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Lactulose as a potential additive to enhance the growth performance, nutrient digestibility, and microbial shedding, and diminish noxious odor emissions in weaning pigs

Vetriselvi Sampath, Jae Hong Park, Sureshkumar Shanmugam, In Ho Kim*

Department of Animal Resource and Science, Dankook University, Cheonan, Chungnam 31116, Korea

*Corresponding author: inhokim@dankook.ac.kr

Abstract

The intention of this research is to analyze the effects of lactulose (LAC) supplementation on the growth performance, nutrient digestibility, microbial shedding, and fecal noxious gas emissions on weaning pigs in a 42-day trial. Based on the initial body weight and sex, a total of 255 piglets (21 day old) were randomly allocated into one of three dietary treatments with 15 replications and five pigs (two female and three male) per pen. The dietary treatments were as follows: a corn-soybean meal-based basal diet (CON) supplemented with 0, 1, and 2 g·kg^{-1} of LAC. During phase 1, significant ($p < 0.05$) increases in the average daily feed intake and average daily gain (ADG) were observed, whereas during phase 2 and overall experimental period, significant improvements ($p < 0.05$) in the body weight, ADG, and gain to feed ratio were observed in pigs fed a graded level of LAC compared to those fed the CON diet. Additionally, dietary LAC supplementation significantly improved ($p < 0.05$) the nutrient digestibility dry matter, nitrogen, and gross energy in both phase 1 and phase 2. Moreover, the inclusion of LAC supplementation significantly increased ($p < 0.05$) the fecal *Lactobacillus counts* and reduced ($p > 0.05$) the *E. coli* counts in pigs. Furthermore, LAC supplementation reduced ($p > 0.05$) fecal ammonia and hydrogen sulfide gas emissions during phase 2. The results here indicate that the addition of lactulose at 1 g·kg^{-1} and/or 2 g·kg^{-1} would be optimal to improve the performance outcomes of weaning piglets.

Keywords: growth performance, lactulose, weaning pigs

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Introduction

Rearing a healthy piglet with an adequate growth rates is essential for the success of swine production. However, during the first week of weaning, young piglets may face several stressors due to their immature immune system that are ensuing with high susceptibility to digestive diseases (Campbell et al., 2013). Besides, post-weaning diarrhea becomes the most prevalent disorder in piglets that mainly leads to mortality. Over the past decades antibiotic growth promoters (AGP) were widely used to overcome this circumstance. However, the overuse of AGP cause an antibacterial

resistance in animals and leads to severe health issues in humans through food chain. Thus, the use of AGP in livestock feed has been strictly prohibited by many countries including South Korea since 2011 (Shanmugam et al., 2021). Consequently, this problem provoked animal nutritionist to find an effective alternative with innovative strategies. For instance, omega-3 fatty acids were found to enhance the immunity of weaned piglets (Qizhang et al., 2014) whereas pre- and probiotics were discovered to promote the gut microorganisms (Peera and James, 2013) and considered as effective additives for many years.

Prebiotics are defined as non-digestible food ingredients that have a beneficially effect on host by stimulating their growth performance (Yoon and Shin, 2017). Besides they can be used by host to stimulate the beneficial bacterial growth by stabilizing the intestinal microbiota (Roberfroid, 2000). The administration of oligosaccharides (OS) which comprise the major prebiotic group, has been reported to enhance the growth and feed efficacy in young ruminants (Cangiano et al., 2020). Also, prebiotic effect of lactulose has played a vital role in improving the growth performance of pigs at weaning age due to its palatability and become a readily digestible energy source to eases the transition from milk to solid feed (Zhao et al., 2021). On the other hand, Krueger et al. (2002) reported that the inclusion of lactulose (LAC) supplement has improved the intestinal microflora of sows. In addition, Cho and Kim (2014) addressed that LAC supplement had increased the body weight gain, apparent metabolizable nitrogen as well as reduced the ammonia and acetic acid gas emission in broilers. Furthermore, Schumann (2002) and Chen et al. (2011) reported that LAC supplement could be used as therapeutic for hepatic encephalopathy and to cure constipation in children. Though, above studies had addressed the positive effects of LAC on colonic metabolism in pigs, broilers, and humans to date, the application of 2 $g \cdot kg^{-1}$ LAC in weaning piglets' diet is still scarce. Previously, Zhao et al. (2021) stated that piglets are born with high intestinal lactase activity which helps to fully digest dietary lactulose. In this study we used lactulose as a promising alternative and hypothesized that 1 $g \cdot kg^{-1}$ and/or 2 $g \cdot kg^{-1}$ lactulose supplementation would play a vital role in enhancing the growth performance, nutrient digestibility, bacterial counts, and reduce noxious odor emission in weaning pigs. Hence, the intention of this study was to determine the effect of lactulose on growth performance, nutrient digestibility, fecal microbial, and fecal noxious gas emission in weaning pigs.

Materials and methods

Ethical clearance

This experiment was conducted at "Dankook University experiment farm" located at Jeonui (Sejong, Korea). Prior to the trial, the research protocols were revised well and permitted (Permit No: DK-4-1755) by the Ethical Committee of Dankook University, Korea and all animals were humanely treated in according to the guidelines of Animal Care and Use Committee.

Trial design

Prior to the trial, all equipment and fostering room was disinfected. A total of 225, three weeks old crossed ([Yorkshire × Duroc] × Landrace) healthy weaning piglets with an initial body weight (BW) of 5.77 ± 1.22 kg were commercially procured from Gene-pig farm (Gongju, Korea). On the arrival day, all piglets were weighed individually and housed in an environmentally-controlled room. Ambient temperature of the piggery was setup at 30℃ at first, then gradually reduced up to 24℃ and 60% humidity was maintained throughout the experiment.

Diets and dietary schedules

This experiment had lasted for 42 days. The feeding program consist of 2 phases: Phase 1 [0 - 14 days] and Phase 2 [15 - 42 days]. Based on initial BW and sex piglets were randomly (complete block design) allocated into 1 of 3 dietary treatments with 15 replication and 5 pigs (2 female and 3 male) per pen, and the dietary treatments includes: CON, corn-soybean meal based basal diet without lactulose and basal diet incorporated with 1 g or 2 g lactulose per kg feed and designated as LAC1 and LAC2, respectively. The lactulose powder used in this study was commercially purchased from Easy Bio Co., Ltd. (Seoul, Korea) and added to piglets' diet at the prescribed level. All diets were formulated according to the recommendation of NRC (2012) (Table 1) and the feed ingredients were finely mixed with DDK-801 feed mixer (Daedong Tech, Anyang, Korea) and provided for piglets at the same time intervals (14:00 - 15:00). Piglets had free access to clean water and feed until the end of the experiment.

Sampling and measurements

Individual piglets (BW) were weighed using a GL-6000S weigh machine (G-Tech Inc., LTD., Seoul, Korea) at beginning and end of each phase to determine the average daily gain (ADG), whereas the amount of feed intake and refusals (pen basis) were recoded to determine gain to feed ratio (G : F) and the average daily feed intake (ADFI).

Chromium oxide (0.3%) as an indigestible marker was added to the piglet's diet on day 35 and provided for about one week until end of the experiment to measure the nutrient digestibility. The representative feed samples were collected using the sterilized plastic bags from each treatment groups right after mixing the marker. At the end of each phase, the fresh fecal samples were randomly collected from 30 piglets·treatment^{-1} (2 pigs - 1 gilt and 1 barrow·pen^{-1}) by rectal palpation. Within half an hour the collected fecal samples were transported to laboratory and stored at -20℃ to prevent changes in nutrients. Before starting the analysis, fecal samples were placed in WOF-L800 convection oven dryer (Daehan Scientific Co., Ltd., Wonju, Korea) at 105℃ for 2 days. The samples were taken out from the dryer and grounded well (Willey mill, Swedesboro, USA) to pass through a 1-mm screen sieve. The nutrient digestibility of dry matter (DM), nitrogen (N), and gross energy (GE) were analyzed according to the procedures of AOAC (2000) Previously described by Thamaraikannan and Kim (2021). The chromium concentrations were determined using Ultraviolet (UV-1201) spectrophotometer (Shimadzu Co., Kyoto, Japan). The nitrogen content was determined using Tector Kjeltec^{TM} 8600 (FOSS Co., LTD., Hilleroed, Denmark) analyzer (Method 981.10). whereas, Parr 6400 oxygen bomb calorimeter (Moline, IL, USA) was used to determine GE. The calculation of digestibility was done based on the relative chromium concentration of feed and feces samples, using the following equation: ATTD (%) = 100 - [(NF/ND) × (CrD/CrF)] × 100], ATTD is the apparent total tract digestibility, whereas NF, ND, CrD, and CrF were nutrient concentration in the feces sample, nutrient concentration in the diet, chromium concentration in the diet, and chromium concentration in the feces sample, respectively.

At the end of each phase, fresh fecal samples were randomly collected from 30 piglets·treatment^{-1} (2 pigs·pen^{-1} - 1 gilt and 1 barrow) using micro-tubes and placed in sterile plastic bags. The samples were then placed in an insulated ice container and immediately taken to the research laboratory for microbial study. To confirm the existence of microbes, 1gm of fresh fecal sample was taken and diluted with 9 mL of 1% peptone broth and mixed well with a vortex mixer. Then 0.02% of peptone solution was poured into MacConkey agar plates and *Lactobacilli* medium III agar plates, respectively. The MacConkey agar plates were incubated at 37℃ for 1 day and *Lactobacilli* medium III agar plates were incubated at 39℃, for 2 days. Finally, *Lactobacilli* and *E. coli* colonies were enumerated immediately and log transformed for statistical analysis.

Table 1. Diet composition (as-fed basis).

Item	Phase 1[x]	Phase 2[x]
Ingredient (g·kg^{-1})		
Extruded corn	444.9	619.7
Soybean meal, 480 g CP·kg^{-1}	162.0	253.0
Fermented soybean meal, 450 g CP·kg^{-1}	50.0	25.0
Fish meal, 660 g CP·kg^{-1}, Brazil	35.0	-
Soy oil	25.5	10.5
Lactulose	83.0	-
Whey	100.0	50.0
Di calcium phosphate	15.0	15.0
Sugar	30.0	-
Plasma powder, AP 920	30.0	-
L-Lys HCl	3.9	4.6
DL-Met	3.0	2.4
L-Thr	1.9	2.0
Choline chloride	1.0	1.0
Vitamin premix[y]	1.0	1.0
Trace mineral premix[z]	2.0	2.0
Limestone	9.8	11.3
Salt	2.0	2.5
Total	1,000.00	1,000.00
Calculated composition (g·kg^{-1})		
ME (kcal·kg^{-1})	3,540	3,410
CP	200.0	190.0
Lys	15.0	13.5
Met	6.2	5.3
Met + Cys	9.7	8.4
Ca	9.5	9.0
Total P	7.5	7.0
Avail P	5.5	4.3
Crude fat	50.2	39.8
Crude fiber	18.7	24.5
Analyzed composition (g·kg^{-1})		
CP	199.9	189.7
Lys	14.9	13.4
Ca	9.4	9.0
Total P	7.4	6.9

ME, metabolizable energy; CP, crude protein; Lys, lysine; Met, methionine; Cys, cysteine.

[x] Phase 1, provided during d 0 to 14; phase 2, provided during d 15 to 42. Replaced the same amount of corn with lactulose to create dietary treatments.

[y] Provided per kilogram of diet: 15,000 IU of vitamin A, 3,750 IU of vitamin D3, 37.5 mg of vitamin E, 2.55 mg of vitamin K3, 3 mg of thiamin, 7.5 mg of riboflavin, 4.5 mg of vitamin B6, 24 μg of vitamin B12, 51 mg of niacin, 1.5 mg of folic acid, 0.2 mg of biotin, and 13.5 mg of pantothenic acid.

[z] Provided per kilogram of diet: 37.5 mg of Zn, 37.5 mg of Mn, 37.5 mg of Fe, 3.75 mg of Cu, 0.83 mg of I, 62.5 mg of S, and 0.23 mg of Se.

At the end of each phase, approximately 300 g of fecal samples were collected from 30 piglets·treatment^{-1} (2 pigs·pen^{-1} - 1 gilt and 1 barrow) and placed in a plastic box (2.6 L) with a small hole in the middle and sealed with plaster. The samples were fermented for seven days at 25℃, and 100 mL of sample was taken away from the headspace (approximately 2.0 cm) of the fecal sample for air circulation. Later the box was re-sealed and manually shaken for around 30 $_S$ to measure the crust formation on the surface. Finally, the concentrations of NH_3 and H_2S was determined within the scopes of 5.0 to 100.0 ppm and 2.0 to 20.0 ppm (No. 3La and 4LK, detector tube; Gastec Corp, Kanagawa, Japan).

Statistical analysis

All the data were analyzed as a completely randomized block design using the GLM procedure of SAS (version 9.2 SAS Institute, Cary, NC, USA). When significant differences were identified among treatment means and they were separated using T-test. Pens were considered as experimental units. Variability in the data was expressed as the SEM. $p < 0.05$ was considered to be statistically significant and $p < 0.10$ was considered as trends.

Results

The effects of increasing level of lactulose supplementation on the growth performance of weaning pigs is shown in Table 2. The piglets receiving lactulose supplement showed a higher body weight ($p < 0.05$) compared to those fed CON diet. Also, during phase 1, LAC1 and LAC 2 group pigs showed higher ($p < 0.05$) ADG and ADFI, whereas during Phase 2 ADG and G : F was significantly increased ($p < 0.05$) in pigs fed lactulose supplement compared to CON. Moreover, during the overall experimental period, BW, ADG and G : F were significantly ($p < 0.05$) increased with graded level of lactulose supplementation.

Table 2. Effects of lactulose additive on growth performance in weaning pigs.

Item	CON	LAC1	LAC2	SEM	p-value
IBW (kg)	5.64	5.65	5.68	0.05	0.12
FBW (kg)	28.25b	29.24a	29.12a	0.78	0.03
Phase 1 (0 - 14)					
ADFI (kg)	0.408b	0.427a	0.429a	0.005	0.04
ADG (kg)	0.317b	0.337a	0.344a	0.006	0.01
G : F	0.777	0.789	0.802	0.017	0.42
Phase 2 (15 - 42)					
ADFI (kg)	0.832	0.821	0.819	0.008	0.12
ADG (kg)	0.649b	0.674a	0.665ab	0.006	0.02
G : F	0.780b	0.821a	0.812a	0.009	0.01
Overall					
ADFI (kg)	0.691	0.690	0.689	0.006	0.72
ADG (kg)	0.538b	0.562a	0.558a	0.004	0.02
G : F	0.779b	0.814a	0.810a	0.007	0.01

CON, basal diet; LAC1, basal diet + 1 g·kg^{-1} lactulose; LAC2, basal diet + 2 g·kg^{-1} lactulose; SEM, standard error means; IBW, initial body weight; FBW, final body weight; ADFI, average daily feed intake; ADF, average daily gain; G : F, feed efficiency.
a, b: Means in the same row with different superscripts differ ($p < 0.05$).

The effects of lactulose supplementation on the apparent total track nutrient digestibility of weaning pigs is presented in Table 3. The dietary inclusion of lactulose supplement has significantly ($p < 0.05$) increased the DM and N at phase 1, whereas during phase 2 the nitrogen digestibility and energy was significantly increased ($p < 0.05$) in pigs fed lactulose supplement compared to CON. Moreover, the fecal *lactobacillus* count was significantly increased in pigs receiving lactulose supplementation during phase 1 and 2. Whereas, *E. coli* count was significantly reduced ($p > 0.05$) in pigs fed lactulose diet during phase 2 (Table 4).

In addition, dietary lactulose supplementation has significantly decreased ($p > 0.05$) NH_3 and H_2S odor emission in weaning pig compared to CON during phase 2 (Table 5).

Table 3. Effects of lactulose additive on apparent total tract nutrient digestibility (%) in weaning pigs.

Item	CON	LAC1	LAC2	SEM	p-value
DM					
d 14	84.80b	85.83ab	86.55a	0.69	0.03
d 42	82.61	83.03	83.04	0.25	0.11
N					
d 14	83.56b	85.01a	84.82a	0.36	0.02
d 42	81.53b	82.40a	82.55a	0.17	0.03
GE					
d 14	82.91	84.72	84.73	1.05	0.39
d 42	82.55b	84.55a	84.62a	0.38	0.04

CON, basal diet; LAC1, basal diet + 1 $g \cdot kg^{-1}$ lactulose; LAC2, basal diet + 2 $g \cdot kg^{-1}$ lactulose; SEM, standard error means; DM, dry matter; N, nitrogen; GE, gross energy.

a, b: Means in the same row with different superscripts differ ($p < 0.05$).

Table 4. Effects of lactulose additive on microbial shedding on weaning pigs.

Item	CON	LAC1	LAC2	SEM	p-value
Lactobacillus					
d 14	6.81b	6.88ab	7.01a	0.07	0.02
d 42	6.83b	7.41a	7.15a	0.16	0.01
E. coli					
d 14	5.25	5.22	5.01	0.07	0.32
d 42	5.62a	5.35b	5.49b	0.10	0.04

CON, basal diet; LAC1, basal diet + 1 $g \cdot kg^{-1}$ lactulose; LAC2, basal diet + 2 $g \cdot kg^{-1}$ lactulose; SEM, standard error means.

a, b: Means in the same row with different superscripts differ ($p < 0.05$).

Table 5. Effects of lactulose additive on noxious gas emission (ppm) in weaning pigs.

Item	CON	LAC1	LAC2	SEM	p-value
NH_3					
d 14	20.61a	18.43b	19.24b	1.81	0.34
d 42	28.53a	25.41b	23.37b	1.14	0.03
H_2S					
d 14	2.14	1.92	1.79	0.21	0.61
d 42	2.98a	2.87ab	2.79b	0.06	0.02

CON, basal diet; LAC1, basal diet + 1 $g \cdot kg^{-1}$ lactulose; LAC2, basal diet + 2 $g \cdot kg^{-1}$ lactulose; SEM, standard error means.

a, b: Means in the same row with different superscripts differ ($p < 0.05$).

Discussion

Lactulose (LAC) is a kind of non-digestible oligosaccharide (NDO) (Schumann, 2002; Cho and Kim, 2014) and produced by isomerization of lactose by regrouping the glucose residue to a fructose molecule. Besides, piglets are born with high intestinal lactase activity that aids to easily digest the dietary lactulose (Zhao et al., 2012). The present study showed increased BW, ADG, ADFI, and G : F in weaning pigs fed lactulose (2 $g \cdot kg^{-1}$) supplement was consistent with the studies of O'Doherty et al. (2004) and Krueger et al. (2002) who found an increased growth performance in piglets fed lactulose supplement. Similarly, Fleige et al. (2007) reported that 30 $g \cdot kg^{-1}$ LAC supplementation has increased the growth performance of calves. However, Konstantinov et al. (2004) reported that 10 $g \cdot kg^{-1}$ of lactulose with other fermentable carbohydrates has no effect on the growth performance. Hence the main reason for increased body weight, daily gain, and G : F of weaning pigs in this study are mainly due to the palatability of lactulose supplement that observed through the increased digestibility and fecal microbiota.

In the earlier studies, Choct and Kocher (2000) and Montagne et al. (2003) had addressed the benefits of short-chain fatty acids in host organism that include the use of acetic acid in peripheral tissues, the use of propionic acid by the liver for gluconeogenesis, and the use of butyric acid in colon cells as the primary energy fuel that helps to increase the nutrient digestibility. Abrams et al. (2005) stated that prebiotics as *Lactobacillus and Bifidobacterium* can easily ferment by the gut microbes and aids to increase the mineral absorption. In this study, pigs fed graded level of LAC supplement has significantly increased the nutrient digestibility of DM, N, and E was correlated with O'Doherty et al. (2004) and Cho and Kim (2014) who observed an increased nutrient digestibility in weaning piglets and broilers, respectively. Furthermore, (Xu et al., 2002) stated that fructooligosaccharides (FOS) supplementation had stimulate the digestive enzyme activity (total protease, trypsin, and amylase) in the small intestine of growing pigs which corroborates with the current findings. Lactulose is an NDO (Natural disaccharide-octyl) that cannot be hydrolyzed by digestive enzymes but fermented to short chain fatty acids, such as acetate, propionate and butyrate in the lower gut (Klaschik et al., 2003). Previously, Cho and Kim (2014) have suggested that lactulose inclusion in the basal diet had reduce *E. coli*, and improve the *Lactobacillus* counts in the broiler. Similarly, Zhao et al. (2013) reported that inclusion of NDO in growing- finishing pigs' diet has enhanced the growth of *Lactobacillus* and reduce *E. coli*. In our study, pigs fed lactulose supplement has significantly increased the population of beneficial *Lactobacillus* counts and suppress the *E. coli* concentration was agreed with Hossain et al. (2015) who observed a same finding in growing-finishing pigs. We believe that increased growth performance and digestibility in this study is mainly due to the presence of abundance *lactobacillus* bacteria in weaning pigs' gut.

The high concentrations of hazard odor ammonia (NH_3) and Hydrogen sulfide (H_2S) emission from the farm may cause adverse effect on animals and humans (Zhang and Kim, 2014). Such odors are highly correlated with nutrient utilization and the intestinal microbial ecosystem (Ferket et al., 2002). In this study, pigs fed LAC supplementation has significantly decreased the NH_3 and H_2S gas emission in weaning pigs was correlated with the literature of Zhao et al. (2013) who observed similar results in growing-finishing pigs fed fructo-oligosaccharides supplementation. Similarly, Cho and Kim (2014) reported that inclusion of lactulose supplementation in the diet of broilers has reduced NH_3 and H_2S gas emission. In 2011, Panesar and Kumari reported that lactulose supplement could be metabolized by LAB (lactic acid bacteria) and live in the hind gut to produce short chain fatty acids and carbon-dioxide that leads to a low pH in gut contents. Apart from this, Hoeksma et al. (1993) study showed that fecal pH has greatly affects NH_3 emission thereby decrease NH_3 and H_2S emissions. The decreased NH_3 and H_2S odor release from weaning pigs fed LAC supplement is mainly because of increased nutrient digestibility and improved microbial ecology.

Conclusions

Our data revealed that administration of 1 g·kg^{-1} and/or 2 g·kg^{-1} LAC diet in weaning diet could be beneficial to improve the growth performance, nutrient digestibility of dry matter, nitrogen, and energy. Moreover, it plays a vital role in modulating the gut microbial as increase *Lactobacillus* population and decrease in *E. coli* counts. Moreover, the graded level of LAC supplementation has reduced NH_3 and H_2S toxic odor emissions from a swine farm. Therefore, we infer that LAC supplementation at a level of 1 g·kg^{-1} and/or 2 g·kg^{-1} would be optimal level of alternative feed additive to enhance the performance of weaning pigs.

Conflict of Interests

The authors declare that there is no conflict of interest.

Authors Information

Vetriselvi Sampath, https://orcid.org/0000-0002-6726-8568

Jae Hong Park, https://orcid.org/0000-0002-2025-0141

Sureshkumar Shanmugam, https://orcid.org/0000-0002-3632-3114

In Ho Kim, https://orcid.org/0000-0001-6652-2504

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