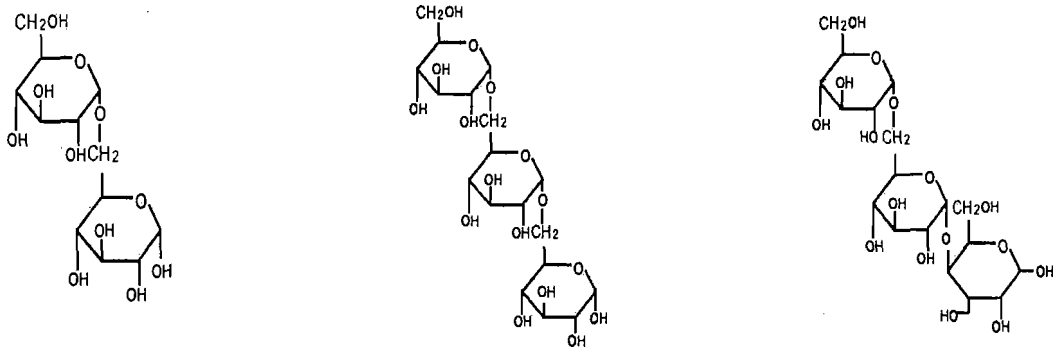

Figure 1. Main structures of oligosaccharides

Diets

Oligosaccharides were provided by YICTORY CO., LTD of ZHUHAI S.E.Z, whose ingredients are mainly Isomalto-oligosaccharides and the structures are as follows:

Table 1 gives the composition of the control diet, and oligosaccharides was added to the basal diet at the level of 0.05%, 0.10%, 0.20%, 0.35% instead of zeolite in premix respectively to form four experimental diets. Pigs from 7-28 d of age were provided with a creep feed .

Table 1. The composition and nutrient level of basal diets

Period	7~28 d of age (creep feed)	28~56 d of age (weanling feed)
Ingredients (%)		
Corn	54.45	61.65
Soybean meal	30.00	23.50
Fish meal	5.00	5.00
Dried whey	5.00	5.00
Corn oil	1.50	1.00
Dicalcium Phosphate	1.50	1.50
Calcium Carbonate	0.90	0.70
Salt	0.30	0.30
L-Lysine-HCl	0.35	0.35
Premix[1]	1.00	1.00
Total	100	100
Nutrient Level[2]		
DE (Mcal/kg)	3.30	3.25
CP (%)	20.50	18.00
Ca (%)	1.01	0.90
P (%)	0.72	0.70
Lys (%)	1.30	1.25
Met+cys (%)	0.68	0.66

[1] Supplied per kg diet: vitamin A, 10,000 IU; vitaminD_3, 3,000 IU; vitamin E, 30 IU; menadione, 4 mg; vitaminB_{12}, 0.02 mg; thiamin, 4 mg; riboflavin, 6.1 mg; d-pantothenic acid, 12 mg; niacin, 12 mg; choline, 500 mg; Fe, 100 mg; Mn, 100 mg; Zn, 100 mg; I, 0.4 mg; Se, 0.3 mg.

[2] Calculated values.

Observation

Body weights and feed refusals were recorded at 7, 21, 28, 42, 56 d of age. The occurrence of diarrhea were recorded every day.

Fresh fecal samples were collected from the rectum at 21 d of age and put on ice until they were transported to the lab for enumeration of microbial populations. Fresh samples were assessed for populations of *Escherichia coli*, pH and moisture content. At 42 d of age, 15 piglets (3 piglets per group) selected from the average-weight pig in each pen were killed and the cecum, colon and rectum were aseptically isolated and removed. Samples of content were assessed for Bifidobacteria and *Escherichia coli* numbers.

For bacterial assays, 10-fold serial dilutions were made from 1 g aliguots of sample contents, using PBS as a diluent. Bifidobacteria were enumerated on BS (Bifidobacteria Selective) plate after being incubated for 48 h. *Escherichia coli* were enumerated on MacConkey agar plates after incubation for 24 h. All plates were incubated at 37°C. Bacterial numbers are expressed as $\log_{10}$cfu/g fresh sample. pH was measured via inserting a point-shaped electrode from a digital pH meter in the faecal sample (pH-HJ90, China). Faecal dry matter content was determined by drying at 105°C until no further weight losses occurred.

Statistical analysis

Data were analyzed by comparing means according to Duncan's multiple range test (Duncan, 1955), using the GLM (General Linear Models) Procedure of SAS (1986) package program.

RESULTS

Table 2 shows the growth performance of pigs during suckling period. There was no significant difference in ADG (Average Daily Gain) or ADFI (Average Daily Feed

Table 2. Effects of oligosaccharides on growth performance of suckling piglets

Group	Control	Oligosaccharides			
		0.05%	0.1%	0.2%	0.35%
Initial weight (kg)	2.30±0.12	2.27±0.17	2.29±0.07	2.27±0.18	2.30±0.16
7~21 d of age					
ADG (g/d)	178.61±16.22	174.33±27.34	187.78±4.43	177.11±22.27	185.88±19.06
ADFI (g)	6.99±0.78	7.44±1.43	7.67±0.28	7.73±2.21	8.12±1.38
Diarrhea (%)	13.26±7.52	12.06±0.88	7.50±0.51	10.33±6.53	3.58±0.46
21~28 d of age					
ADG (g/d)	254.37±26.38	249.21±11.22	254.16±28.10	268.83±13.77	267.86±14.23
ADFI (g)	52.22±9.21	51.83±1.46	52.28±16.90	57.73±7.25	58.77±8.73
Diarrhea (%)	13.65 ± 3.14^{Aa}	9.76 ± 0.34^{ABab}	5.72 ± 2.02^{ABbc}	3.38 ± 0.74^{Bc}	3.25 ± 2.75^{Bc}
7~28 d of age					
ADG (g)	203.86±19.60	198.20±23.23	209.67±11.99	207.69±19.44	213.21±17.44
ADFI (g)	22.06±3.59	21.66±2.06	22.54±5.85	24.40±3.88	25.00±3.83
Diarrhea (%)	13.39 ± 3.97^{a}	11.32 ± 0.67^{a}	6.80 ± 0.18^{ab}	8.01 ± 4.60^{ab}	3.47 ± 0.62^{b}

Means without a common capital superscript letter within rows differ high significantly ($p<0.01$), a common small superscript letter differ significantly ($p<0.05$).

Intake) among treatments. Indeed, Small piglets fed the diets containing 0.1%, 0.2%, 0.35% oligosaccharides decrease the occurrence of diarrhea ($p<0.05$) during 21 to 28 d of age.

Table 3 shows the fecal *Escherichia coli* concentrations, moisture content and pH. Total *Escherichia coli* concentrations were numerically lower ($p<0.01$) in piglets fed 0.1%, 0.2%, 0.3% oligosaccharides.

Piglets fed diets containing 0.1%, 0.2%, 0.35% had lower ($p<0.05$) fecal pH. There was no significant effect of oligosaccharides on moisture content.

Table 4 shows the growth results from four weeks after weanling. Inclusion of oligosaccharides at 0.1% of diet increase ADG from d 0 to 28 after weanling compared with the control diet. Dietary oligosaccharides at 0.05%, 0.1% resulted in a significantly ($p<0.05$) lower feed/gain and diarrhea compared with control group.

Table 5 shows the effect of oligosaccharides on intestinal microbial populations, moisture and pH of weanling piglets. 0.1% and 0.2% oligosaccharides for weanling piglets had a suppression to *Escherichia coli* colonization in rectum, and an enrichment to Bifidobacterium in colon ($p<0.05$). Dietary oligosaccharides decreased significantly ($p<0.05$) rectum moisture content, but did not affect cecum, colon, and rectum pH.

DISCUSSION

Certain NDOs may improve growth performance of young pigs. Several authors reported increased growth and improved feed conversion ratio together with a reduction of diarrhea or loose faeces as a consequence of oligosaccharides inclusion in young piglets' diets (Hidaka et al., 1985; Fukuyasu and Oshida, 1986; Hidaka et al., 1986; Katta et al., 1993). Other authors, however, reported no or slightly negative effects of oligosaccharides on young pigs' growth performance (Kornegay et al, 1992; Farnworth et al, 1992). Antibiotics or additional copper may have been part of these diets. These additives can suppress normal gastrointestinal microflora. Therefore, NDOs may have less or different effects in diets containing microflora-suppressing agents. In the present study, diets did not contain additional copper, antibiotics, or probiotics. The data from our experiment suggest that dietary level of oligosaccharides correlates with performance response and higher levels would not be practiced. We observed no benefit increasing growth performance of supplementing weanling-pig diets with 0.2% or 0.35% oligosaccharides,

Table 3. Effect of oligosaccharides on intestinal microbial populations, moisture and pH of suckling piglets

Group	Control	Oligosaccharides			
		0.05%	0.1%	0.2%	0.35%
E.coli	7.50 ± 0.34^{A}	7.43 ± 0.28^{A}	6.78 ± 0.36^{B}	6.27 ± 0.20^{B}	6.31 ± 0.39^{B}
pH	5.76 ± 0.17^{a}	5.82 ± 0.08^{a}	5.61 ± 0.26^{b}	5.64 ± 0.32^{b}	5.54 ± 0.26^{b}
moisture (%)	63.68±9.82	63.27±6.11	64.83±7.71	66.72±9.63	57.66±6.55

Bacterial numbers are expressed as $\log_{10}$cfu/g fresh samples.

Table 4. Effect of oligosaccharides on growth performance of weanling pigs

Group	Control	Oligosaccharides			
		0.05%	0.1%	0.2%	0.35%
Initial weight (kg)	6.85±0.08	6.83±0.12	6.84±0.16	6.83±0.18	6.82±0.33
0~14 d after weaning					
ADG (g)	141.90±5.02^{b}	153.10±14.23^{ab}	173.25±6.81^{a}	170.78±15.83^{ab}	142.62±5.95^{b}
ADFI (g)	261.43±17.51	254.52±20.27	244.26±10.16	249.37±25.53	257.86±10.00
F/G	1.84±0.06^{a}	1.66±0.03^{b}	1.41±0.02^{c}	1.48±0.08^{bc}	1.81±0.06^{a}
Diarrhea (%)	21.91±2.18^{a}	12.38±4.36^{ab}	9.60±3.12^{b}	10.49±2.21^{b}	17.14±5.15^{a}
14~28 d after weaning					
ADG (g)	420.98±20.75^{b}	458.93±35.58^{ab}	515.18±33.91^{a}	417.96±14.46^{b}	419.09±22.25^{b}
ADFI (g)	788.09±26.85^{ABab}	739.71±47.51^{Bb}	801.95±61.22^{ABab}	818.73±33.89^{ABa}	862.87±18.20^{Aa}
F/G	1.88±0.15^{b}	1.61±0.05^{c}	1.56±0.07^{c}	1.96±0.10^{ab}	2.06±0.13^{a}
Diarrhea (%)	12.40±1.94^{b}	5.16±1.82^{c}	2.58±2.26^{d}	11.11±5.50^{b}	18.85±11.57^{a}
0~28 d after weaning					
ADG (g)	256.24±13.55^{b}	281.32±9.82^{ab}	302.64±30.01^{a}	270.79±7.52^{b}	252.65±10.09^{b}
ADF I (g)	476.36±13.41	458.59±10.76	453.89±55.56	481.56±8.27	461.67±56.51
F/G	1.86±0.12^{a}	1.63±0.04^{b}	1.50±0.04^{b}	1.78±0.04^{ab}	1.83±0.19^{ab}
Diarrhea (%)	17.95±1.05^{a}	9.46±2.63^{b}	7.20±1.55^{b}	10.74±1.14^{ab}	17.18±3.18^{ab}

Table 5. Effect of oligosaccharides on intestinal microbial population, moisture and pH of weanling pigs

Group	Control	Oligosaccharides			
		0.05%	0.1%	0.2%	0.35%
Escherichia coli					
Cecum	7.41±0.28	7.36±0.23	7.03±0.07	6.88±0.37	7.36±0.25
Colon	7.25±0.50	7.06±0.40	6.80±0.21	6.82±0.40	7.00±0.31
Rectum	7.59±0.27^{a}	7.36±0.22^{ab}	6.67±0.58^{b}	6.56±0.74^{b}	7.17±0.16^{ab}
Bifidobaccilli					
Cecum	9.06±0.06^{b}	9.16±0.14^{b}	9.22±0.14^{ab}	9.42±0.25^{a}	9.11±0.07^{b}
Colon	9.11±0.24^{b}	9.16±0.11^{ab}	9.38±0.04^{a}	9.32±0.19^{a}	9.10±0.10^{b}
Rectum	9.17±0.13	9.20±0.18	9.39±0.15	9.48±0.37	9.23±0.16
pH					
Cecum	5.43±0.20	5.46±0.26	5.41±0.24	5.28±0.14	5.48±0.13
Colon	6.40±0.09	6.29±0.27	6.15±0.19	6.03±0.11	6.34±0.18
Rectum	6.44±0.09	6.32±0.06	6.15±0.42	6.28±0.42	6.32±0.11
Moisture					
Cecum	89.98±1.28	88.13±4.33	83.47±3.82	83.85±6.99	88.37±1.57
Colon	83.89±6.66	78.35±1.04	77.76±3.29	78.67±1.62	82.83±1.54
Rectum	84.59±3.89^{a}	75.93±1.85^{b}	75.11±2.50^{b}	74.88±1.82^{b}	78.88±5.77^{ab}

however, weanling-pig diets supplemented with 0.1% oligasaccha-rides had increased growth performance. Growing pig studies with STOC (Sucrose Thermal Oligosaccharide) (Orban et al., 1997) show similar results.

Ingestion of certain non-digestible oligosaccharides (NDOs) may affect composition and/or activity of the normal intestinal flora. Useful function of the normal intestinal flora include resistance against potential pathogens, vitamin production, providing energy from non-digestible components, and suppression of intestinal putrefaction and may be enhance through NDO-ingestion (Mitsuoka, 1990). We provided oligosacchrides to the young pigs before weanling and after weanling, suggest that a discrete dose relationship exists between the dietary levels of oligosaccharides and intestinal microflora. 0.1% oligosaccharides in the weanling-pig diet was the most

effective in altering the gut microflora. It is possible that low concentration of oligosaccharides (0.05%) was not adequate to alter microbial populations, but microbial populations in the present study were not significantly altered by inclusion of 0.35% oligosaccharides in weanling-pig diet. The research could not clarity the reason for the results.

In our study, the oligosaccharides existing feed ingredients weren't considered. In many grain legumes the most common oligosaccharides is stachyose, followed by raffinose and verbascose (Carre et al., 1995). Iji et al. (1998) reviewed the influence of natural raffinose series oligosaccharides and synthetic (industrial) products on the productivity of broiler chicken. There is evidence of negative effects on animal health and productivity from the use of raffinose series oligosaccharides, but beneficial effects from synthetic oligosaccharides. Part of this contradiction may be due to differences in the chemical nature of the supplements, the level of supplementation or the duration of feeding.

Further studies would be necessary to determine the effects of NDOs on nutrient digestion and microflora ecology in young pigs, and elucidate the role of dietary NDOs as functional feed ingredients by compared with antibiotics and probiotics.

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