



Dietary supplementation of delta-aminolevulinic acid to lactating sows improves growth performance and concentration of iron and hemoglobin of suckling piglets

S I LEE¹, T S LI² and I H KIM³

Dankook University, Cheonan, Chungnam, 330 714 Republic of Korea

Received: 11 August 2015; Accepted: 16 November 2015

ABSTRACT

To confirm whether dietary supplementation of delta-aminolevulinic acid (δ -ALA) to lactating sow improves iron concentration of suckling piglets, 24 gestating sows [(Yorkshire \times Landrace), before 14-day of farrowing] and their litters were evaluated on growth performance, blood characteristics, and milk composition. Dietary treatments were as follows: (i) CON, corn-soybean meal based diet; (ii) CON + 0.05 % δ -ALA; and (iii) CON + 0.1 % δ -ALA. Lactating sows fed with δ -ALA had increased backfat thickness and decreased backfat thickness at weaning. Also, dietary supplementation of δ -ALA increased protein concentration in the milk of lactating sows (linear). In addition, lactating sows fed with δ -ALA had increased BW (linear), ADG (linear), iron (linear), and hemoglobin (linear) concentration in suckling piglets. Based on the results, we suggested that dietary supplementation of δ -ALA to lactating sows improved growth performance and concentration of iron and hemoglobin in blood of suckling piglets. Also, dietary supplementation of δ -ALA had positive effects on backfat thickness and protein concentration in milk of lactating sows.

Key words: Delta-aminolevulinic acid, Iron, Lactating sow, Suckling piglets

Iron plays an important role related to the metabolism of oxygen in the body, and it is a major component of hemoglobin which carries oxygen to all parts of the body. As low hepatic irons are stored in newborn piglets and low irons are transferred through the placenta and milk in gestating and lactating sows, iron is commonly deficient in newborn piglets (NRC 2012, Yu *et al.* 2000). To overcome iron deficiency that results in anemia, iron injection is commonly given to piglets via intramuscular injection between 1–7 days after birth. As an alternative to direct iron injection to piglets, feed additives have received much interest as possible utilization of iron in the pig research and industry (Cho and Kim 2011, Wang *et al.* 2009, Wang and Kim 2012, Wang *et al.* 2011b, Yan and Kim 2011).

The delta-aminolevulinic acid (δ -ALA) is the first compound in the porphyrin synthesis pathway, which leads to heme in mammals. It is a non-protein amino acid with a widespread distribution in living organisms (Doering *et al.* 1998). The δ -ALA plays an important role in oxygen transport since the function of δ -ALA is related to biosynthesis of heme, which is the primary functional form of iron, and acts as a prosthetic group of hemoglobin (Brown *et al.* 2004). Dietary exogenous δ -ALA may stimulate the production of heme, which is a better source of iron than non-heme compounds, and it can be absorbed and utilized

more efficiently in the body (Hallberg *et al.* 1989). Dietary supplementation of δ -ALA showed beneficial effects on the growth performance, concentrations of iron, hemoglobin, lymphocyte and immune response in layer and broiler (Wang *et al.* 2011a, Wang *et al.* 2011b). Chen *et al.* (2008a) reported a positive effect on plasma iron status and immune response during an inflammatory challenge in weaned pig fed dietary supplementation of δ -ALA. Wang and Kim (2012) reported that sows fed with δ -ALA during gestation had larger litter size born alive and piglets with higher birth body weight.

In the present study, to test whether the hypothesis that dietary supplementation of δ -ALA to lactating sows can positively influence productivities including iron concentration of suckling piglets, we investigated the effects of dietary supplementation of δ -ALA to lactating sows on growth performance, blood characteristics, and composition of milk in lactating sows and their litters.

MATERIALS AND METHODS

The animal care and protocol used in this study were approved by the Animal Care and Use Committee of Dankook University.

Experimental design, animals, and diets: Gestating sows [24 (Yorkshire \times Landrace), before 14-day of farrowing] and their litters were used in this 42d trial. Sows were assigned randomly to 1 of 3 feeding groups, with an average parity of 1.5 ± 0.2 . At day 107 of gestation, the sows were

Present address: ³Professor (inhokim@dankook.ac.kr), Department of Animal Resource and Science.

moved to farrowing crates (2.4 m×1.8 m), which were constructed with 1.95 m² of solid floor and 2.37m³ of slatted floor. This space included a piglet nest equipped with an infrared lamp, a piglet drinking nipple, and a piglet feeder placed on dimpled rubber matting to collect any spillage from the feed. The temperature in the farrowing house was maintained at a minimum of 20°C. Drinking nipples provided water *ad lib.* to both sows and the piglets. At weaning, the sows were relocated to a mating room, with the piglets remaining in the pen for 3d, and the number of weaning piglets was recorded. Litter numbers at birth varied from 8 to 12 piglets, which was standardized to 10 piglets per litter within 2 day after birth by cross-fostering within each treatment. Dietary treatments were as follows: (i) CON, corn-soybean meal based diet; (ii) CON+ 0.05 % δ -ALA; and (iii) CON+ 0.1 % δ -ALA (Table 1). Sows were fed on lactation feeding strategies as their weight increased to 7 kg, and weaning of piglets was at 24d.

Sampling and measurements: On the day before farrowing, the backfat of sows was measured at 6 cm off the midline on the 10th rib, using a real-time ultrasound instrument. After farrowing, piglets were ear notched and weighed individually. The individual BW and number of piglets per litter were recorded at weaning, and at this time, the BW and last rib fat depth of sows were recorded again. Feed intake of sows was recorded daily to determine the daily feed intake during lactation.

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

	Lactation diet
<i>Ingredients (%)</i>	
Corn	51.00
Soybean meal, 48% CP	26.73
Wheat bran	1.00
Rice bran	5.00
Rapeseed meal, 43% CP	3.50
Tallow	6.05
Molasses	3.50
Dicalcium phosphate	1.64
Limestone	0.76
Salt	0.50
L-lysine-HCl, 78%	0.12
Vitamin ¹	0.10
Mineral ²	0.10
Total	100
<i>Calculated composition, %</i>	
Metabolizable energy, kcal/kg	3,456
Crude protein	18.34
Crude fat	9.16
Lysine	1.08
Calcium	1.06
Phosphorus	0.73

¹Provided per kg of complete diet: 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; pantothenic acid, 29 mg; choline, 166 mg; and vitamin B12, 33 μ g. ²Provided per kg of complete diet: Cu, 12 mg; Zn, 85 mg; Mn, 8 mg; I, 0.28 mg; and Se, 0.15 mg.

Milk (60 mL) was collected at 15d of lactation from each lactating sows. The milk samples were stored at -20 °C to be analyzed for protein and fat (Kjeldahl N \times 6.8). The lactose concentration was analyzed by an automatic biochemistry analyzer.

Per treatment, 20 piglets were selected and blood samples were taken from selected piglets at weaning. Blood samples were collected from the cervical vein into clot activator vacuum tubes. The serum was separated by centrifugation at 4,000 \times g for 30 min at 4°C, and an aliquot was stored at -4°C for subsequent determination of the hemoglobin and iron concentrations by using an automatic biochemistry analyzer.

Statistical analysis: All data were subjected to statistical analyses as a randomized complete block design, using the GLM procedure of the SAS software (SAS Institute 1996). Means were separated using orthogonal polynomial contrasts to examine the effects of δ -ALA diets. Variability in the data was expressed as the pooled standard error means (SEM). P<0.05 were considered to indicate statistical significance.

RESULTS AND DISCUSSION

Performance in sows and suckling piglets: The effects of dietary supplementation of δ -ALA on the performance during lactation are shown in Table 2. There were no differences observed in the number of piglets born alive and weaning, BW, BW loss, ADFI, and estrus interval among treatments (P>0.05). Lactating sows fed with δ -ALA showed increased backfat thickness and decreased backfat thickness loss at weaning (linear; P= 0.019 and <0.001, respectively). The effects of dietary supplementation of δ -ALA to lactating sows on the growth performance of suckling piglets are shown in Table 3. There was no difference observed in piglet survival among the treatments (P>0.05). Dietary supplementation of δ -ALA to lactating sows increased BW and ADG of piglets during suckling (linear; P<0.001).

Earlier it was reported that dietary supplementation of δ -ALA can improve growth performance of broilers and egg laying performance of layers (Chen *et al.* 2008b, Wang *et al.* 2011a, Wang *et al.* 2011b). However, in previous pig trials, effect of dietary supplementation of δ -ALA on growth performance showed inconsistent results. Min *et al.* (2004) reported that dietary supplementation of δ -ALA with antibiotics improved growth performance of nursery pig during 20d trials. However, Mateo *et al.* (2006) found that weaned pigs fed with 0.05% δ -ALA had no adverse effects on growth performance. Also, Chen *et al.* (2008a) demonstrated that dietary supplementation of increasing level (0, 5, 10, and 15 mg/kg) of δ -ALA had no beneficial effect on growth performance during overall period, even though ADG was affected by 15 mg/kg δ -ALA during 22 to 35d of feeding trials. Inconsistent results of these reports might be experimental sanitary conditions and different supplemented level of δ -ALA. In addition to direct supplementation of δ -ALA to pigs, Wang *et al.* (2009)

Table 2. Effects of dietary supplementation of δ -ALA on productivity of sows during lactation

Item	δ -ALA			SEM	P-value	
	0%	0.05%	0.10%		Linear	Quadratic
Parity	1.60	1.50	1.40	0.20	0.637	0.417
<i>Litter</i>						
No. of pigs	9.30	10.00	9.10	0.50	0.856	0.188
Weaned Pigs	8.60	9.40	8.90	0.50	0.717	0.305
<i>Body weight, kg</i>						
Before farrowing	192.90	198.60	198.40	2.30	0.112	0.302
After farrowing	176.00	180.70	180.70	2.20	0.156	0.401
Weaning	157.80	159.70	160.60	2.50	0.442	0.858
<i>Body weight loss, kg</i>						
After farrowing	16.90	18.00	17.70	1.77	0.757	0.766
After weaning	18.15	20.94	20.11	1.35	0.322	0.293
<i>Backfat thickness, mm</i>						
Before farrowing	20.10	20.00	20.30	0.40	0.839	0.725
After farrowing	17.30	17.50	17.90	0.40	0.058	0.362
After weaning	14.80	15.40	16.80	0.50	0.019	0.489
<i>Backfat loss, mm</i>						
After farrowing	2.80	2.50	2.40	0.30	0.407	0.872
After weaning	2.50	2.10	1.10	0.20	<0.001	0.228
<i>Average feed intake, kg</i>						
From farrowing to weaning	7.37	7.30	7.30	0.04	0.234	0.53
Estrus interval, d	4.40	4.30	4.30	0.20	0.570	0.742

Table 3. Effects of dietary supplementation of δ -ALA to lactating sows on growth performance of suckling piglets

Item	δ -ALA			SEM	P-value	
	0%	0.05%	0.10%		Linear	Quadratic
Piglet survival, %	93.62	94.31	96.42	2.41	0.438	0.805
<i>Body weight, kg</i>						
Birth	1.57	1.58	1.59	0.07	0.878	0.921
Weaning	6.56	6.89	7.05	0.07	<0.001	0.323
Average daily gain, g	216.83	230.53	237.38	2.52	<0.001	0.228

demonstrated that effect of dietary supplementation of 10 mg/kg δ -ALA to sows during gestation and lactation enhances growth performance of their litters at birth and weaning. Also, Wang and Kim (2012) reported that sows fed with 90 mg/kg δ -ALA during gestation and lactation improved larger litter size born alive and piglets with higher body weight at birth and weaning. In agreement with previous report, the present study used to evaluate effect of dietary supplementation of 0.05 and 0.01% δ -ALA to lactating sows on growth performance of suckling piglets showed increased BW and ADG, compared with piglets from the sows fed without δ -ALA. In the present study, we used 0.05 and 0.1% δ -ALA for optimization of δ -ALA concentration in lactating sows diets, because it is known that iron concentration must be avoided from an excessive level, since the unbound serum iron has negative effects on growth performance and can cause diarrhea (Chen *et al.* 2008a, Knight *et al.* 1983). The present study suggested

Table 4. Effects of dietary supplementation of δ -ALA to lactating sows on iron and hemoglobin concentration in blood plasma of piglets

Item	δ -ALA			SEM	P-value	
	0%	0.05%	0.10%		Linear	Quadratic
Iron, ug/dL	63.07	77.41	90.62	1.41	0.001	0.907
Hemoglobin, g/dL	11.11	14.52	15.70	0.52	0.031	0.422

that lactating sows fed with up to 0.1% δ -ALA had positive effect on growth performance of suckling piglets. In addition to effect of suckling piglet, we found that dietary supplementation of δ -ALA had no effect on productivity except for backfat thickness and its loss in the lactating sows. In agreement with the present study, supplementation of 10 and 90 mg/kg δ -ALA to gestating and lactating sows had no effect on BW, feed intake, and wean-to-estrus interval in sows (Wang *et al.* 2009, Wang and Kim 2012). However, supplementation of δ -ALA to lactating sows had positive effect on backfat thickness and its loss after farrowing in the present study. In agreement with present study, sows fed with 10 mg/kg δ -ALA had less ultrasound backfat loss during lactation, compared with those that were not fed with δ -ALA (Wang and Kim 2012).

Iron and hemoglobin concentration in blood plasma of suckling piglets and composition of milk of lactating sows: The effects of dietary supplementation of δ -ALA to lactating sows on the iron and hemoglobin concentration in the blood of suckling piglets are shown in Table 4. Dietary supplementation of δ -ALA to lactating sows increased iron

Table 5. Effects of dietary supplementation of δ -ALA on composition of milk in lactating sows

Item	δ -ALA			SEM	P-value	
	0%	0.05%	0.10%		Linear	Quadratic
Protein, %	5.49	5.83	5.96	0.13	0.010	0.415
Lactose, %	4.78	4.92	4.87	0.08	0.453	0.343
Fat, %	7.02	7.22	7.21	0.14	0.360	0.575

(linear; $P=0.001$) and haemoglobin (linear; $P=0.031$) concentration in the blood of suckling piglets. The effects of dietary supplementation of δ -ALA to lactating sows on the composition of milk from lactating sows are shown in Table 5. Lactating sows fed with δ -ALA showed increase in protein concentration of the milk (linear; $P=0.01$). There were no differences observed on the lactose and fat concentration in milk among the treatments ($P<0.05$).

In previous report, it was reported that dietary supplementation of 10 mg/kg (0.001%) δ -ALA enhances iron transfer efficiency from lactating sows to suckling piglets, through elevated milk iron concentration and increased blood iron concentration of lactating sows and piglets (Wang *et al.* 2009). In the present study, dietary supplementation of δ -ALA to lactating sows increased iron and hemoglobin concentration in the blood of suckling pigs and protein concentration in the milk of lactating sows. It is well known that heme and hemoglobin concentrations in blood are considered to be useful criteria for evaluating the effect of δ -ALA, which can affect iron metabolism (Cho and Kim 2011, Laftah *et al.* 2008, Zhu *et al.* 2002). It seems to be improving iron status in the piglets occurred by increasing concentration of iron in the blood and milk of the sows, because lactating sows fed with δ -ALA elevated milk iron concentration and increased blood iron concentration of lactating sows in previous report (Wang *et al.* 2009). Indeed, newborn piglets declined plasma hemoglobin concentration in blood from birth to second of age because a plasma hemoglobin concentration in neonates was diluted by increasing postnatal circulatory fluid from the water in colostrum (Peters and Mahan 2008). It is well accepted that simple dietary supplementation of iron to sows did not change the milk iron concentration (Veum *et al.* 1965). However, Wang *et al.* (2009) reported increase in the concentration of iron in the milk in sows fed with δ -ALA prior to parturition. It might be explained that δ -ALA is able to stimulate heme generation, which is more efficient than simple dietary iron supplementation (Chen *et al.* 2008a, Hallberg *et al.* 1989, Mateo *et al.* 2006). However, because dietary supplementation of δ -ALA did not prevent iron deficiency in piglets through the milk from lactating sows, further research is necessary to overcome iron deficiency in piglets by treatment of δ -ALA combined with other component that can be utilize iron metabolism.

Iron deficiency can induce anemia, which is a serious problem for newborn piglets, and this is caused by low iron transferring through the placenta and milk in gestating and lactating sows. Thus, we hypothesized that dietary

supplementation of δ -ALA to lactating sow can positively influence the iron concentration of suckling piglets by transferring of iron through the milk of lactating sows. To test this hypothesis, we investigated the effects of dietary supplementation of δ -ALA had to lactating sows on growth performance, blood characteristics, and composition of milk in lactating sows and suckling piglets. We found that lactating sows fed with δ -ALA had increased backfat thickness after farrowing and weaning and decreased backfat thickness loss after farrowing. Also, lactating sows fed δ -ALA had increased protein concentration in the milk. In addition, we found that lactating sows fed with δ -ALA had increased BW, ADG, iron, and hemoglobin concentration of suckling piglets. Based on the results, we suggested that dietary supplementation of δ -ALA to lactating sows improved growth performance and concentration of iron and hemoglobin in blood of suckling piglets but also had positive effects on backfat thickness and protein concentration in milk of lactating sows. However, further research is necessary to overcome iron deficiency in piglets by treatment of δ -ALA combined with other components that can utilize iron metabolism.

REFERENCES

- Brown K R, Brown B M, Hoagland E, Mayne C L and Hegg E L. 2004. Heme A synthase does not incorporate molecular oxygen into the formyl group of heme A. *Biochemistry* **43**: 8616–24.
- Chen Y J, Kim I H, Cho J H, Min B J, Yoo J S and Wang Q. 2008a. Effect of δ -aminolevulinic acid on growth performance, nutrient digestibility, blood parameters and the immune response of weanling pigs challenged with *Escherichia coli* lipopolysaccharide. *Livestock Science* **114**: 108–16.
- Chen Y J, Kim I H, Cho J H, Yoo J S, Kim H J and Shin S O. 2008b. Utilization of δ -aminolevulinic acid for livestock: Blood characteristics and immune organ weight in broilers. *Journal of Animal Feed Science* **17**: 215–23.
- Cho J H and Kim I H. 2011. δ -Aminolevulinic Acid (ALA) as a potential feed additive in pig: A review. *Journal of Animal Veterinary Advances* **10**: 1627–31.
- Doering F, Walter J, Will J, Foecking M, Boll M, Amasheh S and Daniel H. 1998. Delta-aminolevulinic transport by mammalian peptide transporters. *Federation of American Societies for Experimental Biology Journal* **12**: 2761–67.
- Hallberg L, Brune M and Rossander L. 1989. The role of vitamin C in iron absorption. *International Journal for Vitamin and Nutrition Research Supplement* **30**: 103–08.
- Hertrampf E and Olivares M. 2004. Iron amino acid chelates. *International Journal of Vitamin Nutrition Research* **74**: 435–43.
- Knight C D, Klasing K C and Forsyth D M. 1983. *E. coli* growth in serum of iron dextran-supplement pigs. *Journal of Animal Science* **57**: 387–95.
- Laftah A H, Simpson R J, Peters T J and Raja K B. 2008. The effect of haem biosynthesis inhibitors and inducers on intestinal iron absorption and liver haem biosynthetic enzyme activities. *Toxicology and Applied Pharmacology* **229**: 273–80.
- Mateo R D, Morrow J L, Dailey J W, Ji F and Kim S W. 2006. Use of δ -aminolevulinic acid in swine diet: Effect on growth performance, behavioral characteristics and hematological/immune status in nursery pigs. *Asian Australasian Journal of*

- Animal Science* **19**: 97–101.
- Min B J, Hong J W, Kwon O S, Kang D K and Kim I H. 2004. Influence of dietary δ -aminolevulinic acid supplement on growth performance and hematological changes in weaned pigs. *Korean Society of Food Science Nutrition* **33**: 1606–10.
- NRC.2012. *Nutrient Requirements of Swine*.11th edn. National Academy Press, Washington DC, USA.
- Peters J C and Mahan D C. 2008. Effects of neonatal iron status, iron injections at birth, and weaning in young pigs from sows fed either organic or inorganic trace minerals. *Journal of Animal Science* **86**: 2261–69.
- Veum T L, Gallo J T, Pond W G and Van Vleck L D. 1965. Effect of ferrous fumarate in the lactation diet on sow milk iron, pig hemoglobin and weight gain. *Journal of Animal Science* **24**: 1169–73.
- Wang J P, Kim H J, Chen Y J, Yoo J S, Cho J H, Kang D K, Hyun Y and Kim I H. 2009. Effects of delta-aminolevulinic acid and vitamin C supplementation on feed intake, backfat, and iron status in sows. *Journal of Animal Science* **87**: 3589–95.
- Wang J P and Kim I H. 2012. Effects of iron injection at birth on neonatal iron status in young pigs from first-parity sows fed delta-aminolevulinic acid. *Animal Feed Science and Technology* **178**: 151–57.
- Wang J P, Lee J H, Jang H D, Yan L, Cho J H and Kim I H. 2011a. Effects of δ -aminolevulinic acid and vitamin C supplementation on iron status, production performance, blood characteristics and egg quality of laying hens. *Journal of Animal Physiology and Animal Nutrition* **95**: 417–23.
- Wang J P, Yan L, Lee J H, Zhou T X and Kim I H. 2011b. Effects of dietary delta-aminolevulinic acid and vitamin C on growth performance, immune organ weight and ferrum status in broiler chicks. *Livestock Science* **135**: 148–52.
- Yan L and Kim I H. 2011. Evaluation of dietary supplementation of delta-aminolevulinic acid and chitooligosaccharide on growth performance, nutrient digestibility, blood characteristics, and fecal microbial shedding in weaned pigs. *Animal Feed Science and Technology* **169**: 275–80.
- Yu B, Huang W J and Chiou P WS. 2000. Bioavailability of iron from amino acid complex in weanling pigs. *Animal Feed Science and Technology* **86**: 39–52.
- Zhu Y, Hon T, Ye W and Zhang L. 2002. Heme deficiency interferes with the Ras-mitogen-activated protein kinase signaling pathway and expression of a subset of neuronal genes. *Cell Growth Difference* **13**: 431–39.