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Effect of mixed organic acid and levan type fructan supplementation on improving performance and reducing gas emission of weaned pigs

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ABSTRACT

The objective of this experiment was to evaluate the effect of mixed organic acids (MOAs) and levan type fructan (LYF) supplementation on the growth performance and fecal gas emission of weaned pigs. A total of 72 weaned pigs (Duroc × [Yorkshire × Landrace], average weight of 21.00 ± 0.78 kg) were randomly allotted to one of three dietary treatments with six replicate pens per treatment two barrows and two gilts per pen. The dietary treatments were as follows: (1) corn-soybean basal diet (CON); (2) CON + 0.1% MOAs (MOAs); (3) CON + 0.05% LYF (LYF). The experiment period lasted for 14 days. Dietary inclusion of MOAs and LYF increased ($p = 0.036$) body weight (BW) at day 14, ($p = 0.001$, $p = 0.030$) as well as average daily gain (ADG) and gain: feed (G : F) at day 14. However, dietary supplementation of MOAs and LYF reduced ($p = 0.018$) the fecal score at day 14. In addition, dietary inclusion of MOAs and LYF to the basal diet significantly reduced ($p = 0.016$) fecal NH_3 emission on day 14 compared with pigs fed the control. Thus, MOAs and LYF supplementation positively affected growth performance and fecal NH_3 emission in weaning pigs.

Key words: fecal gas emission, fecal score, levan type fructan, mixed organic acid, performance

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Introduction

After weaning, pigs are easily exposed to weaning stress due to their undeveloped immune and digestive systems and the sudden changes on environment and diet, which may result in a reduction of feed intake and nutrient absorption, as well as an increase of gastrointestinal disorder, diarrhea rate and mortality rate and stunted growth of piglets (Ma et al., 2021). Therefore, antibiotics have often been added to the feed of weaned piglets to prevent diarrhea and improve growth performance (Liu et al., 2018, Sureshkumar and Kim, 2021). However, the abuse of antibiotics in feed can increase the risk of bacterial cross-resistance and residue of antibiotics in animal products, which might be harmful for the human health (Suiryanrayna and Ramana, 2015). In 2006, the European Union banned the addition of antibiotics to feed, and the United States and China have gradually begun to ban the use of antibiotics. Consequently, safe and green feed additives replacing antibiotics were found to solve the negative effect of antibiotics and also improve performance in pigs. Previous studies in our laboratory had proved that organic acids (Muniyappan et al., 2021), Probiotic (Balasubramanian et al., 2017; Sampath et al., 2022), β-mannanase (Balasubramanian et al., 2018), Yeast (Sampath et al., 2021), Xylanase (Lee et al., 2018), and levan-type fructan (Li et al., 2019a) could be used as substitutes of antibiotics in weaned pigs. Organic acids (OAs) are widely used in livestock production because of their advantages such as lowering the pH value of the gastrointestinal tract, improving the structure of the intestinal microbial flora, immune function and performance (Ahmed et al., 2014; Yang et al., 2019a). Several researchers have demonstrated that the application of a small amount of mixed organic acids (MOAs) has had a wide range of effects compared with that of a single OAs in weaning pigs (Kim et al., 2005; Partanen et al., 2007; Balasubramanian et al., 2016). Fructans, which are normally isolated from plants under natural conditions and can also be produced by some microorganisms, are nonstructural carbohydrates that differ in molecular structure and molecular weight. They may be classified into 3 main types: the inulin group, the levan group, and the branched group. The inulin group consists of the material that is mostly or exclusively the β-2,1 fructosyl-fructose linkage, and nearly all fructans found in plants are of this type. The levan group fructan is the material that contains mostly or exclusively the β-2,6 fructosyl-fructose linkage. The branched group has both β-2,1 and β-2,6 fructosylfructose linkages in substantial amounts. Levan-type fructan (LYF) is considered to be a prebiotic and has a variety of nutritional and pharmaceutical functions, including promoting the absorption of metallic ions (Ohta et al., 1993), having bifidogenic (Banguela and Hernández, 2006), anti-inflammatory (Vigants et al., 2001), and immunomodulatory effects (Calazans et al., 1997), improving nutrient digestibility (Zhao et al., 2012) and having health-promoting effects, such as reducing serum cholesterol, preventing colon cancer, and producing B vitamins (Banguela and Hernández, 2006).

However, additional research is needed to assess the effect of MOAs and LYF on growth performance, fecal score, and gas emission traits of weaning pigs. Thus, the objective of the current experiment was to investigate the effects of MOAs and LYF on growth performance, fecal score and fecal gas emission in weaned pigs.

Materials and Methods

Animal ethics

The experimental protocols describing the management and care of animals were reviewed and approved by the Animal Care and Use Committee of Dankook University, Cheonan, South Korea (DK-1-1908).

Source of MOAs and LYF

The protected MOAs used in the current experiment is provided by a commercial company (Morningbio Co., Ltd., Cheonan, Korea). The active substance is 17% fumaric acid, 13% citric acid, 10% malic acid, benzoic acid 10%, and 1.2% MCFAs (capric and caprylic acid), and carrier vegetable oil 48.8%.

LYF was prepared via enzyme reaction using levansucrase from Zymomonas mobilis, purchased from the RealBioTech Co. (Daejeon, Korea). This source of fructan is different from inulin, which consists of β-(2,1)-linked fructose units and its partial hydrolysate, fructooligosaccharides. The average degree of polymerization is 10 and the average molecular weight is 700 kDa.

Animals and diets

A total of 72 weaned pigs (Duroc × [Landrace × Yorkshire] average weight of 21.44 ± 0.78 kg) were randomly allocated to 1 of 3 dietary treatments with 6 replicate pens per treatment (2 barrows and 2 gilts per pen). As shown in Table 1, nutrients in the diet met the recommended requirements (NRC, 2012). The dietary treatments were as follows: (1) corn-soybean basal diet (CON); (2) CON + 0.1% MOAs (MOAs); (3) CON + 0.05% LYF (LYF). The experiment period was lasted for 14 days. The pigs were kept in 1.2 m × 2 m experimental pens with plastic slatted floors. Each pen was fitted with an adjustable stainless steel feeder and a duckbill drinker. The temperature, humidity, CO_2 and ammonium concentration of the air in a pig house was automatically controlled. The temperature was maintained at 24 - 28℃, and the relative humidity was controlled at 60 - 70%. The pigs had free access to water and feed ad libitum.

Table 1. Composition of basal diets (as-fed basis).

Ingredient ($g \cdot kg^{-1}$)	Experimental diet
Extruded corn	55.83
Soybean meal (dehulled)	24.00
Fermented soybean meal	5.00
Soy oil	3.25
Dicalcium phosphate	1.63
Limestone	0.82
Sugar	2.00
Whey protein	3.00
Lactose	3.00
L-Lysine – HCL	0.48
DL-Met	0.19
Threonine	0.20
Choline Chl 50%	0.10
Salt	0.10
Vitamin premix[y]	0.20
Mineral premix[z]	0.20
Nutrient levels	
Protein	18.50
Fat	4.20
Calcium	0.75
Phosphorus	0.65
Digestible energy ($kcal \cdot kg^{-1}$)	3,800.00
Lysine	1.40
Methionine	0.42
Lactose	5.00

[y] Provided per kg diet: Fe, 115 mg as ferrous sulfate; Cu, 70 mg as copper sulfate; Mn, 20 mg as manganese oxide; Zn, 60 mg as zinc oxide; I, 0.5 mg as potassium iodide; and Se, 0.3 mg as sodium selenite.
[z] Provided per kilograms of diet: vitamin A, 13,000 IU; vitamin D3, 1,700 IU; vitamin E, 60 IU; vitamin K3, 5 mg; vitamin B1, 4.2 mg; vitamin B2, 19 mg; vitamin B6, 6.7 mg; vitamin B12, 0.05 mg; biotin, 0.34 mg; folic acid, 2.1 mg; niacin, 55 mg; D-calcium pantothenate, 45 mg.

Growth performance traits

Piglets were weighed on day 1 and day 14 of the experiment as well as recorded the feed consumption to calculate average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR = ADFI/ADG). The fecal score was recorded daily following the method recommended by (Long et al., 2018).

Fecal gas emission

For the analysis of the fecal $NH_{3,}$ methyl mercaptans, and H_2S, and acetic acid the fresh feces were collected from randomly selected 6 pigs per treatment (1 gilt and 1 barrow per pen per treatment) on the day 1 and day 14. 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 sealed with adhesive plaster. The samples were fermented for 24 h at room temperature (25℃), and 100 mL of the headspace air was sampled from approximately 2.0 cm above the fecal sample. After the collection, the 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, methyl mercaptans, acetic acid 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., Kanagawa, Japan).

Statistical analysis

The data were analyzed as a completely randomized Duncan's multiple range test using GLM procedures of SAS (SAS Institute Inc., Cary, NC, USA), the pen was served as the experimental unit. Variability in the data was expressed as pooled standard error, and a probability level of $p < 0.05$ was considered significant and $p < 0.10$ as trends.

Results

Growth performance and fecal score

The effect of MOAs and LYF supplementation in weaning pig's growth performance is shown in Table 2. During day 14, pigs fed the diet supplemented with a MOAs and LYF have significantly improved ($p = 0.036$) BW gain compared to pigs fed CON. In addition, MOAs and LYF supplementation have significantly increased ($p = 0.001$, $p = 0.030$) ADG and G : F ratio without any effects ($p > 0.05$) on ADFI. Compared with CON, MOAs and LYF had significantly decreased ($p = 0.016$) the fecal score in day 14 (Table 3).

Fecal gas emission

Dietary MOAs and LYF supplementation have significantly reduced fecal NH_3 emission ($p = 0.016$). However there was no difference of fecal H_2S, methyl mercaptans and acetic acid emissions (Table 4).

Table 2. Effect of dietary mixed organic acid and levan fructan supplementation on growth performance in weaning pigs.

Item	CON	MOAs	LYF	SEM	p-value
Body weight (kg)					
Day 1	21.00	21.45	21.49	0.401	0.258
Day 14	29.12	29.90	29.99	0.428	0.036
Overall (day 14)					
ADG (g)	580	683	690	18.29	0.001
ADFI (g)	1,005	1,042	1,053	22.30	0.292
G : F	1.730	1.755	1.746	0.016	0.030

CON, corn-soybean basal diet; MOAs, mixed organic acids; LYF, levan type fructan; ADG, average daily gain; ADFI, average daily feed intake; G : F, gain : feed; SEM, standard error of means.

Table 3. Effect of dietary mixed organic acid and levan fructan supplementation on fecal score in weaning pigs.

Item	CON	MOAs	LYF	SEM	p-value
Fecal score[z]					
Day 1	3.20	3.25	3.24	0.196	0.403
Day 14	3.13	2.88	2.47	0.189	0.018

CON, corn-soybean basal diet; MOAs, mixed organic acids; LYF, levan type fructan; SEM, standard error of means.

[z] Fecal score = 1 hard, dry pellet; 2 firm, formed stool; 3 soft, moist stool that retains shape; 4 soft, unformed stool that assumes shape of container; 5 watery liquid that can be poured.

Table 4. Effect dietary mixed organic acid and levan fructan supplementation on gas emission in weaning pigs.

Item (ppm)	CON	MOAs	LYF	SEM	p-value
Day 1					
NH_3	1.1	0.9	1.4	0.187	0.625
H_2S	3.3	2.8	2.7	0.101	0.206
Methyl mercaptan	6.3	5.9	5.7	0.577	0.111
Acetic acid	0.7	1.0	0.8	0.129	0.666
Day 14					
NH_3	1.4	0.5	0.4	0.259	0.016
H_2S	2.7	3.2	2.6	0.274	0.885
Methyl mercaptan	11.4	10.4	10.8	0.382	0.589
Acetic acid	0.7	1.1	0.9	0.209	0.747

CON, corn-soybean basal diet; MOAs, mixed organic acids; LYF, levan type fructan; NH_3, ammonia; H_2S, hydrogen sulfide; SEM, standard error of means.

Discussion

Growth performance

The application of MOAs in pig production was suggestion in review article published by (Nguyen et al., 2020). Many studies have proved the utility of MOAs as a potential alternative to antibiotic in developing performance and enhancing microbial flora in pigs (Upadhaya et al., 2014a; Li et al., 2019b). In the present study, we demonstrated that MOAs supplementation improved BW, ADG and G : F in weaning pigs compared with CON, which was similar to the results of Upadhaya et al. (2018) who reported that piglets fed MOAs 0.1% and 0.5% respectively could show improved the growth performance, which positive effect on preventing fecal score in weaning pigs. Similarly, Upadhaya et al. (2016) and Yang et al. (2019b) also reported that weaned pigs fed 0.4% MOAs such as fumaric, lactic, propionic, citric, and benzoic acid have significantly improved ADG and G : F compared with pigs fed a CON diet. On the other hand, Zentek et al. (2013) did not observe significant difference in the growth performance due to supplementation of MOAs. The inconsistent result on growth performance among different studies may be due to several diet complexity, types of acid, age and the health status of pigs.

In the present research, supplementation of the LYF significantly improved weaned piglets performance. The positive performance response could be attributed to the synergistic effect between different active ingredients in this blend which are characterized by possessing antioxidant, anti-inflammatory and antimicrobial activities beside the stimulant property of the digestive enzymes with subsequent positive impact on the gut microbial ecosystem, nutrient utilization, and performance parameters (Li et al., 2019a). Significantly improvements in the weaned pigs performance were observed due to feeding on diets supplementation by LYF at levels of 0.1 - 1.0% diet (BW, ADG and G : F during day 42; Lei et al., 2018), LYF at a level of 0.5 - 2.0% diet (ADG and G : F; Li and Kim, 2013) and levan at a levels 0.5 - 1.0 $g \cdot kg^{-1}$ diet (ADG and G : F; Zhang and Kim, 2014).

Fecal gas emission

Due to intensification and large scales of production, harmful gases are produced in livestock farms. These harmful substances produced in feces contribute to climate change and may cause adverse effects on the health of humans and animals (Nguyen et al., 2020; Muniyappan et al., 2022). The addition of probiotics to feed can effectively decrease the level of ammonia, fecal pH, and volatile organic matter in feces of growing pigs. For instance, Chu et al. (2011) noted that the supplementation of prebiotics and OAs in the diet diminished the production of harmful gases, reduced the sulfur compounds and ammonia compounds in feces, thus bringing down the toxicity and smell of feces. It has been agreed that nitrogen and sulfur are one of the major environmental pollutants. In the study, we found that supplementing the diet with 0.1% MOAs significantly decreased fecal NH_3 gas emission in weaned pigs. This is in agreement with Upadhaya et al. (2014b), who reported the addition of MOAs 0.2% had significantly reduces fecal NH_3 gas emission. Hossain et al. (2015) also reported that pigs fed with diet blends of organic acid have significantly reduced fecal NH_3 gas emission compared with a CON diet. In contract, Devi et al. (2016) reported that the dietary inclusion of blends of organic acid had no influence on NH_3, methyl mercaptans, and acetic acid in weaning pigs and sows. Similarly, Upadhaya et al. (2018) reported that the MOAs supplementation had no significant effect on fecal gas emission in weaning pigs.

Supplementation of LYF significantly decreased fecal NH_3 gas emission. Similarly, Zhao et al. (2013a) reported a significantly decreased in the fecal NH_3 gas emission of pigs fed due to dietary LYF supplementation. Zhao et al. (2013b) reported a significantly decreased in the fecal NH_3 gas emission of pigs fed due to dietary supplementation of LYF at levels of 0.25 - 0.50%. On the other end, Lei et al. (2018) did not observe significant difference in the fecal gas emission due to supplementation of LYF. The contradictory results between the various experiments may be due to the age of weaning pigs, variations in amounts of LYF levels and variations in dietary compositions. Thus, the addition of MOAs and LYF is propitious to maintain the balance of the intestinal flora through increasing the benefical bacteria, thereby reducing the concentration of ammonia in the feces.

Conclusion

The present research demonstrated that the growth performance improved and fecal score and fecal NH_3 gas emission reduced in pigs fed diet supplemented with LYF when compared with CON. Hence, LYF can be considered as a potential alternative to antibiotics in order to positive effects on the growth performance in weaning pigs.

Conflict of Interests

No potential conflict of interest relevant to this article was reported.

Ethical Approval

All procedures involving animals were conducted in line with the protocol approved by the Animal Ethics Committee of Dankook University. (Protocol number: DK-2-2114).

Data availability Statement

The data presented in this study are available on request from the corresponding author.

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Author Contributions

MM, HSH and IHK: Conceptualization and designed the trials. MM: writing – original draft preparation, performed the animal trials, MM, HSH and KH: Software, Methodology, Formal analysis, Writing – review and editing, IHK: Supervision. All authors contributed to the article and approved the submitted version.

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