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Growth performance, nutrient digestibility, fecal microbiota and fecal noxious gas emission in weaning pigs fed high and low density diet with and without protected organic acid blends

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ABSTRACT

This study was conducted to evaluate the effects of dietary supplementation of protected organic acid blends including medium chain fatty acids and different nutrient density diets on growth performance, nutrient digestibility, fecal microbiota and fecal noxious gas emission in weaning pigs. A total of 90 crossbred [(Landrace × Yorkshire) × Duroc] weaning pigs with an initial body weight (BW) of 6.54 ± 0.78 kg were used in a six-week trial. Pigs were randomly allocated into one of six treatment groups in a 2 × 3 factorial arrangement with two nutrient density diets; high density (HD) vs low density (LD) supplemented with or without two levels (0.1 and 0.2%) of protected organic acids (OA) according to their sex and BW (3 replicate pens per treatment with 2 gilts and 3 barrows per pen). The inclusion of OA (0.1%, 0.2%) improved ($P < 0.05$) the average daily gain (ADG) during week 6. Likewise, during the overall experimental period, the ADG was higher ($P < 0.05$) in pigs fed HD diet than LD diet as well as in OA supplemented diet, but average daily feed intake (ADFI) was significantly improved ($P < 0.05$) only in diet supplemented with OA than non-supplemented diet. The gain: feed (G:F) ratio was higher ($P < 0.05$) in pigs fed HD than LD diet during week 3 and week 6 and overall experimental periods. The supplementation of OA (0.2%) led to a higher ($P < 0.05$) apparent total tract digestibility (ATTD) of energy during week 3 and week 6 compared with non-supplemented diet. In week 3, dry matter (DM) digestibility was higher in pigs fed diet supplemented with 0.2% OA but in week 6, DM digestibility was higher in both 0.1% and 0.2% OA treatments compared with control. The ATTD of energy was higher ($P < 0.05$) in pigs fed HD than LD diet during week 6. The supplementation of OA (0.2%) in the diet reduced fecal *E.coli* during week 3 and increased *Lactobacillus* counts during week 6. However, the fecal noxious gas emissions were not affected either by OA or diets. In conclusion, protected OA and different density diets improved growth performance, nutrient digestibility but no interactive effects with different density diets and OA were observed.

Abbreviations: LD, low density diet; ADFI, average daily feed intake; ADG, average daily gain; ATTD, apparent total tract digestibility; BW, body weight; DM, dry matter; N, nitrogen; GE, gross energy; G:F, gain:feed; HD, high density diet

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1. Introduction

Weaning is one of the most challenging events that piglets undergo in their life time. It includes numerous acute and chronic stressors including separation from sows, changes in their diet from liquid to solid, alteration in housing, mixing with unfamiliar pigs, and transportation. The dietary alteration has a great influence on gut local immune status, gut microflora and digestive enzyme activity. The problematic effects of delayed feeding and increased behavioral problems have been reported due to the change in diet after weaning. At the initial phase of weaning, the secretion of hydrochloric acid and digestive enzymes in the stomach is not enough to lower gastric pH leading to digestion problems (Tsiloyiannis et al., 2001). Digestion is further compromised by the administration of complex diets with low quality protein sources which eventually contribute to the proliferation of pathogenic microorganisms and development of intestinal disorders (Viola and Vieira, 2007). Taken together, all of these factors contribute to post-weaning diarrhea leading to reduced growth performance and in certain cases it results in the death of animals (Corassa et al., 2006)

In order to prevent diarrhea and improve the performance, the prophylactic doses of antimicrobial feed additive and antibiotic growth promoter were added in weaner diets for over 50 years. However, the ban of antibiotics as growth promoters in European Union (EU) since January 2006 due to the risk of bacteria acquiring resistance to specific antibiotics has posed a big challenge to swine producers for raising pigs specially the weaning pigs that are susceptible to microbial infection. To address these different problems, in recent days researchers are focusing on to the evaluation of the effectiveness of different feed additives. Among several additives, organic acids are proposed as one of the generally recognized as effective alternatives to antibiotics. Organic acids possess both bacteriostatic and bactericidal properties depending on the dosage used. They can effectively be used with other additives (Grilli et al., 2010). The working mechanism of organic acids includes the lowering of gut pH, stimulation of enzyme secretion, inhibition of pathogenic bacteria, improving nutrient digestibility and retention, leading to improved growth performance (Baustadt, 1993; Papatsiros et al., 2012). Previous reports indicated that organic acids reduce coliform population in the gut thereby reducing the incidence of post-weaning diarrhea in weaning pigs (Piva et al., 2002; Papatsiros et al., 2011). Likewise, the inclusion of different levels of fumaric acid in the diet (Miguel et al., 2011), as well as the use of different combinations of formic acid, lactic acid, propionic acid and sodium butyrate (Braz et al., 2011), significantly improved different performance parameters, including average daily gain, in post weaning piglets. In contrast, Boas et al. (2016) reported that organic acids (mixture of lactic, formic and citric acid) with or without sodium butyrate, did not have any effect on growth performance and nutrient digestibility of weaned pigs.

The use of the micro-encapsulation technology with organic acids for effective delivery of OA is limited. Organic acids have to be used in feeds with a reduced acid binding capacity and of high digestibility in order to be effective. Generally, organic acids get dissociated and lose most of their antibacterial capacity before reaching to the distal part of the digestive system. For this reason, a microencapsulation (protection) technology was developed to allow organic acids to reach the distal parts of the gastro-intestinal tract progressively without being totally dissociated and maintaining their efficacy. The protected organic acid could be used in reduced inclusion rate compared with non- protected ones due to their effectiveness. Protected organic acids are reported to be more effective in retarding absorption of dietary acids and allowing more effective delivery of the acids to the distal ileum, cecum, and colon of piglets (Piva et al., 2007). There are very limited studies on protected organic acid supplementation to weaning pigs. In addition, we were interested in exploring the opportunity for least cost feed formulations, by supplementing protected organic acid blends in low nutrient diet (LD) versus high nutrient density diets (HD) to see if they have interactive effects as well as to compare the supplementation of HD diet versus LD diets.

Therefore, the aim of this study was to evaluate the effect of protected OA on growth performance, nutrient digestibility, fecal microbiota and fecal noxious gases emission in weaning pigs fed both high and low density diets.

2. Materials and methods

The experimental protocols describing the management and care of animals were reviewed and approved by the Animal Care and Use Committee of Dankook University.

2.1. Source of organic acid

The protected organic acid used in the current experiment is provided by a commercial company (Morningbio Co., Ltd., Cheonan, Korea). This protected organic acid consists of medium chain fatty acid and composite organic acids. The active ingredients are 17% fumaric acid, 13% citric acid, 10% malic acid and 1.2% medium chain fatty acid (capric and caprylic acid) and carrier vegetable oil 58.8%).

2.2. Animals and diets

A total of 90 crossbred weanling pigs [(Yorkshire × Landrace) × Duroc, 28 days old] with an average body weight (BW) of 6.54 ± 0.78 kg were used in a 6-week experiment trial. Pigs were blocked based on BW and sex and randomly allotted to 1 of 6 dietary treatments (3 replicate pens per treatment with 2 gilts and 3 barrows per pen) in a 2 × 3 factorial arrangement with two nutrient density diets; high density vs low density supplemented with or without two levels (0.1 and 0.2%) of protected OA. All nutrients in diets were formulated to meet or exceed the recommendation of NRC National Research and Council (2012) except for low density diets which were below NRC recommendations for weanling pigs and fed in mash form (Table 1). The animals were fed two phases diet (first phase diet were fed from day 1- day 21 and phase 2 diet were fed from day 22-day 42). The additive was

Table 1
Composition of basal diets[c] (as- fed basis; g/kg).

	Phase 1 (day 1- 21)		Phase 2 (day 22–42)	
Ingredients	High	Low	High	Low
Extruded corn	373.9	479.0	479.0	559.3
Soybean meal (Dehulled)	120.0	180.0	180.0	240.0
Fermented soybean meal	100.0	80.0	80.0	50.0
LT Fish meal	76.0	27.0	27.0	–
Soy oil	31.3	32.0	32.0	32.5
DCP	12.4	13.4	13.4	16.3
Limestone	6.0	7.4	7.4	8.2
Sugar	30.0	20.0	20.0	20.0
Whey protein	110.0	80.0	80.0	30.0
Lactose	128.0	67.0	67.0	30.0
L-Lysine − HCL	3.5	4.6	4.6	4.8
DL-Methionine	1.8	1.7	1.7	1.9
Threonine	2.1	2.9	2.9	2.0
Choline Chloride 50%	1.0	1.0	1.0	1.0
Vitamin premix[a]	2.0	2.0	2.0	2.0
Mineral premix[b]	2.0	2.0	2.0	2.0
Calculated composition				
Digestible energy, MJ/kg	16.75	16.33	16.33	15.91
Crude Protein	200.0	190.0	190.0	185.0
Crude Fat	54.0	48.0	48.1	42.0
Calcium	8.0	7.5	7.5	7.5
Phosphorus	7.0	6.5	6.5	6.5
Lysine	16.0	15.0	15.0	14.0
Methionine	4.8	4.5	4.5	4.2
Lactose	200.0	120.0	120.0	50.0
Calculated SID of amino acids				
Lysine	10.0	10.1	10.1	9.7
Methionine	3.2	3.2	3.2	3.1
Threonine	6.4	7.1	7.1	6.5
Tryptophan	1.2	1.3	1.3	1.4
Analyzed composition				
Crude Protein	200.8	190.3	189.6	184.7
Lysine	16.2	15.1	15.2	14.1
Methionine	4.8	4.5	4.4	4.0
Calcium	7.9	7.5	7.4	7.5
Phosphorous	7.0	6.5	6.5	6.5
Lactose	199.8	120.1	119.9	51.0

[a] Provided per kilogram of complete diet: vitamin A, 11,025 IU; vitamin D3, 1103 IU; vitamin E, 44 IU; vitamin K, 4.4 mg; riboflavin, 8.3 mg; niacin, 50 mg; thiamine, 4 mg; d-pantothenic acid, 29 mg; choline, 166 mg and vitamin B12, 33 μg.

[b] Provided per kilogram of complete diet: Fe (as FeSO4 × 7H2O), 80 mg; Cu (as CuSO4 × 5H2O), 12 mg; Zn (as ZnSO4), 85 mg; Mn (as MnO2), 8 mg; I (as KI), 0.28 mg and Se (as Na2SeO3 × 5H2O), 0.15 mg.

[c] Diets were in mash form.

supplemented into the diet by replacing the same amount of corn. All pigs were housed in an environmentally-controlled room. Each pen had an area of 0.26 × 0.53 m^2 for each pig. A stainless steel feeder and a nipple drinker were provided in each pen, and pigs were allowed *ad libitum* access to feed and water throughout the experiment. Ventilation was provided by a mechanical system. Twelve hours of artificial light per day was automatically regulated. Approximately 30 °C of ambient temperature was maintained within the room during the first week and was decreased by 1 °C each week of the experiment.

2.3. Sampling and measurement

The individual pig BW and feed consumption of each pen were determined at the start of the experiment, day 21 and day 42 to calculate the average daily gain (ADG), average daily feed intake (ADFI), and gain:feed ratio (G:F). Pigs were fed diets mixed with chromic oxide (0.2%) as an indigestible marker 5 days prior to fecal sample collection to determine the apparent total tract digestibility (ATTD). Fecal samples were collected from 6 pigs per treatment (1 gilt and 1 barrow per pen per treatment) by direct rectal massage on day 21 and day 42 of the experiment to determine the apparent total tract digestibility (ATTD) of dry matter (DM), nitrogen (N) and energy (E). The collected fecal samples and feed samples were dried at 70 °C for 72 h, after which they were ground to pass through a 1 mm screen. Diet and fecal samples were analyzed for dry matter (method 930.15; AOAC International, 2000), N (method 968.06; AOAC International, 2000), Ca (method 984.01; AOAC International, 2000), P (method 965.17; AOAC International, 2000), gross energy using a bomb calorimeter (Parr 1281 Bomb Calorimeter, Parr Instrument Co., Moline, IL, USA).

Lysine was measured using an AA analyzer (Beckman 6300; Beckman Coulter Inc.,Fullerton, CA) after a 24-h hydrolysis in HCl). Methionine and cysteine were determined as Met sulfone and cysteic acid after cold performic acid oxidation overnight before hydrolysis (method 982.30 E (b); AOAC International, 2007). Nitrogen was determined (Kjectec 2300 Nitrogen Analyzer; Foss Tecator AB, Hoeganaes, Sweden), and CP was calculated as N x 6.25. Chromium levels were determined via UV absorption spectrophotometry (UV-1201; Shimadzu, Kyoto, Japan) following the method described by Williams et al. (1962). The ATTD of DM, N and E were calculated using indirect-ratio methods using the following formula:

$$\text{Apparent total tract digestibility} = (1\text{-}((Nf \times Cd)/Nd \times Cf)) \times 100\%),$$

where Nf = nutrient concentration in feces (% DM), Nd = nutrient concentration in diet (%DM), Cf = chromium concentration in feces (% DM), and Cd = chromium concentration in diet (% DM).

For the analysis of the fecal NH_3, total mercaptans, and H_2S, the fresh feces were collected from randomly selected 6 pigs per treatment (1gilt and 1 barrow per pen per treatment) at the end of day 21 and day 42. The total sampled feces was then thawed and homogenized. Then, the stock feces (300 g) were stored in 2.6-L plastic boxes with a small hole in the middle of one side that was sealed with adhesive plaster. The samples were fermented for 24 h at room temperature (25 °C), and 100 mL of the headspace air was sampled from approximately 2.0 cm above the fecal sample. After the collection, box was re-sealed with adhesive plaster to measure the fecal noxious content. The fecal samples were manually shaken for approximately 30 s before measurement to disrupt any crust formation on the surface of the fecal sample and to homogenize the samples. Concentrations of NH_3, H_2S, and total mercaptans were measured within the scopes of 5.0–100.0 ppm (No. 3La, detector tube; Gastec Corp. Kanagawa, Japan) and 2.0–20.0 ppm (4LK, detector tube; Gastec Corp.).

For fecal microbial analysis, fecal samples were collected from randomly selected 6 pigs per treatment (1gilt and 1 barrow per pen per treatment), at day 21 and day 42 via rectal massage. The samples were then pooled on pen basis and placed on ice for transportation to the lab. One gram of the composite fecal sample from each pen was diluted with 9 mL of 1% peptone broth (Becton, Dickinson and Co., Franklin Lakes, NJ), and it was homogenized. Viable counts of bacteria in the fecal samples were then conducted by plating serial 10-fold dilutions (in 1% peptone solution) onto MacConkey agar plates (Difco Laboratories, Detroit, MI) and lactobacilli medium III agar plates (Medium 638, DSMZ, Braunschweig, Germany) to isolate the *E. coli* and *Lactobacillus*, respectively. The MacConkey agar plates were incubated for 24 h at 37 °C, and the lactobacilli medium III agar plates were incubated for 48 h at 39 °C, under anaerobic conditions. The *E. coli* and *Lactobacillus* colonies were counted immediately after removal from the incubator.

2.4. Statistical analysis

The data were analyzed as a 2 × 3 factorial arrangement using the GLM procedure of SAS (SAS Institute, 2009), with the pen as the experimental unit. The model included the main effects of OA and diet density, as well as the interaction between OA and diet density. Before carrying out statistical analysis of the microbial counts, logarithmic conversion of the data was performed. Least square means were separated with the PDIFF option and adjusted with Duncan multiple comparison procedure. Variability in the data was expressed as pooled SE and probability level of $P < 0.05$ was considered significant and $P < 0.10$ as trends.

3. Results

3.1. Growth performance

As shown in Table 2, the inclusion of 0.2% OA in the diet improved ($P < 0.05$) the ADG during day 42. During the overall experimental period, the ADG was higher ($P < 0.05$) in pigs fed HD diet than LD diet as well as in OA supplemented diet, but ADFI was significantly improved ($P < 0.05$) only in diet supplemented with 0.1 and 0.2% OA than non-supplemented diet. A trend ($P = 0.09$) in increment of G: F ratio was observed in pigs fed 0.2% OA supplemented diet compared with 0.1% OA during overall period. The G:F ratio was higher ($P < 0.05$) in pigs fed HD than LD diet during week 3, week 6 and overall experimental periods. No interactive effects between OA and diet density were observed in the growth performance parameters.

3.2. Apparent total tract nutrient digestibility

A higher ($P < 0.05$) ATTD of DM and energy digestibility was observed in pigs fed 0.2% OA supplemented diet during week 3 compared with control. In week 6, DM digestibility was higher in pigs fed diet supplemented with both 0.1% and 0.2% OA, but energy digestibility was higher only in pigs fed diet supplemented with 0.2% OA compared with non-supplemented diet. The ATTD of energy was higher ($P < 0.05$) in pigs fed HD than LD diet during week 6 (Table 3). No interactive effects between OA and diet density were observed in nutrient digestibility.

3.3. Fecal noxious gas emission

The fecal noxious gases (NH_3, H_2S and total mercaptans) emission were not affected ($P > 0.05$) either by diet density or by OA supplementation, and no interactive effects were observed (Table 4).

Table 2
Effect of dietary organic acid supplementation on growth performance in weaning pigs.[1]

Items	HD			LD			SE[2]	P-value		
	0	0.10%	0.20%	0	0.10%	0.20%		Den	OA	Den × OA
BW, kg (initial)	6.46	6.49	6.55	6.6	6.56	6.64	0.471	0.82	0.98	1.00
BW, kg (wk 3)	14.46	14.71	14.92	14.35	14.43	14.68	0.693	0.71	0.85	0.99
BW, kg (wk 6)	25.2	25.93	26.43	24.6	25.34	25.81	0.796	0.37	0.34	0.99
3 wk										
ADG, g	381.0	391.6	398.6	369.0	374.6	383.0	12.57	0.17	0.47	0.98
ADFI, g	540.6	554.3	560.6	556.0	559.3	556.7	25.01	0.79	0.91	0.93
G/F	0.705	0.706	0.711	0.664	0.670	0.688	0.02	0.03	0.65	0.86
6 wk										
ADG, g	511.3[b]	534.0[a]	547.7[a]	488.3[b]	519.7[a]	530.0[a]	11.9	0.08	0.02	0.93
ADFI, g	789.0	827.3	819.7	813.7	838.0	840.3	19.1	0.25	0.23	0.93
G/F	0.648	0.646	0.668	0.600	0.620	0.630	0.02	0.02	0.35	0.79
Overall										
ADG, g	446.3[b]	463.0[ab]	473.3[a]	428.3[b]	447.0[ab]	456.3[a]	9.18	0.04	0.03	0.99
ADFI, g	664.7[b]	690.7[a]	690.3[a]	685.0[b]	698.7[a]	698.7[a]	7.99	0.09	0.04	0.69
G/F	0.671[b]	0.670[ab]	0.686[a]	0.626[b]	0.640[ab]	0.653[a]	0.008	< 0.001	0.07	0.60

[a,b]Means in the same row with different superscripts differ (P < 0.05). Values of means represent 5 pigs per replicate pens (n = 15 pigs) per treatment.
[1] Abbreviation: HD = high nutrient density diet with 0%, 0.1% and 0.2% organic acid; LD = low nutrient density diet with 0%, 0.1% and 0.2% organic acid; Den = density;)A = organic acid; Den x OA = Interaction between diet density and Organic acid.
[2] Pooled standard error.

Table 3
Effect of dietary organic acid supplementation on apparent total tract nutrient digestibility in weaning pigs.[1]

Items, %	HD			LD			SE[2]	P-value		
	0	0.10%	0.20%	0	0.10%	0.20%		Den	OA	Den × OA
3 wk										
Dry Matter	80.69[b]	81.79[ab]	82.66[a]	80.49[b]	81.88[ab]	82.38[a]	0.73	0.82	0.04	0.97
Nitrogen	80.55	80.97	81.86	81.37	82.55	82.57	0.71	0.08	0.22	0.8
Energy	81.75[b]	82.63[ab]	83.32[a]	81.44[b]	82.79[ab]	83.21[a]	0.67	0.87	0.05	0.94
6 wk										
Dry Matter	78.30[b]	80.87[a]	80.98[a]	78.17[b]	79.25[a]	79.32[a]	0.72	0.06	0.02	0.49
Nitrogen	78.79	78.67	78.86	79.24	79.17	79.63	0.86	0.90	0.64	0.55
Energy	79.35[b]	81.79[ab]	81.92[a]	79.19[b]	79.67[ab]	79.94[a]	0.74	0.02	0.06	0.34

[a,b]Means in the same row with different superscripts differ (P < 0.05). Values of means represent 2 pigs per replicate pens (n = 6 pigs) per treatment.
[1] Abbreviation: HD = high nutrient density diet with 0%, 0.1% and 0.2% organic acid; LD = low nutrient density diet with 0%, 0.1% and 0.2% organic acid; Den = density;)A = organic acid; Den x OA = Interaction between diet density and Organic acid.
[2] Pooled standard error.

Table 4
Effect of dietary organic acid supplementation on fecal noxious gases emission in weaning pigs.[1]

Items, ppm	HD			LD			SE[2]	P-value		
	0	0.10%	0.20%	0	0.10%	0.20%		Den	OA	Den × OA
3 wk										
NH_3	5.21	5.17	5.13	5.17	5.13	5.11	0.26	0.87	0.96	1.00
H_2S	6.23	6.17	6.10	6.20	6.17	6.13	0.17	1.00	0.84	0.98
R.SH	3.83	3.8	3.77	3.83	3.77	3.70	0.15	0.79	0.79	0.98
6 wk										
NH_3	7.40	7.23	7.37	7.30	7.30	7.28	0.42	1.00	0.94	0.98
H_2S	5.27	5.23	5.23	5.23	5.23	5.21	0.12	0.82	0.96	0.99
R.SH	3.07	3.03	3.07	3.10	3.10	3.13	0.11	0.82	0.84	0.80

Values of means represent 2 pigs per replicate pens (n = 6 pigs) per treatment.
[1] Abbreviation: HD = high nutrient density diet with 0%, 0.1% and 0.2% organic acid; LD = low nutrient density diet with 0%, 0.1% and 0.2% organic acid; Den = density;)A = organic acid; Den x OA = Interaction between diet density and Organic acid.
[2] Pooled standard error.

Table 5
Effect of dietary organic acid supplementation on fecal microflora in weaning pigs.[1]

Items, log 10 cfu	HD			LD			SE[2]	P-value		
	0	0.10%	0.20%	0	0.10%	0.20%		Den	OA	Den × OA
3 wk										
Lactobacillus	7.13	7.19	7.24	7.12	7.13	7.17	0.06	0.30	0.32	0.85
E.coli	6.34[a]	6.17[ab]	6.10[b]	6.26[a]	6.15[ab]	6.11[b]	0.07	0.59	0.02	0.82
6 wk										
Lactobacillus	7.04[b]	7.07[b]	7.30[a]	7.00[b]	7.02[b]	7.20[a]	0.07	0.28	0.01	0.89
E.coli	6.16	6.15	6.08	6.19	6.2	6.14	0.05	0.32	0.41	0.95

[a,b] Means in the same row with different superscripts differ (P < 0.05).
Values of means represent 2 pigs per replicate pens (n = 6 pigs) per treatment.
[1] Abbreviation: HD = high nutrient density diet with 0%, 0.1% and 0.2% organic acid; LD = low nutrient density diet with 0%, 0.1% and 0.2% organic acid; Den = density;)A = organic acid; Den x OA = Interaction between diet density and Organic acid.
[2] Pooled standard error.

3.4. Fecal microbial enumeration

The supplementation of 0.2% OA in the diet reduced fecal *E.coli* during week 3 and increased *Lactobacillus* counts during week 6. The density of diet did not have any effect in fecal microbial counts during both phases (week 3 and week 6) (Table 5).

4. Discussion

4.1. Effects of protected organic acid blends

Organic acids have been widely used as a partial substitution of antibiotic growth promoters due to their acidifying and antimicrobial properties. In the current study, the supplementation of 0.1% and 0.2% OA blends led to higher ADG during the second phase of experiment and inclusion of 0.2% OA blends increased digestibility of DM and energy during both phases of experiment compared with non-supplemented diet. In line with our studies, previous studies also confirmed the positive effects of organic acids in weaning pigs. For instance, Hanczakowska et al. (2011) demonstrated that when piglets were fed diet supplemented with a mixture of 1.5% fumaric acid and 0.2% capric or 0.2% caprylic acid significantly improved bodyweight gain compared with control, and Li et al. (2008) reported that 0.5% blends of organic acids such as butyric, fumaric and benzioic acid in the diet of weanling piglet improved growth performance. Franco et al. (2005) noted that mixture of formic: lactic acid or formic with fumaric had a slightly higher DM digestibility than control. Wang et al. (2009) demonstrated that inclusion of 0.5% phenyl lactic acid improved nutrient digestibility in weanling pig. Our previous studies on growing pigs and finishing pigs fed 0.1 and 0.2% protected OA blends having 17% fumaric acid, 13% citric acid, 10% malic acid and 1.2% medium chain fatty acid (capric and caprylic acid) also showed improved growth performance and nutrient digestibility (Upadhaya et al., 2014a,b; Upadhaya et al., 2016). The improvement in ADG may be associated with antimicrobial property of OA that might have increased the availability of dietary energy to host animal due to reduced pathogenic microbes in the gastrointestinal tract. In contrast Zentek et al. (2013) indicated that diets supplemented with organic acids (0.416% fumaric and 0.328% lactic acid), with medium chain fatty acids (MCFA; 0.15% caprylic and capric acid) had no growth enhancing effects in weaning piglets. The positive effect of OA blends on ADG and nutrient digestibility in the current study at relatively lower dose compared with other studies could be due to the encapsulation of OA blends that reduce the dissociation of OA in the stomach and allow the delivery of protected OA in an undissociated form to the distal ileum, cecum, and colon of piglets (Piva et al., 2007). The inconsistent result on growth performance and nutrient digestibility among different studies could be due to diet complexity, types of acids, inclusion level of acids, age and the health status of the pigs.

The pH in upper gastro intestinal tract of weaning pig is high because they are usually not ready to produce enough hydrochloric acid. This high pH promotes the proliferation of certain bacteria particularly coliform bacteria (Sissons, 1989). Organic acids are considered as a feed additive having the ability to lower gastric pH thereby inhibiting the growth of pathogenic bacteria (Giesting and Easter, 1985; Bosi et al., 1999). A study by Zentek et al. (2013) also noted that combination of OA such as 0.416% fumaric and 0.328% lactic acid and medium chain fatty acid (0.15% capric and caprylic acid) reduced *E.coli* counts in the colon of weaning pigs. Ahmed et al. (2014) also indicated that 0.4% acidifier blend (17.2% formic acid, 4.1% propionic acid, 10.2% lactic acid, 9.5% phosphoric acid, SiO2 34.0%) in the basal diet significantly reduced fecal counts of pathogenic gram-negative *Salmonella* and *E. coli* and increased beneficial *Lactobacilli* and *Bacilli* concentrations compared to control. In the current study, we observed the significant reduction of *E. coli* population in the feces from pigs fed protected 0.2% OA supplemented diet during the first phase and then increase in *Lactobacillus* population in the second phase of the experiment. This result is apparently associated with the fact that organic acids can maintain a lower pH in the gastric digesta of weaned pigs and therefore reduce the proliferation of pathogenic bacteria such as *E.coli* in the gastro intestinal tract (Richards et al., 2005) because coliform bacteria cannot tolerate acidic condition. The noxious gases from the feces of pigs fed OA were not affected despite the reduction of pathogenic bacterial load.

4.2. Effects of diet density

Prior to weaning, piglets are fed liquid diet that contains approximately 30% protein, 35% fat, and 25% lactose on a dry matter basis. After weaning, pigs are normally fed drastically different diet composition consisting of low fat, low lactose, high carbohydrate diet composed of cereal grain and soybean meal in a dry form. The change in the form and nutrient constituent of diet compromises the growth of weaning during the early phase of post weaning. Thus, we designed our experiment to evaluate the effect of high and low nutrient diet supplemented with or without protected organic acid blends in weaning pigs. Thus, for high nutrient diet, a portion of corn was substituted by fish meal, lactose and whey protein, which increased CP, digestible energy and fat. As a result of our study, we observed the G:F ratio in pigs fed high density diet was significantly increased than low density diet during week 3, week 6 and during overall. The ADG tended to increase during week 6 and significantly improved during overall in pigs receiving high nutrient density diet. This result is in agreement with the finding by Ball and Aherne (1987) who also demonstrated that high density diet led to higher G: F ratio and greater gains in weaning pigs compared with normal diet. Our previous study on the growing pigs fed 0.1% protected organic acids blends (17% fumaric acid, 13% citric acid, 10% malic acid, and 1.2% medium chain fatty acid (capric and caprylic acid) demonstrated that ADG, G:F ratio was significantly higher and DM digestibility was slightly higher in pig fed HD compared to LD diets (Upadhaya et al., 2014a). Likewise, in the current study, the energy digestibility was significantly increased and a tendency in DM digestibility was observed during the later phase of the experiment in pigs fed HD diet than LD diet. These results suggest that ad libitum intake of the HD diet might have met the daily nutrient requirements of the weaned pigs in this experiment which may explain the increased growth performance in the present study. However, the fecal microbiota and fecal noxious gases emission were not influenced by the diet density. In the current study, there were no clear interaction between nutrient density and protected organic acid blends on any of growth performance parameters, nutrient digestibility, fecal microbiota counts, and noxious gases emission.

5. Conclusion

In conclusion, protected OA blends supplementation improved growth performance, nutrient digestibility and fecal *Lactobacillus* counts. Among two doses of OA, 0.2% OA showed better results than 0.1% OA in terms of nutrient digestibility and fecal microbiota. Likewise, the high density diets showed better growth performance and nutrient digestibility compared with low density diets. No additive effects were observed between diet density and protected organic acid blends indicating that the mechanism for improved growth performance and nutrient digestibility were independent and not additive.

Conflict of interest statement

We confirm that there are no known conflicts of interest associated with this publication.

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