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**Effects of dietary protected organic acids on growth performance, nutrient digestibility,
fecal microflora, diarrhea score and fecal gas emission in weanling pigs**

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Abstract: A total of 112 weanling pigs with an average body weight of 6.70 ± 1.31 kg were allotted to one of four experimental diets for a 6-week feeding trial to evaluate the effects of protected organic acids on growth performance, nutrient digestibility and fecal microbial counts. Diets consisted of basal diet (CON); CON + 0.2% unprotected organic acid (UOA); CON + 0.1% protected organic acid (POA1); and CON + 0.2% POA (POA2). Feeding POA diets to weaning pigs increased ($P < 0.05$) average daily gain (ADG) during 0 - 2 week and overall experimental period and also increased ($P < 0.05$) the apparent total tract digestibility (ATTD) of dry matter (DM) compared with UOA. The addition of increasing levels of POA showed greater (linear effect, $P < 0.05$) ADG, gain: feed ratio (G: F) and ATTD of DM. Additionally, dietary inclusion of increasing levels of POA linearly increased ($P < 0.05$) the fecal *Lactobacillus* counts, whereas the *E. coli* and *Salmonella* counts, diarrhea score, fecal NH_3 and acetic acid emissions were reduced (linear effect, $P < 0.05$). In conclusion, dietary supplementation of 0.2% POA to weaning pigs has potential to improve growth performance, reduce diarrhea incidence while balancing microbial counts.

Key words: Protected organic acid, growth performance, nutrient digestibility, fecal microflora, diarrhea score.

Abbreviations: ADG, average daily gain; ADFI, average daily feed intake; ATTD, apparent total tract digestibility; BW, body weight; DM, dry matter; GE, gross energy G/F, ratio of gain to feed; ME, metabolizable energy; N, nitrogen; POA, protected organic acids; UOA, unprotected organic acids.

Introduction

The weaning period is one of the most critical stages in the life cycle of pigs. A combination of stressful circumstance can generally result in condition favoring the occurrence of different bacterial and viral illnesses (Wallgren et al., 2001). Early-weaned piglets are exposed to several stress factors, including nutrition stress and stressful indoor environment of housing (Laine et al., 2008). Among a variety of candidates for the replacing antibiotics, organic acids (OA) have been broadly applied worldwide with reasonable success (Mroz et al., 2005). Several previous studies have demonstrated that, under normal physiological condition, dietary inclusion of OA is beneficial to weanling pigs (Lalles et al. 2004; Øverland et al. 2008). The OA can improve their growth performance and reduce the post-weanling diarrhea (Kiarie et al., 2008; Wang et al., 2009). In our previous studies, we have noted that medium chain fatty acids (MCFA) combined with OA can be considered as an alternative of additive to improve the performance growing pigs (Upadhaya et al., 2016), finishing pigs (Upadhaya et al., 2014) and sows (Devi et al., 2016). With the development of coating technology, protected OA (POA) by matrix-coating for targeted delivery to different gut segments has gained considerable attention (Upadhaya et al., 2014). However, studies on the effect of protected OA (POA) supplementation in weanling pigs is limited. Thus, the objective of this study was to determine the effects of POA supplementation on growth performance, nutrient digestibility, fecal microflora, diarrhea score, and fecal gas emission in comparison with an unprotected OA (UOA) in weanling pigs.

Materials and Methods

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

Source of protected organic acid

The matrix coated organic acid, used in the current experiment was provided by a commercial company (Morningbio Co., Ltd., Cheonan, Korea). This POA consists of MCFA and composite organic acids. The active ingredients were 17% fumaric acid, 13% citric acid, 10% malic acid, 1.2% MCFA (capric and caprylic acid) and carrier.

Animal management, experimental design, and diets

A total of 112 crossbred weanling pigs [(Yorkshire × Landrace) × Duroc] with an average body weight (BW) of 6.70 ± 1.31 kg (28 ± 1 d of age) were used in a 42-d experiment. Pigs were allotted into 4 dietary treatments according to initial body weight and sex in a randomized block design experiment. Each treatment comprised 7 pens, with 4 pigs (2 barrows and 2 gilts) each. The dietary treatments included: (1) Control, basal diet (CON); (2) CON + 0.2% UOA (UOA); (3) CON + 0.1% POA (POA1); (4) CON + 0.2% POA (POA2). Pigs were subjected to a 2-period feeding program, consisting of Phase 1 (d 0–14) and phase 2 (d 15–42). Pigs were kept on slatted plastic floors of an environmentally-controlled room. Each pen (1.65 m²) was equipped with a one-side feeder. All experimental diets in mash form were formulated to meet the NRC (2012) nutrient requirements (Table 1). All pigs were provided *ad libitum* access to feed and water through a self-feeder and nipple drinker, respectively, throughout the experiment period.

Sample collection and measurements

Pigs were weighed at day 0 and weeks 2, and week 6 of the experimental period. Feed intake was recorded on a per-pen basis to calculate average daily gain (ADG), average daily feed intake (ADFI), and gain: feed (G: F) ratio. Chromium oxide (Cr₂O₃, 2 g/kg) was added to diet as an indigestible marker to measure digestibility. Fresh fecal samples were collected directly via rectal massage from at least two pigs in each pen at end of the experiment to determine DM (dry

matter), N (nitrogen) and GE (gross energy) as described before. All fecal and feed samples were stored at -20°C until analyzed.

Fecal samples were dried at 70°C for 72 h. Both fecal samples and feed samples, were finely ground to pass through a 1-mm screen. All samples were analyzed for dry matter (DM) (method 934.01; AOAC 2000), Nitrogen (N) (method 968.06; AOAC 2000) by a Nitrogen analyzer (Kjtec 2300 N Analyzer; Foss Tecator AB, Hoganas, Sweden), and gross energy (GE) using an oxygen bomb calorimeter (Parr Instrument Co., Moline, IL). Basal diets were also analyzed for crude fat (method 920.39; AOAC 2000), calcium (Ca, method 984.01; AOAC 1995), and phosphorus (P, method 965.17; AOAC 1995) contents. Individual amino acid (AA) composition was measured using an AA analyzer (Beckman 6300; Beckman Coulter Inc., Fullerton, CA, USA) after a 24-h hydrolysis in HCl. Chromium was analyzed via ultraviolet absorption spectrophotometry (Shimadzu UV-1201; Shimadzu, Kyoto, Japan) following the method described by (Williams et al. 1962).

For microbial analysis, about 5 g fresh fecal samples were collected from 2 randomly selected pigs (1 gilt and 1 barrow) per pen via rectal massage, pooled and then placed on ice for transportation to the laboratory (Becton, Dickinson and Co., Franklin Lakes, NJ, USA). Microbial analysis was immediately carried out according to the method described by Hossain et al. (2016). Briefly, one gram of composite fecal sample from each pen was diluted with sterile saline (10^{-7} to 10^{-3}) and homogenized. Viable counts of bacteria in fecal samples were determined by plating serial dilutions (10-fold in 1% peptone solution) onto MacConkey agar plates, MRS agar plates (Difco, USA), MRS-NPNL (MRS agar + nalidixic acid, paromomycin + neomycin sulphate + lithium chloride), sheep blood agar base plates (Oxoid, Basingstoke, UK), and *Salmonella-Shigella* agar plates to isolate *Escherichia. coli*, *Lactobacillus*, *Bifidobacterium*

spp., *Clostridium perfringens*, and *Salmonella*, respectively. Numbers of colonies of *E. coli* and *Lactobacillus* were counted immediately after incubation at 37 °C for 48 h. The incidence of diarrhea in piglets was observed. Diarrheal score of each piglet was recorded on week 2 and week 6 of the trial. In order to assess the severity of diarrhea, feces from each pen were scored as follows: 1 = hard, dry pellets in a small, hard mass; 2 = hard, formed stool that remains firm, and soft; 3 = soft, formed, and moist stool that retains its shape; 4 = soft, unformed stool that assumed the shape of the container; and 5 = watery, liquid stool that could be poured. A cumulative diarrhea score per day was then assessed (Smiricky et al. 2002).

To the analyze of fecal ammonia (NH₃), hydrogen sulfide (H₂S), total mercaptans (R.SH) and acetic acid, 200 g of fresh feces were randomly collected from 2 pigs in each pen by rectal palpation on d 42 according to the method described by Hossain et al. (2016). Fecal samples were transferred to a sealed box and fermented in an incubator at 35 °C for 36 h (35 °C). Fermented samples were then analyzed with a gas search probe (Gastec Model GV-100, detector tube No. 3La for NH₃ and acetic acid, No. 4LK for H₂S; No. 70L for R.SH, Gastec Corp., Kanagawa, Japan).

Statistical Analyses

The data were analyzed using the GLM procedure of SAS (SAS Institute, 1996) in a randomized complete block design using pen as the experimental unit. Pre-planned contrast was used to test the following: the basal diet versus UPOA diets. Furthermore, linear and quadratic polynomial contrasts were used to examine responses to supplemental graded levels of POA at 0%, 0.10% and 0.2 % 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.

Results

Growth performance and nutrient digestibility

Results of growth performance, nutrient digestibility of weanling pigs are shown in Table 2. Dietary supplementation with UOA showed no difference ($P > 0.10$) on growth performance and nutrient digestibility compared with CON. Feeding POA diet to weaning pigs increased ADG compared with UOA during 0 - 2 week ($P = 0.0368$) and during the overall experimental period ($P = 0.0095$). The dietary supplementation of POA increased the ATTD of DM ($P < 0.05$) compared with UOA. Increasing the concentration of POA linearly improved the ADG ($P = 0.0092$) and G: F ($P = 0.0342$) during 0 - 2 weeks. During week 2-6, there was linear increase in ADG ($P = 0.0039$), ADFI ($P = 0.0345$) and G: F ($P = 0.0055$) of weaning pigs fed diet supplemented with graded levels of POA. Increasing the concentration of POA linearly increased the ADG ($P = 0.0003$) and G: F ($P = 0.0024$) during the overall experiment period. Moreover, supplementing the diet with different levels of POA linearly increased ATTD of DM ($P = 0.0152$) and GE ($P = 0.0044$) in weaning pigs.

Fecal microflora, diarrhea score and noxious gas emission

The UOA diet did not have any effect ($P > 0.05$) on fecal microbial counts, diarrhea score and gas emission compared with CON diet. The supplementation of POA increased ($P < 0.05$) the *Lactobacillus* and reduced *Salmonella* counts compared with weaning pigs fed with UOA diet. Supplementing diet with different levels of POA increased *Lactobacillus* (linear, $P = 0.0038$), and reduced *E. coli* (linear, $P = 0.0125$), *Salmonella* (linear, $P = 0.0244$) counts, diarrhea score (linear, $P = 0.0022$; quadratic $P = 0.0431$), ammonia (linear, $P = 0.0006$; quadratic $P = 0.0458$) and acetic acid emissions (linear, $P = 0.0277$) in weaning pigs.

Discussion

Livestock production can make good use of resources and it can contribute high quality nutrient to the human diet (Devi et al. 2014). Weaning is a complex period involving dietary, environmental, social and psychological stresses that can deeply interfere with feed intake, gastro intestinal tract development and adaptations to the weaning diet (Lalles et al. 2004). Post-weaning diarrhea is one of the most frequent causes of heavy economic losses in pig herds. Major bacteria that caused diarrhea include *E. coli*, members of the genera *Clostridium*, *Lawsonia*, and *Brachyspira* bacteria, viral agents, and rotaviruses (Thomsson et al. 2008) due to irregular feed intake (Laine et al. 2008) and feed structure (Amezcuca et al. 2002). Before 2006, health strategies have widely used antibiotic growth promoters to reduce enteric infections and the occurrence and ability of pathogens to adhere to intestinal mucosa (Budino et al. 2005). These antibiotics were added to feed for piglets during the weaning period with the aim to improve the composition of intestinal microflora in piglets and thus ameliorating the potential consequences (Sorensen et al. 2009).

Growth Performance

Acidifiers have received much attention in pig production due to their positive effects on growth performance of pigs (Papatsiros et al. 2011). A beneficial effect of POA on the growth performance, feed intake and general health condition was observed in the present investigation, resulting in higher ADG and G: F in pigs fed POA diet compared to those fed with other diets. These results are in agreement with our previous studies showing that dietary supplementation of POA can improved the growth performance of growing and finishing pigs (Upadhaya et al., 2014; Upadhaya et al., 2016). Consistent with our findings, some researchers have shown positive effects with single or blends of dietary acidifiers. For instance, supplementation of single acidifier such as formic or sorbic acid has improved the growth rate and feed efficiency on

weanling pigs (Overland et al. 2008). Likewise, Li et al. (2008) have reported that blends of organic acids such as botanic acid, fumaric acid and benzoic acid at the rate of 5 g/kg in the diet of weanling piglets can numerically improve their growth performance. However, Canibe et al. (2005), have reported that, ADG and ADFI are not affected by diet containing 1.8% formic acid. However, G: F tends to be greater for growing pigs fed with the diet containing 1.8% formic acid (Canibe et al. 2005). In contrast, other reports have indicated no or negative responses of weaned pigs to single acidifiers such as fumaric acid, citric acid or formic acid (Radecki et al. 1988; Manzanilla et al. 2004) or blend of acidifiers such as formic acid, lactic acid and volatile fatty acids (Lee et al. 2007). These inconsistent results and highly variable responses might be due to several factors such as stage of growth, diet complexity, type of acid, inclusion level of acid and health status of pigs. Improvements in growth performances might be due to the antimicrobial activity of OA that can reduce of harmful microflora, thereby reducing the metabolic need of microbes which in turn enhances the availability of dietary energy and nutrients to pigs.

Nutrient digestibility

The current study showed that POA improved nutrient digestibility during the experimental period. The addition of organic acids to pig diets has been examined for decades, clearly demonstrating that organic acids can improve the growth performance and nutrient digestibility of pigs (Kluge et al., 2006). Mroz et al. (2000) have suggested that, OA has a beneficial effect on the apparent ileal and total tract digestibility of nutrients in growing pigs. This might be due to the fact that OA diet supplementation can reduce the acidity through lowering the dietary pH which might have increased the solubility of minerals. In agreement with our study, Upadhaya et al. (2014) have demonstrated that, the dietary POA can improved the nutrient digestibility in finishing pigs. Positive effects of organic acid on digestibility and growth performance in

weanling pigs which reported in a previous study has been found to be primarily a result of the inhibition of proliferation of pathogenic bacteria successfully (Dibner and Buttin 2002). It is expected that the action of the OA might be higher in the proximal portions of the digestive tract (Canibe et al. 2001). The increase in nutrient digestibility might have resulted from the increased microbial activity in the gastro intestinal tract (Yin et al., 2001). However, other reports have indicated that dietary supplementation of OA has no positive response on nutrient digestibility in weanling pigs (Kil et al., 2006) and growing pigs (Upadhaya et al., 2016).

Fecal microflora and diarrhea score

The present study showed that the number of *Lactobacillus* counts was increased while numbers of *E. coli* and *Salmonella* counts were reduced in the feces obtained from pigs fed with diet supplemented with POA compared to those in other treatments. The addition of OA to the diets of pigs altered the total microbial load. It has been found that OA can particularly effect *E. coli* and other acid-intolerant organisms (Dibner and Buttin 2002). The increase in *Lactobacillus* population could benefit intestinal function by reducing the survival rate of enteric pathogens due to the low pH resulting from the acid produced by the fermentation of *Lactobacillus*. Similarly, Li et al. (2008) and Ahmed et al. (2014) have reported that blends of organic acid supplementation can reduce *E. coli* but increase *Lactobacilli* numbers in weaned piglets and growing pigs (Upadhaya et al., 2017).

Possible modes of actions of organic acids includes reducing the pH value of digesta in the gastrointestinal tract (Ravindran and Kornegay, 1993), regulating the balance of microbial populations in the gut, and stimulating the secretion of digestive enzyme (Thaela et al., 1998). Thus, the survival rate of *E. coli* in the present study might have been influenced by low pH because *E. coli* is less tolerant of acid pH. Thus, OA might have inhibited the proliferation of

pathogenic bacteria in the gastrointestinal tract. It can prevent or reduce the incidence of diarrhea in pigs (Papatsiros et al. 2011). It can also shift microbiota by increasing fecal *Lactobacillus* counts but reducing *E. coli* and Salmonella counts in weanling pigs.

Gas emission

We found that POA exhibited reduced noxious gas emissions such as NH_3 , and acetic acid compared to other diets, in agreement with results of Upadhaya et al (2014) showing that dietary inclusion of POA reduced levels of NH_3 and acetic acid in finishing pigs. Cortus (2006) had observed that urinary pH has a major impact on the volatilization potential of NH_3 . As pH is reduced, the concentration of NH_4^+ is increased. However, the concentration NH_3 is decreased within a solution. Reduction in these odorous gases could possibly be due to a reduction of the pathogenic bacterial population in the gastrointestinal tract or due to enhanced activity of beneficial microbial activity, leading to changes in end products of microbial fermentation and a shift in the ecosystem towards more anabolic status. Our experiment results showed that the POA numerically increased counts of *Lactobacillus* but reduced the *E. coli* counts in pigs.

Conclusions

In conclusion, our results suggest that dietary supplementation with 0.2% POA in weaning pigs can improve their growth performance, increase nutrient digestibility of DM and N, and exert beneficial effects on fecal microflora with reduced incidence of diarrhea. It can also reduce the fecal gas emissions of NH_3 and acetic acid. Thus, dietary supplementation with 0.2% protected organic acid is efficacious in weaning pigs, and it can improve the health status and performance of weanling pigs.

References

- Ahmed, S.T., Hwang, J. A., Hoon, J., Mun, H. S., and Yang, C. J. 2014. Comparison of single and blend acidifiers as alternative to antibiotics on growth performance, fecal microflora, and humoral immunity in weaned piglets. *Asian-Aust. J. of Anim. Sci.* **27**(1): 93.
- Amezcuca, R., Friendship, R. M., Dewey, C. E., Gyles, C., and Fairbrother, J. M. 2002. Presentation of postweaning *Escherichia coli* diarrhea in southern Ontario, prevalence of hemolytic *E. coli* serogroups involved, and their antimicrobial resistance patterns. *Can.J of Vet. Res.* **66**(2): 73.
- Budiño, F. E. L., Thomaz, M. C., Kronka, R. N., Nakaghi, L. S. O., Tucci, F. M., Fraga, A. L., Scandolera, A. J., and Huaynate, R. A. R. 2005. Effect of probiotic and prebiotic inclusion in weaned piglet diets on structure and ultra-structure of small intestine. *Braz. Arch. Biol. Techn.* **48**(6): 921-929.
- Canibe, N., Højberg, O., Højsgaard, S., and Jensen, B. B. 2005. Feed physical form and formic acid addition to the feed affect the gastrointestinal ecology and growth performance of growing pigs. *J.Anim. Sci.* **83**(6): 1287-1302.
- Canibe, N., Steien, S., Overland, M., and Jensen, B. B. 2001. Effect of K-diformate in starter diets on acidity, microbiota, and the amount of organic acids in the digestive tract of piglets, and on gastric alterations. *J. Anim. Sci.* **79**(8): 2123-2133.
- Cortus, E. L. 2006. A dynamic model of ammonia production within grow-finish swine barns. University of Saskatchewan.
- Devi, S. M., Balachandar, V., Lee, S. I., and Kim, I. H. 2014. An outline of meat consumption in the Indian population-A pilot review. *Korean. J. Food. Sci. An.* **34**(4): 507.

- Devi, S. M., Lee, K. Y., and Kim, I. H. 2016. Analysis of the effect of dietary protected organic acid blend on lactating sows and their piglets. *Rev. Bras. Zootecn.* **45**(2): 39-47.
- Dibner, J., and Buttin, P. 2002. Use of organic acids as a model to study the impact of gut microflora on nutrition and metabolism. *J. Appl. Poult. Res.* **11**(4): 453-463.
- Hossain, M., Begum, M., and Kim, I. H. 2016. Effect of *Leuconostoc mesenteroides* KCCM35046 fermented aged garlic extract on egg production, egg quality, odour gas emissions, targeted *E. coli* colony, haematological characteristics and fatty acids composition of egg yolk in laying hens. *J. Appl. Anim. Res.* **44**(1): 458-465.
- Kiarie, E., Krause, D., and Nyachoti, C. 2008. Net fluid and electrolyte losses in enterotoxigenic *Escherichia coli* infected piglet small intestine upon perfusion with fumaric acid, zinc oxide, egg yolk antibodies or carbadox. *Can. J. Anim. Sci.* **88**(3): 485-488.
- Kil, D. Y., Kwon, W. B., and Kim, B. G. 2011. Dietary acidifiers in weanling pig diets: a review. *Rev. Colomb. Cienc. Pec.* **24**(3): 231-247.
- Laine, T. M., Lyytikäinen, T., Yliaho, M., and Anttila, M. 2008. Risk factors for post-weaning diarrhoea on piglet producing farms in Finland. *Acta. Vet. Scand.* **50**(1): 21.
- Lallès, J.-P., Boudry, G., Favier, C., Le Floch, N., Luron, I., Montagne, L., Oswald, I. P., Pié, S., Piel, C., and Sève, B. 2004. Gut function and dysfunction in young pigs: physiology. *Anim. Res.* **53**(4): 301-316.
- Lee, D., Liu, S., Chen, Y., Wang, R., Lin, S., and Weng, C. 2007. Effects of diets supplemented with organic acids and nucleotides on growth, immune responses and digestive tract development in weaned pigs. *J. Anim. Physiol. An. N.* **91**(11-12): 508-518.
- Li, Z., Yi, G., Yin, J., Sun, P., Li, D., and Knight, C. 2008. Effects of organic acids on growth

- performance, gastrointestinal pH, intestinal microbial populations and immune responses of weaned pigs. *Asian-Aust. J. Anim. Sci.* **21**(2): 252.
- Manzanilla, E., Perez, J., Martin, M., Kamel, C., Baucells, F., and Gasa, J. 2004. Effect of plant extracts and formic acid on the intestinal equilibrium of early-weaned pigs. *J. Anim. Sci.* **82**(11): 3210-3218.
- Mroz, Z. 2005. Organic acids as potential alternatives to antibiotic growth promoters for pigs. *Adv. Pork Prod.* **16**(1): 169-182.
- Mroz, Z., Jongbloed, A., Partanen, K., Vreman, K., Kemme, P., and Kogut, J. 2000. The effects of calcium benzoate in diets with or without organic acids on dietary buffering capacity, apparent digestibility, retention of nutrients, and manure characteristics in swine. *J. Anim. Sci.* **78**(10): 2622-2632.
- NRC. 2012. Nutrient Requirements of swine. 11th ed. National Academic Press, Washington, DC.
- Øverland, M., Kjos, N., Borg, M., Skjerve, E., and Sørum, H. 2008. Organic acids in diets for entire male pigs: Effect on skatole level, microbiota in digesta, and growth performance. *Livest. Sci.* **115**(2-3): 169-178.
- Papatsiros, V., Tassis, P., Tzika, E., Papaioannou, D., Petridou, E., Alexopoulos, C., and Kyriakis, S. 2011. Effect of benzoic acid and combination of benzoic acid with a probiotic containing *Bacillus cereus* var. *toyoi* in weaned pig nutrition. *Pol. J. Vet. Sci.* **14**(1): 117-125.
- Radecki, S., Juhl, M., and Miller, E. 1988. Fumaric and citric acids as feed additives in starter pig diets: effect on performance and nutrient balance. *J. Anim. Sci.* **66**(10): 2598-2605.
- Ravindran, V. and Kornegay, E. 1993. Acidification of weaner pig diets: a review. *J. Sci. Food.*

- Agr. **62**(4): 313-322.
- Smiricky, M., Grieshop, C., Albin, D., Wubben, J., Gabert, V., and Fahey Jr, G. 2002. The influence of soy oligosaccharides on apparent and true ileal amino acid digestibilities and fecal consistency in growing pigs. *J. Anim. Sci.* **80**(9): 2433-2441.
- Sørensen, M. T., Vestergaard, E.-M., Jensen, S. K., Lauridsen, C., and Højsgaard, S. 2009. Performance and diarrhoea in piglets following weaning at seven weeks of age: Challenge with *E. coli* O 149 and effect of dietary factors. *Livest. Sci.* **123**(2): 314-321.
- Thaela, M., Jensen, M., Pierzynowski, S., Jakob, S., and Jensen, B. 1998. Effect of lactic acid supplementation on pancreatic secretion in pigs after weaning. *J. Anim. Feed. Sci.* **7**(Suppl 1): 181-183.
- Thomsson, A., Rantzer, D., Botermans, J., and Svendsen, J. 2008. The effect of feeding system at weaning on performance, health and feeding behaviour of pigs of different sizes. *Acta. Agr. Scan. A* **58**(2): 78-83.
- Upadhaya, S. D., Lee, K. Y., and Kim, I. H. 2014. Protected organic acid blends as an alternative to antibiotics in finishing pigs. *Asian-Aust. J. Anim. Sci.* **27**(11): 1600.
- Upadhaya, S. D., Lee, K. Y., and Kim, I. H. 2016. Effect of protected organic acid blends on growth performance, nutrient digestibility and faecal micro flora in growing pigs. *J. Appl. Anim. Res.* **44**(1): 238-242.
- Upadhaya, S. D., Li, T. S., and Kim, I. H. 2017. Effects of protected omega-3 fatty acid derived from linseed oil and vitamin E on growth performance, apparent digestibility, blood characteristics and meat quality of finishing pigs. *Anim. Prod. Sci.* **57**(6): 1085-1090.
- Wallgren, P., and Melin, L. 2001. Weaning system in relation to disease. The weaner pig Nutrition and Management: 309-316.

- Wang, J., Yoo, J., Lee, J., Jang, H., Kim, H., Shin, S., Seong, S., and Kim, I. 2009. Effects of phenyllactic acid on growth performance, nutrient digestibility, microbial shedding, and blood profile in pigs. *J. Anim. Sci.* **87**(10): 3235-3243.
- Williams, C., David, D. J., and Iismaa, O. 1962. The determination of chromic oxide in faeces samples by atomic absorption spectrophotometry. *J. Agr. Sci.* **59**(3): 381-385.
- Yin, Y., Baidoo, S., Schulze, H., and Simmins, P. 2001. Effects of supplementing diets containing hulless barley varieties having different levels of non-starch polysaccharides with β -glucanase and xylanase on the physiological status of the gastrointestinal tract and nutrient digestibility of weaned pigs. *Livest. Pro. Sci* **71**(2-3): 97-107.

Table 1. Compositions of basal weanling pig diets (as- fed basis)

Item	Phase 1 (week 0~2)	Phase 2 (week2~6)
Ingredient (g kg ⁻¹)		
Extruded corn	111.5	349.2
Extruded oat	100.0	-
Biscuit meal	-	50.0
Soybean meal (44% CP)	80.0	200.0
Fermented soybean meal	78.0	82.0
Fish meal	50.0	40.0
Soy oil	41.5	48.0
Lactose	100.0	60.0
Whey	170.0	107.0
Milk product	130.0	20.0
Monocalcium phosphate	12.5	10.0
Sugar	40.0	20.0
Plasma powder	65.0	
L-Lys·HCl (78%)	1.2	2.5
DL-Met (50%)	2.6	1.5
L-Thr (89%)	7.7	0.8
Choline chloride (25%)	2.0	1.0
Vitamin premix ^a	1.0	1.0
Trace mineral premix ^b	2.0	2.0
Limestone	2.0	2.0
Salt	3.0	3.0
Analyzed composition (g kg⁻¹)		
Gross Energy (kcal kg ⁻¹)	14.8	14.8
Crude Protein	226.3	215.4
Lys	16.1	15.1
Met + Cys	8.0	8.9
Ca	8.7	7.9
P	7.8	7.4
Fe (mg kg ⁻¹)	45	40

^aProvided per kg of premix: vitamin A, 11,025 IU; vitamin D₃, 1,103 IU; vitamin E, 44 IU; vitamin K, 4.4 mg; riboflavin, 8.3 mg; niacin, 50 mg; thiamine, 4 mg; d-pantothenic, 29 mg; choline, 166 mg; and vitamin B₁₂, 33 µg.

^bProvided per kg of complete diet: 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.

Table 2. Effect of protected organic acid supplementation on growth performance and nutrient digestibility in weanling pigs^a

Item	CON	UOA	POA1	POA2	SEM ^b	CON vs UOA	UOA vs POA	P value	
								Linear	Quadratic
Weeks 0 ~ 2									
ADG (g)	307	311	319	324	3.8	0.5315	0.0368	0.0092	0.5621
ADFI (g)	397	395	401	402	3.783	0.6743	0.1917	0.3925	0.8435
G/F	0.775	0.788	0.796	0.807	0.009	0.3086	0.2061	0.0342	0.5879
Weeks 2 ~ 6									
ADG (g)	502	507	508	514	2.21	0.1736	0.1137	0.0039	0.8618
ADFI (g)	762	757	755	750	3.613	0.3275	0.2937	0.0345	0.7544
G/F	0.659	0.669	0.67	0.681	0.005	0.1554	0.1177	0.0055	0.7471
Week 0 ~ 6									
ADG (g)	437	441	443	452	1.99	0.1583	0.0095	0.0003	0.8232
ADFI (g)	641	636	637	634	3.132	0.3620	0.7973	0.1736	0.8236
G/F	0.683	0.694	0.696	0.71	0.005	0.1339	0.0596	0.0024	0.7483
Nutrient digestibility									
Dry matter (%)	80.4	80.24	82.87	83.11	0.842	0.8924	0.0111	0.0152	0.2295
Nitrogen (%)	74.01	75.83	75.25	76.79	0.98	0.1953	0.8777	0.0844	0.9138
Gross Energy (%)	79.91	81.14	81.72	82.59	0.744	0.2490	0.2718	0.0044	0.5325

Note: There were 7 replicate pens per treatment with 4 pigs per pen: ^a CON, basal diet; UOA, CON + unprotected organic acid 0.2%; POA1, CON+ protected organic acid 0.1%; POA2, CON+ protected organic acid 0.2%;

ADG, average daily gain; ADFI, average daily feed intake; G/F, gain: feed ratio.

^b Standard error of the mean.

Table 3. Effects of protected organic acid supplementation on fecal microbial shedding, diarrhea score and gas emission in weanling pigs^a

Item	CON	UOA	POA1	POA2	SEM ^b	CON vs UOA	UOA vs POA	P value	
								Linear	Quadratic
Microbial counts (log ₁₀ cfu/g)									
<i>Lactobacillus</i>	7.43	7.44	7.58	7.60	0.11	0.8108	0.0290	0.0038	0.1274
<i>Bifidobacterium</i> spp.	8.49	8.75	8.79	8.86	0.20	0.2869	0.1610	0.0649	0.4701
<i>E. coli</i>	5.70	5.65	5.60	5.49	0.11	0.5303	0.0600	0.0125	0.8755
<i>Clostridium perfringens</i>	8.25	7.74	7.70	7.61	0.15	0.1714	0.2400	0.0866	0.4283
<i>Salmonella</i>	2.53	2.42	2.39	2.34	0.04	0.1320	0.0470	0.0244	0.4627
Diarrhea Score ^c	1.61	1.33	1.27	1.24	0.06	0.0576	0.0900	0.0022	0.0431
Items (ppm)									
Ammonia	23.3	21.4	19.8	17.4	0.2	0.0828	0.5200	0.0006	0.0458
Total mercaptans	9.0	8.2	7.3	6.7	1.7	0.5780	0.9100	0.1613	0.6394
Hydrogen sulfide	10.7	10.6	9.1	8.8	1.3	0.9678	0.8070	0.1695	0.5223
Acetic acid	18.7	18.3	17.8	17.2	0.5	0.2691	0.2610	0.0277	0.6556

Note: There were 7 replicate pens per treatment with 4 pigs per pen: ^a CON, basal diet; UOA, CON + unprotected organic acid 0.2%; POA1, CON+ protected organic acid 0.1%; POA2, CON+ protected organic acid 0.2%;

^b Standard error of the mean.

^c Diarrhea score: 1 = hard, dry pellet in a small, hard mass, 2 = hard, formed stool that remains firm and soft, 3 = soft, formed, and moist stool that retains its shape, 4 = soft, unformed stool that assumes shape of the container, 5 = watery, liquid stool that can be poured.