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Coated sodium butyrate supplementation to a reduced nutrient diet enhanced the performance and positively impacted villus height and faecal and digesta bacterial composition in weaner pigs

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ABSTRACT

A study was conducted to evaluate the effects of dietary supplementation of different levels of coated sodium butyrate (CSB) on growth performance, nutrient digestibility, faecal and intestinal coliform and lactic acid bacteria counts, gas emission and small intestinal villi length in weaning pigs. A total of 160 weaning pigs [(Landrace x Yorkshire) x Duroc, 28 days old] with an average initial body weight (BW) of 7.04 ± 1.15 kg were randomly allotted to 4 treatments (8 replicates per treatment with 5 pigs; 3 barrows and 2 gilts per replicate pen) according to their initial body weight for a 6-week trial in three phases. Treatments consisted of corn-soybean meal-based diet with slight reduction in lactose (approximately 5 %) as well as sodium content relative to National Research Council (NRC, 2012) requirement as the control (CON) and CON diet supplemented with low dose CSB (0.5 g/kg feed), medium dose CSB (1.5 g/kg feed for first 3 weeks followed by reduction to 0.75 g/kg feed thereafter) and high dose CSB (3 g/kg feed for first 3 weeks followed by reduction to1.5 g/kg feed thereafter). As a result of this study, the supplementation of increasing levels of CSB increased final BW (linear, $P = 0.04$, quadratic $P = 0.02$ effects) at day 42, linearly increased average daily gain (ADG) and gain: feed (G:F) ratio ($P = 0.05$, $P = 0.01$ respectively) during day 8 – 21. The ADG showed trends ($P = 0.073$) in increment and a significant linear increase in G:F ($P < 0.05$) during days 1 – 21 were observed with the increase in the supplemental levels of CSB. The overall ADG was linearly increased ($P < 0.05$). The co-efficient of apparent total tract digestibility (ATTD) of dry matter (DM) was higher (linear effect, $P = 0.001$; quadratic effect, $P = 0.01$) with the increase in the levels of CSB during day 21 but not during day 42. A trend in reduction of coliform counts (linear effect, $P = 0.08$, quadratic effect, $P = 0.07$) was observed during day 21. The lactic acid bacteria count was increased (linear and quadratic effects, $P = 0.01$) in pigs fed diet supplemented with increasing levels of CSB at day 42. The coliform counts were reduced (linear and quadratic effects, $P < 0.05$) in the digesta obtained from duodenum of pigs fed high; medium and low doses of CSB supplemented diet. The supplementation of low, medium and high doses of CSB showed reduction (linear effect, $P < 0.001$, quadratic effect, $P = 0.01$) in fecal H_2S concentration. The inclusion of increasing dose of CSB in the diet increased (linear effect, $P < 0.05$) the villi length of duodenum. In addition, linear ($P < 0.05$) and quadratic ($P < 0.05$) responses in villi length were observed in jejunum and ileum from pigs fed graded levels of CSB. Taken together, low or medium levels of

Abbreviations: ADF, acid detergent fiber; ADFI, average daily feed intake; ADG, average daily gain; ATTD, apparent total tract digestibility; BW, body weight; CON, control; CSB, coated sodium butyrate; DM, dry matter; G:F, gain:feed; H_2S, hydrogen sulphide; N, nitrogen; NH_3, ammonia; NDF, neutral detergent fiber; SB, sodium butyrate; SCFAs, short chain fatty acids

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CSB supplementation can have a beneficial role in enhancing performance, and digestibility of weaning pigs.

1. Introduction

Weaning is the greatest challenge encountered by a piglet in its lifetime (Campbell et al., 2013). During weaning pigs are confronted with different stressors such as change in diet composition, environmental stress and social stress resulting in reduced feed intake. The reduction in feed intake consequently leads to the onset of intestinal inflammation characterized by the atrophy in villous, reduction in the secretion of digestive enzymes, reduced weight gain and post-weaning diarrhea (Rodas, 2010). Over the last few decades, antimicrobial growth promoters were used to enhance growth performance of weaning pigs. The use of antibiotics at subtherapeutic concentrations has been banned in the European Union since January 2006 as a result of the increasing prevalence of resistance to antibiotics in pigs after being routinely used for several decades as growth promoters in piglets. The ongoing increase in multidrug resistant bacterial infections (Blair et al., 2015) has emphasized an increased need for prudent use of antibiotics. This has initiated the surge for alternatives. Among several alternatives, the importance of short-chain fatty acids (SCFAs) particularly butyrate in maintaining gut health has attracted significant research attention to its application for animal production for the enhancement of performance and health of weaning pigs.

The short-chain fatty acids (SCFAs) are saturated aliphatic organic acids that consist of one to six carbons of which acetate (C2), propionate (C3), and butyrate (C4) are the most abundant (95 %) (Cook and Sellin, 1998). Acetate, propionate, and butyrate are present in an approximate molar ratio of 60:20:20 in the colon and stool (Hijova and Chmelarova, 2007; Binder, 2010). These SCFAs are produced in the hindgut of mammals as well as poultry by microbial fermentation and have been shown to exert multiple beneficial effects on their energy metabolism as well as promotion of health by improving immune function (den Besten et al., 2013; Maki et al., 2019). Comparison studies showed that the pro-absorptive and anti-secretory effects of butyrate are significantly higher than those of all other SCFAs (Binder and Mehta, 1989). The mechanisms of action of butyrate are different; many of these are related to its potent regulatory effects on gene expression. Butyric acid is available in the salt form of Na, K, Mg or Ca. Butyrate has been used as a direct energy source for cellular metabolism via β-oxidation, as a modulator of genetic activity as well as a ligand for transmembrane receptors (den Besten et al., 2013; Bedford and Gong, 2018). These biological effector functions of butyrate influence several cell types, many of which are associated with the digestive tract. The dietary supplementation of sodium butyrate (SB) in weaning pigs has been reported to promote the growth of piglets; improve gain: feed ratio during the first two weeks after weaning; inhibit the proliferation of pathogenic bacteria; and enhance nutrient digestion, absorption and gut barrier function of piglets (Piva et al., 2002; Biagi et al., 2007; Mazzoni et al., 2008; Le Gall et al., 2009). A recent study by Yang et al. (2018) demonstrated that the dietary supplementation of butyrate glyceride to broiler chickens led to the significant increase of *Bifidobacterium* in both diversity and abundance, *Bifidobacterium spp*. are well-recognized probiotic bacteria with a wide spectrum of benefits (Arboleya et al., 2016) indicating dietary butyrate likely promotes the health and well-being of animals by favoring the growth of beneficial bacteria. Wang et al. (2016) noted that butyrate changed the diversity and relative abundance of the microbes which thereby altered the fermentation characteristics leading to reduction in ammonia production in the cecal contents of laying hens indicating sodium butyrate can contribute in mitigating ammonia emission.

The supplementation of sodium butyrate in the diet of weaned pigs increased gastric mucosa thickness as reported by Mazzoni et al. (2008). The mucosa thickness in the jejunum, ileum, colon, and cecum of piglets was also increased when butyrate was given by cecal infusion (Kien et al., 2007; Kumari et al., 2013; Van Immerseel et al., 2005). Some other studies have also shown positive effects of butyric acid on the ileal villi and cecal crypts structure (Galfi and Bokori, 1990; Piva et al., 2002; Lu et al., 2008).

The volatile fatty acids are the main metabolites of hindgut fermentation in pigs and are rapidly absorbed in the hind gut. Jha et al. (2019) reported that energy provided by butyrate plays an influential role in pigs' health and in maintaining the gut ecosystem. The cells of large intestine can use butyrate as a metabolism substrate (Jozefiak et al., 2004). A slow release of sodium butyrate over the gastro intestinal tract has been suggested in order to exert the influence in large intestine (Hu and Guo, 2007). Moreover, there is reduced production of butyrate by the colonic microflora resulting from intestinal infections, chronic inflammatory disorders and use of antibiotics (Kumari et al., 2013). It has been demonstrated that coated butyrate is more effective than uncoated butyric acid in reducing smell and in reducing intestinal Salmonella colonization in swine, as well as in chickens (Van Immerseel et al., 2005; Boyen et al., 2008). To provide additional amounts of butyrate to the colon, the addition of microencapsulated butyrate to the diet could be a viable option.

Thus, the aim of coating or encapsulation is to reduce the typical unpleasant smell of butyrate and induce the slow release of sodium butyrate in the whole digestive tract. The amount of SB in the gastric content of piglets fed standard weaning diets is very low (Manzanilla et al., 2006).There are limited studies evaluating the effects of coated sodium butyrate (CSB) supplementation on the performance, digestibility, intestinal microbiota and gases emission as well as intestinal structure in weaning pigs. In the current study, the effect of supplemental CSB was evaluated by slightly reducing lactose and sodium levels in the standard weaning diet. In addition, the opportunity for least cost feed formulations was explored by supplementing different doses (low, medium, and high) of CSB to low nutrient diet as well as reducing the dosage of medium and high CSB by half of their respective doses after three weeks of experiment. Thus, the objective of this study was to assess the efficacy of CSB as a feed additive on overall performance and gut health of weaning pigs.

2. Material 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, Republic of Korea (DK-2-1608).

2.1. Source of tested product

The coated sodium butyrate (Intest-Plus sc 40) used in the current experiment was the commercial product of Palital Feed Additives, De Tweede Geerden, Velddriel, the Netherlands and supplied by a local company (Morningbio Co., Ltd., Cheonan, Korea). As per the company's information, this product was protected using lipid matrix coating and contained 400 g/kg sodium butyrate (having 51.1 g/kg Na) and 600 g/kg hydrogenated palm oil (having fatty acid composition as C 14:0, 11 g/kg; C16:0, 613.4 g/kg and C18:0, 375.5 g/kg).

2.1.1. Experimental design, animals, housing and diets

A total of 160 crossbred weaning pigs (96 barrows and 64 gilts) [(Landrace x Yorkshire) x Duroc], 28 days old, with an average initial BW of 7.04 ± 1.15 kg were used in a 6-week feeding trial to evaluate the effect of dietary supplementation of different levels of CSB. Growth performance, fecal microflora, small intestinal microflora, nutrient digestibility, gas emission and small intestinal villi

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

	Phase 1 (day 1 – 7)	Phase 2 (day 8 – 21)	Phase 3 (day 22 – 42)
Ingredient			
Extruded corn	479.00	559.30	624.00
Soybean meal (Dehulled)	180.00	240.00	285.60
Fermented soybean meal	80.00	50.00	0.00
LT Fish meal	27.00	0.00	0.00
Soy oil	32.00	32.50	36.50
Di-calcium phosphate	13.40	16.30	13.60
Limestone	7.40	8.20	8.00
Sugar	20.00	20.00	20.00
Whey protein	80.00	30.00	0.00
Lactose	67.00	30.00	0.00
L-Lysine – HCL	4.60	4.80	3.80
DL-Methionine	1.70	1.90	1.60
Threonine	2.90	2.00	1.90
Choline chloride (50 %)	1.00	1.00	1.00
Vitamin premix[a]	2.00	2.00	2.00
Mineral premix[b]	2.00	2.00	2.00
Calculated composition			
Digestible energy, MJ/kg	14.75	14.9	14.92
Metabolisable energy, MJ/kg	14.27	14.13	14.28
Net energy, MJ/kg	8.75	7.67	9.97
Lactose	113.7	48.3	0
dig Lysine	11.90	11.90	11.20
dig Methionine + Cysteine	9.40	9.40	9.00
dig Threonine	7.30	7.20	7.00
dig Tryptophan	2.10	2.10	2.00
dig Valine	7.50	7.40	7.50
Available Phosphorus	4.30	3.90	4.10
Sodium	1.10	0.90	1.00
Analyzed composition (g/kg)			
Gross Energy, MJ/kg 18.44 18.62			18.65
Dry matter	906	898	891
Crude fat	56.3	56.8	62.6
Crude protein	197.9	194.5	190.2
Lysine	14.4	13.9	12.8
Methionine	4.7	4.7	4.5
Threonine	1.04	0.93	0.90
Calcium	8.7	8.3	7.4
Phosphorus	6.8	6.6	6.1
Ash	56.7	54.2	40.7
ADF	23.3	28.8	33.2
aNDF	58.4	70.7	81

Abbreviation: ADF, acid detergent fiber; aNDF, neutral detergent fiber; LT Fish meal, low temperature fish meal.

[a] Provided per kilogram of complete diet: 1.3 mg vitamin A (Retinol); 0.022 mg vitamin D3 (Cholecalciferol); 45 mg vitamin E (Tocopherol); 4.2 mg vitamin K3 (Menodione); 24.6 mg vitamin B5 (d-Ca-pantothenate); 8.6 mg vitamin B2 (Riboflavin); 0.04 mg vitamin B12 (Cobalamine).

[b] Provided per kilogram of complete diet: 15 mg Cu; 80 mg Fe; 56 mg Zn; 73 mg Mn; 0.3 mg I; 0.5 mg Co; 0.4 mg Se.

length were assessed. Pigs were randomly allotted to 4 treatments according to their initial body weight. There were 8 replicates per treatment with 5 pigs (3 barrows, 2 gilts) per replicate pen. Treatments consisted of corn-soybean meal-based lactose and sodium reduced diet as the control diet (CON) and CON diet supplemented with low dose CSB (0.5 g/kg feed), medium dose CSB (1.5 g/kg feed for first 3 weeks followed by reduction to 0.75 g/kg thereafter) and high dose CSB (3 g/kg feed for first 3 weeks followed by reduction to 1.5 g/kg feed thereafter). The reason for the reduction of medium and high dose CSB to half of their respective doses after three weeks was to reduce diet costs. Coated sodium butyrate in the form of powder was added to the diet at the expense of corn using a mixing machine. Diets were fed in mash form in 3 phase feeding scheme. All nutrients in diets were formulated according to the recommendation of the National Research Council (NRC, 2012) (Table 1) except for a slight reduction in lactose (approximately 5 %) as well as sodium content were below NRC recommendation. Within 24 h after farrowing, all litters were standardized to nine piglets per litter in the farrowing unit throughout the suckling period. At day 28, the experimental pigs were transported to the weaning building and housed in an environmentally controlled room. An area of $0.26 \times 0.53\ m^2$ was provided to each pig. Each pen was provided a stainless steel feeder and a nipple drinker with *ad libitum* access to feed and water throughout the experiment. Creep feed was not provided to the pigs. Ventilation was provided by a mechanical system. Lighting was automatically regulated to provide 12 h of artificial light per day. Ambient temperature within the room was approximately 30 °C. It was decreased 1 °C each week of the experiment.

2.2. Experimental procedures, sampling and assay

The BW of pigs was measured at the beginning (day 1) and on days 7, 21 and 42. Feed intake was recorded on a pen basis during the experimental period to calculate average daily gain (ADG), average daily feed intake (ADFI), and gain:feed ratio (G:F).

Chromium oxide was added to the diet as an indigestible marker at 2 g/kg of the diet for 7 days prior to fecal collection at the 3rd and 6th week to calculate apparent dry matter (DM), nitrogen (N), and energy digestibility. Fecal samples were collected from at least 2 pigs (1 barrow and 1gilt) from each pen by rectal massage, mixed and pooled and a representative sample was stored in a freezer at −20 °C until analyzed. All feed and fecal samples were freeze-dried and finely ground to pass through a 1 mm screen. Diets samples were analyzed for dry matter (method 930.15), crude protein (N × 6.25; method 988.05), crude fat (954.02), ash (method 942.05), acid detergent fiber (ADF, method 973.18), calcium (method 984.01), phosphorous (method 965.17) and amino acids (method 982.30E) following the procedures established by the Association of Official Analytical Chemists (AOAC International, 2000). And neutral detergent fiber assayed with heat stable amylase (aNDF) was determined using the method of Van Soest et al. (1991). Fecal samples were analyzed for dry matter (method 930.15), nitrogen (method 988.05) following the procedures established by the Association of Official Analytical Chemists (AOAC International, 2000). Chromium levels were determined via UV absorption spectrophotometry (UV-1201, Shimadzu, Kyoto, Japan). Gross energy was determined by measuring the heat of combustion in the samples using a Parr 6100 oxygen bomb calorimeter (Parr Instrument Co., Moline, IL, USA). The coefficient of apparent total tract digestibility of nutrient was calculated using the following formula

Apparent total tract digestibility coefficient (ATTD) = 1 – [(Chromium oxide in feed / Chromium oxide in faeces) x (Nutrient in faeces / Nutrient in feed)].

At day 7, day 21 and day 42 of experiment, fresh faecal samples were collected randomly from two pigs (one gilt and one barrow) in each pen via rectal massage, then pooled and placed on ice for transportation to the laboratory, where analysis was carried out immediately. A 1 g mixed fecal sample from each replication was diluted with 9 mL of 10 g L − 1 peptone broth (Becton, Dickinson and Co., Rutherford, NJ, USA) and then homogenized. Viable counts of bacteria in the fecal samples were then conducted by plating serial tenfold dilutions onto Mac-Conkey agar plates (Difco Laboratories, Detroit, MI, USA) and lactobacilli medium III agar plates (Medium 638, DSMZ, Braunschweig, Germany) to isolate coliform and lactic acid bacteria respectively. The lactobacilli medium III agar plates were then incubated for 48 h at 39 °C under anaerobic conditions. The MacConkey agar plates were incubated for 24 h at 37 °C. Colonies were counted immediately after removal from the incubator from respective plates. Coliform colonies were differentiated from the background growth based on the morphology and color of the colony as well as on the ability of coliforms to ferment lactose. The microbial populations were log transformed before statistical analysis.

At the end of the experiment, fresh faecal samples were collected randomly from two pigs (one gilt and one barrow) in each pen via rectal massage and pooled on a pen basis. For gas emissions measurement, 300 g fecal samples were stored in 2.6 L sealed plastic boxes, in duplicates. These samples were allowed to ferment at 32 °C for 30 h. After the fermentation period, an instrument (Gas Detector GV-100S, Gastec Corp., Kanagawa, Japan) was used for gas detection. The plastic boxes were punctured and headspace air was sampled approximately 2 cm above the samples at a rate of 100 ml min^{-1}. Levels of ammonia (NH_3), hydrogen sulfide (H_2S) and total mercaptans were measured using Gastec Detector Tubes No. 3La, No. 4 L K and No. 70 L (Gastec Corp.) respectively.

At the end of the feeding trial, eight pigs consisting of 5 barrows and 3 gilts from each treatment (1 pig per replicate pen), selected based on similar body weight, were killed and evisceration was done immediately to collect the digesta samples from the small intestine and proximal colon into glass containers under CO_2. The collected digesta samples were then sealed and put on ice until they were transported to the lab for enumeration of microbial populations.

The tissue samples were collected from the middle part of duodenum, jejunum and ileum from the slaughtered pigs by excision. The samples were then flushed with physiological saline and fixed in 10 % formalin. Three cross-sections for each intestinal sample were prepared followed by staining with hematoxylin and eosin using standard paraffin embedding procedures. A total of 10 intact, well-oriented crypt-villus units were selected in triplicate for each intestinal cross-section. Villus length was determined using an image processing and analysis system.

2.3. Statistical analysis

The data were analyzed using the GLM procedure of SAS (SAS Institute, 2002) in a randomized complete block design. Pen served as the experimental unit. Linear and quadratic polynomial contrasts were used to examine responses to supplemental graded levels of coated sodium butyrate in the basal diet. Variability in the data was expressed as the standard error of means (SEM) and $P < 0.05$ was considered to be statistically significant and $P < 0.10$ was considered as a trend.

3. Results

All pigs were in good health before and throughout the experimental period, and mortality rate was zero. Firm feces were observed throughout the study with no occurrence of diarrhea.

3.1. Growth performance

The effect of supplementation of CSB (low, medium and high dose) to basal diet on growth performance is presented in Table 2. The supplementation of increasing levels of CSB increased final BW (linear, $P = 0.04$, quadratic $P = 0.02$ effects) at day 42, linearly increased ADG and G:F ratio ($P = 0.05$, $P = 0.01$ respectively) during day 8 − 21. The ADG showed trends ($P = 0.073$) in increment and a significant linear increase in G:F ($P < 0.05$) during days 1 − 21 were observed with the increase in the supplemental levels of CSB. The overall ADG was linearly increased ($P < 0.05$) and ADFI tended ($P = 0.062$) to increase in pigs fed increasing levels of CSB during day 1 − 42.

3.2. Apparent total tract nutrient digestibility

The co-efficient of ATTD of DM was higher (linear effect, $P = 0.001$; quadratic effect, $P = 0.01$) with the increase in the levels of CSB during day 21 but not during day 42. The co-efficient of ATTD of N and energy was unaffected by treatment (Table 3).

Table 2
Effects of supplementing the diet offered to weaner pigs with different doses of coated sodium butyrate on growth performance.[a].

Items	CON	Coated sodium butyrate			SEM[b]	P-value	
		Low	Medium	High		Linear	Quadratic
Body weight, kg							
Initial	7.03	7.04	7.04	7.04	0.006	0.763	0.219
Final	25.09	26.26	26.25	26.01	0.276	0.037	0.018
1 − 7 day							
ADG, g	187	225	213	205	11.15	0.420	0.050
ADFI, g	245.4	278.5	264.0	259.0	15.30	0.703	0.225
G:F	0.763	0.811	0.811	0.795	0.0245	0.379	0.209
8 − 21 day							
ADG, g	305.3	328.2	346.4	339	13.03	0.053	0.258
ADFI, g	489.3	508	499	504.4	22.94	0.727	0.779
G:F	0.625	0.648	0.696	0.674	0.0145	0.006	0.134
1 − 21 day							
ADG, g	265.8	294.5	301.6	293.4	10.69	0.073	0.098
ADFI, g	408.1	431.4	420.4	422.5	16.78	0.673	0.535
G:F	0.693	0.731	0.756	0.736	0.0121	0.005	0.0401
22 − 42 day							
ADG, g	590	619	613	613	11.5	0.233	0.219
ADFI, g	954	1001	991	1003	14.8	0.053	0.249
G:F	0.618	0.618	0.619	0.611	0.0079	0.609	0.631
1 − 42 day							
ADG, g	429	456	456	451	6.5	0.035	0.018
ADFI, g	640.13	669.87	661.87	669.5	9.09	0.062	0.237
G:F	0.669	0.681	0.689	0.673	0.0066	0.533	0.046

Values represent the means of eight pens with five pigs per pen.

[a] Abbreviation: CON, basal diet (lactose and sodium reduced); Low, CON + 0.5 kg/ton coated sodium butyrate; Medium, CON + 1.5 kg/ton coated sodium butyrate and after 3week CON + 0.75 kg/ton; High, CON + 3.0 kg/ton coated sodium butyrate and after 3week CON + 1.50 kg/ton. ADG, average daily gain; ADFI, average daily feed intake; G:F, gain : feed ratio.

[b] Standard error of means.

Table 3
Effects of supplementing the diet offered to weaner pigs with different doses of coated sodium butyrate on co-efficient of apparent total tract nutrient digestibility.[a].

Items	CON	Coated sodium butyrate			SEM[b]	P-value	
		Low	Medium	High		Linear	Quadratic
Day 21							
Dry matter	0.802	0.825	0.824	0.826	0.0041	0.001	0.014
Nitrogen	0.806	0.817	0.818	0.819	0.0046	0.090	0.283
Energy	0.804	0.816	0.820	0.807	0.0066	0.645	0.081
Day 42							
Dry matter	0.797	0.806	0.805	0.805	0.0118	0.684	0.731
Nitrogen	0.778	0.799	0.793	0.792	0.0142	0.579	0.441
Energy	0.784	0.793	0.799	0.783	0.0182	0.972	0.497

Values represent the means of eight pens with two pigs per pen.
[a] Abbreviation: CON, basal diet (lactose and sodium reduced); Low, CON + 0.5 kg/ton coated sodium butyrate; Medium, CON + 1.5 kg/ton coated sodium butyrate and after 3week CON + 0.75 kg/ton; High, CON + 3.0 kg/ton coated sodium butyrate and after 3week CON + 1.50 kg/ton.
[b] Standard error of means.

3.3. Faecal coliform and lactic acid bacteria enumeration

The coliform counts in the faeces of pigs fed diets with increasing levels of CSB remained unaffected during day 7 and 42, but a trend in reduction of coliform counts (linear effect, $P = 0.08$, quadratic effect, $P = 0.07$) was observed on day 21. The Lactic acid bacteria count was increased (linear and quadratic effects, $P = 0.01$) in pigs fed diets supplemented with increasing levels of CSB at day 42 (Table 4).

3.4. Faecal gases emission

The effect of CSB on fecal gases (NH_3, H_2S and mercaptans) emission is shown in Table 5. The supplementation of low, medium and high doses of SB showed reduction (linear effect, $P < 0.001$, quadratic effect, $P = 0.01$) of H_2S concentration but had no significant effect in NH_3 and total mercaptans concentration.

3.5. Intestinal digesta coliform and Lactic acid bacteria enumeration

The coliform counts at day 42 were reduced (linear and quadratic effects, $P < 0.05$) in the digesta obtained from duodenum of pigs fed high, medium and low doses of CSB supplemented diet. The coliform counts in the digesta of ileum showed quadratic response ($P = 0.01$) with the increase in the levels of CSB in the diet (Table 6).

3.6. Intestinal morphology

The inclusion of increasing dose of CSB in the diet increased (linear effect, $P < 0.05$) the villi length of duodenum. Linear

Table 4
Effects of supplementing the diet offered to weaner pigs with different doses of coated sodium butyrate on fecal microflora.[a].

Items, Log 10 cfu/g	CON	Coated sodium butyrate			SEM[b]	P-value	
		Low	Medium	High		Linear	Quadratic
Day 7							
Coliform	4.53	4.33	4.36	4.38	0.061	0.167	0.110
Lactic acid bacteria	7.11	7.19	7.32	7.20	0.084	0.313	0.229
Day21							
Coliform	4.66	3.78	3.95	3.98	0.217	0.088	0.067
Lactic acid bacteria	7.08	7.04	7.20	6.84	0.177	0.482	0.380
Day 42							
Coliform	4.28	4.34	4.11	4.13	0.178	0.407	0.924
Lactic acid bacteria	7.09	7.48	7.67	7.48	0.084	0.005	0.008

Values represent the means of eight pens with two pigs per pen.
[a] Abbreviation: CON, basal diet (lactose and sodium reduced); Low, CON + 0.5 kg/ton coated sodium butyrate; Medium, CON + 1.5 kg/ton coated sodium butyrate and after 3week CON + 0.75 kg/ton; High, CON + 3.0 kg/ton coated sodium butyrate and after 3week CON + 1.50 kg/ton.
[b] Standard error of means.

Table 5
Effects of supplementing the diet offered to weaner pigs with different doses of coated sodium butyrate on gas emission at day 42.[a].

Items, mg/kg	CON	Coated sodium butyrate			SEM[b]	P-value	
		Low	Medium	High		Linear	Quadratic
Mercaptans	3.38	3.13	3.08	2.93	0.250	0.242	0.846
H_2S	7.85	5.38	5.38	4.83	0.290	< 0.001	0.009
NH_3	13.08	12.55	11.28	11.30	0.847	0.115	0.753

Values represent the means of eight pens with two pigs per pen.
[a] Abbreviation: CON, basal diet (lactose and sodium reduced); Low, CON + 0.5 kg/ton coated sodium butyrate; Medium, CON + 1.5 kg/ton coated sodium butyrate and after 3week CON + 0.75 kg/ton; High, CON + 3.0 kg/ton coated sodium butyrate and after 3week CON + 1.50 kg/ton.
[b] Standard error of means.

Table 6
Effects of supplementing the diet offered to weaner pigs with different doses of coated sodium butyrate on small intestinal microflora at day 42.[a].

Items. Log 10 cfu/g	CON	Coated sodium butyrate			SEM[b]	P-value	
		Low	Medium	High		Linear	Quadratic
Duodenum							
Coliform	3.83	3.67	3.63	3.66	0.037	0.010	0.026
Lactic acid bacteria	7.39	7.43	7.56	7.42	0.088	0.572	0.306
Jejunum							
Coliform	3.87	3.77	3.79	3.73	0.079	0.316	0.829
Lactic acid bacteria	7.40	7.44	7.66	7.56	0.080	0.077	0.417
Ileum							
Coliform	4.74	4.43	4.53	4.69	0.064	0.912	0.005
Lactic acid bacteria	7.60	7.59	7.58	7.59	0.034	0.825	0.832

Values represent the means of eight pens with one pig per pen.
[a] Abbreviation: CON, basal diet (lactose and sodium reduced); Low, CON + 0.5 kg/ton coated sodium butyrate; Medium, CON + 1.5 kg/ton coated sodium butyrate and after 3week CON + 0.75 kg/ton; High, CON + 3.0 kg/ton coated sodium butyrate and after 3week CON + 1.50 kg/ton.
[b] Standard error of means.

($P < 0.05$) and quadratic ($P < 0.05$) responses in villi length were also observed in jejunum and ileum obtained from pigs fed graded levels of CSB (Table 7).

4. Discussion

The weaning transition is challenging for piglets and often results in post-weaning growth lag. The major factor contributing to growth lag is the inability to properly absorb and digest nutrients due to underdeveloped gastrointestinal tract. To ease the weaning transition, there have been many studies investigating different feed additives, including short-chain fatty acids (Lalles et al., 2007; de Lange et al., 2010; Heo et al., 2013; Thacker, 2013).

In the current study, we evaluated the efficacy of the supplementation of CSB to corn-soybean meal-based diets with slight reduction in lactose and sodium compared with standard weaning diet. Growth performance and nutrient digestibility were improved to some extent with CSB supplementation in the diet. The final BW of pigs fed diet supplemented with different levels of CSB linearly

Table 7
Effects of supplementing the diet offered to weaner pigs with different doses of coated sodium butyrate on villi length at day 42.[a].

Items, μM	CON	Coated sodium butyrate			SEM[b]	P-value	
		Low	Medium	High		Linear	Quadratic
Duodenum	340.8	341.9	348.3	364.0	6.14	0.021	0.266
Jejunum	306.8	353.6	363.0	354.1	11.99	0.019	0.045
Ileum	303.7	360.3	363.0	352.3	11.28	0.016	0.015

Values represent the means of eight pens with one pig per pen.
[a] Abbreviation: CON, basal diet (lactose and sodium reduced); Low, CON + 0.5 kg/ton coated sodium butyrate; Medium, CON + 1.5 kg/ton coated sodium butyrate and after 3week CON + 0.75 kg/ton; High, CON + 3.0 kg/ton coated sodium butyrate and after 3week CON + 1.50 kg/ton.
[b] Standard error of means.

increased and a trend in linear increment in ADG was observed during the second phase (8 − 21 day) as well as during days 1 − 21, and a significant linear increment of ADG during 1 − 42 days was also observed in pigs receiving graded levels of CSB. A significantly higher feed efficiency with medium level of CSB occurred during the second phase and day 1 − 21. Manzanilla et al. (2006) also reported that supplementation of 3 g SB/kg improved the gain:feed in the first 2 week post weaning but did not affect feed intake. Piva et al. (2002) showed that supplementation of 0.8 g/kg SB led to 20 % higher ADG and 16 % higher FI during 0–14 days and 10 % higher FI during 15–35 days compared to pigs fed control diet. The reason for improved growth performance was suggested by these authors to be due to the beneficial effect of butyric acid on the proliferation of the intestinal epithelium. However, Fang et al. (2014) reported that dietary SB (1 g/kg) had no effects on growth performance which was supported by other studies (Biagi et al., 2007; Weber and Kerr, 2008). A higher apparent total tract DM digestibility was observed only during week 3 of the current experiment in pigs fed low, medium and high CSB diets. In contrast, studies by Manzanilla et al. (2006) and Le Gall et al. (2009) reported decreased ileal and faecal digestibilities of DM and N with the supplementation of 3 g/kg SB in post-weaning piglets. These authors speculated that sodium butyrate had an inhibiting effect on amylolytic bacteria and, consequently, depressed starch utilization. The varied response of growth performance and nutrient digestibility to SB addition among different studies may result from the differences in dietary composition, dose of SB in the diet and the status of gut maturation.

It has been demonstrated that VFAs are able to eliminate pathogenic microorganisms without affecting the intestinal microflora (Ricke, 2003). This is because the undissociated form of these acids can freely diffuse across the bacterial membrane (Warnecke and Gill, 2005). Once inside the cytoplasm of the bacteria, the acid dissociates, thus releasing free hydrogen ion and reducing the pH, which causes internal cell damage exhibiting higher bactericidal activity (Leeson et al., 2005). In the present study, the faecal lactic acid bacteria count was linearly increased at day 42 with the supplementation of graded levels of CSB, whereas faecel coliform counts showed trends in linear reduction in pigs with increasing level of CSB. Coliform counts were also reduced in the small intestine digesta (duodenum) of pigs fed low, medium and high levels of CSB. In agreement with our finding, Galfi et al. (1991) reported that butyrate-fed pigs had increased intestinal lactic acid levels and lactobacilli counts and reduced number of coliforms and *E. coli* counts.

Odorous gas emission from pig slurry is associated with the incomplete anaerobic decomposition of protein and fermentable carbohydrates by the intestinal microbes (Le et al., 2008). It has been reported that the major precursors of sulfurous, indolic and phenolic compounds, VFAs, NH_3, and volatile amines in the slurry are proteins (Mackie et al., 1998). The physiological effects of ammonia and H_2S gases are dual and dose dependent. The signaling induced by small amounts of H_2S in the distal GIT contributes to gut homeostasis (Motta et al., 2015). However, higher levels of H_2S gas production not only cause chronic intestinal inflammation but also exert negative effect on air quality (Loubinoux et al., 2002). Moreover, high millimolar concentrations of ammonia likely inhibit short-chain fatty acid oxidation and basal oxygen consumption in colonic epithelial cells (Cremin et al., 2003; Andriamihaja et al., 2010). Besides ammonia, other bacterial metabolites, including hydrogen sulfide, p-cresol, may interfere with colonocyte oxygen consumption. Therefore, reducing higher levels of ammonia, H_2S and mercaptans, may have a positive effect on the intestinal health of the pigs and may contribute in mitigating the negative environmental effect. The emission of such odorous gases from pig facilities should be reduced by proper management and dietary modification. Ammonia concentrations in the gut lumen can be reduced by active carbohydrate fermentation, which stimulates the bacterial requirement for N due to increased growth. The inclusion of CSB has been reported to mitigate ammonia emission (Wang et al., 2016). In the current study, supplementation of CSB at low, medium and high doses linearly reduced the emission of H_2S, however, no significant effects on total mercaptans and ammonia emissions were observed.

Changes in gut morphology are important as they can affect growth rate. Short chain fatty acids produced by microbial fermentation from dietary fiber stimulate epithelial cell proliferation resulting in a larger absorptive surface (Sakata, 1988). At weaning, the capacity of absorption of nutrients by the small intestine is decreased resulting in a marked reduction in villous height and crypt depth. As a consequence of these changes, a reduction in feed intake and poor growth are noted (Piva et al., 2002; Pluske et al., 1996). Butyrate supplementation stimulates epithelial cell proliferation resulting in a larger absorptive surface. It also preserves villus length and thereby helps to maintain feed intake (Guilloteau et al., 2010). The indirect effect of CSB on villi height could consist of increased pancreatic fluid secretion and, consequently, higher nutrient absorption (Kaczmarek et al., 2016). The changes in villus height might have led to the improvement of feed efficiency during second phase, increase in ADG as well as enhancement of DM digestibility at day 21 in CSB fed pigs. The supplementation of protected butyrate led to numerical changes in villus height of pig as reported by Galfi and Bokori (1990). The increase in villi height may be linked with improved health status of animals.

5. Conclusion

Supplementation of lactose and sodium reduced diets with low and medium doses of coated sodium butyrate increased final body weight and growth rate over the 42-day post-weaning period. The improvement in growth rate was associated with an improvement in feed efficiency during phase 2 (8 − 21 days) and in DM digestibility measured at day 21 but not at day 42. Villus height and the small intestinal microflora were also positively affected by the coated sodium butyrate suggesting the material at low and medium doses has the potential to improve gastro intestinal tract function and animal performance and health.

CRediT authorship contribution statement

Santi Devi Upadhaya: Conceptualization, Methodology, Writing - original draft, Investigation. **Yang Jiao:** Formal analysis, Investigation. **Yong Min Kim:** Formal analysis, Investigation. **Kwang Yong Lee:** Conceptualization, Methodology, Writing - review & editing. **In Ho Kim:** Conceptualization, Methodology, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that there is no conflict of interest.

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