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δ-Aminolevulinic Acid (ALA) as a Potential Feed Additive in Pig: A Review

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Abstract: Delta-Aminolevulinic Acid (ALA) which is the precursor of is synthesized by the condensation of glycine and succinyl-CoA with ALA synthetase as a coenzyme. This reaction is a mandatory step in heme synthesis and is rate-determining for the pathway. After several intermediate reactions, ALA is transformed into protoporphyrin IX. Subsequently, an iron atom is inserted into the porphyrin ring of protoporphyrin IX with the help of ferrochelatase forming heme. According to this reaction mechanism, supplementation of ALA in livestock can affect heme synthesis, positively influence the iron contentor hemoglobin status of animals. By increasing iron transfer efficiency from sow to piglets, through elevated milk iron concentrations immune system response could improve during inflammatory challenge.

Key words: Non-ruminant animal, iron utilization, δ-Aminolevulinic Acid (ALA), influence, iron contento, porphyrin

INTRODUCTION

δ-Aminolevulinic Acid (ALA) is a compound which is widely present in the biosphere and plays an important role as an intermediate of the tetrapyrrole compound biosynthesis pathway for vitamin B12, heme, chlorophyll, etc. ALA has been commonly used in photodynamic therapy for several years.

Previous studies have also suggested that ALA has the potential to be used as both a biodegradable herbicide and insecticide (Sasaki *et al*., 2002) that while being inhibitory to weeds is not harmful to crops, animals or humans. In addition, ALA has been found to be effective in increasing cold temperature and salt tolerances in and in promoting the growth of several crops (Watanabe *et al*., 2000).

A recent hypothesis suggests that dietary supplementation of ALA for pigs can affect the synthesis of heme and subsequently improve immune response and the hemoglobin concentration or other blood components such as iron concentration. Those probable effects may provide better general health and resistance to disease, especially for nursery pigs with the insufficient immune ability.

Furthermore, the health status of pigs also relates to growth performance as a compromised immune system will limit the production efficiency. Investigations of ALA provide a new concept and potential strategy for optimizing production in the modern livestock industry.

BIOSYNTHESIS OF HEME

Heme is the prosthetic group of hemoglobin, myoglobin and the cytochromes. Aside from its importance as the prosthetic group of hemoglobin and a small number of enzymes, it is vitally important as a number of genetic disease states are also associated with deficiencies of the enzymes used in its biosynthesis. Heme biosynthesis involves eitht enzymes, four localized to the cytoplasm with the remaining being in the mitochondrial matrix (Ponka, 1999; Ryter and Tyrrell, 2000). Its biosynthesis pathway also requires which is derived from the plasma in the intestinal crypt by the activity of a transferrin receptor operating in collaboration with the hemochromatosis protein (Lebron *et al*., 1998). The preliminary reaction in the synthesis of heme is the condensation of glycine and succinyl-CoA by to form ALA (C_4 pathway, Fig. 1). Pyridoxal phosphate serves as coenzyme for δ-Aminolevulinate synthase (ALA synthase). The enzyme is evolutionarily related to transaminases. ALA synthase is the committed step of the heme synthesis and is usually rate determining for the overall pathway.

The amount of the enzyme is regulated through gene transcription control. Heme functions as a feedback inhibitor, repressing transcription of the gene for ALA synthase in most cells. The C_4 pathway has been found in animals, fungi (including yeasts) and δ-proteobacteria such as the photosynthetic genera Rhodobacter and

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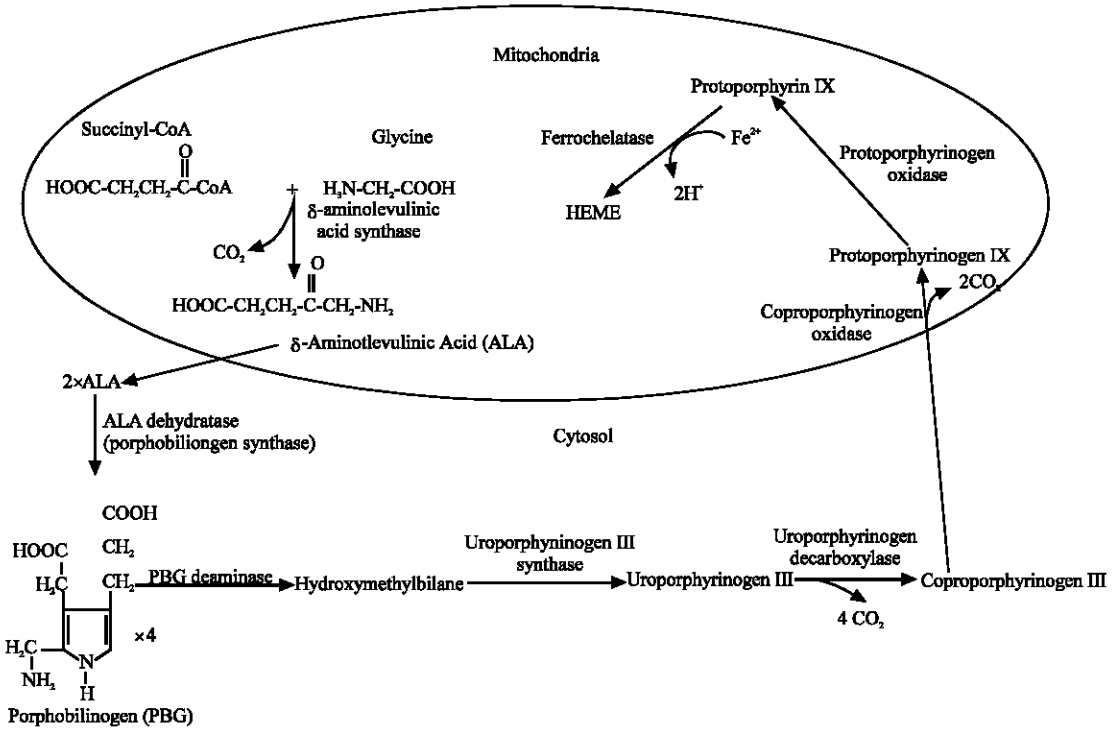

Fig. 1: Pathway of heme biosynthesis (King, 2008)

Bradyrhizobium. Another pathway to form ALA, the C_5 pathway has been found in plants (including algae) and in all other bacteria examined to date. In this pathway, glutamate is coupled with a cognate tRNA in a reaction catalyzed by glutamyl-tRNA synthase and is then reduced to GSA in a reaction catalyzed by glutamyl-tRNA reductase. Finally, the transamination of GSA, catalyzed by GSA aminomutase, yields ALA (Piao *et al*., 2004). After the ALA is formed in the mitochondrial matrix, it is transported to the cytosol where ALA dehydratase dimerizes two molecules of ALA to produce the pyrrole ring compound PBG. The next step in the pathway involves the head-to-tail condensation of four molecules of porphobilinogen to produce the linear tetrapyrrole intermediate, hydroxymethylbilane. The enzyme for this condensation is PBG deaminase, also referred to as hydroxymethylbilane synthase or uroporphyrinogen I synthase. In the cytosol, the acetate substituents of uroporphyrinogen (normal uroporphyrinogen III or abnormal uroporphyrinogen I) are all decarboxylated by the enzyme uroporphyrinogen decarboxylase. The resultant products have methyl groups in place of acetate and are known as coproporphyrinogens with coproporphyrinogen III being the important normal intermediate in heme synthesis. Coproporphyrinogen III is transported to the interior of the where two propionate residues are decarboxylated yielding vinyl substituents on the two pyrrole rings. In the mitochondrion, protoporphyrinogen IX is converted to protoporphyrin IX by protoporphyrinogen IX oxidase. The final reaction in heme synthesis also takes place in the mitochondrion and involves the insertion of the iron atom into the ring system generating heme b. The enzyme catalyzing this reaction is known as ferrochelatase (King, 2008).

BIOCHEMICAL FUNCTIONS OF IRON

Iron serves numerous important functions in the body relating to the metabolism of oxygen, not the least of which is its role in hemoglobin's transport of oxygen. It is an essential component for proper cell differentiation and cell growth. In addition, iron is a critical component of peroxide-generating enzymes and nitrous oxide-generating enzymes that are critical for the proper enzymatic functioning of immune cells. Finally, iron is likely involved in the regulation of cytokine production and mechanism of action through its influence on 2nd-messenger systems (Hershko, 1993). Four major classes of iron-containing proteins carry out these reactions in the mammalian system iron-containing nonenzymatic proteins (hemoglobin and myoglobin), ironsulfur enzymes, heme-containing enzymes and iron containing enzymes that are noniron-sulfur, nonheme enzymes (Fig. 2). Therefore, almost two-third of the iron in the body is

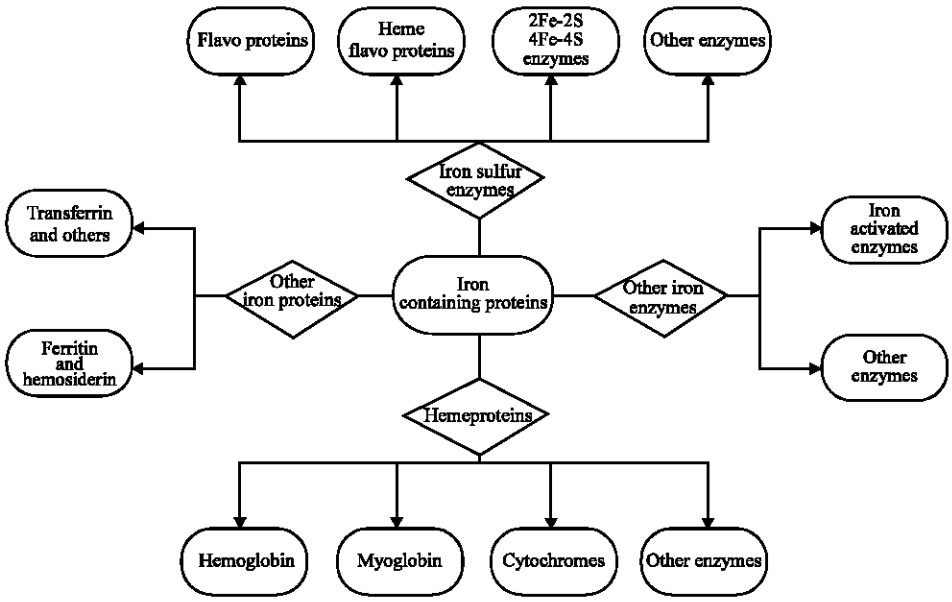

Fig. 2: Classification of major iron-containing proteins (Beard, 2001)

found in hemoglobin, the protein in red blood cells that carries oxygen to the body's tissues. Smaller amounts of iron are found in myoglobin, a protein that helps supply oxygen to muscle tissue and in enzymes that assist biochemical reactions within cells (Fig. 2). Within the body iron exist in two oxidation states: Ferrous (Fe^{2+}) and Ferric (Fe^{3+}). Iron has an affinity for electronegative atoms such as oxygen, nitrogen and sulfur, these atoms are found at the heart of the iron-binding centers of macromolecules.

Adult men and post-menopausal women lose very little iron except through bleeding. Women with heavy monthly periods can lose a significant amount of iron. For animals, anemia is also presented frequently. Within pigs for example, the total body quantity of iron is low but the daily requirement is highest among young nursing pigs whose rapid growth rates and high hemoglobin synthesis increases the demand for this mineral (Mahan, 1990). Another important reason behind this dietary requirement is that iron transfer through the placenta is very low, regulated largely by uteroferrin (Ducsay *et al*., 1984). There are numerous factors which affect iron absorption and bioavailability such as age, iron status, species, dosage level and other nutrient components of the diet both organic and inorganic.

The physical or chemical form of iron can also influence absorption. Iron from animal sources is more available for absorption than that from plant due to the large proportion of heme iron in animal sources. Heme iron is absorbed as an intact porphyrin complex whereas nonheme iron must be removed from its protein-bound complexes prior to absorption (Morris, 1987). Vitamin C or ascorbic acid also has been shown to have beneficial effects upon iron absorption. Greenberg *et al.* (1957) concluded that iron-deficient rats had increased efficiency of absorption of iron when ascorbic acid was given with the iron supplement. This research supports the previous findings of Moore and Dubach (1951) who reported increased food iron absorption in human through the dietary addition of ascorbic acid or foods containing ascorbic acid. This may be mainly due to the ability of ascorbic acid to act as both a reducing agent and a chelating agent.

UTILIZATION OF ALA IN NON-RUMINANT ANIMALS

As ALA dose not influence the animal bodies directly, medium substances such as heme or hemoglobin are considered to be useful criteria to evaluate the effect of ALA. In addition, several previous studies have indicated that ALA can affect iron metabolism (Laftah *et al*., 2008). Synthesized by condensing succinyl-CoA and glycine, ALA is the precursor of heme which contains iron and has catalytic and regulatory roles in all cells (Zhu *et al*., 2002). As mentioned before, iron performs many functions within the animal body. Heme biosynthesis makes iron convert from non-heme iron to heme iron which has a much higher bioavailability. McGowan and Chrichton (1937) reported that the efficiency of absorption was attributed primarily to the iron status of the animal.

Bothwell *et al.* (1958) proposed that the two most important factors concerning iron absorption were iron stores and rate of erythropoiesis. This research indicated that an animal only absorbs the amount of iron that it needs or requires. Increasing levels of dietary iron lead to higher total amounts absorbed, however the iron status of the animal is still more influential in determining iron absorption (Van Campen, 1974). Excess iron entering the mucosal cells of iron adequate pigs is incorporated into ferritin only to be lost later in feces as a product of sloughed mucosal cells (Harmon *et al.*, 1974).

Yu *et al.* (2000) suggested that organic iron (Availa-Fe®) supplementation improved the iron status of weanling pigs, however growth performance was found not to be significantly influenced. Park *et al.* (2004) also investigated organic iron (Availa-Fe®) supplementation in hens' diets increased iron concentration in eggs. Vahl and Van 'T Klooster (1987) reported that the growth performance of broiler increased to a plateau of between 20 and 60 mg added Fe kg^{-1} diet but further additions of Fe depressed growth. Therefore, it is considered that the intercellular iron as a part of the iron reutilization process is more important than intestinal absorption with regards to growth performance.

The use of ALA is in agreement with such a concept and will theoretically improve iron utilization rather than exogenous supplementation of iron. In fact, recent studies have been conducted under practical feeding conditions as it is a new concept to use ALA as functional feed additive for animals. Wang *et al.* (2009) reported that dietary supplementation (10 mg kg^{-1}) of ALA can increase the blood iron status of sows and sucking as well as increase iron transfer efficiency from sows to piglets through elevated milk iron concentrations. Chen *et al.* (2008a) did a further experiment which demonstrated supplement of dietary supplement of 15 mg kg^{-1} of ALA improved DM and N digestibilities and iron status in blood. Chen *et al.* (2008b) also found that 10 mg kg^{-1} ALA had a beneficial effect on the immune response during an inflammatory (LPS: escherichia coli lipopolysaccharide) challenge situation.

Chen *et al.* (2008a, b) reported that supplementation of ALA (0, 5, 10 and 15 mg kg^{-1}) in commercial broiler diets could partially improve hemoglobin concentration and immune organ weights linearly, without influencing the growth performance and other blood characteristics of broilers. Min *et al.* (2004) conducted a feeding trial in weanling pigs and suggested that 0.02% of ALA supplementation could improve growth performance, nutrient digestibility and blood profiles. Min *et al.* (2004) also obtained a comparable result between ALA and antibiotic mixture (apramycin and oxyteetracycline) which indicated that ALA may have the potential to act as an antibiotic alternative. However, Mateo *et al.* (2006) also conducted similar feeding trials, however they did not observe a growth promotion effect with ALA. In addition, most of the pigs blood profiles were not influenced by ALA supplementation with the exception of RBC counts.

CONCLUSION

Due to the processing costs of ALA, its utilization has been limited to the clinic medical area of human research. However, the development of a new fermentation method has lead to a dramatic decrease in ALA production cost. Such new technical advances also make ALA appropriate for use as a feed-grade additive in the livestock industry. The new concept of improving the health conditions of animals via dietary ALA supplementation is largely different from traditional iron supplemental which is focused on improving iron metabolism and utilization rather than simple exogenous supplementation. Iron deficiency induced anemia is a serious problem in milk consuming animals such as pigs; this is mainly caused by a low transfer efficiency of iron from sow to offspring. Therefore, the prevention of iron deficiency continues to be a challenge to the swine industry. On the other hand if ALA utilization can be applied successfully to economic animals such as laying hens, functional food of iron enriched eggs may be available and have a beneficial influence for human.

REFERENCES

Beard, J.L., 2001. Iron biology in immune function, muscle metabolism and neuronal functioning. J. Nutr., 131: 568S-580S.

Bothwell, T., G. Pirzio Biroli and C. Finch, 1958. Iron absorption 1. Factors influenci absorption. J. Lab. Clin. Med., 51: 24-36.

Chen, Y.J., I.H. Kim, J.H. Cho, B.J. Min and J.S. Yoo *et al.*, 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. Liv. Sci., 114: 108-116.

Chen, Y.J., I.H. Kim, J.H. Cho, J.S. Yoo and H.J. Kim *et al.*, 2008b. Utilization of δ-aminolevulinic acid for livestock: blood characteristics and immune organ weight in broilers. J. Anim. Feed. Sci., 17: 215-223.

Ducsay, C.A., W.C. Buhi, F.W. Bazer, R.M. Robert and G.E. Comb, 1984. Role of uteroferrin in placental iron transport: Effect of maternal iron treatment on fetal iron and uteroferrin content and neonatal hemoglobin. J. Anim. Sci., 59: 1303-1308.

Greenberg, S.M., R.G. Tucker, A.E. Heming and J.K. Mathues, 1957. Iron absorption and metabolism. I. Interrelationship of ascorbic acid and vitamin E. J. Nutr., 63: 19-31.

Harmon, B.G., S.G. Cornelius, J. Totsch, D.H. Baker and A.H. Jensen, 1974. Oral iron dextran and iron from steel slats as hematinices for swine. J. Anim. Sci., 39: 699-702.

Hershko, C., 1993. Iron, infection and immune function. Proc. Nutr. Soc., 52: 165-174.

King, M.W., 2008. Iron, heme and porphyrin metabolism. http://www.intute.ac.uk/cgi-bin/fullrecord.pl?handle=2028468.

Laftah, A.H., R.J. Simpson, T.J. Peters and K.B. Raja, 2008. The effect of haem biosynthesis inhibitors and inducers on intestinal iron absorption and liver haem biosynthetic enzyme activities. Toxicol. Appl. Pharmacol., 229: 273-280.

Lebron, J.A., M.J. Bennett, D.E. Vaughn, A.J. Chirino and P.M. Snow *et al*., 1998. Crystal structure of the hemochromatosis protein HFE and characterization of its interaction with transferrin receptor. Cell., 93: 111-123.

Mahan, D.C., 1990. Mineral nutrition of the sow: A review. J. Anim. Sci., 68: 573-582.

Mateo, R.D., J.L. Morrow, J.W. Dailey, F. Ji and S.W. Kim, 2006. Use of δ-aminolevulinic acid in swine diet: Effect on growth performance, behavioral characteristics and hematological/ immune status in nursery pigs. Asian-Aust. J. Anim. Sci., 19: 97-101.

McGowan, J.P. and A. Chrichton, 1937. Absorption and excretion of iron. Lancet., 233: 680-684.

Min, B.J., J.W. Hong, O.S. Kwon, D.K. Kang and I.H. Kim, 2004. Influence of dietary ä-aminolevulinic acid supplement on growth performance and hematological changes in weaned pigs. J. Korean. Soc. Food. Sci. Nutr., 33: 1606-1610.

Moore, C. and R. Dubach, 1951. Observations on the absorption of iron from foods tagged with radioiron. Trans. Assac. Am. Phys., 64: 245-256.

Morris, E.R., 1987. Trace Elements in Human and Animal Nutrition. Academic Press, New York.

Park, S.W., H. Namkung, H.J. Ahn and I.K. Paik, 2004. Production of iron enriched eggs of laying hens. Asian-Aust. J. Anim. Sci., 17: 1725-1728.

Piao, Y.Z., P. Kiatpapan, M. Yamashita and Y. Murooka, 2004. Effects of Expression of hemA and hemB genes on production of porphyrin in propionibacterium freudenreichii. Appl. Environ. Microbiol., 70: 7561-7566.

Ponka, P., 1999. Cell biology of heme. Am. J. Med. Sci., 318: 241-256.

Ryter, S.W. and R.M. Tyrrell, 2000. The heme synthesis and degradation pathways: role in oxidant sensitivity. Heme oxygenase has both pro- and antioxidant properties. Free. Radical. Bio. Med., 28: 289-309.

Sasaki, K., M. Watanabe, T. Tanaka and T. Tanaka, 2002. Biosynthesis, biotechnological production and applications of 5-aminolevulinic acid. Appl. Microbiol. Biotechnol., 58: 23-29.

Vahl, H.A. and A.T. Van 'T Klooster, 1987. Dietary iron and broiler performance. Br. Poultry. Sci., 28: 567-576.

Van Campen, D., 1974. Regulation of iron absorption. Fed. Proc., 33: 100-105.

Wang, J.P., H.J. Kim, Y.J. Chen, J.S. Yoo and J.H. Cho *et al*., 2009. Effects of delta-aminolevulinic acid and vitamin C supplementation on feed intake, backfat and iron status in sows. J. Anim. Sci., 87: 3589-3595.

Watanabe, N., F.S. Che, K. Terashima, S. Takayama, S. Yoshida and A. Isogai, 2000. Purification and properties of protoporphyrinogen oxidase from spinach. Plant Cell Physiol., 41: 880-892.

Yu, B., W.J. Huang and P.W.S. Chiou, 2000. Bioavailability of iron from amino acid complex in weanling pigs. Anim. Feed Sci. Technol., 86: 39-52.

Zhu, Y., T. Hon, W. Ye and L. Zhang, 2002. Heme deficiency interferes with the Ras-mitogen-activated protein kinase signaling pathway and expression of a subset of neuronal genes. Cell. Growth. Differ., 13: 431-439.