Use of δ-Aminolevulinic Acid in Swine Diet: Effect on Growth Performance, Behavioral Characteristics and Hematological/Immune Status in Nursery Pigs*

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ABSTRACT : Certain amino acids are essential precursors of a variety of important biomolecules in addition to their major function as protein building blocks. δ-Aminolevulinic acid (ALA) is synthesized from the condensed form of succinyl-CoA with glycine after decarboxylation catalyzed by ALA synthase. The objective of the study was to determine the effects of ALA supplementation on growth performance, behavioral characteristics and hematological/immune status in nursery pigs. A total of 144 pigs weaned at 21 d of age were allotted to three dietary treatments representing (-) control (w/o antibiotics; NC), (+) control (w/carbadox at 50 mg/kg; PC), and the treatment group with ALA supplementation (0.05%; TA). Each treatment had 6 pens (replicates) with 8 pigs per pen. Pigs were fed phase 1 (21.9% CP, 1.40% Lys) and 2 (20.6% CP, 1.15% Lys) experimental diets for 3 and 2 wks, respectively. Feed intake and weight gain were measured weekly during phase 1 and at the end of phase 2. At the end of phase 2, blood samples were taken and analyzed using an automated hematology analyzer. Skin color and activity of pigs (48 h) from all pens in each treatment were measured at the second week of phase 2. Growth performance was not affected ($p>0.05$) by the dietary supplementation of ALA during the 5 wk nursery period. Pigs in the TA (6.46) and PC (6.68) had a higher ($p<0.05$) number of red blood cells (10^6 cell/μL) than pigs in the NC (6.15). Pigs in PC (12.16) had a higher ($p<0.05$) hemoglobin level (g/dL) than pigs in the NC group (11.29) and the TA group (11.47). Pigs in the TA and PC had darker ($p<0.05$) and less ($p<0.05$) yellow skin color than pigs in the NC. Pigs in the PC tended ($p = 0.081$) to be less active than pigs in the other groups. There were no differences in behavioral characteristics between the NC and the TA. The data suggest that ALA supplementation has no adverse effects on growth performance of nursery pigs. Moreover, ALA supplementation increased red blood cell counts which may be beneficial to pigs. ***(Asian-Aust. J. Anim. Sci. 2006. Vol 19, No. 1 : 97-101)***

Key Words : δ-Aminolevulinic Acid, Antibiotics, Behavior, Hematological Status, Nursery Pigs

INTRODUCTION

Certain amino acids are essential precursors of a variety of important biomolecules such as heme in addition to their major function as building blocks of protein. δ-Aminolevulinic acid (ALA) is a chemical produced from amino acids in the liver and is catalyzed by the pyridoxal phosphate (PLP) dependent enzyme, ALA synthase, from glycine and succinyl CoA. This reaction is known as the Shemin pathway and is a rate limiting step for heme synthesis (Jover et al., 2000). The next series of steps in the synthesis of heme occurs in the cytosol wherein ALA dehydratase converts two molecules of ALA to monopyrrol porphobilinogen (PBG). Two subsequent enzymatic steps convert four molecules of PBG into uroporphyrinogen III, which is then decarboxylated to form coproporphyrinogen III. The final steps which include the insertion of ferrous iron into protoporhyrin IX by ferrochelatase occurs in the mitochondria (Ponka, 1997).

Heme is a regulatory molecule that controls metabolic pathways and the biosynthesis of various proteins (Jover et al., 2000) called heme-proteins. These heme-proteins are involved in a broad spectrum of crucial biologic functions including oxygen binding and oxygen metabolism (Ponka, 1997). Compared with other cells in the organism, rapid heme biosynthesis occur in liver and erythroid cells where large amounts of heme are needed not only for mitochondrial cytochromes but also as prosthetic groups for cytochrome P450 and hemoglobin, respectively. Under physiological conditions, ALA is required for the synthesis of protoporphyrin IX, a direct precursor of heme (Neumann and Brandsch, 2003) and has been used *in vitro* and clinical studies as an endogenous photosensitizer for photodynamic therapy in the treatment of various tumors in humans (Doring et al., 1998). However, the potential beneficial effects of ALA supplementation on growth and immune status of pigs have not been tested. Thus, it was hypothesized that the supplementation of ALA to nursery pigs improves heme synthesis that will in turn increase the synthesis of heme-proteins such as hemoglobin. These heme-proteins would then increase the number of red blood cells thereby improving the immune status of nursery pigs.

MATERIALS AND METHOD

A total of 144 pigs (Camborough 22×PIC boar, Pig Improvement Company, Franklin, KY) were used in the

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Table 1. Experimental diets

Ingredients (%)	Phase 1[a] (21 to 42 d of age)	Phase 2[a] (43 to 56 d of age)
Corn	35.00	62.10
SBM (dehulled)	26.00	32.70
Dried whey	25.00	0.00
Salt	0.40	0.25
Vitamin-mineral premix[b]	4.00	2.00
Fat (vegetable oil)	2.60	1.00
Dicalcium phosphate	0.90	1.15
Limestone	0.70	0.80
Plasma protein (APC-920)	2.50	0.00
Fish meal (Menhaden)	2.50	0.00
Zinc oxide	0.40	0.00
Total	100.00	100.00
Chemical composition		
Dry matter (%)	91.4	90.0
ME (Mcal/kg)	3.20	3.30
Crude protein (%)	21.9	20.6
Lysine (%)	1.40	1.15
Cysteine+methionine (%)	0.76	0.68
Tryptophan (%)	0.29	0.25
Threonine (%)	0.99	0.78
Calcium (%)	0.93	0.71
Available phosphorus (%)	0.55	0.32
Total phosphorus (%)	0.74	0.59

[a] Either carbadox (0.1%) or δ-aminolevulinic acid (0.05%) was added for PC or TA treatment, respectively by replacing the same amount of corn.

[b] Vitamin-mineral premix provided the following per kilogram of complete phase 1:62.2 mg of manganese as manganous oxide, 100 mg of iron sulfate, 138.4 mg of zinc as zinc oxide, 12.6 mg of copper as copper oxide, 0.96 mg of iodide as ethylenediamine dihydroiodide, 0.30 mg of selenium as sodium selenite, 10,074 IU of vitamin A as vitamin A acetate, 1,100 IU of vitamin D_3, 82.6 IU of vitamin E, 3.6 IU of vitamin K as menadione sodium bisulfate, 73.2 μg of vitamin B_{12}, 18.4 mg of riboflavin, 58.6 mg of D-pantothenic acid as calcium pantothenate, 73.2 mg of niacin, and 2,210 mg of choline as choline chloride. Phase 2 diet contained 50% levels of vitamin and mineral provided to the phase 1 diet.

study. Pigs were weaned at 21 d of age and allotted to one of three dietary treatments that consisted of a control group without any antibiotics (NC), control group with antibiotics using carbadox at 50 mg/kg (PC), and a treatment group with the ALA supplementation (0.05%) without any antibiotic included (TA). Each treatment had six replicates with eight pigs per replicate. The pigs were housed in raised-deck pens (2×2 m). Ventilation was provided by a mechanical system, and lighting was automatically regulated to approximate the seasonal day length. Ambient temperature within the room was approximately 30°C immediately after weaning and was adjusted downward to approximately 22°C by the end of the experiment. The animal care and use protocol was approved by the Texas Tech University Animal Care and Use Committee. Pigs were fed the phase 1 experimental diets for 3 wks and the phase 2 experimental diets for the following 2 wks (Table 1).

Table 2. Growth performance of pigs fed the experimental diets during the 5 wk nursery period

Treatment	NC[a]	PC[a]	TA[a]	SEM
Body weight (kg)				
Initial	6.09	6.1	6.13	0.18
Week 1	7.42	7.28	7.31	0.25
Week 2	9.61	9.23	9.26	0.27
Week 3	11.66	11.77	11.47	0.32
Week 5	19.55	20.43	20.05	0.45
Average daily gain (g)				
Week 1	190.3	168.1	168.1	29.7
Week 2	312.8	279.3	278.5	12.3
Week 3	293.3	361.8	315.0	17.3
Week 5	563.4	619.2	613.3	16.4
Overall	384.5	409.5	397.7	11.8
Average daily feed intake (g)				
Week 1	256.5	228.8	240.1	30.8
Week 2	406.5	371.7	359.3	13.5
Week 3	478.0	528.0	479.9	14.2
Week 5	1,304.5	1,344.2	1,289.3	23.7
Overall	750.0	763.4	731.5	15.4
Gain:feed ratio				
Week 1	0.665	0.671	0.683	0.043
Week 2	0.773	0.748	0.789	0.027
Week 3	0.603	0.689	0.665	0.031
Week 5	0.433	0.463	0.477	0.013
Overall	0.512	0.537	0.545	0.012

[a] NC: control group without any antibiotics, PC: control group with antibiotics using carbadox at 50 mg/kg, TA: treatment group with the ALA supplementation (0.05%) without any antibiotic included.

The experimental diets provided 21.9% CP and 1.40% total lysine during the phase 1 and 20.6% CP and 1.15% total lysine during the phase 2. All the nutrients were provided to meet the nutrient requirement suggested by the NRC (1998).

Pigs had free access to feed and water. Feed intake and weight gain were measured weekly during the phase 1 and at the end of phase 2. At the end of phase 2, blood samples (10 ml) were obtained as described by Kim et al. (2004) and were then analyzed for the number of white blood cells, neutrophils, lymphocytes, monocytes, eosinophils, basophils, red blood cells, hemoglobin, platelets, etc using an automated hematology analyzer (CELL-DYN 3200, Abbott Lab, Abbot Park, IL).

Eighteen camcorders were placed at the ceiling of each pen to monitor the movement of all pigs in each pen. Individual pigs had their own unique number (one to eight) on their backs written with a black permanent marker making it possible to identify the behavior of individual pigs. Pig behavior was recorded for 48 h using video recorders with monitors that were located outside of the nursery room. Although, no one was allowed to enter the nursery room during the 48 h period, we were still able to observe the pigs through the monitors. After recording, tapes were played to measure pig behavior data. Number of pigs for each behavioral category was counted for 48 h with a 10 min interval. Behavioral category included standing,

Table 4. Color measurement by Hunter Color System from the pigs' skin fed experimental diets during the 5 wk nursery period

	NC[a]	PC[a]	TA[a]	SEM
L*	74.23[b]	73.08[c]	73.16[c]	0.23
a*	2.82	2.74	2.67	0.08
b*	9.39[b]	8.46[c]	8.29[c]	0.20

[a] NC: control group without any antibiotics, PC: control group with antibiotics using carbadox at 50 mg/kg, TA: treatment group with the ALA supplementation (0.05%) without any antibiotic included.
[b, c] Means in the same row with different superscripts differ (p<0.05).

lying, sitting, and eating. Behaviors including standing and eating were combined and named 'active behavior' whereas lying and sitting were combined and named 'inactive behavior'. Average percentages of each behavior from all pens from each treatment were obtained and recorded.

After behavioral measurement, the left side ham area of all pigs from each treatment group was shaved to remove any hair. On the following morning (0900 h), pig skin color was measured from the shaven spot using a Minolta color recorder (MiniScan XE Plus, Hunter, Reston, VA). The L*, a*, and b* values were obtained. Colored pigs and pigs with spots on their skin were excluded from the data.

The statistical analysis was performed with the General Linear Models procedure (PROC GLM) in SAS/STAT® software (SAS Inst. Inc., Cary, NC). Treatment was the main effect and the initial pig weight was used as covariate. Least-square means, probability of differences, and standard errors were used to evaluate the differences among the different treatment groups.

RESULTS

Growth performance of pigs fed the experimental diets during the 5 wk nursery period is shown in Table 2. Average daily gain (g) of nursery pigs did not differ (p>0.05) among the treatments during the 5 wk nursery period. Average daily feed intake (g) of nursery pigs did not differ (p>0.05) among the treatments as well as gain: feed ratio. Thus, growth performance was not affected (p>0.05) by the dietary supplementation of ALA during the 5 wk nursery period.

Table 5. Behavioral measurements of pigs fed experimental diets during the 5 wk nursery period

	NC	PC	TA	SEM
48 h period				
Standing	7.6	7.2	7.7	0.6
Eating	11.3	6.1	10.4	1.4
Sitting	2.3	3.5	2.9	1.2
Lying	78.7[b]	82.9[a]	78.9[b]	1.0
Total active[c]	18.9	13.4	18.1	1.8
Total inactive[d]	81.0	86.4	81.8	1.8
12 h period (0800-2000 h)				
Standing	7.9	8.1	8.0	0.6
Eating	10.6	6.2	10.6	1.4
Sitting	2.0	3.5	3.2	1.3
Lying	7.9	8.2	7.8	8.9
Total active[c]	18.5	14.2	18.6	1.8
Total inactive[d]	81.4	85.5	81.3	1.8

[a, b] Means within a row with different superscripts differ (p = 0.081).
[c] Behavior measurements including standing and eating.
[d] Behavior measurements including lying and sitting.

Hematological evaluation of pigs fed the experimental diets during the 5 wk nursery period is presented in Table 3. Pigs from both the TA and PC groups had higher (p<0.05) numbers of red blood cells than pigs in the NC group. However, no differences (p>0.05) were noted between the TA and PC. Pigs in the PC had higher (p<0.05) hemoglobin levels compared to those pigs from both the TA and the NC. Significant differences (p<0.05) were noted in terms of basophil counts between the NC and the PC. Basophil counts of the TA, however, did not differ (p>0.05) from that of either the NC or the PC. No differences (p>0.05) were observed on all other hematological measures in the study including white blood cells, neutrophils, lymphocytes, monocytes and eosinophils.

Pigs in the TA and PC had lower (p<0.05) L* values and lower (p<0.05) b* values indicating that they had darker and less yellow skin color than pigs in the NC (Table 4). There were no differences in behavioral characteristics between the NC and the TA. However, the pigs in the PC tended (p = 0.081) to be less active than pigs in other groups (Table 5).

Table 3. Hematological evaluation of pigs fed the experimental diets during the 5 wk nursery period

	NC[a]	PC[a]	TA[a]	SEM
White blood cell (10^3 cell/μl)	18.35	18.16	19.80	0.56
Red blood cell (10^6 cell/μl)	6.15[b]	6.68[c]	6.46[c]	0.06
Hemoglobin (g/dl)	11.29[b]	12.16[c]	11.47[b]	0.12
Neutrophil (10^3 cell/μl)	6.45	5.33	6.88	0.26
Lymphocyte (10^3 cell/μl)	8.66	10.32	9.91	0.40
Monocyte (10^3 cell/μl)	2.32	1.80	2.17	0.15
Eosinophil (10^3 cell/μl)	0.37	0.35	0.42	0.03
Basophil (10^3 cell/μl)	0.54[b]	0.35[c]	0.42[bc]	0.04

[a] NC: control group without any antibiotics, PC: control group with antibiotics using carbadox at 50 mg/kg, TA: treatment group with the ALA supplementation (0.05%) without any antibiotic included.
[b, c] Means in the same row with different superscripts differ (p<0.05).

DISCUSSION

This study indicates that the pigs fed a diet supplemented with ALA or carbadox had increased number of red blood cells as compared to the pigs fed a diet without both ALA and carbadox supplementation. The fraction of whole animal fasting oxygen consumption used by the portal vein drained organs (PVDO) was found to be reduced (Yen and Nienaber, 1992), and improved retention of dietary protein accretion have been reported in pigs fed carbadox supplemented diet (Roof and Mahan, 1982). Yen and Nienaber (1992) suggested that although carbadox reduces oxidative demand by PVDO, it does not decrease O_2 consumption of the whole animal, because carbadox probably also causes an increase O_2 consumption as a result of enhancement of muscle protein accretion. This increase in oxygen consumption would have required an increase in the production of red blood cells. However, in our experiment, pigs fed diets from all three groups did not show any significant response in terms of growth performance. Although pigs from both the PC and TA obtained higher red blood cell counts compared to the NC, all values were still within the normal range found in pigs (Merck Veterinary Manual, 1991). Pigs in the TA and the PC had darker ($p<0.05$) and less ($p<0.05$) yellow skin color than pigs in the NC**.** This is probably due to the higher numbers of red blood cells as shown in our experiment which may have contributed largely to the darker skin color observed in pigs from both the PC and TA.

The lack of response of nursery pigs in both TA and PC to growth performance may have been due to the prevailing research facility conditions used in the study. It was noted that during the study period all experimental pigs used were raised under completely controlled environment wherein pathogenic and other stress factors were possibly maintained at very low levels. Yen and Pond (1990) reported that the main mode of action of carbadox is that it suppresses the microbial production of NH_3 in the gastrointestinal tract. This effect would probably have been more apparent in conditions where higher pathogen or stress load is evident. Numerous studies have indicated that responses to antibiotics under farm conditions may be twice as great as those occurring in a research station environment since the disease challenge and other stressors are generally lesser (Cromwell, 2001) and that the level of growth performance depends on the management and housing conditions (Cromwell, 2000). This would suggest that the nursery environment may have been significant in terms of the response of pigs towards supplemental treatments. This may have also been the case for the TA.

In the study, both PC and TA showed higher red blood cell counts compared to the NC. However, hemoglobin content did not show the same trend as red blood cell counts did. Pigs from both the NC and TA showed lower hemoglobin content compared to the PC. It has been previously reported that ALA synthase is the rate limiting enzyme in heme synthesis and its expression is regulated by heme levels through a negative feedback mechanism (Doring et al., 1998; Jover et al., 2000). Human erythroid cells have been reported to be subjected to end product feedback regulation, which occurs at one or more rate limiting steps which lead to the formation of ALA (Gardner and Cox, 1988). However, it has also been reported that when ALA is applied topically or systemically (Collaud et al., 2004) or after oral application (Neumann and Brandsch, 2003), ALA bypasses the negative feedback control that heme exerts on the enzyme ALA synthase, which catalyses the natural production of ALA. This would suggest that in our experiment, the feed back inhibition brought about by heme would not be much of a factor since exogenous ALA was provided in the diet.

A probable explanation for the conflicting response between RBC number and hemoglobin content in both the PC and TA would involve the iron uptake by erythroid cells. According to Ponka (1997), heme-mediated repression of ALA synthase-1 is responsible for rendering this enzyme the rate-limiting step in the non-erythroid heme biosynthetic pathway, however in erythroid cells iron acquisition or the availability of iron for ferrochelatase, rather than ALA production, is the rate-limiting step in heme synthesis. Gardner and Cox (1988) reported that negative feedback by heme markedly affects iron utilization within human cells and control of its own synthesis must be exerted either at steps preceding the formation of protoporphyrin IX or at the level of intracellular iron metabolism, including the insertion of ferrous iron into the protoporphyrin nucleus by ferrochelatase. Similarly, several reports (Ponka and Schulman, 1985; Hradilek and Neuwrit, 1989) suggest that heme blocks the uptake of iron from transferrin by erythroid cells. *In vitro* and *in vivo* studies indicate that transferrin is the only physiologic source of iron for erythroid cell heme synthesis (Ponka, 1997). *In vitro* studies with erythroid cells have shown that the only physiologically active chelate that can provide iron for their hemoglobin synthesis is transferrin (Ponka, 1997). Moreover, a heme-induced block of heme synthesis in erythroid cells could not be restored by added ALA (Ponka et al., 1973). A study by Gallo (1967) suggests that even with pharmacological quantities of ALA, there is insignificant entrance into erythroid precursors or that the site of hemin inhibition of heme synthesis is not ALA synthase. This mode of regulation of heme synthesis may be a specific characteristic of the hemoglobin biosynthetic pathway (Ponka and Schulman, 1985) since heme does not inhibit iron uptake in non-erythroid cells. This would suggest that the exogenous supplementation of ALA done in the present study may not have prevented the

inhibition brought about by heme in erythroid cells (red blood cells) since ALA synthase may not be the rate limiting step in erythroid cells. This would also encourage further investigation on the effect of ALA supplementation on non-erythroid cells, ALA synthase being the rate limiting step in these cells.

In terms of white blood cell differential counts measured in this experiment, only the basophil counts responded to the different treatments. Pigs from the TA obtained values that were not significantly different from either NC or PC. However, between the PC and the NC, the former obtained lower basophil counts. Although, differences were observed, the values were still within the normal range found in pigs. No significant differences were observed in terms of lymphocyte counts among treatments which may explain why there were also no differences obtained in WBC count among treatment groups. Other hematological values measured did not show any significant difference among treatment groups. There were no differences noted in terms of behavioral characteristics between the NC and the TA. However, it is interesting to note that pigs in the PC group tended ($p = 0.081$) to be less active than pigs from the other treatment groups.

IMPLICATIONS

Our results indicate that at 0.05% inclusion, ALA had no adverse effects on growth performance of nursery pigs. Moreover, ALA supplementation had increase red blood cell counts which may be beneficial to pigs. In addition, the results lead us to investigate further regarding the potential effects of ALA supplementation especially on non-erythroid cells.

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