



δ -Aminolevulinic acid, and lactulose supplements in weaned piglets diet: Effects on performance, fecal microbiota, and in-vitro noxious gas emissions



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ABSTRACT

δ -Aminolevulinic acid (ALA) is a non-protein amino acid that plays a rate limiting role in the process of heme biosynthesis. Lactulose (LAC) is a kind of non-digestible oligosaccharides which has been shown to improve growth performance in weaning pigs through prebiotic actions. This study evaluated the efficacy of ALA, and LAC in weaned piglets. The study was conducted with one hundred seventy five [(Yorkshire \times Landrace) \times Duroc] weaned piglets in a 33 d feeding trial, and one of five diets: 1) CON (basal diet, no antibiotic); 2) ALA05 (CON + 0.5 g ALA/kg of diet); 3) ALA10 (CON + 1 g ALA/kg of diet); 4) LAC05 (CON + 0.5 g LAC/kg of diet); 5) LAC10 (CON + 1 g LAC/kg of diet). All data were statistically analyzed using the PROC MIXED procedure of SAS. Orthogonal contrasts were used to the effects of treatments. Weaning pigs fed diets with the ALA, and LAC had higher body weight (BW) compared with pigs fed the CON diet on d 19 ($P=0.028$, and 0.011), and d 33 ($P=0.031$, and 0.015), respectively. In addition, LAC supplementation had higher BW than ALA supplementation ($P=0.046$) on d 19. Piglets fed diets with ALA, and LAC had higher average daily growth (ADG), and feed efficiency (G:F) compared with piglets fed CON diet during phase 2 (d 6–19), and overall (d 1–33), respectively ($P<0.05$). Besides, LAC diets improved ADG ($P=0.037$), and G:F ($P=0.024$) compared with ALA diets during phase 2. Weaned piglets fed LAC increased dry matter (DM; d 19, and 33, respectively), nitrogen (d 33), and energy (d 19) digestibility compared with those fed CON diet ($P<0.05$). ALA supplementation increased DM digestibility compared with CON diet ($P=0.041$) on d 33. Piglets fed with the ALA diet increased serum total iron-binding capacity, hemoglobin, and hematocrit, and blood red blood cell compared with those fed the CON diet ($P<0.05$). Piglets fed with the LAC diet increased fecal *Lactobacillus*, and reduced *E. coli* counts ($P<0.05$) when compared with those fed the CON on d 19, and 33, respectively. Moreover, piglets fed with the LAC diets had higher fecal *Lactobacillus* (d 19, and 33), and lowered *E. coli* (d 33) than pigs those fed the ALA diets ($P<0.05$). The fecal moisture, and diarrhea score were not affected by dietary supplementation with ALA or LAC during the whole experiment. Piglets fed the LAC diets had reduced ammonia gas emissions compared with the CON diet on d 33 ($P<0.001$). In conclusion, results indicated that dietary supplementation of ALA, and/or LAC improved performance, and reduced noxious gas emissions in weaned piglets.

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1. Introduction

Weaning is a critical stage for pigs because of alterations in the gastrointestinal tract structure, and function, changes to the histology, and biochemistry of the small intestine in adapting to enteric microbiota, and immune response challenges, as well as weaning stresses, such as nutritional, environmental, and social that are responsible for depressed growth performance, and nutrient malabsorption (Pluske et al., 1997; Wang et al., 2009; Zhang

et al., 2013). Iron (Fe) is the main deficient mineral in nursery pigs due to poor efficiency of Fe transfer through the placenta (Wang et al., 2009), and low Fe concentrations in the milk of sows (NRC, 2012); furthermore, weaning pigs rapidly increase red blood cell volume, and body mass (Yu et al., 2000). Therefore, direct intramuscular (IM) injection of Fe preparations in nursery pigs has become popular in anemia prevention (Bruininx et al., 2000). Dietary δ -aminolevulinic acid (ALA) supplementation is a new approach that is expected to have beneficial effects on Fe utilization, and hemoglobin (Hb) synthesis in pigs. δ -Aminolevulinic acid plays an important role in oxygen transport through the biosynthesis of heme, which initially occurs in the mitochondrion, and involves the condensation of glycine, and succinyl CoA to form

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ALA, and primary functional form of Fe that acts as a prosthetic group of Hb (Mateo et al., 2006; Wang et al., 2009; 2011). Based on previous study, the addition of 500 mg/kg of ALA to the diets of weaned pigs improves growth performance, serum Fe, Hb, and lymphocyte concentrations (Min et al., 2004). Our previous studies with relatively low doses (3, 10, and 50 mg/kg) suggested that ALA supplementation could affect the synthesis of Hb, and positively influence the Fe status without any significant overall growth performance in weaned pigs (Chen et al., 2008a; Wang et al., 2011; Yan and Kim, 2011).

Lactulose (LAC) is a kind of non-digestible oligosaccharide (NDO, Schumann, 2002; Cho and Kim, 2014), which is produced by isomerization of lactose by regrouping the glucose residue to a fructose molecule. Previous studies showed that lactulose elicits a prebiotic effect by increasing *Lactobacillus*, and *Bifidobacteria* counts in pigs (Konstantinov et al., 2006). Animal studies further showed that LAC has various physiological functions including reducing fermentative diarrhea by reducing *E. coli* during the pig rearing period (Krueger et al., 2002), improving apparent metabolizable nitrogen, as well as reducing ammonia, and acetic acid gas emissions in broilers (Cho and Kim, 2014), and enhancing growth performance in nursery pigs, and broilers (Miguel et al., 2004; Cho and Kim, 2014; Hossain et al., 2014). However, several supplemental NDO ingredients, such as galacto-oligosaccharide (GOS), manno-oligosaccharide (MOS), and chito-oligosaccharide (COS) were shown to improve growth performance in nursery pigs via prebiotic mechanisms (Miguel et al., 2004; Yan and Kim, 2011; Zhao et al., 2012; Zhao et al., 2013). Moreover, it was previously suggested that NDO could accelerate the absorption of Fe by chelating Fe at low pH thus increasing its solubility in the intestines (Liao et al., 2007; Xia et al., 2011). Therefore, this study was designed to evaluate the effects of ALA, and LAC on performance, and fecal characteristics in weaning pigs, and examine the orthogonal contrast effect of dietary ALA versus LAC.

2. Materials, and methods

All animals received human care as outlined in the guide for the care, and use of experimental animals (Dankook University, South Korea, Animal Care Committee).

2.1. Animals, and diets

A total of 175 crossed healthy weaning pigs [(Yorkshire × Landrace) × Duroc] with an average body weight (BW) of 8.04 ± 0.92 kg (28d of age) were used in a 33 days experiment. Pigs were randomly allotted to 1 of 5 experimental diets according to initial BW in a randomly complete block design. There were 7 replicated pens per treatment with 5 pigs (3 gilts, and 2 borrows) per pen. Dietary treatments were: 1) CON: basal diet, no antibiotic 2) CON+0.5 g ALA/kg of diet (ALA05), 3) CON+1 g ALA/kg of diet (ALA10), 4) CON+0.5 g LAC/kg of diet (LAC05), and 5) CON+1 g LAC/kg of diet (LAC10). The experiment included 3 phases (5–14–14 day phase feeding; phase 1, provided during d 1 to 5; phase 2, provided during d 6 to 19, and phase 3, provided during d 20 to 33). All diets were formulated to meet or exceed the recommendation of NRC (2012) for weaning pigs, and were fed in a mash form (Table 1). ALA, and LAC as powder form were supplied by EasyBio, Seoul, Korea, and added at 0.5, and 0.1 g/kg to the basal diet, respectively. All pigs were housed in an environmentally controlled nursery room. The stainless steel pens were 0.5 m × 0.6 m × 2.0 m with a slatted plastic floor. Each pen was provided with a stainless steel feeder and a nipple waterer that allowed ad libitum access to feed and water throughout the experiment. Ventilation was provided by a mechanical system and

Table 1
Basal diet composition (as-fed basis).

Items	Phase 1 ^a	Phase 2 ^a	Phase 3 ^a
Ingredients (g/kg)			
Extruded corn	111.5	349.2	451.0
Extruded oat	100.0	–	–
Biscuit meal	–	50.0	90.0
Soybean meal, 440 g crude protein/kg	80.0	200.0	296.5
Fermented soybean	78.0	82.0	–
Fish meal	50.0	40.0	25.0
Soy oil	41.5	48.0	30.0
Lactose	100.0	60.0	–
Whey	165.0	100.0	–
Milk product	130.0	20.0	20.0
MCP	12.5	10.0	6.0
Sugar	40.0	20.0	–
Plasma powder	65.0	–	–
L-Lys HCl	1.2	2.5	1.6
DL-Met	2.6	1.5	1.4
L-Thr	7.7	0.8	–
Choline chloride	2.0	1.0	1.0
Vitamin premix ^b	1.0	1.0	1.0
Trace mineral premix ^c	2.0	2.0	2.0
Limestone	–	2.0	3.0
Salt	–	2.0	3.0
Calculated values (g/kg)			
ME (MJ/kg)	14.8	14.8	14.6
SID ^d Lysine	14.8	14.7	13.4
SID Methionine	4.7	4.7	4.2
SID Tryptophan	2.7	2.7	2.8
SID Threonine	7.8	7.7	6.8
Ca	8.1	7.8	7.5
Avail P	5.5	5.5	4.3
Analyzed values (g/kg)			
pH	6.05	6.02	6.03
CP	219.3	210.1	204.7
Ca	8.1	7.7	7.4
Total P	7.5	7.6	7.4
Crude fat	51.1	50.1	39.8

^a Phase 1, provided during 1–5 days; phase 2, provided during 6–19 days, and phase 3, provided during 20–33 days.

^b 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.

^c 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.

^d SID = Standardized ileal digestible.

lighting was automatically regulated to provide 12 h of artificial light per day. The ambient temperature within the room was approximately 30 °C at the start of the experiment and decreased by 1 °C each wk.

2.2. Chemical analysis

Feed, and fecal samples were ground to pass through a 1-mm screen, after which they were analyzed for DM (method 934.01; AOAC, 2000), crude protein (CP, method 990.03; AOAC, 2000), crude fat (method 920.39; AOAC, 1995), calcium (Ca, method 984.01; AOAC, 1995), and phosphorus (P, method 965.17; AOAC, 1995). Nitrogen (N) was determined (Kjeltec 2300 Nitrogen Analyzer; Foss Tecator AB, Hoeganaes Sweden), and CP was calculated as $N \times 6.25$. Gross energy (GE) was analyzed by oxygen bomb calorimeter (Parr 1600 Instrument Co., Moline, IL, USA). The pH of the feed sample was measured by a calibrated, glass electrode pH meter (WTW pH 340-A, WTH Measurement Systems, Ft. Myers, FL, USA).

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