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Chito-oligosaccharides as an Alternative to Antimicrobials in Improving Performance, Digestibility and Microbial Ecology of the Gut in Weanling Pigs

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ABSTRACT : A total of 126 crossbred weanling pigs (average body weight of 6.3±0.3 kg) were used to investigate the effect of chito-oligosaccharide (COS) on growth performance, nutrient digestibility, pH of gastro-intestinal tract (GI), intestinal and fecal microflora of young piglets. Pigs were allocated to three dietary treatments based on body weight and gender in a single factorial arrangement. Treatments were control (No COS), T1 (0.2% COS during starter (6-13 kg) and 0.1% COS during grower (13-30 kg) phases, and T2 (0.4% COS during starter (6-13 kg) and 0.3% COS during grower (13-30 kg) phases, respectively. Each treatment had 3 replicates and 14 pigs were raised in each pen. COS is a low molecular weight water-soluble chitosan that can be obtained from chitin of the crab shell after deacetylation with concentrated sodium hydroxide at high temperature and then further decomposition by chitosanase enzyme in the presence of ascorbic acid. For the starter and grower periods, there were no significant differences ($p>0.05$) in average daily gain (ADG) and feed to gain ratio among treatments. However, during the overall period (6-30 kg), T2 showed better ($p<0.05$) feed to gain ratio than other treatments. A digestibility study was conducted at the end of grower phase which showed improvement ($p<0.05$) in DM and crude fat digestibility in T2 over the control. At 25 kg body weight, 6 pigs per treatment (2 per replicate) were sacrificed to determine the effect of diets on pH and microbial count at different sections of the GI tract. The pH of the cecal contents in pigs fed 0.1% COS was higher ($p<0.05$) than in the other treatments. Total anaerobic bacterial number increased from cecum to rectum in all treatments. The weekly total bacterial counts showed higher ($p<0.05$) in feces of pigs fed COS than that of untreated pigs at the 8th week. The number of fecal *E. coli* in untreated pigs at 4th wk was 7.35 log CFU/g compared to 6.71 and 6.54 log CFU/g in 0.1 and 0.3% COS-treated pigs, respectively. Similarly, at 8th wk, fecal *clostridium spp.* were lower in pigs fed 0.3% COS (5.43 log CFU/g) than in untreated pigs (6.26 log CFU/g). In conclusion, these results indicated that chito-oligosaccharide could improve feed efficiency in young pigs and inhibited the growth of harmful bacteria. (**Key Words :** Chito-oligosaccharides, Piglets, Growth Performance, Microbial Counts, Digestibility)

INTRODUCTION

It is well known that antibiotic supplementation in the diet usually improves growth rate and feed efficiency in swine. Because of the regulatory pressure and public perception of the need to remove antibiotics from animal feeds, it is necessary to identify alternatives to antibiotics to maintain growth performance benefits (Bae et al., 1999). Chitosan is a copolymer, consisting of β-(1→4)-2-acetamido-D-glucose and β-(1→4)-2-amino-D-glucose units, which is derived from chitin by deacetylation with alkali (Arvanitoyannis et al., 1998). Recent studies indicated that converting chitin and chitosan to water-soluble oligosaccharides improved its biological activities. Studies showed that chitosans had antitumor activity (Suzuki et al., 1986; Tsukada et al., 1990), antifungal activity (Park et al., 2005) and antimicrobial activity (Uchida et al., 1989; Jeon et al., 2001). Chitosans possessed a lot of polycationic amines that interacted with the negatively charged residues of macromolecules on the cell surface of bacteria and inhibit their growth (Young and Kauss, 1983). There are few reports about chitosan use in animal feeding. Chitosan could increase dry matter digestibility by broilers (Lim et al., 2006). Chitin was degraded in the rumen of sheep (Yoshino et al., 1990), while chitosan remained intact. Chitosan was found to be degraded in the stomach and large intestine of dogs (Okamoto et al., 2001) but not in the small intestine.

In spite of much attention given to these materials as

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Table 1. Basal formula and chemical composition of experimental diets for feeding trial

Ingredients (%)	Starter (6-13 kg)	Grower (13-30 kg)
Corn	14.54	49.18
SBM	14.00	34.72
Bakery-by product	10.00	6.00
Rice bran	-	4.00
Milk replacer	36.00	-
Spray-dried porcine plasma	6.00	-
Fish meal	4.00	-
Animal-fat	2.00	2.80
Tri-calcium phosphate	0.60	1.75
Limestone	-	0.31
Five star-booster	2.50	-
Lactose	5.00	-
Sucrose	4.00	-
L-lysine	0.13	0.25
DL-methionine (50%)	0.23	0.17
Vitamin-mix[a]	0.30	0.12
Trace mineral-mix[b]	0.20	0.20
Salt	0.10	0.25
Choline (25%)	0.10	0.05
Mecadox	0.10	0.10
Chlortetracycline	0.10	0.10
Apramycin	0.10	-
Total	100.00	100.00
Chemical composition[c]		
ME (kcal/kg)	3,420	3,400
CP (%)	21.17	20.50
Lys (%)	1.50	1.35
Met+cys (%)	0.84	0.75
Ca (%)	0.80	0.80
Av. P (%)	0.59	0.42

[a] Supplied per kg diet: 12,000 IU vitamin A, 3,000 IU vitamin D_3, 30 IU vitamin E, 3.45 mg vitamin K_3, 1.8 mg vitamin B_1, 14.4 mg vitamin B_2, 3 mg vitamin B_6, 0.045 mg vitamin B_{12}, 30 mg pantothenic acid, 90 mg niacin, 0.105 mg biotin, 0.75 mg folic acid.

[b] Supplied per kg diet: 143 mg Cu, 125 mg Fe, 102 mg Zn, 38.74 mg Mn, 0.75 mg Co, 0.75 mg I, 0.23 mg Se.

[c] Calculated values.

functional foods, information regarding the digestion and absorption of chitin and chitosan in the gastrointestinal tract is limited. The chitosan derivatives like chitosan lactate and chitosan hydroglutamate were reported to be superior antibacterial substances against general coliform bacteria (Sudarshan et al., 1992). Recent *in vitro* studies showed antimicrobial activities of chito-oligosaccharides against *Escherichia coli*, *Staphylococcus aureus* and *Salmonella cholera* (Jung et al., 2006), and *Vibrio* species causing fish diseases (Jeon et al., 2005). Although the mechanism of such antibacterial action is not yet fully discovered, the possible causes of reaction are specificity of chitosan amino group bonding with cell walls of pathogens preventing their multiplication (Uchida, 1989), influence of antibacterial action of chitosan on surface structure of bacillus (Young et al., 1982), or inhibitory action on DNA formation in metabolism of pathogens (Stossel and Jeuba, 1984). Moreover, the reports also claimed that desirable lactic acid bacteria and bifidobacteria in intestines were increased (Benno et al., 1987; Kawaguchi et al., 1993; Martin, 1994). Various efforts were being made to implement such antibacterial function of chitosan (Kendra et al., 1984; Amako et al., 1987; Shimojoh et al., 1996) in food preservatives (Sudarshan et al., 1992) as new organic food materials.

Based on their reported antibacterial effects, chito-oligosaccharide has a promising future as an alternative to antibiotics thereby promoting the productivity of animals, but sufficient research has not yet been done. In this context, the present study was an attempt to elucidate the effect of feeding chito-oligosaccharide on growth performance, nutrient digestibility and intestinal microflora in weanling pigs.

MATERIALS AND METHODS

Experimental design, animals and feeding

A total of 126 crossbred weanling pigs (Landrace×Yorkshire×Duroc; average body weight of 6.3±0.3 kg; 25±3 d of age) were used to investigate the effect of chito-oligosaccharide on the growth performance, nutrient digestibility, intestinal and fecal microflora of young piglets. Pigs were randomly allotted to three treatments based on body weight and gender in a single factorial arrangement. Each treatment had 3 replications with 14 pigs per replicate. Treatments were control (No COS), T1 (0.2% COS for starter (6-13 kg) and 0.1% for grower (13-30 kg) stages, and T2 (0.4% COS for starter (6-13 kg) and 0.3% for grower (13-30 kg) stages. COS is a low molecular weight water-soluble chitosan that can be obtained from chitin of the crab shell after deacetylation with concentrated sodium hydroxide at high temperature and then further decomposition by chitosanase-KPB enzyme in the presence of ascorbic acid (Kunpoong Bio Co., Seoul, Company brochure, 2000). This product, named Biovita-P®, was used in the present study.

The basal formula and chemical composition of experimental diets is presented in Table 1. All nutrients met or exceeded NRC (1998) requirements. COS replaced corn on an equal percent basis for both phases. The anti-bacterial products used in the basal diet were also added in experimental diets. The pigs were housed in partially slotted and concrete floored pens of 1.9×2.54 m size with a self-feeder and nipple drinker to allow *ad libitum* access to feed and water. The pigs were weighed and feed intake was recorded at the end of starter and grower stages. A digestibility trial was performed using chromic oxide (0.25%) as an indicator. All the pigs were fed diets mixed with chromic oxide during the grower phase after 10 days

Table 2. Growth performance of weanling pigs as affected by chito-oligosaccharide

	Control	T1[1]	T2[2]	SEM	p-value	
					Linear	Quadratic
Starter (6-13 kg)						
ADG (g)	341	371	340	31.94	0.9746	0.9072
ADFI (g)	494	445	413	64.58	0.1638	0.9292
FCR (F/G)	1.45	1.20	1.22	0.26	0.3637	0.9417
Grower (13-30 kg)						
ADG (g)	636	638	629	65.41	0.9069	0.9926
ADFI (g)	1,207[a]	1,210[a]	1,063[b]	112.62	0.1018	0.5083
FCR (F/G)	1.90	1.92	1.69	0.17	0.1064	0.4522
Overall (6-30 kg)						
ADG (g)	507	501	507	39.12	0.9895	0.8730
ADFI (g)	869[a]	855[ab]	758[b]	69.50	0.0385	0.3952
FCR (F/G)	1.72[a]	1.71[a]	1.50[b]	0.13	0.0095	0.1613

[a,b] Values with different superscripts in the same row differ significantly.
[1] T1: 0.2% (6-13 kg), 0.1% (13-30 kg); [2] T2: 0.4% (6-13 kg), 0.3% (13-30 kg) chito-oligosaccharaide.

of experimental feeding; then fecal samples were collected from day 16 to 18 during the grower phase and pooled. The fecal samples were dried in an air-forced drying oven at 60°C for 72 h and ground with a 1 mm mesh Wiley mill for chemical analysis.

Chemical and microbiological analysis

Analysis of the experimental diets and excreta was done according to the methods of the AOAC (1990). Gross energy was measured by a bomb calorimeter (Model 1261, Parr Instrument Co., Molin, IL), and chromium was determined with an automated spectrophotometer (Shimadzu, Japan) according to the procedure of Fenton and Fenton (1979).

Six pigs per treatment (2 per replicate, randomly selected), reflecting average body weights, were sacrificed by electrocution at the end of experimental feeding for microbiological analysis. Immediately after slaughter, the digestive system was excised. Digesta from the stomach, duodenum, jejunum, ileum, cecum, colon and rectum were aseptically isolated and removed. The contents were collected in sterilized plastic bottles in an icebox and then brought to the laboratory for analysis. A pH meter measured the pH of the different samples of digesta collected. The bacterial count in the intestinal contents, especially from the cecum, colon and rectum, was measured. At regular intervals (end of 1st, 4th, 6th and 8th week) fresh fecal samples were randomly collected from each pen and pooled and grab sample was utilized for measuring fecal bacterial counts. The microbiological assay was carried out by the procedure suggested by Torrallardona et al. (2003).

Statistical analysis

Data collected were subjected to statistical analysis using the GLM procedure of SAS (1985) and a completely randomized design to compare the treatments, which were the main effects. The pens were the experimental units for all analyses except for microbiological assays in which each pig was the experimental unit. When significant differences were noted, the means were compared using LSD by multiple range test. The level of significance was accepted at $p<0.05$, unless otherwise noted.

RESULTS AND DISCUSSION

Growth performance

During the starter stage (6-13 kg), there were no significant differences ($p>0.05$) in average daily gain (ADG), average daily feed intake (ADFI) and feed to gain (F/G) ratio between dietary treatments (Table 2). However, at the grower stage (13-30 kg) pigs fed diets containing 0.3% chito-oligosaccharide (T2 treatment) showed a trend for lowering feed intake as compared to other groups ($p<0.10$). For the overall period, ADFI and F/G ratio were not only significantly lower ($p<0.05$) but also linearly decreased ($p = 0.0385$ and $p = 0.0095$, respectively) as dietary COS level increased, although no significant differences were detected in ADG between treatments. The reduction in feed intake improved the F/G ratio in the T2 group compared with pigs in T1 and control groups. Kim et al. (1999) reported significantly higher ADG in an antibiotic (chlortetracycline)-added diet than non-antibiotics and chitosan- added diets from 0-21 d in weanling pigs, and the effect was due to higher feed intake in the positive control group than in the other groups. Reports of *in vitro* studies showed that chitosans became a gel in artificial gastric juice but there were no such physical changes when placed in artificial intestinal juice (Okamoto et al., 2001). Chickens fed a commercial broiler diet containing 20% dried whey and 2 or 0.5% chitin had significantly improved weight gain compared to controls (Zikasis et al., 1982), and the feed efficiency ratio shifted from 2.5 to 2.38 due to incorporation of chitin in the feed. The results for growth performance showed that feeding COS in weaning pig is generally

Table 3. Effects of chito-oligosaccharide on nutrient digestibility in weanling pigs

	Control	T1[1]	T2[2]	SEM	p-value
DM	77.98[ab]	77.23[b]	79.05[a]	0.99	*
GE	76.83	76.14	77.71	0.94	NS
CP	74.36	74.21	75.62	2.47	NS
EE	59.13[b]	58.64[b]	62.84[a]	3.38	*
Ca	46.32	45.77	47.80	4.82	NS
P	44.00	50.45	50.21	0.83	NS

[a, b] Values with different superscripts in the same row differ significantly ($p<0.05$).
[1] T1: 0.2% (6-13 kg), 0.1% (13-30 kg); [2] T2: 0.4% (6-13 kg), 0.3% (13-30 kg) chito-oligosaccharide.
NS: Not significant.

Table 4. Effect of chito-oligosaccharide supplementation on pH of contents in different regions of the gastrointestinal tract of piglets

	Chito-oligosaccharide (%)			SEM	p-value
	0	0.1	0.3		
Stomach	4.50	4.57	4.53	0.26	NS
Duodenum	5.93	6.27	5.37	0.59	NS
Jejunum	6.30	6.47	5.97	0.63	NS
Ileum	5.57	6.10	5.53	0.32	NS
Cecum	4.90[b]	5.37[a]	5.17[b]	0.26	*
Colon	5.10	5.33	5.40	0.26	NS
Rectum	5.63	5.70	5.67	0.12	NS

[a, b] Values with different superscripts in the same row differ significantly ($p<0.05$).
NS: Not significant.

desirable. However, regardless of an improvement in F/G ratio, the reasons for decrease in ADFI must be explained. In a previous study, chitosan formed a highly viscous solution in the gastrointestinal tract of animals because of its melting properties in weak acid (Sugano et al., 1988), and caused a full stomach in animals by expanding the duodenum region (Razdan and Petterson, 1994). There is a scarcity of reports on the effects of chitosans on performance of weanling pigs. Therefore, further research is required to investigate the effect of chitosan and its derivative on feed intake in pigs.

Nutrient digestibility

There were no significant differences ($p>0.05$) in gross energy (GE), crude protein (CP), calcium (Ca), and phosphorus (P) digestibility between dietary treatments (Table 3). However, pigs fed the T2 diet generally showed the best nutrient digestibility and higher crude fat (EE) digestibility ($p<0.005$). This effect on digestibility may explain why pigs fed the T2 diet showed improvement in growth performance. Thus, these nutrient digestibilities were closely related with the improvement in F/G ratio. There were no differences in nutrient digestibility in weanling pigs between positive (antibiotic added), negative (no antibiotic) and chitosan-added diets reported by Kim et al. (1999).

pH of contents in different sections of digestive tract

The effect of chito-oligosaccharide on pH of contents in different regions of the gastrointestinal tract of piglets is shown in Table 4 and Figure 1. There were no significant differences ($p>0.05$) between the treatments in pH of various sections of the gastrointestinal tract except for the cecum. Pigs fed 0.1% COS had a significantly higher pH in the cecal contents compared to the control and 0.3% COS treatment ($p<0.05$), but the reasons remain obscure. The data revealed an increasing trend of pH in contents from stomach to jejunum, and a drop at the ileal region; and then pH began to rise again until the rectum in all groups.

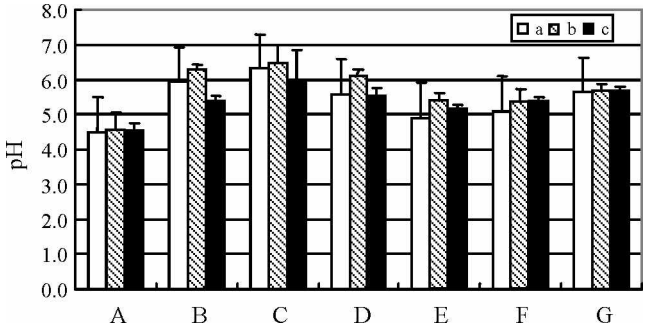

Figure 1. Effect of chito-oligosaccharide supplementation on pH of contents in different regions of the gastrointestinal tract of piglets. * A: Stomach, B: Duodenum, C: Jejunum, D: Ileum, E: Cecum, F: Colon, G: Rectum * a: 0%, b: 0.1%, c: 0.3% chito-oligosaccharide.

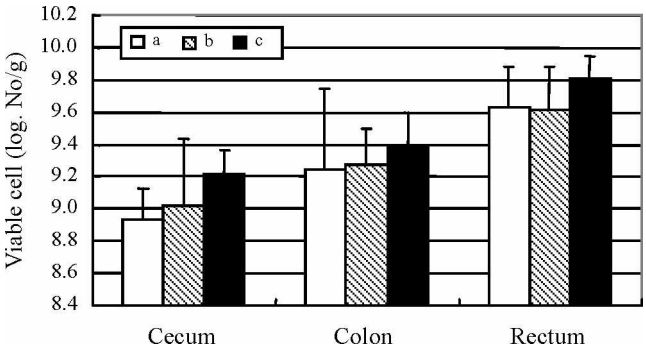

Figure 2. Effect of chito-oligosaccharide supplementation on total anaerobic bacterial counts in different regions of the large intestine of piglets. * a: 0%, b: 0.1%, c: 0.3% chito-oligosaccharide.

Bacterial counts in intestinal tract

Total anaerobic bacterial counts (TBC) in the large

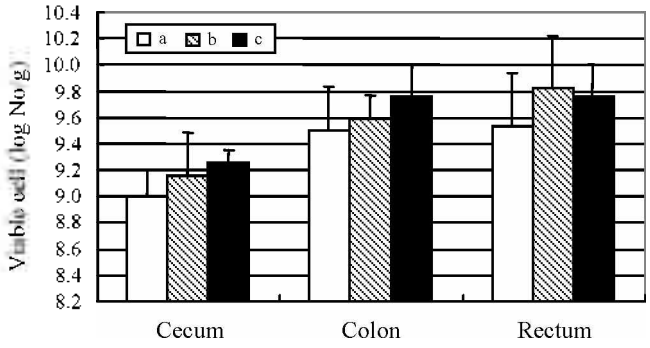

Figure 3. Effect of chito-oligosaccharide supplementation on lactic acid bacterial counts in different regions of the large intestine of piglets. * a: 0%, b: 0.1%, c: 0.3% chito-oligosaccharide.

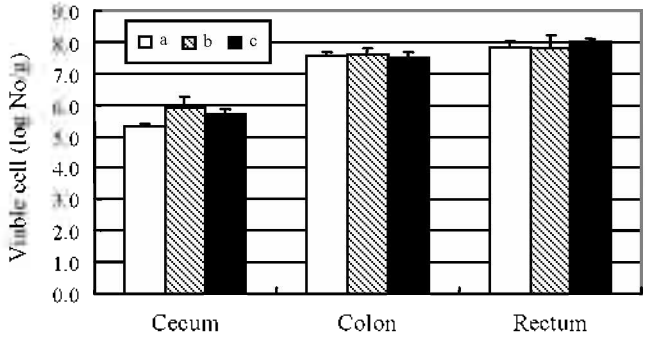

Figure 4. Effect of chito-oligosaccharide supplementation on *Enterococcus spp.* counts in different regions of the large intestine of piglets. * a: 0%, b: 0.1%, c: 0.3% chito-oligosaccharide.

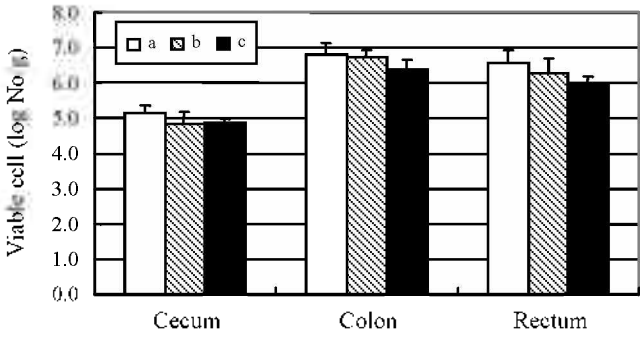

Figure 5. Effect of chito-oligosaccharide supplementation on *E. coli* counts in different regions of the large intestine of piglets. * a: 0%, b: 0.1%, c: 0.3% chito-oligosaccharide.

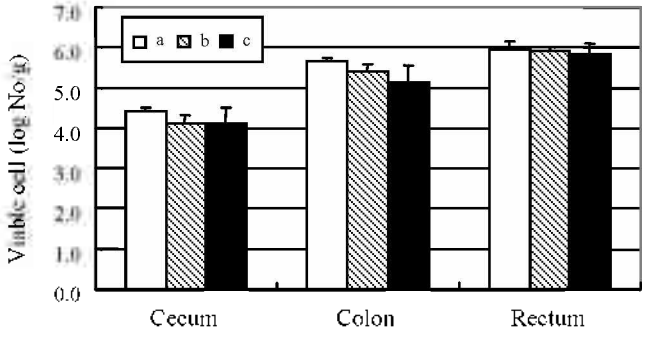

Figure 6. Effect of chito-oligosaccharide supplementation on *Clostridium spp.* counts in different regions of the large intestine of piglets. * a: 0%, b: 0.1%, c: 0.3% chito-oligosaccharide.

intestine increased from cecum to rectum in all the groups (Figure 2). In addition, pigs fed 0.3% COS had numerically higher total anaerobic bacterial counts along the large intestine compared to the control and 0.1% COS treatments. However, there were no significant differences ($p>0.05$) in total anaerobic bacterial counts between various sections of the gastrointestinal tract. The effects of COS supplement on lactic acid bacterial counts also revealed a similar trend to TBC (Figure 3). On reaching the rectum, lactic acid bacterial counts in the large intestine increased. There were no significant differences in lactic acid bacterial counts between various sections of the gastrointestinal tract. However, previous studies showed that the three strains of lactic acid bacteria *L. bulgaricus*, *L. casei* and *L. fermentum* were effectively inactivated most sensitively by all chitosan preparations (Jeon et al., 2001), which was contrary to the present study. However, Austin et al. (1981) reported that addition of 10% chitin to the diet of chickens resulted in normal growth and increased the growth of *bifidobacterium spp.* in the gut. For *enterococcus spp.* in different regions of the gastrointestinal tract of piglets, an increasing trend was noticed from cecum to rectum, although no significant differences were noted between groups (Figure 4). The effects of COS on *E. coli* in different regions of the gastrointestinal tract of piglets showed increased *E. coli* counts in the colon compared to the cecum and rectum, and lower counts were noted in supplemented groups when compared with the control, although differences were non-significant (Figure 5). Numerous previous studies have shown that chitosans were effective for *E. coli* inhibition, although the concentration required for complete inhibition of *E. coli* growth varied according to the degree of acetylation, molecular weight and functional groups (Wang, 1992; Darmadji and Izumimot, 1994). With respect to *clostridium spp.* in different regions of the gastrointestinal tract of piglets, similarly to other bacterial counts an increasing trend was noted from cecum to rectum, and no significant differences were observed between the treatments (Figure 6). That COS has more effective activity against pathogens than non-pathogens, except in the case of lactic acid bacteria; was also reported by Jeon et al. (2001).

Fecal pH and microflora

The effects of COS supplement on weekly fecal pH showed no significant differences in pH (average 6.5-6.6) between different groups (Figure 7). The pH of fecal contents was lower at 6 weeks on all treatments, and this may be by chance as no reasons could be attributed for such a change. The effects of chito-oligosaccharide supplement

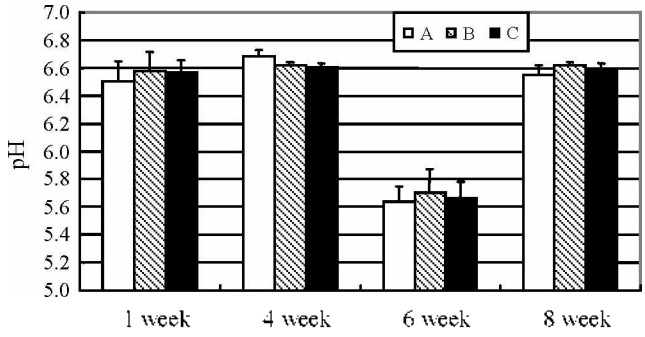

Figure 7. Effect of chito-oligosaccharide supplementation on fecal pH at different weeks. * A: 0%, B: 0.1%, C: 0.3% chito-oligosaccharide.

Table 5. Effect of chito-oligosaccharide supplementation on viable bacterial cell counts (log CFU/g) in the feces of piglets at different weeks

	Chitooligosaccharide (%)			SEM
	0	0.1	0.3	
Total anaerobic bacterial counts				
1wk	9.33	9.41	9.63	0.29
4wk	9.69	9.74	9.81	0.20
6wk	9.63	9.62	9.81	0.22
8wk	9.85[b]	10.14[a]	10.08[a]	0.15
Lactobacilli counts				
1	9.45	9.36	9.70	0.23
4	9.95	10.02	10.31	0.27
6	9.53	9.83	9.76	0.33
8	9.46	9.76	9.69	0.19
Enterococcus spp. counts				
1	7.83	7.97	8.12	0.29
4	8.03	8.05	8.10	0.11
6	7.84	7.82	7.98	0.19
8	7.63	7.92	8.12	0.39
E. coli counts				
1	7.11	6.99	6.87	0.19
4	7.35[a]	6.71[b]	6.54[b]	0.47
6	6.55	6.30	5.93	0.36
8	7.17	7.08	7.18	0.14
Clostridium spp. counts				
1	5.95	5.92	5.81	0.1
4	6.04	5.98	5.95	0.11
6	5.94	5.9	5.84	0.11
8	6.26[a]	6.16[a]	5.43[b]	0.48

[a,b] Values with different superscripts in the same row differ significantly ($p<0.05$).

on total anaerobic bacterial count in the feces of piglets showed significantly higher counts on COS treatment diets at 8 weeks ($p<0.05$) than in the control, but a similar trend was not evident at other weeks (Table 5). The lactic acid bacterial count in the feces of piglets did not show any effect of dietary treatment, although higher counts were noted at 4 weeks than at other times (Table 5). The higher *lactobacilli* count in chito-oligosaccharide-fed pigs agrees with the results of others who have reported numerically higher *lactobacilli* and *bifidobacterium spp.* counts in dogs and pigs fed oligosaccharides (Mathew et al., 1998; Flickinger et al., 2000; Strickling et al., 2000). The effects of chito-oligosaccharide supplement on *Enterococcus spp.* in the feces of piglets showed no differences between the groups at different weeks and the values were almost identical at all measurements (Table 5). The effects of treatment on *E. coli* count in the feces of piglets showed that at 4 weeks chito-oligosaccharide supplement significantly lowered ($p<0.05$) the *E. coli* counts compared with the control, but there were no significant changes after 4 weeks. The effects of treatment on *Clostridium spp.* in the feces of piglets showed that at 8 weeks the 0.3% COS supplement had significantly lower ($p<0.05$) *clostridium spp.* concentrations compared with the control and 0.1% COS treatments (Table 5). In the intestine, this bacterium is representative of a bacterial population producing noxious compounds such as indol, mercaptan, H_2S and ammonia. In conclusion, these results indicated that chito-oligosaccharide could improve feed efficiency in young pigs and reduce the growth of harmful bacteria. However, further experiments are needed to assess the effect of COS on feed intake in pigs.

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