Journal of Applied Animal Research

ISSN: 0971-2119 (Print) 0974-1844 (Online) Journal homepage: https://www.tandfonline.com/loi/taar20

Effects of different levels of metabolite combination produced by *Lactobacillus plantarum* on growth performance, diarrhoea, gut environment and digestibility of postweaning piglets

Teck Chwen Loh , Tran Van Thu , Hooi Ling Foo & Mohd Hair Bejo

To cite this article: Teck Chwen Loh , Tran Van Thu , Hooi Ling Foo & Mohd Hair Bejo (2013) Effects of different levels of metabolite combination produced by *Lactobacillus plantarum* on growth performance, diarrhoea, gut environment and digestibility of postweaning piglets, Journal of Applied Animal Research, 41:2, 200-207, DOI: 10.1080/09712119.2012.741046

To link to this article: https://doi.org/10.1080/09712119.2012.741046

Published online: 23 Jan 2013.

Journal of Applied Animal Research, 2013
Vol. 41, No. 2, 200–207, http://dx.doi.org/10.1080/09712119.2012.741046

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

Effects of different levels of metabolite combination produced by *Lactobacillus plantarum* on growth performance, diarrhoea, gut environment and digestibility of postweaning piglets

Teck Chwen Loh[a,b]*, Tran Van Thu[a], Hooi Ling Foo[c,d] and Mohd Hair Bejo[e]

[a]*Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Malaysia;* [b]*Institute of Tropical Agriculture, Universiti Putra Malaysia, Serdang, Malaysia;* [c]*Department of Bioprocess Technology, Faculty of Biotechnology and Biomolecular Science, Universiti Putra Malaysia, Serdang, Malaysia;* [d]*Institute of Biosciences, Universiti Putra Malaysia, Serdang, Malaysia;* [e]*Department of Veterinary Pathology, Faculty of Veterinary Medicine, Universiti Putra Malaysia, Serdang, Malaysia*

(*Received 3 February 2012; final version received 9 October 2012*)

A study was conducted to investigate the effects of different levels of metabolite combinations produced by *Lactobacillus plantarum* on growth performance, diarrhoea incidence, gut environment and nutrient digestibility in postweaning piglets. A total of 40 piglets were assigned into one of five treatments: (1) negative control (free antibiotic); (2) positive control (0.03% antibiotic of chlortetracycline); (3) Met 1 (0.1% metabolite combination of TL1, RG14 and RS5 strains); (4) Met 3 (0.3% metabolite combination of TL1, RG14 and RS5 strains); and (5) Met 5 (0.5% metabolite combination of TL1, RG14 and RS5 strains). After 5 weeks of the trial, the average daily gain (ADG) and daily feed intake (DFI) was significantly ($P < 0.05$) improved in Met 5 group compared to the negative control and Met 1 group. The diarrhoea incidence was significantly ($P < 0.05$) reduced when were piglets fed with positive control and Met 5 dietary treatment. The pH value and *Enterobacteriaceae* (ENT) in the gut were reduced in Met 5 treatment as compared to negative control and Met 1 group. However, lactic acid bacteria (LAB) counts and short chain fatty acids (SCFA) in the gut were significantly higher ($P < 0.05$) when piglets were fed with Met 5 dietary treatment. In contrast, feeding of metabolite combination to piglets did not improve energy utilisation. In addition, piglets fed with Met 5 dietary treatment improved protein digestibility compared to the negative control and Met 1 group. The results obtained in this study showed that feeding of 0.5% metabolite combinations could improve growth performance, gut health environment and protein digestibility in postweaning piglets.

Keywords: metabolite combination; microflora; short chain fatty acids; diarrhoea incidence; postweaning piglets

1. Introduction

Postweaning piglets suffer several stress factors including nutritional, environmental and social conditions. A gut imbalance frequently occurs in postweaning piglets due the increased population of ENT and decreased population of LAB (Loh et al. 2002; Taras et al. 2006; Thu et al. 2011). Over the past few years, the utilisation of probiotics, prebiotics (Marinho et al. 2007; Giang et al. 2010) and fermented products (Loh et al. 2003; Canibe et al. 2007) in animal feeding has been carried out to improve gut health and growth performance. Potentially, this could be an alternative approach to in-feed antibiotics in animal livestock, particularly in piglets. The LAB has many positive effects in reducing pH value, ENT population and increasing SCFA concentration in the gastrointestinal tract (Franklin et al. 2002; Niba et al. 2009; Hong et al. 2009; Loh et al. 2010). Furthermore, the live organisms in probiotics may be able to protect villi height atrophy, crypt hyperplasia as well as capable of removing toxic substances (Holzapfel et al. 1998; Marinho et al. 2007). These effects could reduce diarrhoea incidence in postweaning piglets (Niven et al. 2004; Giang et al. 2010). However, a recent study indicated that even probiotics such as LAB have acquired resistance to antimicrobials that are commonly used in human and animal health sciences (Shalini and Rameshwar 2005).

Metabolites produced by *L. plantarum* strains have been known to have probiotic effects which contain mainly organic acids and bacteriocins (Foo et al. 2005; Thanh et al. 2009). These substances encourage favourable bacteria development through the reduction of pH and the production of SCFA in the gut. Therefore, LAB metabolites can have beneficial effects in the gastrointestinal tract and provide available nutrients to the host animal (Loh et al. 2003; Thu et al. 2011). Other studies showed that the feeding of metabolites produced by *L. plantarum* strains improved growth performance, villi height and faecal LAB in rats (Foo et al. 2003), chickens (Thanh et al. 2009; Loh et al. 2010) and piglets

*Corresponding author. Email: tcloh@putra.upm.edu.my

(Thu et al. 2011). Additionally, low pH value of metabolites (Thanh et al. 2010) also provides suitable conditions for pepsin activity to degrade protein in the stomach (Longland 1991). However, there is limited information on metabolite concentration effects on nutrient utilisation and gut environment in pigs. Therefore, the objective of this study was to evaluate the effects of different concentrations of metabolite combination produced by *L. plantarum* strains on growth performance, diarrhoea incidence, gut environment and nutrient digestibility in postweaning piglets.

2. Materials and methods

2.1 Preparation of LAB metabolites

The metabolites were produced from three strains of *L. plantarum*, namely TL1, RG14 and RS5. The *L. plantarum* strains were isolated from Malaysian foods (Foo et al. 2003; Lim et al. 2006) and kept at -20°C in Man Rogosa Sharpe broth (MRS; Merck, Darmstadt, Germany) containing 20% (v/v) glycerol. The stock culture was revived twice in MRS broth and incubated anaerobically at 30°C for 48 h. The liquid metabolites were prepared according to the method as described by Foo et al. (2003). Metabolites were collected by separating the producer cell by centrifugation at 12,000 rpm for 15 min at 4°C and kept at 4°C until mixing in piglet diets.

2.2. Animal diets and experimental design

This study was carried out at the commercial research unit in a pig farm, Tanjung Sepat, Selangor, Malaysia. A total of 40 postweaning crossbred piglets (Large White $\times$ Landrace $\times$ Duroc) of six litters from the third to fourth parity of sows, at 26 days of age with an average initial body weight (BW) of 6.53 ± 0.25 kg were used in this study. The piglets were kept in raised slatted floor pens (1.2×1.6 m) at a temperature ranging from 29°C to 35°C with humidity $86 \pm 4\%$. Heater was provided from 20:00 h to 08:00 h to keep the piglets warm. Each pen (replication) consisted of two piglets and four replications were assigned per treatment. Water and feed were offered *ad libitum* throughout the experimental period. The piglets were randomly allocated into one of five treatments. The feed compositions of dietary treatments are shown in Table 1. The basal control diet was formulated to meet the nutrient requirement of the piglet according to the National Research Council (1998). The five treatments were: (1) negative

Table 1. Composition of piglet dietary treatments (% as fed basic).

	Dietary of treatments				
Ingredients, %	Negative control	Positive control	Met 1	Met 3	Met 5
Corn	38.5	38.5	38.5	38.5	38.5
Soybean	30	30	30	30	30
Fine corn meal	12.75	12.75	12.75	12.75	12.75
Wheat pollard	5.15	5.12	5.05	4.85	4.65
Monocalcium phosphorus	0.5	0.5	0.5	0.5	0.5
Salt	0.5	0.5	0.5	0.5	0.50
Milk powder	5.00	5.00	5.00	5.00	5.00
Lactose	5.50	5.50	5.50	5.50	5.50
Calcium carbonate	0.50	0.50	0.50	0.50	0.50
L-lysine	0.15	0.15	0.15	0.15	0.15
DL-methionine	0.12	0.12	0.12	0.12	0.12
L-triptophan	0.05	0.05	0.05	0.05	0.05
Vitamin premix*	0.28	0.28	0.28	0.28	0.28
Minerals premix**	0.50	0.50	0.50	0.50	0.50
Choline chloride 50%	0.50	0.50	0.50	0.50	0.50
Chlotetracyline	–	0.03	–	–	–
Metabolite	–	–	0.1	0.3	0.5
Total	100.00	100.00	100.00	100.00	100.00
Calculated analyses					
Crude protein, %	21.36	21.24	21.47	21.34	21.27
ME, MJ/kg	14.64	14.68	14.63	14.72	14.70

Note: The negative control is a group of free antibiotics; the positive control is a group of antibiotics; Met 1 is a treatment with 0.1% metabolite combination; Met 3 is a treatment with 0.3% metabolite combination; Met 5 is a treatment with 0.5% metabolite combination.
*The vitamin premix provided the following amounts per kilogram of diet: retinol 5.8 mg; cholecalciferol 75 μg; α-tocopherol 36 mg; thiamine 4.0 mg; riboflavin 6.0 mg; calpan 40 mg; niacin 38 mg; pyridoxine 4.2 mg; menadione, 4.5 mg; folic acid 1.5 mg; cyanocobalamin 42 μg and biotin 0.1 mg.
**The mineral premix provided the following amounts per kilogram of diet: manganese 100 mg; iron 80 mg; zinc 80 mg; copper 100 mg; cobalt 80 mg; iodine 70 mg and selenium 50 mg; Monocalcium phosphorus: phosphorus 21%; Calcium 17% and Fluorine 0.21%.

control (free antibiotic); (2) positive control (0.03% chlortetracycline); (3) Met 1 (0.1% metabolite combination of TL1, RG14 and RS5 strains); (4) Met 3 (0.3% metabolite combination of TL1, RG14 and RS5 strains); and (5) Met 5 (0.5% metabolite combination of TL1, RG14 and RS5 strains). The study was investigated for five consecutive weeks. At the end of trial period, four male piglets with similar BW from each treatment were sacrificed for ileal digesta and rectal faeces samples. All of the animal experiments were carried out according to the guidelines of the Committee of Animal Research and Ethics, Universiti Putra Malaysia for animal experiments.

2.3. Growth, diarrhoea recording and sampling

The body weight and feed intake were measured weekly. The diarrhoea score of each piglet was recorded on days 3, 5, 10, 12, 17 and 24. Diarrhoea was assessed visually from the scale of 0–3, based on the consistency of the faeces form according to the score: 0 = pellet faeces; 1 = semi-pellet faeces; 2 = soft faeces; and 3 = watery faeces. This observation was followed using the method described by Loh et al. (2002).

The digesta samples were collected from ileum intestine and faecal samples were collected from the rectum of sacrificed piglets at slaughter and the pH was measured immediately after the collection. Samples were then stored at $-20^{\circ}C$ until further analyses for microfloral counts and SCFA contents.

2.4. LAB, ENT counts and SCFA contents

Ten per cent (w/v) of the sample was diluted in sterile peptone water as prepared by 25.5 g/L and homogenised to stand at room temperature for 1 h. The samples were diluted (10% v/v) for a further 10-fold series with peptone water. The dilution was vortexed and spread using a sterile glass spreader. Enumerations of bacteria were performed on MRS agar for LAB and incubated in an anaerobic jar at $30^{\circ}C$ for 48 h. ENT enumeration were performed on Eosin Methylene Blue (EMB; Merck, Darmstadt, Germany) agar and incubated in anaerobic conditions at $37^{\circ}C$ for 24 h. The number of colony forming units (CFU) were expressed as $\log_{10}$ CFU per gram. The SCFA contents were determined using gas chromatography as described by Thanh et al. (2009).

2.5. Digestibility measurement

Nutrient digestibility was measured based on the amount of nutrients in a diet and nutrient lost through excreta by using inert marker of titanium dioxide (TiO_2) following the equation described by Adedokun and Adeola (2005). Apparent metabolisable energy was calculated based on the following formula:

$$\text{AME of diets (kcal/kg} = (\%\text{energy utilisation}/100) \times \text{diet energy}$$

Inert marker of TiO_2 was added @0.3% (w/w) in diets of piglets at the fifth week of the trial period. The protocol of TiO_2 determination in diet and digesta was followed as per the method described by Lercari et al. (1983). The crude protein and gross energy determination were conducted following the protocol of AOAC (2000).

2.6. Data analysis

The data were analysed by one-way analysis of variance using the General Linear Model procedure by SAS (1998) (SAS Inst., Inc., Cary, NC) to determine effects of dietary treatments. Duncan's Multiple Range Test was used to compare the significant difference of treatments at $P<0.05$. The number of piglets experiencing diarrhoea was analysed using Chi-square Test by Minitab (2000) at $P<0.1$. The data were presented as mean $\pm$ standard error of the mean (SEM).

3. Results

3.1. Growth performance and diarrhoea score

Initial BW of piglets was selected in a similar fashion ($P>0.05$) among treatments but after 5 weeks of the experiment, Met 5 had the highest ($P<0.05$) final BW among the treatments (Table 2). However, the ADG of piglets in the Met 5 treatment was significantly higher ($P<0.05$) than the negative control and Met 1 groups. The DFI in Met 3 and Met 5 dietary treatments were significantly higher ($P<0.05$) than the other three treatments, in particular the Met 5, which appeared to be the highest ($P<0.05$) among the treatment groups. In contrast, the FCR was significantly lower ($P<0.05$) in the positive control and Met 5 groups than those in the Met 1 group but no significant differences ($P>0.05$) were found in the negative control, or the Met 1 and Met 3 groups.

The piglets fed with diets in positive control and Met 5 groups had a significantly a lower ($P<0.05$) diarrhoea score than the negative control group but no significant differences ($P>0.05$) were observed in Met 1 and Met 3 treatment groups. However, the trend of diarrhoea incidence in Figure 1 showed that almost all of the piglets had diarrhoea beginning on

Table 2. Growth performance, diarrhoea score and nutrient digestibility of piglets fed with different dietary treatments.

Items	Dietary treatments					SEM	*P*-value
	Negative control	Positive control	Met 1	Met 3	Met 5		
Growth							
Initial BW, kg	6.63[a]	6.49[a]	6.64[a]	6.54[a]	6.34[a]	0.26	0.92
Final BW, kg	15.43[b]	16.75[ab]	15.85[ab]	16.83[ab]	17.39[a]	0.59	0.15
ADG, g/day	251.8[c]	293.2[ab]	263.2[bc]	293.9[ab]	315.7[a]	13.51	0.01
DFI, g/pig/day	465.4[c]	474.5[bc]	476.0[bc]	485[ab]	4.93[a]	5.48	0.02
FCR	1.87[ab]	1.62[b]	2.02[a]	1.74[ab]	1.59[b]	0.11	0.07
Diarrhoea score							
Days 0 to 17	0.40[a]	0.09[b]	0.25[ab]	0.16[ab]	0.13[b]	0.07	0.12
Nutrient digestibility							
Protein digestibility, %	65.48[ab]	64.41[b]	65.13[ab]	66.32[ab]	68.06[a]	0.94	0.08
Energy digestibility, %	68.76[a]	68.33[a]	68.10[a]	68.95[a]	70.12[a]	0.65	0.24
AME of diets, kcal/kg	2761.3[a]	2751.2[a]	2724.0[a]	2745.4[a]	2841.5[a]	38.89	0.27

Note: The results were presented as mean values ± SEM. Means expressed with different superscripts letters within the same row was significantly different at $P < 0.05$.
Met 1 is a treatment with 0.1% metabolite combination; Met 3 is a treatment with 0.3% metabolite combination; Met 5 is a treatment with 0.5% metabolite combination.

the third day. The diarrhoea scores achieved maximum at the on the fifth day ($P < 0.1$) and then finished at the 17th day of the experimental period.

3.2. The pH and microfloral counts

The ileal digesta pH of piglets in Met 3 and Met 5 groups was significantly lower ($P < 0.05$) than those in both controls and the Met 1 group. However, no significant differences ($P > 0.05$) were found in the Met 1, negative and positive control groups (Table 3). In contrast, piglets fed on Met 3 and Met 5 dietary treatments had significantly higher ($P < 0.05$) LAB counts than both controls and Met 1 group in digesta. The LAB counts in digesta were the lowest ($P < 0.05$) observed in positive control among treatment groups. The results for ENT counts in ileum digesta were not significantly different ($P > 0.05$) among treatment groups. However, the ratio of LAB and ENT in digesta was significantly higher ($P < 0.05$) in Met 3 and Met 5 treatments as compared to both control groups.

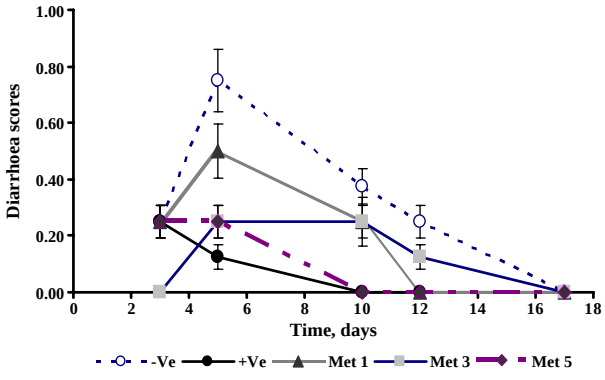

Figure 1. Diarrhoea score in piglets fed with different dietary treatments.

The faecal pH was significantly lower ($P < 0.05$) in Met 3 and Met 5 groups compared to both controls and Met 1 group. No significant differences ($P > 0.05$) were found in Met 1, negative and positive control groups. The piglets fed with Met 3 and Met 5 dietary treatments had significantly higher ($P < 0.05$) faecal LAB counts than both controls and Met 1 group (Table 3). Moreover, there was significantly higher ($P < 0.05$) faecal LAB in Met 1 than in control groups, and surprisingly, the faecal LAB counts was the lowest ($P < 0.05$) in positive control. On the other hand, the results for faecal ENT counts in Met 3 and Met 5 treatments were significantly lower ($P < 0.05$) than those in the negative control and Met 1 groups. No significant differences ($P > 0.05$) were noted between controls and Met 1 group, as well as Met 3 and Met 5 for faecal ENT counts (Table 3). In addition, the ratio of LAB and ENT in faeces was significantly higher ($P < 0.05$) in Met 3 and Met 5 than Met 1 and both control groups.

3.3. Short chain fatty acids

There was significantly higher ($P < 0.05$) acetic acid in Met 3 and Met 5 treatments than those observed in both control groups. No significant differences ($P > 0.05$) were found among three metabolite treatment groups (Table 4). There were also no significant differences ($P > 0.05$) for propionic and butyric acids in ileal digesta of piglets after feeding on different dietary treatments. Iso-butyric, iso-valeric and valeric acids were not detected in ileal digesta of piglets in current study. However, the total SCFA in ileal

Table 3. The pH value, LAB and ENT population (log_{10} CFU/g) in piglets fed with different dietary treatments.

	Dietary treatments						
Items	Negative control	Positive control	Met 1	Met 3	Met 5	SEM	*P*-value
Digesta							
pH	6.25[a]	6.22[a]	6.23[a]	6.06[b]	6.06[b]	0.05	0.02
LAB	6.40[bc]	6.25[c]	6.64[b]	6.98[a]	7.11[a]	0.11	0.00
ENT	4.59[a]	4.52[a]	4.62[a]	4.62[a]	4.56[a]	0.03	0.26
Ratio LAB:ENT	1.39[c]	1.38[c]	1.44[bc]	1.51[ab]	1.56[a]	0.02	0.00
Faeces							
pH	6.54[a]	6.54[a]	6.50[a]	6.24[b]	6.23[b]	0.08	0.02
LAB	6.51[c]	6.12[d]	6.92[b]	7.29[a]	7.32[a]	0.10	0.00
ENT	5.34[a]	5.05[ab]	5.24[a]	4.77[bc]	4.57[c]	0.11	0.00
Ratio LAB:ENT	1.22[c]	1.22[c]	1.32[b]	1.53[a]	1.60[a]	0.02	0.00

Note: The results were presented as mean values ± SEM. Means expressed with different superscripts letters within the same row was significantly different at $P < 0.05$.

Met 1 is a treatment with 0.1% metabolite combination; Met 3 is a treatment with 0.3% metabolite combination; Met 5 is a treatment with 0.5% metabolite combination.

digesta was significantly higher ($P < 0.05$) in Met 3 than those in Met 1 and both controls.

The piglets fed on Met 3 and Met 5 had significantly higher ($P < 0.05$) acetic acid than three other treatments. No significant differences ($P > 0.05$) were observed in the negative control, the positive control and Met 1 groups. There were also no significant differences ($P > 0.05$) among treatments for propionic, iso-butyric, iso-valeric and valeric acids in faeces of piglets. In contrast, faecal butyric acid in Met 3 and Met 5 was significantly higher ($P < 0.05$) than negative control group. Therefore, total faecal SCFA was significantly higher ($P < 0.05$) in Met 3 and Met 5 than Met 1 and both control groups.

3.4. Protein and energy digestibility

The piglets fed with Met 5 dietary treatment had significantly higher ($P < 0.05$) values than those in the positive control group for digestibility of protein, but no significant differences ($P > 0.05$) were found in Met 1, Met 3, Met 5 metabolite groups and the negative control group. Moreover, there were also no significant differences ($P > 0.05$) in Met 1, Met 3 and both control groups for protein digestibility (Table 2). On the other hand, energy digestibility of piglets was not significantly different ($P > 0.05$) among treatment groups. No significant differences ($P > 0.05$) were observed among dietary treatment groups for ileal apparent metabolisable energy in this study.

4. Discussion

4.1. Growth performance and diarrhoea score

The results indicated that feeding of different metabolite concentrations had different growth performance of piglets. The piglets fed with 0.5%

Table 4. The SCFA concentrations in digesta and faeces of piglets fed with different dietary treatments.

	Dietary treatments						
Items	Negative control	Positive control	Met 1	Met 3	Met 5	SEM	*P*-value
Ileal digesta short chain fatty acids, mM/L							
Acetic	18.57[b]	18.29[b]	18.92[ab]	19.70[a]	19.47[a]	0.30	0.01
Propionic	4.34[a]	4.06[a]	4.02[a]	4.45[a]	4.39[a]	0.22	0.53
Butyric	1.06[a]	1.09[a]	1.08[a]	1.05[a]	1.14[a]	0.04	0.71
Total	23.98[bc]	23.44[c]	24.02[bc]	25.21[a]	25.00[ab]	0.39	0.01
Rectum faecal short chain fatty acids, mM/L							
Acetic	30.15[b]	29.67[b]	30.03[b]	31.61[a]	31.68[a]	0.36	0.00
Propionic	11.36[a]	11.16[a]	11.17[a]	11.95[a]	11.96[a]	0.38	0.35
Butyric	3.83[b]	4.29[ab]	4.24[ab]	4.90[a]	4.78[a]	0.22	0.02
Others*	1.44[a]	1.46[a]	1.52[a]	1.58[a]	1.42[a]	0.06	0.26
Total	46.77[b]	46.58[b]	46.96[b]	50.05[a]	49.83[a]	0.61	0.00

Note: The results were presented as mean values ± SEM. Means expressed express with different superscripts letters within the same row was significantly different at $P < 0.05$.

Met 1 is a treatment with 0.1% metabolite combination; Met 3 is a treatment with 0.3% metabolite combination; Met 5 is a treatment with 0.5% metabolite combination.

*Other short chain fatty acids are iso-butyric, iso-valeric and valeric acids.

metabolites improved significantly for ADG, DFI and FCR when compared to the group of free antibiotic (negative control) and 0.1% metabolites. This result is in agreement with Loh et al. (2010) when feeding of 0.4% metabolite combinations produced by *L. plantarum* improved significantly the growth performance of broiler chickens. This effect could be explained by feeding of metabolites containing antimicrobial substances produced by *L. plantarum* that provide nutrients and enhance biophysiology activities in the gut of animals. This would have resulted in an improved absorption and mucosal construction in intestinal morphology of the animals (Niba et al. 2009). On the other hand, a similar result was obtained for growth performance in piglets when fed with 0.3% metabolites and 0.03% antibiotic in this study. Thanh et al. (2009) reported that a supplement of 0.3% metabolites in chicken diets improved the ADG and DFI. Many previous studies have reported that the positive effects of antimicrobial compounds produced by *Lactobacillus* spp. on growth performance in pigs due to improvement of gut health (Hale and Newton 1979; Canibe et al. 2008; Thu et al. 2011).

Postweaning piglets suffer several stressors immediately after weaning, which can cause problems. In the current study, the piglets fed on 0.5% metabolites showed reduced diarrhoeal incidence after weaning and almost all of the piglets had a reduction in diarrhoeal incidence at day 10 of the experiment. This result is in keeping with Thu et al. (2011), who reported that diarrhoea of postweaning piglets has reached the highest at day 5 after weaning and then subsided within 2 weeks. Aumaitre (2000) claimed that diarrhoea incidence in piglets can be prevented via the inhibitory activity of LAB to harmful pathogens in the gut. However, feeding of 0.1% and 0.3% metabolites did not reduce the diarrhoea incidence effectively in piglets. This could be explained by the dosages of metabolites (0.1% and 0.3%) not being sufficient enough to contain diarrhoea incidence in piglets. Metabolites contain large quantity of organic acids and bacteriocins, which could be reducing the development of *E. coli* and *Salmonella* in the gut of piglets (Scholten et al. 1999; Hong et al. 2009; Thanh et al. 2010).

4.2. The pH and microfloral population

Feeding of 0.3% and 0.5% of metabolites to piglets reduced pH and increased LAB in digesta, as well as in faeces. The reduction in pH may be due to the production of organic acids such as lactic, acetic, propionic and butyric acids during fermentation process in the gut, particularly in large intestine. A similar result reported by Loh et al. (2010), when supplements of 0.4% metabolites in the diet of broiler chickens increased faecal LAB counts and decreased faecal pH. A low pH level is not a conducive environment for ENT development (Broberg et al. 2007; Lange et al. 2010). However, it is a good environment for LAB development (Holzapfel et al. 1998; Tang et al. 2011). For that reason, the population of faecal ENT counts observed in the group fed with 0.5% metabolites was lower than other groups. Moreover, increasing LAB populations were able to compete with ENT for the surface and nutrients in the gastrointestinal tract (Canibe et al. 2008; Niba et al. 2009). The existing bacteria present in the gut were able to reduce pH further through the production of metabolites via the fermentation process (Foo et al. 2005). This is also in agreement with Broberg et al. (2007), who explained that low pH could stimulate the fermentation process. On the other hand, there was a significantly higher ratio of LAB and ENT population in the gut of piglets when fed with 0.3% and 0.5% metabolites. This is very important to balance the microfloral activity and protect intestinal mucosa membrane in the gut.

4.3. Short chain fatty acids

The SCFA are the main products of microbial fermentation, particularly in the large intestine of the host animal. In this study, piglets fed with 0.3% and 0.5% metabolite combinations increased SCFA in ileal digesta and faeces. However, the total SCFA detected in digesta were fewer than in faeces, so it may relate to area of microbial fermentation activities in the hindgut. The SCFA, comprised of acetic, propionic, iso-butyric, butyric, iso-valeric and valeric acids, are absorbed mostly in the large intestine and provide energy to the host (Franklin et al. 2002; Chiba 2007), particularly butyric acid that can contribute significantly to the health of the colon mucosa (Niba et al. 2009). Interestingly, there is a relationship of SCFA in digesta and faeces, where acetic acid increased accordingly by the concentrations of metabolites. Thanh et al. (2009) also claimed that feeding of 0.3% metabolites produced by *L. plantarum* increased faecal SCFA in broiler chickens. Moreover, piglets treated with Met 3 and Met 5 dietary treatments had a significant increase in faecal butyric acid. Franklin et al. (2002) reported that butyric acid is a main energy source for epithelial cells in the hindgut. An increase in total SCFA concentration results in lowering the gut pH and creating a good environment of LAB to survive, especially in the piglets fed with metabolites.

4.4. Nutrient digestibility

Feeding of 0.5% metabolite combination improved protein utilisation in piglets. This could be explained by piglets fed with 0.5% metabolites having a lower pH and providing a suitable condition for pepsin activity and protein degradation (Longland 1991; Lawlor et al. 2002). Low pH is required to initiate protein digestion by pepsin and prevent harmful bacteria development. Maintenance of optimal pH value throughout the digestive tract would improve the action of digestive enzymes and induce the undigested biomass substances, which become available nutrients for growing piglets (Scholten et al. 1999; Sakata et al. 2003). Furthermore, this also affects on reduction in competition of nutrients between ENT and LAB in the gastrointestinal tract of host animals. Similarly, piglets fed with fermented feed and probiotics improved protein digestibility by reduction in pH and expansion of lactic acid bacteria in the gut (Loh et al. 2003; Castillo et al. 2008; Hong et al. 2009).

Energy utilisation of piglets was not improved by feeding of metabolite combinations, and no significant differences were found in ileal apparent metabolisable energy in this study. This is probably due to fermentation activities from SCFA, that normally happens at the end of the hindgut of the animals (Chiba 2007). Metabolites in the dietary treatments containing lactic acid, SCFA and ethanol can be the energy sources for piglets. However, these energy sources may be evaporated during dry matter preparation at high temperature heating. Scholten et al. (1999) also estimated evaporation of lactic acid, short chain fatty acids and ethanol at 8, 50 and 100%, respectively. The evaporation of these compounds may affect the results of metabolisable energy in piglet diets. A similar study of Lynch et al. (2009) reported that the probiotics containing *Lactobacillus* spp. could not improve the apparent metabolisable energy in piglets.

5. Conclusion

The current results show that the piglets offered with 0.3% and 0.5% metabolites have a positive effect on growth performance, as well as to reduce diarrhoea incidence. Furthermore, a beneficial relationship among pH value, SCFA concentration and microflora population in the gut facilitates the protein digestibility for piglets. This study also showed that the feeding of metabolite combinations would not improve apparent metabolisable energy of piglets. In contrast, the piglets fed with 0.5% metabolite combinations had a reduced pH in the gut and increased protein digestibility. Therefore, feeding of 0.5% metabolite combination of TL1, RG14 and RS5 strains gave the best result in terms of growth performances, protein digestibility and gut environment of postweaning piglets.

References

Adedokun SA, Adeola O. 2005. Apparent metabolizable energy value of meat and bone meal for while pekin ducks. Poultry Science 84:1539–1546.

Association of Official Analytical Chemists (AOAC). 2000. Official Methods of Analysis. 17th ed. Arlington (VA): Association of Official Analytical Chemists.

Aumaitre LA. 2000. Adaptation and efficiency of the digestive process in the gut of the young piglets. Asian–Australasia Journal of Animal Science 13: 227–242.

Broberg A, Jacobsson K, Strom K, Schnurer J. 2007. Metabolite profiles of lactic acid bacteria in grass silage. Applied Environmental Microbiology 73:5547–5552.

Canibe N, Virtanen E, Jensen BB. 2007. Effect of acid addition to pig liquid feed on its microbial and nutritional characteristics. Livestock Science 108: 202–205.

Canibe N, Miettinen H, Jensen BB. 2008. Effect of adding *Lactobacillus plantarum* or a formic acid containing-product to fermented liquid feed on gastrointestinal ecology and growth performance of piglets. Livestock Science 114:251–262.

Castillo M, Martin-Orue SM, Taylor-Pickard JA, Perez JF, Gasa J. 2008. Use of mannanoligosaccharides and zinc chelate as growth promoters and diarrhoea preventative in weaning pigs: Effects on microbiota and gut function. Journal Animal Science 86:94–101.

Chiba LI. 2007. Digestive physiology. Animal Nutrition. Handbook. 2nd rev. ed. Auburn (AL): Auburn University. p. 29–53.

Foo HL, Loh TC, Law FL, Lim YZ, Kufli CN, Rusul G. 2003. Effects of feeding *Lactobacillus plantarum* I-UL4 isolated from Malaysian Tempeh on growth performance, faecal flora and lactic acid bacteria and plasma cholesterol concentrations in postweaning rats. Journal Food Science Biotechnology 12:403–408.

Foo HL, Lim YS, Loh TC, Saleh NM, Raha AR, Rusul G. 2005. Characterization of bacteriocin produced *by Lactobacillus plantarum* I-UL4 isolated from Malaysian fermented tapioca, Tapai Ubi. Proceeding of 4th NIZO Dairy Conference, Papendal, Netherland. p. 33.

Franklin MA, Mathew AG, Vickers JR, Clift RA. 2002. Characterization of microbial populations and volatile fatty acid concentrations in the jejunum, ileum, and cecum of pigs weaned at 17 vs 24 days of age. Journal of Animal Science 80:2904–2910.

Giang HH, Viet TQ, Ogle B, Lindberg JE. 2010. Growth performance, digestibility, gut environment and health status in weaned piglets fed a diet supplemented with potentially probiotic complexes of lactic acid bacteria. Livestock Science 129:95–103.

Hale OM, Newton GL. 1979. Effects of a nonviable *Lactobacillus* species fermentation product on performance of pigs. Journal of Animal Science 48:770–775.

Holzapfel WH, Haberer P, Snel J, Schillinger U, Huis in't VJHJ. 1998. Overview of gut flora and probiotics.

International of. Journal Food Microbiology 41: 85–101.
Hong TTT, Thuy TT, Passoth V, Lindberg JE. 2009. Gut ecology, feed digestion and performance in weaned piglets fed liquid diets. Livestock Science 125:232–237.
Lange CFM, Pluske J, Gong J, Nyachoti CM. 2010. Strategic use of feed ingredients and feed additives to stimulate gut health and development in young pigs. Livestock Science 134:124–134.
Lawlor PG, Lynch PB, Gardiner GE, Caffrey PJ, O'Doherty JV. 2002. Effect of liquid feeding weaned pigs on growth performance to harvest. Journal of Animal Science 80:1725–1735.
Lercari C, Sartorel BL, Sedea L, Toninelli G. 1983. Determination of titanium dioxide in soaps by furnace atomic absorption spectrometry. Journal of American Oil Chemistry Society 6:856–857.
Lim YS, Foo HL, Raha AR, Loh TC, Bejo MH, Gulam Rusul RA. 2006. The probiotic characteristics of *Lactobacillus plantarum* strains isolated from local foods. Proceedings of the 28th Symposium of Malaysian Society for Microbiology, Melaka, Malaysia. p. 88–89.
Loh TC, Choo PY, Cheong YH. 2002. Effects of organic acid and natural herbs on performance and incidence of diarrhoea in postweaning pigs. Malaysian Journal of Animal Science 7:25–30.
Loh TC, Foo HL, Lee KL, Lim YZ, Kufli CN. 2003. Effect of fermented fruits on the growth performance, shedding of *Enterobacteriaceae* and *Lactobacilli* in postweaning pigs. Asian–Australasia Journal of Animal Science 16:1656–1660.
Loh TC, Thanh NT, Foo HL, Hair-bejo M, Azhar BK. 2010. Feeding of different levels of metabolite combinations produced by *Lactobacillus plantarum* on growth performance, faecal microflora, volatile fatty acids and villi height in broilers. Animal Science Journal 81: 205–214.
Longland AC. 1991. Digestive enzyme activities in pigs and poultry. *In vitro* digestion for pigs and poultry. Wallingford, UK: CAB International. p. 3.
Lynch B, Callan JJ, O'Doherty JV. 2009. The interaction between dietary crude protein and fermentable carbohydrate source on piglet post weaning performance, diet digestibility and selected faecal microbial populations and volatile fatty acid concentration. Livestock Science 124:93–100.
Marinho MC, Pinho MA, Mascarenhas RD, Silva FC, Lordelo MM, Cunha LF, Freire JPB. 2007. Effects of prebiotic or probiotic supplementation and ileo rectal anastomosis on intestinal morphology of weaned piglets. Livestock Science 108:240–243.
Minitab. 2000. Minitab reference manual release 12. State College (PA): Pennsylvannia State College.
National Research Council (NRC). 1998. Nutrient Requirements of Swine. 9th rev. ed. Washington (DC): National Academic Press.
Niba AT, Beal JD, Kudi AC, Brooks PH. 2009. Bacterial fermentation in the gastrointestinal tract of non-ruminant: influence of fermented feeds and fermentable carbohydrates. Tropical Animal Health and Production 41:1393–1407.
Niven SJ, Beal JD, Brooks PH. 2004. The simultaneous determination of short chain fatty acid, monosaccharides and ethanol in fermented liquid pig diets. Animal Feed Science and Technology 117:339–345.
Sakata T, Kojima T, Fujieda M, Takahashi M, Michibata T. 2003. Influences of probiotic bacteria on organic acid production by pig caecal bacteria *in vitro*. Proceedings the Nutrition Society 62:73–80.
SAS. 1998. Statistical Analysis System (SAS). Cary (NC): SAS Institute Inc.
Scholten RHJ, Van der Peet-Schwering CMC, Verstegen WA, den Hartog LA, Schrama JW, Vesseur PC. 1999. Fermented co-products and fermented compound diets for pigs: a review. Animal Feed Science and Technology 82:1–19.
Shalini M, Rameshwar S. 2005. Antibiotic resistant in food lactic acid bacteria. International Journal Food Microbiology 105:281–295.
Tang ZR, Sun ZH, Tang XS, Feng ZM, Zhou D, Xiao DF, Zhang B, Li LL. 2011. Dietary bovine lactoferricin improved gut microflora, benefited intestinal mucosal morphology and inhibited circular cytokines delivery in 21-day weaned piglets. Journal of Applied Animal Research 39:36–40.
Taras D, Vahjen W, Macha M, Simon O. 2006. Performance, diarrhoea incidence, and occurrence of *Escherichia coli* virulence genes during long-term administration of a probiotic *Enterococcus faecium* strain to sows and piglets. Journal of Animal Science 84: 608–617.
Thanh NT, Loh TC, Foo HL, Hair-bejo M, Azhar BK. 2009. Effects of feeding metabolite combinations produced by *Lactobacillus plantarum* on growth performance, faecal microbial population, small intestine villus height and faecal volatile fatty acids in broilers. British Poultry Science 50:298–306.
Thanh NT, Loh TC, Foo HL, Hair-bejo M, Azhar BK. 2010. Inhibitory activity of metabolites produced by *Lactobacillus plantarum* isolated from Malaysian fermented food. International Journal Probiotics and Prebiotics 5:37–44.
Thu TV, Loh TC, Foo HL, Yaakub H, Bejo MH. 2011. Effects of liquid metabolite combinations produced by *Lactobacillus plantarum* on growth performance, faeces characteristics, intestinal morphology and diarrhoea incidence in postweaning piglets. Journal Tropical Animal Health and Production 43:69–75.