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Effect of dietary supplementation with essential oils and a *Bacillus* probiotic on growth performance, diarrhoea and blood metabolites in weaned pigs

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Abstract

Context. Dietary supplementation of essential oils or in combination with a *Bacillus* probiotic was investigated as an antibiotic growth promoter for weaned pigs.

Aims. To evaluate the effect of essential oils (i.e. thymol and carvacrol mixture) or in combination with a probiotic strain (i.e. *Bacillus subtilis* PB6) on the growth performance, diarrhoea incidence, ammonia emission and serological profiles of weaned pigs.

Methods. A total of 96 crossbred ([Yorkshire × Landrace] × Duroc) weaned pigs were randomly allotted to one of six treatments based on sex and initial bodyweight. Each group was distributed into four replicates with four pigs each according to a randomised complete block design. The treatments were: (i) positive control, basal diet supplemented with colistin 150 g/tonne and amoxicillin 200 g/tonne; (ii) negative control, basal diet without supplementation; (iii) T3, basal diet supplemented with essential oils 300 g/tonne; (iv) T4, basal diet supplemented with essential oils 600 g/tonne; (v) T5, basal diet supplemented with essential oils 1000 g/tonne; and (vi) T6, basal diet supplemented with essential oils 300 g/tonne diet and *Bacillus* probiotics 1000 g/tonne.

Key results. The piglets fed with supplements had a significantly higher average daily gain and lower incidence of diarrhoea than the piglets in the negative control (P = 0.001). Feeding the essential oils alone or in combination with probiotics significantly reduced faecal ammonia emission (P = 0.027) and blood urea nitrogen (P = 0.039), while markedly increasing the serum immunoglobulin G concentration of weaned pigs compared with the negative control treatment (P = 0.014). The difference in time of blood collection had significant effects on blood urea nitrogen and immunoglobulins (P = 0.001). However, no significant differences emerged in average daily feed intake, gain : feed ratio, feed efficiency and antibody against swine fever among the treatments.

Conclusions. Diet supplementation with essential oils or in combination with probiotics improved growth performance and immunity, and lowered ammonia emissions and diarrhoea incidence of weaned pigs.

Implications. These findings provide a basis for the application of phytogenic compounds and probiotics as antibiotic growth promoter alternatives in post-weaning diets for pigs.

Additional keywords: classical swine fever virus, gas emission, immunity.

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Introduction

Weaned pigs commonly suffer from post-weaning diarrhoea (PWD), which has an economic impact on pig production worldwide. PWD is caused by many factors, such as the weaning period, removal from the sow, gastrointestinal and dietary changes, adaption to new environments, and social stress (Lallès *et al*. 2004; Lallès *et al*. 2007). It has been demonstrated to increase the mortality rate, and contribute to dehydration, gut dysfunction and growth retardation in surviving piglets (Amezcua *et al*. 2002; Rhouma *et al*. 2017). Colistin is an effective antibiotic used to mitigate PWD induced by Gram-negative bacteria, particularly *Escherichia coli* (Nguyen *et al*. 2016; Rhouma *et al*. 2017), whereas amoxicillin is a moderate-spectrum antibiotic, which effects both Gram-positive and Gram-negative bacteria. Despite the effectiveness of colistin in controlling PWD, several studies have reported high rates of colistin-resistant *E. coli* in swine (Amezcua *et al*. 2002; Nguyen *et al*. 2016). In response to this, some farmers have resorted to using a combination of antibiotics. Although co-treatment using colistin and amoxicillin is effective against PWD, the overuse of these antibiotics has raised concerns regarding the spread of multidrug resistant bacteria. Therefore, the European Medicines Agency has recommended reducing the use of colistin and amoxicillin in animal production, and restricting their use to the treatment of unhealthy animals (European Medicines Agency 2016).

To curb the escalating problem of antimicrobial resistance exacerbated by the livestock industry, probiotics and phytogenic compounds, such as essential oils, have been proposed as alternatives to the in-feed antibiotics. Numerous studies have confirmed the positive roles of *Bacillus* probiotic on growth performance and feed efficiency of piglets (Kyriakis *et al*. 1999), growing-to-finishing pigs (Davis *et al*. 2008; Upadhaya *et al*. 2015), and weaning-to-finishing pigs (Giang *et al*. 2012; Jørgensen *et al*. 2016). Lee *et al*. (2014) found that weaned pigs fed a 4500-g/tonne diet of *Bacillus subtilis* had lower faecal *Clostridium* spp. and coliforms counts. *Bacillus* species have also been observed to have positive effects on pig productivity when combined with other substances, such as organic acids and essential oils (Giang *et al*. 2012; Jiang *et al*. 2015). Ahmed *et al*. (2013) demonstrated that oregano oil successfully mitigated PWD caused by *E. coli*. in weaned pigs. Many reports have observed that the active substances present in essential oils, such as carvacrol and thymol, exhibit antimicrobial (Michiels *et al*. 2010) and immunostimulant (Li *et al*. 2012ab) properties, anti-oxidant activity, and safe-guard intestinal morphology (Xu *et al*. 2018). Our hypothesis is that combinations of essential oils and probiotics are more likely to modulate growth performance and immunity than in-feed antibiotics. Consequently, the primary goal of this research was to evaluate the combined or sole effect of essential oils (thymol and carvacrol) and a probiotic as a replacement for colistin and amoxicillin on the growth performance and incidence of diarrhoea in weaned pigs.

Materials and methods

The Institutional Animal Care and Use Committee at King Mongkut's Institute of Technology Ladkrabang reviewed and approved the animal protocol for the current trial.

In vitro *antagonistic test against Gram-negative bacteria*

The minimum inhibitory concentration of essential oils (mixture of 1.1% thymol and 2.2% carvacrol) against Gram-negative bacteria (*E. coli*) was determined using a broth microdilution method. Briefly, *E. coli* ATCC 25922 culture was first prepared by streaking a loopful of stock culture (kept at –80°C) onto Tryptic Soy Agar (Oxoid, Richmond, VA, USA) + 0.6% Yeast Extract (Lab M, Heywood, Lancashire, UK) agar plate and incubated overnight at 37°C. After incubation, several colonies were aseptically picked with a sterile loop, suspended in Tryptone Soy Broth (Oxoid) + 0.6% Yeast Extract (Lab M) broth and vortexed to ensure homogeneity. The bacterial suspension was subsequently diluted with Tryptone Soy Broth Yeast Extract broth until a final working concentration of ~6.7×10^5 cells/mL. A total of 150 µL of essential oil mixture in varying final concentrations (0.10–0.50% with an increase concentration of 0.10%; diluted in culture broth) were first added to individual wells of a 96-well microplate. The bacterial culture was inoculated into each well to yield a density of ~1.0×10^5 cells/well. After 48 h of incubation at 37°C, the minimum inhibitory concentration of *Bacilli*-based probiotic against Gram-negative bacteria was determined. The minimum inhibitory concentration is defined as the minimum concentration of tested product required to inhibit bacterial growth.

Experimental design and diet

A three-phase feeding program was carried out: the diets for Phases I (0–2 weeks), II (3–5 weeks) and III (6–8 weeks) contained 22.7%, 21.26% and 19.71% crude protein, and 1.38%, 1.18% and 1.07% total lysine, respectively. Experimental diets were provided by a local company (Top Feed Mills, Pathum Thani, Thailand), and both feed additives were provided by Kemin Industries, Singapore, and supplemented to the basal diets according to each treatment. The formulae and nutrient compositions of the basal diets are presented in Table 1. All experimental diets were fed to the pigs in a mash form.

A total of 96 crossbred ([Yorkshire × Landrace] × Duroc) pigs averaging 7.25 ± 0.24 kg in initial bodyweight (BW) were randomly assigned to each treatment based on sex and initial BW according to a randomised complete block design. Each treatment consisted of four replicates with four pigs per pen. The treatments were: (i) positive control (PC), basal diet supplemented with colistin 150 g/tonne and amoxicillin 200 g/tonne; (ii) negative control (NC), basal diet without supplementation; (iii) T3, basal diet supplemented with essential oils at 300 g/tonne diet; (iv) T4, basal diet supplemented with essential oils at 600 g/tonne diet; (v) T5, basal diet supplemented with essential oils at 1000 g/tonne diet; and (vi) T6, basal diet supplemented with essential oils at 300 g/tonne diet and *Bacillus* probiotics at 1000 g/tonne. The essentials oils consisted of a mixture of 2.2% carvacrol and 1.1% thymol (marketed as ORSENTIAL), whereas the probiotic contained 4×10^{11} cfu/kg *Bacillus subtilis* PB6 (marketed as CLOSTAT).

All experimental pigs were raised in an evaporative house with half-slatted concrete floors (0.96×2.16 m^2). The pigs were provided with water, and feed *ad libitum* from a self-feeder and a nipple drinker throughout the experimental period. The lighting program was artificial 20 h daily. The temperature was maintained at 31°C during the first 7 days and gradually decreased by 1°C on a weekly basis up to the 6th week, and subsequently maintained at 20°C for the 6th to 8th week. BW and feed intake were recorded on Week 0, 2, 5 and 8 to determine average daily gain, average daily feed intake, gain : feed ratio and feed efficiency.

Diarrhoea score

The occurrence of diarrhoea for each piglet was recorded from Day 1 to 21 during the feeding period. Diarrhoea was assessed

Table 1. Nutrient composition of the basal diets (%, as fed basis)
CP, crude protein; Met, methionine; Cys, cysteine

Ingredient	Phase I (0–2 weeks)	Phase II (3–5 weeks)	Phase III (6–8 weeks)
Broken rice	24.06	55.48	62.22
Full-fat soybean meal	19.25	13.87	9.72
Soybean meal (44% CP)	–	4.62	19.44
Soy protein concentrate	14.44	12.95	3.89
Sweet whey	19.25	9.25	–
Hydrolysed rice	19.25	–	–
L-lysine HCl	0.09	–	–
DL-methionine (98%)	0.19	0.13	0.09
L-threonine (98%)	0.09	–	0.06
Monodicalcium phosphate	1.92	2.22	2.33
$CaCO_3$	0.96	0.92	1.36
Salt	–	0.09	0.39
Vitamin–mineral premix[A]	0.50	0.47	0.50
Total	100.00	100.00	100.00
Calculated value			
Metabolisable energy (kcal/kg)	3393	3373	3321
Crude protein (%)	22.70	21.26	19.71
Fat (%)	4.07	3.37	2.73
Fibre (%)	1.77	2.03	2.61
Calcium (%)	0.84	0.89	1.01
Total phosphorus (%)	0.81	0.85	0.81
Available phosphorus (%)	0.47	0.50	0.46
Lys (%)	1.38	1.18	1.07
Met + Cys (%)	0.86	0.78	0.71
Lactose (%)	13.47	6.47	–

[A]Provided the following per kilogram of diet: vitamin A, 8000 IU; vitamin D_3, 1600 IU; vitamin E, 34 IU; d-biotin, 64 g; riboflavin, 3.4 mg; calcium pantothenic acid, 8 mg; niacin, 16 mg; vitamin B_{12}, 12 g; vitamin K, 2.4 mg, Se, 0.1 mg; I, 0.32 mg; Mn, 25.2 mg; $CuSO_4$, 53.9 mg; Fe, 127.3 mg; Zn, 83.46 mg; Co, 0.28 mg.

visually based on the faecal appearance collected at 0800 hours. A faecal consistency score was assessed visually in a range from 1 to 5 (1 = hard faeces; 2 = slightly soft faeces; 3 = soft, partially formed faeces; 4 = loose, semiliquid faeces; 5 = watery, mucus-like faeces; Gahan *et al*. 2009). Faecal consistency was determined per pen by recording the number of pigs within the category of faecal consistency scores. These data were used to calculate the diarrhoeal rate using the following equation: [total number of diarrhoeal pigs / (total number of pigs × days of experiment)] × 100.

Blood metabolites and immunological assay

Blood samples were collected from the jugular vein of piglets in control and treatment groups at the beginning (5 samples) and the end (24 samples; 4 samples per treatment) of the experiment. Five millilitres of blood was transferred immediately into non-heparinised tubes, and incubated at room temperature for 2 h before centrifugation at 3000*g* for 15 min. The serum was collected for blood urea nitrogen (BUN) and immunoglobulin analyses. The BUN was measured using a commercial test kit (Boehringer Mannheim, Mannheim, Germany), immunoglobulin A (IgA) and immunoglobulin G (IgG) were measured using an enzyme-linked immunosorbent assay (ELISA) kit (ELISA Stater Accessory Package, Pig IgG ELISA Quantitation Kit, Pig IgA ELISA Quantitation Kit, Bethyl Laboratories, Montgomery, TX, USA). For immunoglobulin measurement, each sample was analysed in triplicate with 1 : 10 000 (IgA) and 1 : 100 000 (IgG) fold dilution under the same conditions. The rest of the blood samples were sent directly to a commercial laboratory (Betagro Science Centre, Pathum Thani, Thailand) for field strain classical swine fever virus antibody analysis using a serum neutralisation test (neutralising peroxidase-linked assay against ALD strain).

Ammonia emission

Fresh faecal samples were collected in Weeks 0, 5 and 8 by direct rectal massage to determine the effect of dietary supplementation with essential oils and *B. subtilis* PB6 on ammonia reduction in pig excreta. Faecal samples were collected from four pigs (two gilts and two barrows, n = 24) in each treatment. A total of 50 g of fresh faecal samples were collected in duplicate and kept in a 2.6-L sealed plastic box. The samples were incubated at room temperature for 7 days to allow for fermentation using the method described by Cho *et al*. (2008). A total of 100 μL of headspace air was sampled for ammonia quantification using a gas detector. The ammonia concentration was detected within the ranges of 5–100 mg/kg (model GV-100S; Gastec Corp., Kanagawa, Japan) and 2–20 mg/kg (4 LK Detector tube; Gastec Corp.).

Statistical analyses

Data were analysed in a randomised complete block design using the general linear models procedure from statistical software package SAS (SAS Institute, Cary, NC, USA). Each pen was defined as an experimental unit for detecting growth performance and faecal consistency score, whereas selected individual pig was the experimental unit for BUN, immunological assay and ammonia emission. Duncan's new multiple range test declared statistically significant differences between the dietary treatments at the probabilities of $P < 0.05$ and $P < 0.01$. A split-plot in time was used to show significant differences in collection periods for faeces and blood ammonia, BUN, and immunoglobulin concentrations among treatments.

Results and discussion

The irresponsible use of antibiotics in the livestock industry has contributed to the emergence and widespread of antibiotic-resistant bacteria (Economou and Gousia 2015; Brown *et al*. 2017; Ronquillo and Hernandez 2017). Dietary supplements, such as probiotics and phytogenic compounds, have been proposed as the next-generation antibiotic alternatives (Ri *et al*. 2017; Poulsen *et al*. 2018). The current study reported the impact of dietary supplementation with essential oils and *Bacillus* probiotics on the incidence of diarrhoea and growth performance of weaned pigs.

Animal growth performance

The pigs receiving diet supplements (antibiotics, essential oils or combination of essential oils and probiotics) had better growth performance than the NC, in terms of BW ($P = 0.019$) and average daily gain ($P = 0.001$; Table 2). In addition, piglets treated with essential oils with and without probiotics gained, on average, 25–45 g per day more than the PC. It is important to note that average daily feed intake and feed efficiency were comparable among all groups, suggesting feed intake by piglets was not compromised, even at the highest essential oil concentration (1000 g/tonne). These findings are consistent with previous reports showing the beneficial effects of oregano essential oils on pig growth performance and productivity (Hong *et al*. 2004; Cho *et al*. 2006; Li *et al*. 2012*a*, 2012*b*; Xu *et al*. 2018). Overall, our findings and others support the positive role of essential oils (Michiels *et al*. 2010; Zou *et al*. 2016; Wei *et al*. 2017) and this may be further enhanced by the addition of probiotics to diets.

Diarrhoea incidence

The occurrence of PWD is common among piglets, and mainly results in *E. coli* infection (Kyriakis *et al*. 1999) and poor digestion (Li *et al*. 2012*a*). High rates of diarrhoea have been associated with piglets fed a diet without antibiotics or antibiotic replacement (Fairbrother *et al*. 2005). In this study, we showed that diarrhoea was significantly lower when piglets were given diets supplemented with antibiotics or essential oils alone, or in combination with *Bacillus* probiotics ($P = 0.001$; Table 2). These findings are consistent with previous reports indicating the anti-diarrhoeal effects of many plant extracts (with essential oils as the main ingredients; Jiang *et al*. 2015). For example, thymol and carvacrol, the active components in oregano essential oils, were reported to reduce faecal coliform (*E. coli*; Varel 2002; Jiang *et al*. 2015; Zou *et al*. 2016), suppress biofilm formation by pig faecal isolates (Oh *et al*. 2017) and increase *Lactobacilli* counts (Wei *et al*. 2017; Xu *et al*. 2018) in pigs. Jiang *et al*. (2015) demonstrated that essential oils primarily reduced the pathogen load in pigs' gastrointestinal tracts through anti-microbial activity and by reducing the viscosity of the intestinal digesta. Similarly, our *in vitro* study demonstrated the antimicrobial effect of thymol and carvacrol mixture against *E. coli* (Fig. 1). ORSENTIAL at 0.3% slightly decreased the growth of *E. coli*, and from 0.4%, the growth was totally inhibited. In contrast, previous studies showed that *Bacillus* supplementation could improve nutrient

Table 2. Growth performance of weaned pigs fed diets with essential oils and a *Bacillus* probiotic

PC, basal diet with colistin 150 g/tonne + amoxicillin 200 g/tonne; NC, basal diet without supplementation; T3, basal diet + essential oils 300 g/tonne; T4, basal diet + essential oils 600 g/tonne diet; T5, basal diet + essential oils 1000 g/tonne diet; T6 = basal diet + essential oils 300 g/tonne diet + *Bacillus* probiotics 1000 g/tonne diet; BW, bodyweight; ADG, average daily gain; ADFI, average daily feed intake; G : F, gain : feed ratio; FE, feed efficiency. Means within a row not sharing common lowercase letters differ significantly ($P < 0.01$)

Item	Dietary treatment						s.e.m.	*P*-value
	PC	NC	T3	T4	T5	T6		
				BW (kg)				
Initial	7.24	7.24	7.24	7.25	7.25	7.25	0.236	0.999
2nd week	11.15	11.04	11.48	11.37	11.38	11.30	0.375	0.993
5th week	19.74ab	18.39b	20.18ab	19.76ab	20.24ab	20.73a	0.472	0.001
8th week	29.49ab	27.30b	30.86ab	30.91ab	31.59a	31.97a	0.699	0.019
				ADG (g)				
0–2 weeks	279	271	303	294	295	290	11.327	0.867
3–5 weeks	409ab	350b	414ab	400ab	422a	449a	8.044	0.007
6–8 weeks	464ab	425b	509a	531a	540a	536a	14.135	0.001
0–8 weeks	397ab	358b	422a	422a	435a	442a	9.424	<0.001
				ADFI (g)				
0–2 weeks	381	374	392	392	386	371	12.141	0.992
3–5 weeks	673	676	677	681	674	705	14.788	0.993
6–8 weeks	753	728	774	731	755	741	21.237	0.994
0–8 weeks	630	620	642	624	633	635	14.535	0.999
				G : F ratio				
0–2 weeks	0.736	0.713	0.771	0.755	0.776	0.788	0.022	0.917
3–5 weeks	0.614	0.527	0.617	0.588	0.629	0.648	0.017	0.498
6–8 weeks	0.630	0.597	0.662	0.724	0.724	0.741	0.024	0.457
0–8 weeks	0.736	0.712	0.771	0.755	0.776	0.788	0.016	0.354
				FE				
0–2 weeks	1.39	1.42	1.33	1.33	1.31	1.31	0.039	0.953
3–5 weeks	1.66	1.95	1.64	1.72	1.60	1.58	0.051	0.392
6–8 weeks	1.65	1.71	1.52	1.40	1.41	1.40	0.055	0.413
0–8 weeks	1.61	1.73	1.52	1.49	1.46	1.44	0.040	0.288
Diarrhoea (%)	2.98b	5.16a	3.27b	3.27b	2.08b	2.18b	0.269	0.001

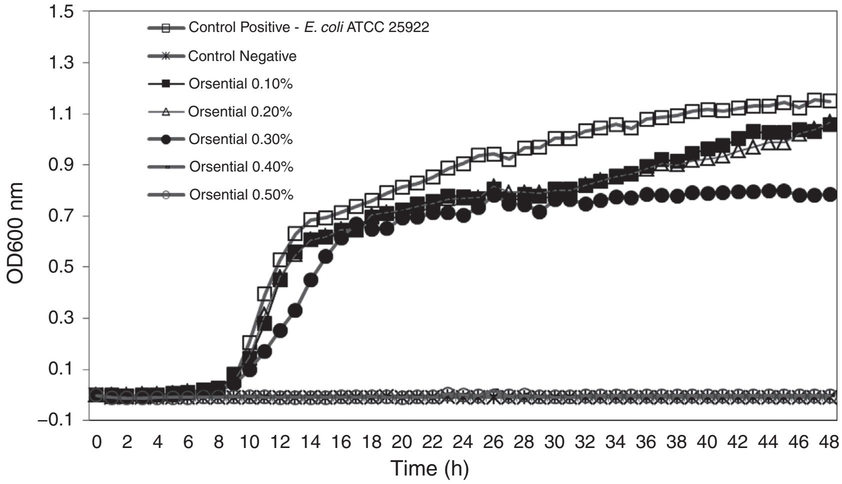

Fig. 1. Growth curve of *Escherichia coli* in response to different concentrations of essential oil mixture in liquid culture broth.

Table 3. Ammonia concentration of weaned pigs fed diets with essential oils and a *Bacillus* probiotic

PC, basal diet with colistin 150 g/tonne + amoxicillin 200 g/tonne; NC, basal diet without supplementation; T3, basal diet + essential oils 300 g/tonne; T4, basal diet + essential oils 600 g/tonne diet; T5, basal diet + essential oils 1000 g/tonne diet; T6, basal diet + essential oils 300 g/tonne diet + *Bacillus* probiotics 1000 g/tonne diet. Means within a row not sharing common lowercase letters differ significantly ($P < 0.05$)

Item	Dietary treatment						s.e.m.	*P*-value
	PC	NC	T3	T4	T5	T6		
				NH₃ (ppm)				
Initial	4.26	4.26	4.26	4.26	4.26	4.26	–	–
5th week	14.11	13.88	12.42	11.47	10.64	9.98	0.687	0.301
8th week	17.39a	15.28ab	13.58abc	12.16bc	11.01bc	9.86c	0.814	0.027
P-value								
Diet			0.018					
Duration			0.681					
Diet × duration			0.213					

digestion efficiency (Hermes *et al*. 2009), and reduce the occurrence and severity of PWD in piglets (Kyriakis *et al*. 1999). It is likely that probiotics play a role in digesting complex feed substrates, such as fibre and insoluble protein, reducing the production of branched chain fatty acids and ammonia (toxic metabolite), which may lead to the increased occurrence of diarrhoea. These observations suggest interactions between essential oils and probiotics, creating a favourable gastrointestinal tract environment for reducing the diarrhoea score of piglets.

Ammonia emission

Piglets fed with essential oils or a combination of essential oils and probiotics had lower faecal ammonia emission compared with the NC and PC (P = 0.027; Table 3). Particularly, treatment with >600 g/tonne of essential oils or lower essential oils (i.e. 300 g/tonne) combined with probiotics showed significantly lower ammonia emission compared with the PC (P = 0.027). Chen *et al*. (2006) observed ammonia concentration reduced in growing pigs treated with the 0.2% complex probiotics (*Lactobacillus acidophilus* 1.0×10^{10} cfu/kg; *Saccharomyces cerevisiae* 4.3×10^{9} cfu/kg; *B. subtilis* 2.0×10^{9} cfu/kg). Zhao and Kim (2015) also observed a reduction in faecal noxious emissions when probiotics were fed directly to weaned pigs. Ammonia emission was probably lowered due to the increased total volatile fatty acids in the caecum following feeding of essential oils (Xu *et al*. 2018). In contrast, Zhao and Kim (2015) observed that direct-fed probiotic could enhance intestinal morphology, leading to better nutrient digestibility. This means fewer substrates are available for microbial fermentation in the large intestine, resulting in lower emissions of faecal noxious gas to the environment (Yan *et al*. 2010). This finding supports the result of decreased BUN in the weaned pigs. Therefore, it appears that both feed additives reduce odour release from pig manure.

Serological profiles

The serological profiles of weaned piglets, including BUN and immunoglobin (i.e. IgG, IgA and classical swine fever virus-specific antibody), were determined (Tables 4 and 5). BUN is widely used to quantify nitrogen utilisation and excretion rates (Kohn *et al*. 2005); a lower BUN may indicate better utilisation of nitrogen for protein synthesis (Lan *et al*. 2016) and muscle generation (Kohn *et al*. 2005). Piglets fed with a diet containing essential oils alone or mixed with probiotics had lower BUN concentrations than the piglets in the NC (P = 0.027; Table 4), which is consistent with observations in broilers and nursery pigs (Zhu *et al*. 2014; Cai *et al*. 2015). However, it remains unknown, mechanistically, how essential oils or probiotics may lower the circulating concentration of BUN.

Serum immunoglobulins are generally considered an indicator of humoral immunity in animals. Dietary supplementation with antibiotics, essential oils or *Bacillus* probiotics did not elicit the classical swine fever virus antibody titre or IgA concentration (Tables 4 and 5, respectively). However, the serum IgG concentration was significantly increased by dietary supplementation with the 600 g/tonne essential oils or combination of 300 g/tonne essentials with probiotics compared with the NC (P = 0.014).

Table 4. Blood metabolites of weaned pigs fed diets with essential oils and a *Bacillus* probiotic

PC, basal diet with colistin 150 g/tonne + amoxicillin 200 g/tonne; NC, basal diet without supplementation; T3, basal diet + essential oils 300 g/tonne; T4, basal diet + essential oils 600 g/tonne diet; T5, basal diet + essential oils 1000 g/tonne diet; T6, basal diet + essential oils 300 g/tonne diet + *Bacillus* probiotics 1000 g/tonne diet. CSFV, classical swine fever virus. Means within a row not sharing common lowercase letters differ significantly (P < 0.01)

Item	Dietary treatment						s.e.m.	*P*-value
	PC	NC	T3	T4	T5	T6		
			Neutralising antibody against CSF virus (SN titre)					
Initial	18	18	18	18	18	18	–	–
8th week	21	16	19	21	18	16	2.286	0.647
			Blood urea nitrogen (mg/dL)					
Initial	6.21	6.21	6.21	6.21	6.21	6.21	–	–
5th week	12.31ab	13.92a	11.24bc	11.21bc	10.28bc	9.56c	0.359	0.002
8th week	23.27ab	24.93a	21.65ab	20.73ab	20.62ab	19.41b	0.556	0.039
P-value								
Diet	<0.001							
Duration	<0.001							
Diet × duration		0.968						

Table 5. Immunological parameters of weaned pigs fed diets with essential oils and a *Bacillus* probiotic

PC, basal diet with colistin 150 g/tonne diet + amoxicillin 200 g/tonne diet; NC, basal diet without supplementation; T3, basal diet + essential oils 300 g/tonne diet; T4, basal diet + essential oils 600 g/tonne diet; T5, basal diet + essential oils 1000 g/tonne diet; T6, basal diet + essential oils 300 g/tonne diet + *Bacillus* probiotics 1000 g/tonne diet; IgA, immunoglobulin A; IgG, immunoglobulin G. Means within a row not sharing common lowercase letters differ significantly (P < 0.01)

Item	Dietary treatment						s.e.m.	*P*-value
	PC	NC	T3	T4	T5	T6		
				IgA (mg/mL)				
Initial	0.66	0.66	0.66	0.66	0.66	0.66	–	–
5th week	0.47	0.39	0.46	0.45	0.49	0.48	0.104	0.204
8th week	0.96	0.89	1.03	1.04	1.07	1.04	0.041	0.114
P-value								
Diet	0.003							
Duration	<0.001							
Diet × duration			0.938					
				IgG (mg/mL)				
Initial	0.78	0.78	0.78	0.78	0.78	0.78	–	–
5th week	0.58ab	0.39b	0.57ab	0.66a	0.61ab	0.62ab	0.027	0.067
8th week	0.63ab	0.52b	0.77ab	0.89a	0.81ab	0.86a	0.037	0.014
P-value								
Diet	0.001							
Duration	0.001							
Diet × duration		0.659						

This finding is consistent with that of Trevisi *et al*. (2007), who demonstrated that thymol supplementation could increase IgG concentration in the sera of weaning pigs and in non-challenged pigs' intestine (Li *et al*. 2012*b*). Thymol supplementations may increase goblet cells in animals' ileum (Zhang *et al*. 2017) and suppress the growth of pathogenic bacteria (Hedegaard *et al*. 2016). Together, these results clearly suggest that dietary supplementation with essential oils and probiotics can enhance pig immunity by modulating the humoral immune system. This may provide additional protection against microbial infection and thus reduce diarrhoea among piglets at weaning.

Conclusions

Dietary supplementation with essential oils can improve growth performance and reduce diarrhoea of weaned pigs in a dosage-dependent manner (i.e. 300 g/tonne to 1000 g/tonne). The positive effects of essential oils on pig growth and diarrhoea control can be further enhanced with the inclusion of *Bacillus* probiotics. These findings provide a basis for the application of phytogenic compounds and probiotics as antibiotic growth promoter alternatives in feed.

Conflicts of interest

The authors declare that they have no conflicts of interest with any financial organisation regarding the material discussed. Tan Boon Fei and Lim Tricia are employees of Kemin Industries (Singapore). Waewaree Boontiam conducted the experiment, collected and analysed trial data, and did not receive any personal benefits from the funder. All authors have read and approved this research article.

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