Original Article

Effects of β-glucan and mannan-oligosaccharide supplementation on growth performance, fecal bacterial population, and immune responses of weaned pigs

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Abstract

The effects of β-glucan and mannan-oligosaccharide (BG + MOS) supplementation in feed on growth performance, bacterial population in feces and immune responses of weaned pigs were investigated. Two hundred and eighty-eight weaning piglets were randomly allocated into 3 groups with 8 replicates per group and 12 pigs per replicate. Group 1, as control, was fed a commercial basal nursery diet. Groups 2 and 3 were fed the same diet supplemented with BG + MOS (Biolex® MB-40) 1 and 2 kg/ton of feed, respectively. Weight gain (WG), average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) were calculated on days 1, 21 and 42 of experiment. Blood samples were analyzed for interleukin (IL)-1, IL-6 and tumor necrosis factor alpha (TNF-α). Total bacteria, total coliform, *Lactobacillus* concentrations and *Lactobacillus* to Coliform ratio (L:C) in feces were also determined. Results showed that groups 2 and 3 had better WG, ADFI and ADG on days 1-21. No significant difference in growth performance was, however, observed among the groups on days 21-42 and 1-42. FCR was not different among the groups throughout the experiment. Both BG + MOS supplemented groups had reduction in total bacteria and coliform concentration in feces but increase in number of *Lactobacillus* spp. and L:C ratio. Similar extent of reduction in diarrhea incidence of both BG + MOS supplemented groups was observed during the first 3 weeks of experiment. Group 3 pigs exhibited higher PRRS antibody response than the others on day 42. No statistically significant difference in serum IL-1, IL-6 and TNF-α among the groups was shown during the experiment. In conclusion, BG + MOS supplementation is potentially beneficial to growth performance on days 1-21, bacterial population balance in feces and diarrhea incidence reduction of weaning pigs.

Keywords: β-Glucan, fecal bacterial population, growth performance, immune responses, mannan-oligosaccharide, pigs

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Introduction

Weaning is a critical stage of pig life due to stress under management, nutritional and environmental conditions. Gut microbial system is another factor that contributes to weaning stress. During this period, function of the gastrointestinal tract and immunity of pigs are incompetent enough to resist infection, resulting in low digestibility and absorption, depressed growth performance and more susceptibility to gastrointestinal diseases (Barnett et al., 1989; Yuan et al., 2006). Antimicrobial uses have been programmed to prevent such infectious diseases and provide improvement in growth performance for a few decades. However, concerns about bacterial resistance and residues are important and thought to be hazardous to consumers (Chae et al., 2006). Therefore, alternative products that may be supplemented in pig production to reduce antimicrobial use have been sought. A combination of prebiotic and immunomodulator appears to be a potential solution, including a byproduct from beer producing industry, the yeast cell wall, in which β-D-glucan and α-D-mannan oligosaccharides are two major components (Reisinger et al., 2012). β-D-glucan polysaccharides had potential effects on activation of macrophages, leading to release of inflammatory mediators (Ljungman et al., 1998). β-D-glucans are also beneficial to antibacterial and antiviral activities, antitumor response and antioxidation. D-mannose can block bacterial attachment to enterocytes via compete mannose-receptors on epithelial cells with some strains of pathogens such as *E. coli* and *Salmonellla* spp. (Linquist et al., 1987). Firon and colleagues (1983) demonstrated that mannan-oligosaccharides (MOS) were more effective in preventing bacterial adhesion than pure D-mannose. Both BG and MOS have their own mechanisms to promote gut health. This could, therefore, support pig performance. Previous studies often refer to one of those two components and there are fewer studies of the use of them in combination. Therefore, the objective of this study was to investigate the effects of BG + MOS supplementation on growth performance, bacterial population in feces and immune responses of weaned pigs.

Materials and Methods

Animal use and experimental protocols were approved by Animal Care and Use Committee, Faculty of Veterinary Science, Chulalongkorn University.

BG + MOS: BG + MOS was feed supplemented in form of Biolex-MB40® (Leiber GmbH, Germany), a yeast cell wall extracted product derived from *Saccharomyces cerevisiae* yeast. Biolex–MB40®, containing 20-25% MOS and 25-30% β-D-glucans, was supplemented at 1 and 2 kg/ton of feed under standard commercial feed manufacturing conditions. The product was mixed with basal diets following experimental dosage in a feed mill before transporting to the farm.

Animals and experimental design: Two hundred and eighty-eight weaned cross-bred piglets (Large White X Landrace X Duroc), 24-26 days of age, initially weighing 7.10±0.54 kg, were used in a 6-week clinical trial in a commercial farrowing to fattening pig producing farm in Ratchaburi province of Thailand. The piglets were allocated in a completely randomized design (CRD) into 3 groups, 8 replicates per group, 12 pigs per replicate (pen), with equal numbers of male and female pigs in each group. The experimental pens were 1.2 × 1.8 m, with slatted metal flooring. In each pen, a manual feeder and two automatic nipple drinkers were equipped to provide feed and water *ad libitum*. All pigs were kept in open housing system throughout the experiment. A commercial feed was used as basal diet. The pigs in group 1 (control group) were fed basal diet while those in groups 2 and 3 were fed basal diet mixed with Biolex-MB40® 1 and 2 kg/ton of feed, respectively. During the first 4 weeks of trial, the pigs were fed diet containing 22.50% Crude Protein (CP) and 4077 kcal/kg of feed gross energy (GE) as requirements for weaned pigs. For the remaining 2 weeks of trial, the diet contained 22.25% CP and 3965 kcal/kg of feed GE as requirements for pre-starter pigs following NRC (2012). The chemical compositions of feed in this experiment were analyzed by proximate analysis, following the method of AOAC (2005) as shown in Table 1.

Table 1 Chemical analysis of nutrient compositions of experimental diets

Nutrient	Weeks 1-4			Weeks 5-6			NRC (2012)
	Group 1	Group 2	Group 3	Group 1	Group 2	Group 3	
GE (kcal/kg)	4053.9	4092.1	4085.2	3971.9	3947.6	3976.2	No data
DE (kcal/kg)[1]	3806.7	3842.5	3837.3	3726.2	3698.1	3725.7	3490-3542
Crude Protein (%)	22.47	22.53	22.49	22.25	22.23	22.26	21-24
Moisture (%)	9.31	9.34	9.30	9.29	9.33	9.28	No data
Crude Fat (%)	10.54	10.63	10.6	10.48	10.42	10.47	No data
Crude Fiber (%)	2.37	2.36	2.30	2.35	2.38	2.40	No data
Ash (%)	2.48	2.49	2.53	2.55	2.60	2.57	No data
Ca (%)	1.05	1.08	1.06	1.04	1.04	1.03	0.7-0.8
Total P (%)	0.73	0.75	0.74	0.71	0.70	0.68	0.6-0.65

Group 1 = Pigs fed a commercial basal diet, Groups 2 and 3 = Pigs fed the same basal diet supplemented with BG + MOS 1 and 2 kg/ton of feed, respectively

[1]DE was estimated following the formula of Noblet and Perez (1993).

Performance and sample collection: Feed offered and refused was weighed daily to calculate average daily feed intake (ADFI). Approximately 100 g of feed samples were randomly collected 3 times at the

beginning, middle and end of experiment, and kept refrigerated for nutrient composition analysis. The experimental pigs were weighed 3 times at 3-week intervals: at the beginning, 3 weeks and the end of experiment in order to calculate body weight (BW), weight gain (WG), average daily gain (ADG) and feed conversion ratio (FCR).

One pig per replicate was randomly selected for blood and fecal collections on the same day of body weighing. Briefly, 5 ml blood was collected from the anterior vena cava of the same pigs using 20 gauge sterile needles and a 5 ml syringe on days 1, 21 and 42 of the trial. The blood was transferred into a container without anticoagulant and centrifuged at 3000 g for 10 minutes. Serum was carefully collected and kept frozen at -80^{o}C for cytokine profile analyses. Approximately 1 g of feces per pig was collected by inserting a sterile cotton bud into the pig's anus to stimulate defecation. The fecal samples were carefully transferred into plastic bags, sealed and stored at 4^{o}C for determination of total bacterial count (TBC), total coliform count (TCC) and *Lactobacilus* spp. count (LC) within 24 hours after collection.

Clinical observation with special interest in diarrhea and mortality was carefully performed daily throughout the experiment. Numbers of diarrheic and dead pigs in each replicate/group were recorded daily. Four levels of fecal consistency were used for diarrhea score: scores 0 = solid and well-formed feces, 1 = soft and formed feces, 2 = semi-liquid, loose feces, and 3 = liquid feces (Rossi et al., 2012). A fecal consistency score of 2 or 3 with prolonged sign of equal or more than 2 consecutive days was defined as diarrhea. Diarrheic pigs longer than 3 days were discarded from the experiment and recorded as culled pigs. Diarrhea incidence (DI) of each treatment was calculated following the formula below (Liu et al., 2010):

$$DI\ (\%) = \frac{\text{Number of pigs with diarrhea in group x diarrhea days}}{\text{Total pigs in group x experiment days}} \times 100$$

Data analysis: Chemical analyses of diets of all experimental groups were carried out following the AOAC (2005) methods. Dried matter content of the feed and fecal samples was measured using an oven. Gross energy was determined by an automatic adiabatic oxygen bomb calorimeter. Crude protein was measured by Kjeldahl method. Crude fiber, crude fat, Calcium (Ca) and Phosphorus (P) were analyzed by AOAC Official Method 962.09, 2003.05, 927.02 and 964.06, respectively.

The blood samples were analyzed for interleukin-1 (IL-1), IL-6 and tumor necrosis factor alpha (TNF-α) using commercial ELISA (Quantikine porcine IL-1, IL-6 and TNF-α immunoassay; R&D Systems, Inc, MN, USA). Antibody against PRRS was examined using IDEXX PRRS X3 Ab Test (IDEXX Laboratories, Inc. Maine, USA).

The fecal samples were diluted as a serial 10-fold dilution and cultured on plate count agar (PCA), violet red bile (VRB) Agar and de Man, Rogosa and Sharpe (MRS) agar using plate culture technique for TBC, TCC and LC. Fecal dilution technique for TBC and TCC was performed according to WHO Global Foodborne Infections Network (2010), whereas bacteria enumeration followed FDA Bacteriological analytic manual (2001). For LC, the fecal samples were diluted, cultured and incubated following the instructions of ISO15214 (1998).

Statistical analysis: Data were analyzed as CRD and displayed as Least Square Means (LSM) and SEM. Effect of treatments and time was analyzed by General Linear Model (GLM). Significant differences among treatment means were tested by Duncan's New Multiple Range Test. Significant differences among means of the same treatment at different times were tested by repeated measures ANOVA. For microbiological parameters, data were converted to $\log_{10}$ (cfu/g) before analysis. For BW analysis, if the BWs on days 1-21 were significantly different, they would be included in the model to analyze for the next period (days 21-42) by ANCOVA. A significant difference was considered when $P<0.05$.

Results

Growth performance and FCR: The effects of BG + MOS supplementation on growth performance and FCR are shown in Table 2. Although the initial BW was similar among the three groups, the pigs in group 3 had better BW on day 21 than the control group by 1.5 kg, whereas group 2 was heavier than the control by 1.0 kg. Both BG + MOS supplemented groups showed significantly better BW than the non-supplemented group on day 21 of experiment. In contrast, no significant difference in BW was shown among the three groups on day 42 of experiment. During the first period of trial (days 1-21), the pigs supplemented with BG + MOS showed significantly increased WG compared to the control pigs ($P<0.01$). Weight gain was 7.46, 7.20 and 6.10 kg in groups 3, 2 and 1, respectively. No significant difference in WG was observed between groups 2 and 3. During this period, groups 2 and 3 had noticeably increased ADFI (395 and 402 g/day, respectively) compared to group 1 (339 g/day; $P<0.05$). Higher ADFI resulted in significantly higher ADG in groups 2 and 3 (360 and 373 g/day, respectively) than in group 1 (305 g/day; $P<0.01$). However, there was no significant difference in WG, ADG and ADFI among the 3 groups on days 21-42 and during the overall experimental period. Although FCR was not significantly different among the groups during all periods, there was a trend that FCR was reduced in the pigs supplemented with BG + MOS compared to the control group over the entire period of experiment ($P=0.07$).

Bacterial population in feces: On the first day of experiment, there was no significant difference in total bacterial, total coliform bacteria, total *Lactobacilli* populations and *Lactobacillus* to Coliform ratio (L:C) among the 3 groups (Table 3). On experimental day 21, the pigs supplemented with BG + MOS 2 kg/ton of feed had a significant reduction in total bacterial population, almost 10 times, compared to the control group (6.66 $\log_{10}$ and 7.52 $\log_{10}$ cfu/g feces, respectively; $P<0.05$), while the total bacterial

population in the pigs fed BG + MOS 1 kg/ton of feed was 7.24 log_{10} cfu/g, which was also lower than that of the control group, but not significantly different. Similarly, the total coliform bacterial population was reduced in the feces of pigs fed BG + MOS 2 kg/ton of feed in comparison with that of the control pigs (4.53 log_{10} and 5.89 log_{10} cfu/g, respectively; P<0.05). In contrast, groups 2 and 3 had increased numbers of total fecal *Lactobacillus* (7.93 log_{10} and 7.79 log_{10} cfu/g, respectively) compared to the non-supplemented group (6.78 log_{10} cfu/g: P<0.05). The L:C ratio was significantly lower in the pigs fed control diet (1.15) than those in the pigs supplemented with BG + MOS 1 and 2 kg/ton of feed (1.52 and 1.72, respectively). On day 42 of the experiment, the pigs in group 2 had significant reduction in total bacteria (7.11 log_{10} cfu/g; P<0.05) and total coliform (4.06 log_{10} cfu/g; P<0.01) in feces compared to the pigs in the control group (7.96 log_{10} and 5.37 log_{10} cfu/g, respectively). The pigs in group 3 showed significant reduction in the total coliform number (4.37 log_{10} cfu/g), but not the total bacteria number in feces compared to the control group. There was no significant difference in total bacterial and total coliform populations between groups 2 and 3. Although the BG + MOS supplementation did not significantly improve the total *Lactobacillus* population in feces, the L:C ratio appeared to increase in the feces of both BG + MOS supplemented pig groups compared to the control group (P<0.01).

Table 2 Effect of BG + MOS supplementation on growth performance and FCR (n=8 replicates per group)

Items	Groups[1]			SEM	P-value
	1	2	3		
Number of pig day 1	(96 pigs)	(96 pigs)	(96 pigs)		
Initial BW, kg	7.08	7.02	7.21	0.11	0.7913
BW on day 21, kg	13.17[a]	14.22[ab]	14.67[b]	0.25	0.0317
BW on day 42, kg	23.25	23.70	23.64	0.36	0.8694
Days 1-21	(92 pigs)	(94 pigs)	(93 pigs)		
WG, kg	6.10[a]	7.20[b]	7.46[b]	0.21	0.0096
ADFI, g/day	339[a]	395[b]	402[b]	10.05	0.0122
ADG, g/day	305[a]	360[b]	373[b]	10.31	0.0097
FCR	1.111	1.097	1.078	0.01	0.4220
Days 21-42	(88 pigs)	(93 pigs)	(89 pigs)		
WG, kg	10.08	9.48	8.97	0.32	0.3742
ADFI, g/day	717	672	610	22.02	0.1340
ADG, g/day	504	474	449	15.83	0.3777
FCR	1.423	1.418	1.359	0.02	0.6568
Days 1-42					
WG (kg)	16.18	16.68	16.43	0.33	0.8367
ADFI, g/day	528	533	506	11.39	0.5981
ADG, g/day	405	417	411	8.26	0.8390
FCR	1.304	1.278	1.231	0.01	0.0733

[1]Group 1 = Pigs fed a commercial basal diet, Groups 2 and 3 = Pigs fed the same basal diet supplemented with BG + MOS 1 and 2 kg/ton of feed, respectively. Culling rates were 4.17% in group 1 (4/96 pigs), 2.08% in group 2 (2/96 pigs) and 3.13% in group 3 (3/96 pigs) on days 1-21; were 4.35% in group 1 (4/92 pigs), 1.06% in group 2 (1/94 pigs) and 4.30% in group 3 (4/93 pigs) on days 21-42.
[a,b] Within a row, means without a common superscript differ (P<0.05).

Table 3 Effect of BG + MOS supplementation on bacterial population in feces (Unit: log_{10} cfu/g; n=8 pigs per group)

	Groups[1]			SEM	P-value
	1	2	3		
Day 1					
Total bacterial count	8.43	7.96	8.50	0.20	0.4938
Total coliform count	7.63	6.34	8.05	0.35	0.1217
Lactobacillus count	5.48	5.89	6.39	0.16	0.0683
L:C ratio	0.72	0.92	0.79	0.04	0.0703
Day 21					
Total bacterial count	7.52[b]	7.24[ab]	6.66[a]	0.15	0.0475
Total coliform count	5.89[b]	5.22[ab]	4.53[a]	0.22	0.0291
Lactobacillus count	6.78[a]	7.93[b]	7.79[b]	0.21	0.0476
L:C ratio	1.15[a]	1.52[b]	1.72[b]	0.08	0.0054
Day 42					
Total bacterial count	7.96[b]	7.11[a]	7.66[ab]	0.13	0.0222
Total coliform count	5.37[b]	4.06[a]	4.37[a]	0.19	0.0063
Lactobacillus count	7.47	7.83	8.25	0.18	0.2011
L:C ratio	1.40[a]	1.93[b]	1.89[b]	0.09	0.0048

[1]Group 1 = Pigs fed a commercial basal diet, Groups 2 and 3 = Pigs fed the same basal diet supplemented with BG + MOS 1 and 2 kg/ton of feed, respectively
L:C = *Lactobacillus* to Coliform ratio
[a,b] Within a row, means without a common superscript differ (P<0.05).

In Figure 1, the total bacterial population in feces of groups 1 and 3 was greater on the first day, reduced on day 21 and then increased again on day 42 of experiment (P<0.05 and P<0.001, respectively).

There was no significant difference in the total bacteria in feces of the pigs in group 2 at different time points. Figure 2 shows the changes of total coliform count (log_{10} cfu/g feces) of the experimental pigs at 3 different times. The number of coliform continuously reduced, was highest on the first day, reduced on day 21 and was lowest on day 42 of experiment. There were significant differences between the coliform number in feces of the pigs in groups 1 and 3 on the first day compared to those on days 21 and 42 (P<0.01 and P<0.001). There was no significant difference between the fecal coliform number on days 21 and 42 in groups 1 and 3. For group 2, the coliform number on the last day was significantly reduced compared to the number on the first day and day 21 of experiment (P<0.05). The *Lactobacillus* population was greater on days 21 and 42 compared to the first day in all groups (P<0.05 for group 1, P<0.001 for groups 2 and 3). No significant difference in the *Lactobacillus* population was observed on days 21 and 42 in all groups (Fig. 3). The effect of BG + MOS supplementation on L:C ratio in feces of the experimental pigs at different time points is illustrated in Figure 4. The L:C ratios on day 21 were remarkably enhanced, compared to those on the first day of trial in all groups (P<0.001). The percentages of L:C ratio increase were 60, 65 and 118% for groups 1, 2 and 3, respectively (data not shown). The L:C ratios numerically increased on day 42 in all groups, however only that of the pigs in group 2 significantly differed, compared to those on day 21.

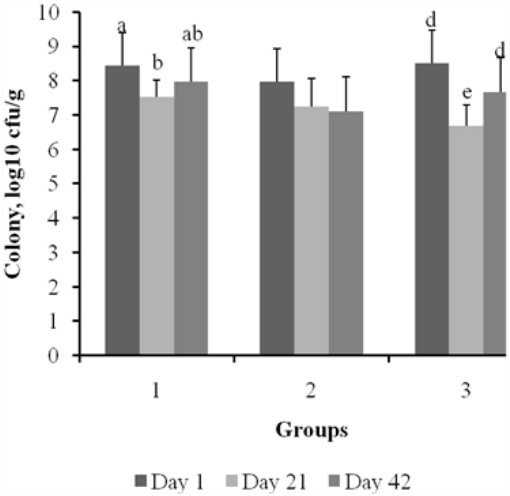

Figure 1 Effect of BG + MOS supplementation on total bacterial population in feces at different time points, log_{10} cfu/g. Group 1 = Pigs fed a commercial basal diet. Groups 2 and 3 = Pigs fed the same basal diet supplemented with BG + MOS 1 and 2 kg/ton of feed, respectively. [a,b] Means in the same group with different superscript letters are statistically significantly different (P<0.05). [d,e] Means in the same group with different superscript letters are statistically significantly different (P<0.001).

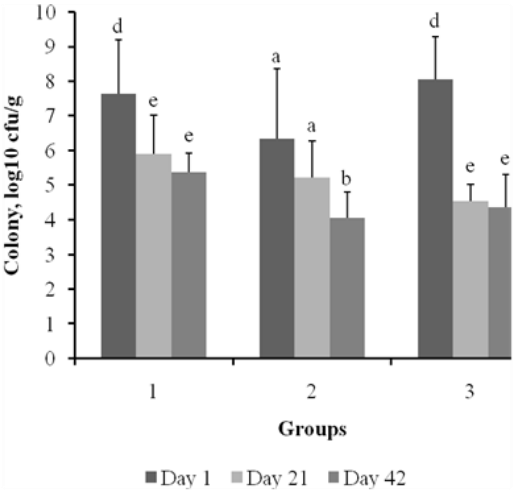

Figure 2 Effect of BG + MOS supplementation on total coliform population in feces at different time points, log_{10} cfu/g. Group 1 = Pigs fed a commercial basal diet. Groups 2 and 3 = Pigs fed the same basal diet supplemented with BG + MOS 1 and 2 kg/ton of feed, respectively. [a,b] Means in the same group with different superscript letters are statistically significantly different (P<0.05). [d,e] Means in the same group with different superscript letters are statistically significantly different (P<0.01).

Diarrhea incidence (DI): Diarrhea was found from the second day after weaning and appeared to reach its peak during the first week of experiment. During the first 3 weeks, the pigs supplemented with BG + MOS showed significantly reduced DI (10.96 and 11.83% for groups 2 and 3 respectively) compared to the pigs without supplementation (15.62%; P<0.05). DI was not different among the groups during the last 3 weeks of trial (2.55, 1.59 and 2.37% for groups 1, 2 and 3, respectively). Throughout the experiment, the BG + MOS supplementation of 1 kg/ton of feed significantly lowered DI (6.32%) compared to the control diet (9.26%; P<0.05). However, no significant difference in DI between the pigs supplemented with BG + MOS 1 and 2 kg/ton of feed was observed (6.32 and 7.27%; Table 4).

Serum IL-1, IL-6 and TNF-α: The IL-1 concentrations in pig sera were similar among the groups at the same time point and remained similar in the same groups at different time points (Table 5). On the first day of experiment, the IL-1 levels in serum of groups 1, 2 and 3 were 18.73, 18.43 and 14.0 pg/ml, respectively. These amounts increased up to 45.16, 36.34 and 28.51 on day 21. The concentrations of serum IL-1 were 25.82, 29.91 and 32.76 pg/ml on day 42.

Similarly, the concentrations of IL-6 in the serum of piglets did not dramatically change among the groups at all time points (Table 6). The levels of serum IL-6 were 24.02, 28.78 and 26.76 pg/ml for groups 1, 2 and 3, respectively, on the first day of experiment. The concentrations of IL-6 in the serum of pigs in groups 1, 2 and 3 were maintained at 28.86, 18.21 and 14.03 pg/ml on day 21; and 15.21, 18.47 and 22.27 pg/ml on day 42 of the experiment, respectively. Within the same group, the concentrations of IL-6 were not statistically different at different time points.

The effect of BG + MOS supplementation on TNF-α levels in the serum of weaned pigs is shown in Table 7. With similar extent to the serum IL-6

concentration, the TNF-α levels in serum of all pig groups were not significantly different. No significant effect of BG + MOS supplementation on serum TNF-α concentration in the same group at different time points was observed.

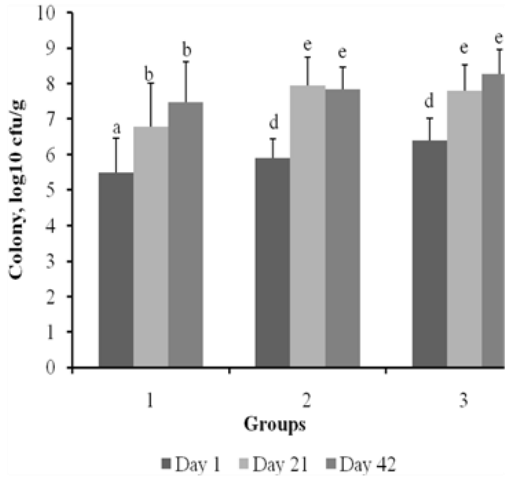

Figure 3 Effect of BG + MOS supplementation on total *Lactobacillus* population in feces of weaned pigs at different time points, log_{10} cfu/g. Group 1 = Pigs fed a commercial basal diet. Groups 2 and 3 = Pigs fed the same basal diet supplemented with BG + MOS 1 and 2 kg/ton of feed, respectively. [a,b] Means in the same group with different superscript letters are statistically significantly different (P<0.05). [d,e] Means in the same group with different superscript letters are statistically significantly different (P<0.001).

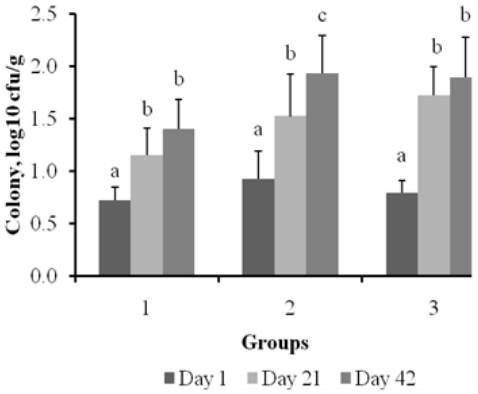

Figure 4 Effect of BG + MOS supplementation on L:C ratio in feces at different time points, log_{10} cfu/g. Group 1 = Pigs fed a commercial basal diet. Groups 2 and 3 = Pigs fed the same basal diet supplemented with BG + MOS 1 and 2 kg/ton of feed, respectively. [a-c] Means in the same group with different superscript letters are statistically significantly different (P<0.001).

Serum antibody against PRRS: The effects of time and BG + MOS supplementation on antibody titer against PRRS in the serum of experimental pigs are shown in Table 8. The antibody titers against PRRS were similar among the groups on days 1 and 21 of the experiment. Noticeably, on day 42, the pigs supplemented with BG + MOS 2 kg/ton of feed had higher antibody titer (1.38±0.04) compared to the pigs fed BG + MOS 1 kg/ton of feed and the pigs fed control diet (1.04±0.12 and 1.07±0.13, respectively; P=0.06).

There was no significant difference between the antibody titer against PRRS in the serum of pigs fed BG + MOS 1 kg/ton of feed and pigs fed control diet in all periods of the experiment. The serum antibody titers of the same group were similar at different time points for the pigs in groups 1 and 2. On the other hand, the antibody titer against PRRS in the serum of pigs in group 3 showed changes at different time points with an intermediate value on the first day, the lowest value on day 21 and the highest value on day 42 (P<0.01).

Table 4 Effect of BG + MOS supplementation on diarrhea incidence (n=8 replicates per group)

DI, %	Groups[1]			SEM	P-value
	1	2	3		
Days 1-21	15.62[b]	10.96[a]	11.83[a]	0.76	0.0221
Days 21-42	2.55	1.59	2.37	0.34	0.4823
Days 1-42	9.26[b]	6.32[a]	7.27[ab]	0.49	0.0372

[1]Group 1 = Pigs fed a commercial basal diet, Groups 2 and 3 = Pigs fed the same basal diet supplemented with BG + MOS 1 and 2 kg/ton of feed, respectively
DI = Diarrhea incidence
[a,b] Within a row, means without a common superscript differ (P<0.05).

Table 5 Effect of BG + MOS supplementation on IL-1 levels in serum at different time points, pg/ml (n=7 per group)

Day	Groups[1]			SEM	P-Value
	1	2	3		
Day 1	18.73	18.43	14.08	1.78	0.4809
Day 21	45.16	36.34	28.51	3.75	0.1982
Day 42	25.82	29.91	32.76	4.82	0.8531
P-Value	0.0864	0.1591	0.1615		

[1]Group 1 = Pigs fed a commercial basal diet, Groups 2 and 3 = Pigs fed the same basal diet supplemented with BG + MOS 1 and 2 kg/ton of feed, respectively

Table 6 Effect of BG + MOS supplementation on IL-6 levels in serum at different time points, pg/ml (n=7 replicates per group)

Day	Groups[1]			SEM	P-Value
	1	2	3		
Day 1	24.02	28.78	26.76	4.60	0.3384
Day 21	28.86	18.21	14.03	3.50	0.0955
Day 42	15.21	18.47	22.27	1.93	0.3423
P-Value	0.2999	0.5114	0.2844		

[1]Group 1 = Pigs fed a commercial basal diet, Groups 2 and 3 = Pigs fed the same basal diet supplemented with BG + MOS 1 and 2 kg/ton of feed, respectively

Table 7 Effect of BG + MOS supplementation on TNF-α levels in serum at different time points, pg/ml (n=7 replicates per group)

Day	Groups[1]			SEM	P-Value
	1	2	3		
Day 1	81.98	85.13	79.04	5.24	0.9029
Day 21	135.6	120.34	119.13	8.91	0.7201
Day 42	92.34	96.05	105.00	5.54	0.6545
P-Value	0.1159	0.0563	0.1055		

[1]Group 1 = Pigs fed a commercial basal diet, Groups 2 and 3 = Pigs fed the same basal diet supplemented with BG + MOS 1 and 2 kg/ton of feed, respectively

Table 8 Effect of BG + MOS supplementation on antibody against PRRS at different time points (n=7 replicates per group)

Day	Groups[1]			SEM	P-Value
	1	2	3		
Day 1	0.85	1.09	0.99^{xy}	0.11	0.6390
Day 21	0.80	0.65	0.48^{x}	0.10	0.4680
Day 42	1.07^{a}	1.04^{a}	1.38^{by}	0.06	0.0601
P-Value	0.2294	0.1533	0.0063		

[1]Group 1 = Pigs fed a commercial basal diet, Groups 2 and 3 = Pigs fed the same basal diet supplemented with BG + MOS 1 and 2 kg/ton of feed, respectively

[a,b] Within a row, means without a common superscript differ .

[x, y] Within a column, means without a common superscript differ (P<0.01).

Discussion

The BG + MOS supplementation had positive effects on the performance of weaned pigs, especially during the first period of experiment. The combined product improved ADFI, leading to better ADG by 16.5-18.5%. Due to the higher average growth rate, groups 2 and 3 also had better WG and appeared heavier on day 21 after weaning compared to the control group. However, no statistical difference in growth performance was observed during the second half of the experiment and the overall period.

In the present study, although the BG + MOS supplementation was not able to significantly generate statistical difference in FCR, there was the trend that FCR was the greatest in the control pigs, lower in the pigs fed BG + MOS 1 kg/ton of feed, and the lowest in the pigs fed BG + MOS 2 kg/ton of feed throughout the experiment. Based on the current results, the beneficial effect of BG + MOS supplementation seems to relate to increase in feed consumption rather than improvement in feed efficiency. Previous studies suggested that healthy intestinal ecology might promote positive effect on voluntary feed intake of animals due to reduction in disorder stress (Pluske et al., 1997; Liu et al., 2008). The higher ADFI in BG + MOS supplemented pigs, the better gut environment observable. In the present study, ADFI of the experimental pigs seemed lower than normal expectation. The most probable explanation is that the crude fat level in the diets (average = 10.52%) was higher than normal level, which leads to concentrated energy in the feed and reduces volunteer feed intake.

In addition, circulation of PRRS virus in the herd during the last two weeks of this experiment might affect the growth performance parameters during the second period. Although no significant difference was observed in growth performance on days 21-42 of the trial and the feed intake was lower than expected level, there was no significant improvement in FCR. However, the increase in ADFI, ADG, WG and BW likely affected the rearing time to reach market weight. From an economical aspect, the numerical reduction in FCR in this study may result in significant advantage in industrial model of animal production.

In the current study, the reduction in total coliform and increase in *Lactobacillus* count in feces of the supplemented pigs could be related to the positive effects of MOS as prebiotic supplementation. In a microbiological point of view, this finding is similar to previous studies. Pigs fed 0.2% MOS had a decline of *enterobacteria* population in jejunal digesta during 14 days after weaning (Castillo et al., 2008). The outcomes of this study supported an earlier report by White and colleagues in 2002 in which weanling pigs fed 0.16% MOS (Brewer yeast product) had increased fecal *Lactobacilli* number on day 28 and numerically reduced total coliform count on days 14 and 28 post weaning. Total coliform count in jejunum and cecum, and feces 10 days after being challenged with pathogenic *Escherichia coli (E. coli)* K88 strain also decreased in the supplemented group compared to control group

(White et al., 2002). However, the finding of the present study was inconsistent with other previous studies which reported that MOS supplementation did not have significantly beneficial effects on manipulation of intestinal microflora (Mathew et al., 1998; Biggs et al., 2007; van der Peet-Schwering et al., 2007). The controversial outcomes may result from different dosages, product purities, given durations and periods of MOS supplementation. An interesting difference among the controversial studies is the dosages of MOS. Although the dosages of MOS used in this study were lower (0.02-0.025 and 0.04-0.05% for groups 2 and group 3, respectively), when compared to the effective dosages from previous reviews, the positive effects remained significantly noticeable. In another study in broilers, BG + MOS derived in form of yeast with similar supplementation levels to those in our study also resulted in better growth performance (Reisinger et al., 2012). A possible explanation for that may relate to the effect of combination of β-glucans and MOS in one product. β-glucans have been considered as potential non-starch polysaccharides which are likely to increase the number of *Lactobacillus*, *Bifidobacteria* (Pieper et al., 2008; O'Shea et al., 2010) and reduce the number of *E. coli* in feces in weaned pigs (Zhou et al., 2013).

Change in microbial population with reduced beneficial bacteria and increased pathogenic bacteria number was suggested as potential risk of diarrhea incidence (Mathew et al., 1998). In the current study, the pigs fed diet containing BG + MOS had dramatically reduced total coliform population and increased *Lactobacillus* number in feces compared to those without supplementation. Similar findings in previous studies suggested that L:C ratio might be an indicator of pig gut health (Mourão et al., 2006; Muralidhara et al., 1977). In the present study, the BG + MOS supplementation increased the L:C ratio in feces of the supplemented pigs on days 21 and 42 compared to those without supplementation. A lower diarrhea incidence was, therefore, found in the supplemented pigs, particularly in the first period of post-weaning. According to a review by Metzler and colleagues (2005), diarrhea was most severe during the first week after weaning. This reason may explain why the beneficial effects of BG + MOS were clearly shown in the first period, but not in the second period in this experiment.

β-glucans have been demonstrated to induce secretion of pro-inflammatory cytokines including IL-1, IL-6 and TNF-α. An *in vitro* study showed the ability of β-glucans in inducing the expression of IL-1β and IL-6 mRNA and TNF-α from macrophages (Ljungman et al., 1998). β-glucans elevated plasma IL-1 after 12, 24 hours, 3, 5, 9 and 12 days in rats (Sherwood et al., 1987). However, the effect of β-glucan supplementation on cytokine response remains controversial. A previous report found that β-glucan supplementation enhanced IL-1β/ IL-1Ra and TNF-α mRNA expression in lung and spleen tissues after being exposed to *E. coli* Lipopolysaccharides (LPS) (Eicher et al., 2006), whereas other reports found that β-glucan supplementation caused a reduction in serum IL-6 and TNF-α concentrations in pigs after being challenged with LPS both *in vitro* and *in vivo* models (Li et at., 2005; Li et al., 2006). Under the present study's conditions, no significant effect of the BG + MOS supplementation on serum IL-1, IL-6 and TNF-α was shown. The contrary findings may result from various experimental conditions, different administration methods and target samples in which cytokines were detected. In earlier studies, experiment was conducted *in vitro* by cell culture technique (Ljungman et al., 1998), or β-glucans were injected intraperitoneally to the animals (Sherwood et al., 1987), which may lead to different responses of the animals, compared to the oral administration in the present study. In addition, IL-1 mRNA measured from other tissues may cause various levels of IL-1 concentrations in the serum.

The report of Li et al. (2005) suggested that β-glucan supplementation to weaned pigs at 50 ppm led to increased production of anti-inflammatory cytokines (IL-10) or secretion of pro-inflammatory cytokine receptor antagonists (IL-1Ra). Both cytokines appeared to inhibit the synthesis of pro-inflammatory cytokines, or availability of their receptors. However, higher doses of β-glucan supplementation possibly promoted the secretion of pro-inflammatory cytokines such as IL-1, IL-6 and TNF-α (Li et al., 2006). Furthermore, the health status of pig may be involved in the effects of β-glucans on the immune responses. In addition, the levels of IL-1, IL-6 and TNF-α detected in the present study appeared to be lower than the expected levels, and did not significantly differ among the groups. The dissimilarity in study conditions may result in different outcomes among studies.

PRRS has been considered as a common viral disease causing a huge economic loss in pig population worldwide. Although specific treatment is not possible for PRRS control and prevention (Dietze et al., 2011), vaccination is considered as an option for disease control and establishes stability of herd immunity. However, vaccination alone is not able to provide satisfaction of PRRS control and prevention under filed conditions, and various outcomes are often seen. Alternatively, β-glucan supplementation was shown to affect increase in antiviral function, cell-mediated or antibody responses in pigs (Jung et al., 2004; Li et al., 2005; Shen et al., 2009; Zhou et al, 2013). In disagreement, other reports could not demonstrate any effects of BG + MOS supplementation on antibody against PRRS or influenza virus in pigs and mice (Hiss and Sauerwein, 2003; Amornlertviman et al., 2008; Hester et al., 2012; Pence et al., 2012). However, BG was supplemented at lower dosages, 0.015 and 0.03% in the study of Hiss and Sauerwein (2003) and 0.03% in the study of Amornlertviman et al. (2008), compared to the higher dosages in present study (0.05-0.06%). In the present study, the pigs supplemented with BG + MOS 2 kg/ton of feed showed increased antibody response to PRRS in the serum compared to the pigs in other groups on day 42. However, the increase in antibody titer in the experimental pigs on day 42 might relate to the circulation of PRRS virus during the study period. The outcome revealed the potential effect of BG + MOS supplementation on modulation of the immune function against PRRS in recently weaned pigs. The pigs supplemented with BG + MOS seemed to have lower antibody titer against PRRS on day 21 of the experiment. At that time, only 23.8% of the pigs

showed positive response to PRRS ELISA test (5 of 21 pigs, data not shown). This might be the remaining maternal derived PRRS antibody in the herd. However, all pigs became positive to PRRS ELISA test by day 42. The results of the current study may support the suggestion of Li et al. (2005) that β-glucans may have potential property of immune-modulator which can prevent overproduction of pro-inflammatory cytokines in the case of low infection stress and promote adaptive immune response when the amount of infectious pathogens is enough to induce immune responses of the animals. To completely achieve the conclusion, further investigation should be conducted to confirm the effects of BG + MOS supplementation on specific immune responses in weaned pigs.

The results of this study showed that the BG + MOS supplementation in feed was able to improve the growth performance of weaned pigs during 3 weeks after weaning. In addition, the BG + MOS supplementation potentially promoted increase in the number of *Lactobacillus*, reduction in the total bacteria and total coliform populations, and increase in the L:C ratio in GIT. Obviously, DI was also significantly lowered in the pigs supplemented with BG + MOS. However, the two different supplementation dosages did not offer significant effect on the growth performance, bacterial population in feces and diarrhea incidence.

In conclusion, the findings of present study suggest that BG + MOS supplementation in form of yeast cell wall product in pig feed has beneficial effects on growth performance during the first 3 weeks after weaning by balancing bacterial population in feces, reducing diarrhea and slightly improving immune responses of weaned pigs.

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บทคัดย่อ

ผลของการเสริมเบต้ากลูแคนและแมนแนนโอลิโกแซคคาไรด์ในอาหารต่อสมรรถภาพการเจริญเติบโต ประชากรแบคทีเรียในอุจจาระ และภูมิตอบสนองของสุกรหย่านม

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ศึกษาผลของการเสริมเบต้ากลูแคนและแมนแนนโอลิโกแซคคาไรด์ในอาหารต่อสมรรถภาพการเจริญเติบโต ประชากรแบคทีเรียในอุจจาระ และภูมิตอบสนองของสุกรหย่านม ทำการทดลองในลูกสุกรหย่านมจำนวน 288 ตัว โดยสุ่มแยกเป็น 3 กลุ่ม ๆ ละ 8 ซ้ำ ๆ ละ 12 ตัว กลุ่มที่ 1 ซึ่งเป็นกลุ่มควบคุม เลี้ยงด้วยอาหารอนุบาลสำเร็จรูป กลุ่มที่ 2 และ 3 เลี้ยงด้วยอาหารอนุบาลสำเร็จรูปผสมเบต้ากลูแคนและแมนแนนโอลิโกแซคคาไรด์ (ไบโอเล็ค เอ็มบี-40) ขนาด 1 และ 2 กิโลกรัมต่อตันอาหาร ตามลำดับ คำนวณน้ำหนักตัวที่เพิ่มขึ้น น้ำหนักตัวที่ได้ต่อวัน ปริมาณอาหารที่กินได้ต่อวัน และอัตราการแลกเนื้อ ณ วันที่ 1, 21 และ 42 ของการทดลอง วิเคราะห์หาความเข้มข้นของอินเตอร์ลิวคิน 1, 6 และทูเมอร์เนคโครติกแฟกเตอร์ แอลฟ่าในตัวอย่างเลือด ตรวจนับปริมาณเชื้อแบคทีเรียทั้งหมด ปริมาณเชื้อโคลัยฟอร์มและปริมาณเชื้อแลคโตบาซิลลัสในอุจจาระ รวมถึงอัตราส่วนของเชื้อแลคโตบาซิลลัสต่อเชื้อโคลัยฟอร์มในอุจจาระ การทดลองพบว่าสุกรกลุ่มที่ 2 และ 3 มีน้ำหนักตัวที่เพิ่มขึ้น น้ำหนักตัวที่ได้ต่อวัน และปริมาณอาหารที่กินได้ต่อวันในช่วงวันที่ 1-21 ดีกว่ากลุ่มควบคุม แต่ไม่พบความแตกต่างอย่างมีนัยสำคัญทางสถิติในช่วงวันที่ 21-42 และ 1-42 ไม่พบความแตกต่างของอัตราการแลกเนื้อในแต่ละกลุ่มตลอดช่วงการทดลอง ทั้งสองกลุ่มทดลองมีปริมาณเชื้อแบคทีเรียทั้งหมดและปริมาณเชื้อโคลัยฟอร์มในอุจจาระลดลง ในขณะที่ปริมาณเชื้อแลคโตบาซิลลัสและอัตราส่วนของเชื้อแลคโตบาซิลลัสต่อเชื้อโคลัยฟอร์มเพิ่มขึ้น พบการลดลงของอุบัติการณ์ท้องเสียในช่วง 3 สัปดาห์แรกของการทดลอง สุกรกลุ่มที่ 3 มีภูมิตอบสนองต่อเชื้อพีอาร์อาร์เอสสูงกว่ากลุ่มอื่นในวันที่ 42 ของการทดลอง ไม่พบความแตกต่างอย่างมีนัยสำคัญทางสถิติของอินเตอร์ลิวคิน 1, 6 และทูเมอร์เนคโครติกแฟกเตอร์ แอลฟ่าในตัวอย่างเลือดของสุกรแต่ละกลุ่มในช่วงทดลอง จากผลการทดลองสรุปได้ว่าการเสริมเบต้ากลูแคนและแมนแนนโอลิโกแซคคาไรด์ในอาหารมีแนวโน้มดีต่อสมรรถภาพการเจริญเติบโตในช่วงวันที่ 1-21 การปรับสมดุลของประชากรแบคทีเรียในอุจจาระ และการลดอุบัติการณ์ท้องเสียในสุกรช่วงหย่านม

คำสำคัญ: เบต้ากลูแคน ประชากรแบคทีเรียในอุจจาระ สมรรถภาพการเจริญเติบโต การตอบสนองทางภูมิคุ้มโรค แมนแนนโอลิโกแซคคาไรด์ สุกร

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