Comparative Effects of Sodium Gluconate, Mannan Oligosaccharide and Potassium Diformate on Growth Performances and Small Intestinal Morphology of Nursery Pigs

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ABSTRACT : This study was conducted to compare the effects of dietary supplementation of Sodium Gluconate (SG), Mannan Oligosaccharide (MOS) and Potassium Diformate (PDF) on growth performance and small intestinal morphology in nursery piglets. One hundred forty four female piglets (11.69 ± 0.71 kg) were divided into 4 treatments with six replicates of six pigs each. The pigs received a control diet or diets supplemented with SG, MOS and PDF at 2,500, 3,000 and 8,000 ppm; respectively, for 6 weeks. Supplementation of SG, MOS or PDF increased final body weight, average daily gain and tended to improve feed to gain ratio ($p = 0.02$, 0.04 and 0.16; respectively), other than average daily feed intake, intestinal pH and the bacterial populations were not influenced by the dietary treatments. SG significantly decreased the ammonia concentration in the caecum ($p<0.05$) and supplementation of SG, MOS or PDF tended to increase lactic acid and total short chain fatty acid concentration in the caecum ($p = 0.08$, 0.09; respectively), in addition SG, MOS or PDF slightly increased butyric acid concentration in the caecum ($p = 0.14$). SG highly significant increased the villous height in jejunum ($p<0.01$) and supplementing SG, MOS or PDF significantly increased crypt depth in jejunum ($p<0.05$), moreover, PDF significantly increased villous height and crypt depth ratio in jejunum ($p<0.05$) compared with control. The dietary treatments did not influence villous height and crypt depth in duodenum and villous height in jejunum ($p>0.05$). It can be concluded that supplementing SG, MOS or PDF as a feed additive has the potential to improve the growth performance, the intestinal lactic acid bacteria population, intestinal short-chain fatty acid concentration and the intestinal morphology of pigs. (**Key Words :** Sodium Gluconate, Mannan Oligosaccharide, Potassium Diformate, Growth Performance, Intestinal Morphology, Pigs)

INTRODUCTION

In the recent years, there has been a considerable interest in using feed additive as an alternative to the antibiotics as growth promoter in feeds. Especially, in the modern or intensive farming system, adding feed additives can improve the production of pigs. Because of, during the post weaning period (4-5 weeks of age), piglets are usually susceptible to pathogenic microorganism by various factors. Therefore, losses of piglet as a result of diarrhea are often seen. Using antibiotics clearly prevents diarrhea during post weaning period. However, these are being banned around the world, and particularly European Union has completely banned use of antibiotics as growth promoters since January 2006. Consequently, alternative feed additives which can be substituted for the antibiotics are intensively focused.

Using feed additive is one of the strategies on feed management; each feed additive has mode of action to promote growth. Supplementing prebiotic to animal feed enhanced growth performance and improved feed utilization by promoting a range of beneficial bacteria and inhibiting pathogenic bacteria (Fuller and Perdigon, 2003). For example, supplementation of prebiotic such Mannan Oligosaccharides (MOS) (derives from cell wall of a specific strain of yeast) or Sodium Gluconate (SG) (derives from uncompleted glucose fermentation) promoted growth performances of nursery pigs by increasing intestinal beneficial bacteria population and intestinal morphology (Davis et al., 2002; Fuller and Perdigon, 2003; Poeikhampha et al., 2007). Organic acids supplementation such Potassium Diformate (PDF) directly disrupted the growth of the pathogenic bacteria, consequently reducing the frequency of post weaning diarrhea and improving growth performance in piglets (Knarreborg et al., 2002; Mroz et al., 2002). Unfortunately, only few trials have been conducted to compare these alternatives under the same

conditions. Therefore, this study was conducted to evaluate the effects of SG, MOS and PDF on growth performances, intestinal microorganisms and intestinal morphology of pigs.

MATERIALS AND METHODS

This study was conducted at Animal Research Farm, Department of Animal Science, Faculty of Agriculture, Kasetsart University and the Faculty of Veterinary Science, Mahidol University, Thailand, and experimental animals were kept, maintained and treated in adherence to accepted standards for the humane treatment of animals.

Animals and managements

One hundred and forty four female commercial crossbred piglets (Duroc×Large White×Landrace; 11.69± 0.71 kg body weight) were used in this trial. The pigs were divided into 4 treatments and each treatment consisted of six pens (six pigs/pen). The piglets were raised in naturally ventilated houses consisting of 24 pens (3×2.5 m^2), and each pen was assigned a crib and two of water nipples. During the feeding trial, the piglets were bathed and the house was cleaned two days interval, while the feces of piglets were removed every day.

Experimental design and diets

The Completely Randomized Design (CRD) was used as the experimental design. Four experimental diets were provided to pigs for 6 weeks as follows; i) basal diet (control), ii) basal diet+2,500 ppm of SG, iii) basal diet+ 3,000 ppm of MOS and iv) basal diet+8,000 ppm of PDF. The basal diets were formulated to provide the same amount of nutrients and met the requirement as commercial recommendation without antimicrobial agent (Table 1). Feed and water were provided *ad libitum*. Body weight and feed intake were recorded two weeks interval.

Parameters

Growth performances : The initial body weight of each piglet was recorded and at the end of feeding trial (6 weeks) the body weight, body weight gain and feed intake were recoded two weeks interval in order to calculation of average daily gain, average daily feed intake and feed to gain ratio and the morbidity and mortality of piglets were observed.

Preparation of bacterial counts, measurement of gastrointestinal pH, intestinal morphology and analysis of the digestal content : At the end of the feeding trial, 6 pigs per treatment were putdown. The pH in the stomach, duodenum, jejunum, ileum and caecum were directly measured by a pH meter (IQ Scientific Instruments, Carlsbad, CA, USA) and middle section of the duodenum and jejunum tissue were collected and used for

Table 1. Composition of the basal diet and chemical analysis

Ingredient (%)	Amount
Broken rice	42.50
Corn	5.08
Full fat soybean	28.00
Soybean meal (CP 48%)	4.00
Rice bran	10.00
Fish meal (CP 60%)	2.00
Skimmed milk	5.00
L-lysine	0.30
DL-methionine	0.10
L-threonine	0.10
Monodicalciumphosphate (P 21%)	1.20
Calcium carbonate	0.90
Salt	0.32
Premix	0.50
Total	100.00
Chemical composition	
Swine ME (Kcal/kg)	3,350.00
Crude protein	20.00 (20.51*)
Calcium	1.00 (1.10*)
Available phosphorus	0.50
Lysine	1.35
Methionine	0.50
Threonine	0.85

Premix content; Vitamin A 4 MIU, D 0.64 MIU, E 24,000 IU, K$_3$ 1.4 g, B$_1$ 0.6 g, B$_2$ 0.3 g, B$_6$ 0.75 g, B$_{12}$ 14 mg, Nicotinic acid 20 g, Pantothenic acid 10 g, Folic acid 0.44 g, Biotin 0.04 g, Choline 60 g, Fe 45 g, Cu 40 g, Mn 15 g, Zn 40 g, Co 0.2 g, I 0.4 g, Se 0.06 g, Carrier Added to 1 kg.
* Chemical analysis.

measurement of intestinal morphology by light microscope in accordance with Nunez et al. (1996). The tissues were taken and immediately fixed in 10% neutral buffer formalin, and then carefully embedded in paraffin. For each specimen, at least 10 sections of 7 ml thickness were prepared. Tissues were then stained with haematoxylineosin for histological evaluation. The morphology of the small intestines in this study included villous height, crypt depth and the villous height to crypt depth ratio. The digesta of pigs were collected from the caecum and rectum. The samples of digesta were divided into 2 parts in order to count the bacterial population and determine lactic acid and SCFAs concentrations. The samples for bacterial counts were immediately pooled, and kept in a sealed plastic bag at 39°C. For the other part, the samples for analysis of SCFAs were mixed with 6 N HCl (ratio 5:1) to stop fermentation and stored at -20°C.

Measurement of intestinal morphology : The intestinal mucosa samples were embedded in paraffin; histological sections were obtained from tissue blocks, cut perpendicular to the mucosal surface, and stained with haematoxylin and eosin. Measurement of intestinal morphology was used by a computer-assisted (image-analysis system) (Biowizard,

Thaitec, Thailand). The microscopic of histological sections were randomized and assessed the height of 10 villi and the depth of 10 crypts in each sample.

Bacterial counts : Ten grams of sample was diluted with 90 ml of 1% peptone solution and homogenized by Stomacher (Stomacher Lab Blender 400, Seward Medical, West Sussex, United Kingdom); the peptone was kept in a sealed plastic bag which filled by CO_2. The bacterial population was determined by using serial 10-fold dilutions with 1% peptone solution onto the Sharpe (MRS) agar (DifcoTM; Becton and Dickinson, Argentina) for determinations of *Lactobacillus spp.* and Mac Conkey Agar (Laboratorios Britania, Mendoza, Argentina) for determinations of *Escherichia coil*. To determine population of microorganisms, Sharpe (MRS) agar plates were incubated under anaerobic conditions at 37.0°C for 24 h, while Mac Conkey agar plates was incubated under aerobic conditions for 24 h at 35.0 and the population of *Escherichia coil* in Mac Conkey Agar were indentified by the presence of pink-red colonies according to Yousef and Carlstrom (2003) and Delost (1997).

Analysis of SCFAs and lactic acid in the digesta : The samples of caecal digesta were centrifuged for 15 min at 14,000×*g* at 4°C and the supernatants were sampled to determine lactic acid concentration using the commercial kit (Megazyme International, Wicklow, Ireland) and the concentration of SCFAs (acetic acid, propionic acid and butyric acid) using gas chromatography; the supernatants were mixed with 6 N HCl (ratio 5:1) to stop fermentation and centrifuged for 15 min at 14,000×*g* at 4°C. Then supernatants were taken in order to determine the concentration of such SCFAs in the following manner; one micro liter of the supernatant was injected into the silica

capillary column (DB-Wax, J&W 30 m×0.25 mm i.d.). The GC-2010 High-end Gas Chromatograph (Shimadzu, Tokyo, Japan) was used. The GC oven was temperature-programmed from 50 to 220°C at a rate of 4°C/min. The carrier gas (He) flow rate was 1.0 ml/min. and a split ratio was 1:20. The temperatures of the injection port and detector were 225 and 250°C, respectively.

Statistical analysis

All data were statistically analyzed using analysis of variance (ANOVA) of SAS (SAS, 1988). The differences between the means of groups were separated by Duncan's New Multiple Range Test (Duncan, 1955) according to the following model;

$$Y_{ij} = \mu + A_i + \varepsilon_{ij}$$

Where; Y_{ij} is the observed response, A_i is the effect of diet and ε_{ij} is experimental error; $\varepsilon_{ij} \sim NID(0, \delta^2)$. Statements of statistical significance were based on $p < 0.05$. All statistical analyses were done in accordance with the method of Steel and Torrie (1980).

RESULTS AND DISCUSSION

Growth performances

The growth performances of animals are shown in Table 2 and 3. All animals remained in good health throughout the trial. The initial body weights of pigs were not significantly difference. At the end of feeding trial, supplementation of SG, MOS or PDF increase final body weight, average daily gain and improved feed to gain ratio (p = 0.02, 0.04 and

Table 2. Growth performance of piglets fed with diet supplementing sodium gluconate, mannan oligosaccharide or potassium diformate during nursery period

Item	Control	Sodium gluconate	Mannan oligosaccharide	Potassium diformate	p-value	SEM
Initial body weight (kg)	11.83	11.97	11.37	11.61	0.51	0.14
Final body weight (kg)	36.72[c]	38.36[a]	37.35[b]	37.53[b]	0.02	0.14
ADG (kg/d)	0.59[b]	0.63[a]	0.62[ab]	0.62[ab]	0.04	0.01
Average feed intake (kg)	1.03	1.04	1.03	1.03	0.82	0.01
Feed to gain ratio	1.75	1.65	1.66	1.67	0.16	0.01

Table 3. pH values in each gastro-intestinal segment of piglets fed with diet supplementing sodium gluconate, mannan oligosaccharide or potassium diformate during nursery period

Item	Control	Sodium gluconate	Mannan oligosaccharide	Potassium diformate	p-value	SEM
Stomach	4.00	3.96	3.86	3.63	0.81	0.13
Duodenum	5.42	4.93	4.98	4.73	0.36	0.13
Jejunum	6.21	5.89	6.30	6.07	0.38	0.09
Ilium	6.98	6.73	6.99	6.68	0.27	0.07
Caecum	6.11	6.10	5.99	6.02	0.41	0.03

0.16; respectively). Whereas, the dietary treatments did not influence the feed intake of pigs.

Improvement of growth rate by SG supplementation confirms positive effect of this prebiotic that has previously reported by Poeikhampha et al. (2007). Similarly, MOS and PDF also promoted growth rate without any effect on feed consumption (Davis et al., 2002; Taube et al., 2009). It can be assumed that supplementation of these feed additives may improve nutrients utilization (digestion and absorption), since FCR was trendy improved (p = 0.11). Although in this study, all feed additives tended to promote the growth performance of piglet (increase of growth rate and decrease of FCR), mode of action of each feed additive (SG, MOS or PDF) in digestive system may differ. Similarly, in this study pigs fed with MOS and PDF presented slightly better growth performance and the results were similar to Davis et al. (2002) and Sutton et al. (1991); reported that growth rate and FCR were improved by supplementation of MOS and PDF. Furthermore, using organic acids or their salts supplementation improved growth performance and depressed the growth of bacteria and mold which widely used as a natural preservative in food and feed (Knarreborg et al., 2002; Taube et al., 2009). The mechanism to promote growth performance of SG, MOS and PDF is probably focused on intestinal morphology and nutrients utilization, there are several investigator agree with this assumption, SG and PDF supplementation inducing growth of intestinal villi and increasing feed utilization of pigs has been reported by Poeikhampha et al. (2007) and Roth and Kirchgessner (1998) and dietary MOS supplementation increased jejunum villi height and the number of goblet cells per villus and improved feed conversion in birds has been reported by Baurhoo et al. (2007).

Intestinal microorganisms

Bacterial populations in the caecum and rectum digesta of pigs were shown in Table 4. the population of lactic acid bacteria contained and *E. coli* count in the caecum or rectum digesta were not affected by SG, MOS or PDF supplementation (p>0.05). In the commercial term of swine production, during nursery period is the critical stage because piglets are usually susceptible to pathogenic

microorganisms by various factors, thus losses of piglet as a result of diarrhea are often seen. Using SG, MOS or PDF as feed additive possible to control the population of intestinal microorganisms and promote the growth of beneficial bacteria such as lactic acid bacteria and may protect pigs from diarrhea. For SG, SG is poorly digested and absorbed in the small intestine, it is utilized by lactic acid bacteria such as *Lactobacillus spp.* and *Bifidobacterium spp.* (Asano et al., 1994), thus SG directly increase the population of intestinal lactic acid bacteria. One another prebiotic, MOS is classified as a prebiotic that 4,000 ppm of dietary MOS inhibits growth of intestinal pathogenic microorganisms through binding to cell walls of bacteria preventing the bacteria from attaching to intestinal epithelial cells (Spring et al., 2000). For acidifiers, organic acid can dissolved and entering the cell in the undissociated form and dissociating in the more alkaline cell interior causing acidification of the cytoplasm and inhibition of cell metabolism of pathogenic microorganism (Hunter and Segel, 1973; Lueck, 1980). Accordingly, Mroz et al. (2002) demonstrated that PDF supplementation predominantly depressed pathogenic bacteria population and enhanced growth performances. In this study, the dietary treatment did not influence the population of intestinal microorganisms. This may be due to under well managed conditions, the expression of treatment on intestinal microorganisms are limit.

Ammonia, short chain fatty acids and lactic acids concentration in caecum

The ammonia, lactic acids and SCFAs concentrations in the caecum of pigs were shown in Table 5. The ammonia concentration in caecum of pigs were significantly decreased by SG, MOS or PDF supplementation (p<0.05). Moreover, supplementation of SG, MOS or PDF tended to increase butyric acid and total SCFAs in the caecum of pigs (p = 0.14, 0.09) and the dietary treatments did not influence the concentration of lactic acid, acetic acid and propionic acid in the caecum of pigs.

Asano et al. (1994) reported that SG significantly increased SCFAs production by increases the population of lactic acid bacteria which produce lactic and acetic acids in the large intestinal tract. In agreement with Poeikhampha

Table 4. Bacterial population (log CFU/g digesta) in the ceacum and rectum digesta of piglets fed with diet supplementing sodium gluconate, mannan oligosaccharide or potassium diformate during nursery period

Item	Control	Sodium gluconate	Mannan oligosaccharide	Potassium diformate	p-value	SEM
Lactic acid bacteria						
Caecum	9.15	9.21	9.20	9.19	0.56	0.04
Rectum	9.13	9.32	9.32	9.34	0.22	0.04
Escherichia coil						
Caecum	7.30	7.27	7.28	7.24	0.59	0.08
Rectum	8.66	8.66	8.85	8.57	0.66	0.09

Table 5. Ammonia, lactic acids and short chain fatty acids (mmol/L) in the caecum of piglets fed with diet supplementing sodium gluconate, mannan oligosaccharide or potassium diformate during nursery period

Item	Control	Sodium gluconate	Mannan oligosaccharide	Potassium diformate	p-value	SEM
Ammonia	2.62[a]	2.11[b]	2.27[a]	2.64[a]	0.04	0.08
Lactic acid	0.51	0.57	0.59	0.52	0.08	0.03
Acetic acid	11.43	13.04	11.65	12.57	0.74	0.57
Propionic acid	15.30	20.96	20.84	20.51	0.20	1.10
Butyric acid	6.09	9.58	9.18	8.47	0.14	0.58
Total SCFA	32.82	43.57	41.68	41.55	0.09	1.68

[a, b] Means within a row with different superscripts differ significantly ($p < 0.05$).

and Bunchasak (2010), Poeikhampha et al. (2007), Biagi et al. (2006) and Van Beers-Schreurs et al. (1998) and the results of current study also showed that SG tended to increase butyric acid and total SCFAs in the caecum of pigs. The study of Savage et al. (1996) reported that supplementation of MOS and PDF also increased the lactic acid bacteria population leading to produce lactic acid and acetic acid. It is believed that increasing acids utilizing bacteria increase SCFAs production (Asano et al., 1994; Tsukahara et al., 2002). The study of Guedes et al. (2009) informed that rabbits fed MOS had higher SCFAs and tended to had lower pH in the caecum than rabbits fed antibiotic (zinc bacitracin) and control diets Therefore, in this study, piglets fed diet containing SG, MOS or PDF tended to increase total SCFAs production as reached to +32.75% in SG, +27.00% in MOS, +26.60% in PDF compared with control group (p = 0.09).

SCFAs are energy source of intestinal epithelial cell growth. In present study, total SCFAs and butyric acid were slightly increased by the feed additives supplementation. Significant enchantment of butyric acid may be produced from lactic acid and acetic acid, since these SCFA can be the substrates to form butyric acid via activities of *Megasphaera elsdenii* (Tsukahara et al., 2002). Unfortunately, we did not determine the population of *Megasphaera*

elsdenii, so the exact processes of butyric acid synthesis is still unclear. In addition, depression of ammonia product in large intestine caused by feed additives supplementation indicates less remaining nitrogen for fermentation. In this study, the dietary treatments significantly increased ammonia concentration in caecum of pigs ($p < 0.05$). Therefore, the positive effect of these feed additives on SCFAs and ammonia production could be the reasons to support the phenomenon of an improvement of growth performance in this trial.

Small intestinal morphology

The effects of dietary treatments on villous height, crypt depth and the ratio of villous height and crypt depth from duodenum and jejunum of piglets are shown in Table 6. In jejunum, the villous height were significantly increased by the supplementation of SG ($p < 0.05$). Supplementation of SG, MOS and PDF significantly increased crypt dept in jejunum ($p < 0.05$) and PDF significantly decreased villous height and crypt depth ratio ($p < 0.05$). The dietary treatment did not influence villous height, crypt depth and villous height and the crypt depth ratio in duodenum.

Factors affecting small intestinal development of pigs includes, stress of weaning, adaptation to solid feed during weaning period, dietary factor and so on (Steven et al.,

Table 6. Villous height, crypt depth (μm) and the ratio of villous height and crypt depth from duodenum and jejunum of piglets fed with diet supplementing sodium gluconate, mannan oligosaccharide or potassium diformate during nursery period

Item	Control	Sodium gluconate	Mannan oligosaccharide	Potassium diformate	p-value	SEM
Villous height (μm)						
Duodenum	443.84	465.25	474.28	472.14	0.17	5.41
Jejunum	335.63[B]	373.77[A]	342.28[B]	318.50[B]	<0.01	6.00
Crypt depth (μm)						
Duodenum	292.42	309.32	302.83	318.84	0.17	4.31
Jejunum	221.47[b]	269.33[a]	259.28[a]	263.26[a]	0.02	6.31
Villous height:crypt depth ratio						
Duodenum	1.53	1.51	1.57	1.48	0.76	0.03
Jejunum	1.52[a]	1.40[ab]	1.34[ab]	1.22[b]	0.03	0.04

[a, b] Means within a row with different superscripts differ significantly ($p < 0.05$).
[A, B] Means within a row with different superscripts differ highly significant ($p < 0.01$).

2001). For these reasons, villous atrophy after weaning is caused by an increased rate of cell loss or a reduced rate of cell renewal has been reported by and this associate with activity of enzymes such as lactase and sucrase (Pluske et al., 1995; Pluske et al., 1997). SCFA production is related to the development of intestinal villous and crypts. Especially, the butyric acid has high potential to stimulate the growth of epithelial cells in the intestine of pigs by providing energy to the cells (Roediger, 1980; Scheppach et al., 1995). In this study, supplementation of SG, MOS or PDF tended to increase butyric acid and total SCFAs and significantly increased crypt dept in jejunum (p<0.05). Consequently, the daily gain and feed to gain ratio were slightly increased. This may be due to the increase in intestinal villous surface and the absorptive efficiency of the small intestine. The function of villous and crypts are very important to the production performances of pigs due to the portion of the digestive and absorptive capacity of the small intestine occurs near and around the villous and crypts (Steven et al., 2001).

Focusing on each feed additives, however, SG supplementation promoted better growth of villous in jejunum than other feed additives, and PDF clearly decreased the ratios of villous height and crypt depth, and SG, MOS and PDF obviously increased crypt dept in jejunum and slightly improved of daily gain and feed to gain ration of pigs, however, the statically differences were not found. In conclusion, under well managed conditions, effects of these feed additives on intestinal bacteria population and production performance may be lesser than that on small intestinal morphology of nursery pigs.

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