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Utilization of δ-aminolevulinic acid for livestock: blood characteristics and immune organ weight in broilers

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ABSTRACT

A study was conducted to evaluate the effects of δ-aminolevulinic acid (ALA) on blood characteristics and immune organ weight in broilers. One-day-old broiler chicks were randomly assigned to one of four dietary treatments with six replicates of 20 chickens each. Treatments were basal diet supplemented with 0, 5, 10 and 15 mg/kg ALA. The two-phase experimental diets were formulated to meet the NRC requirements for chicks and fed for 5 weeks. Growth performance was not affected by supplementation of ALA during any of the experimental periods. Blood cell counts (WBC, RBC and lymphocyte), serum total protein, albumin, iron concentrations, and total iron binding capacity (TIBC) were also not influenced by dietary treatments. Haemoglobin concentration tended to increase with an increased ALA supplementation level (linear effect; $P<0.10$). Dietary ALA addition did not influence liver weight. However, spleen and bursa of fabricius weights were increased with the increased ALA supplementation level (linear effect; $P<0.05$). The current data indicate that supplementation of ALA in commercial broiler diets could partly improve haemoglobin concentration and immune organ weights, without influencing growth performance and other blood characteristics of broilers.

KEY WORDS: δ-aminolevulinic acid, blood characteristics, immunity, broilers

INTRODUCTION

δ-Aminolevulinic acid (ALA) is a non-protein amino acid with widespread distribution in living organisms. It has been well known as a precursor in por-

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phyrin synthesis and has been recently used *in vitro* and in clinical studies as an endogenous photosensitizer for photodynamic therapy in the treatment of various tumors (Döring et al., 1998). In agriculture, ALA has also been used as a biodegradable herbicide and insecticide that is inhibitory to weeds without any harmful influence on crops, animals, or humans (Nishikawa and Murooka, 2001).

Due to ALA being a precursor of haeme synthesis, some investigators suggested that dietary ALA administration may influence the haeme status of animals (Mateo et al., 2006). Iron metabolism is closely related with haeme since iron is involved in its formation. Therefore, a change in haeme status would also influence iron status. In fact, a few studies have already been conducted on livestock models such as weanling pigs. A study conducted by Min et al. (2004) reported that dietary ALA supplementation improved the iron status of weanling pigs. Chen et al. (2008) also did a further experiment and showed that dietary ALA addition improved the immunity of weanling pigs in a bacteria challenge situation. However, to our knowledge, no study has been performed in chickens yet. We therefore attempted to show whether the positive effect of dietary ALA administration also occurs in broilers.

The current study was conducted as a preliminary feeding experiment examining the effects of ALA on blood characteristics and immune organ weight in broilers.

MATERIAL AND METHODS

Experimental design, animals and diets

The experimental protocol was approved by the Animal Care and Use Committee of Dankook University. Four hundred and eighty, one-day-old male broilers (Arbor Acre) obtained from a commercial hatchery were used in this five-week feeding trial. At the beginning of the experiment, the broilers were weighed and randomly assigned to four dietary treatments consisting of six replications. Each cage (1 × 1 m) contained 20 broilers with an average initial body weight of 39.08 g/bird. Dietary treatments included: 1. CON (basal diet), 2. ALA1 (basal diet with 5 mg/kg ALA), 3. ALA2 (basal diet with 10 mg/kg ALA) and 4. ALA3 (basal diet with 15 mg/kg ALA). Basal diets were formulated to meet or exceed all the nutrient requirements of broilers for starter (0-21 day) and finisher (22-35 day) phases (NRC, 1994). Ingredients and chemical composition of the basal diet are shown in Table 1. Broilers were housed in stainless steel cages with concrete floors covered with clean rice bran. The temperature was maintained at 33 ± 1°C during the first week and decreased 3°C per week until reaching 24°C. Artificial light was provided 24 h/day by the use of fluorescent lights. All diets were fed in mash

form with feed and water being provided *ad libitum* throughout the experimental period.

Table 1. Diet composition, as-fed basis

Item	Starter[1]	Finisher[1]
Ingredients, %		
maize	55.67	63.21
soyabean meal (48%)	28.25	24.61
maize gluten meal (60%)	6.50	3.50
soyabean oil	5.50	4.89
tricalcium phosphate	2.46	2.29
limestone	0.89	0.75
salt	0.20	0.20
DL-methionine	0.07	0.07
L-lysine-HCl	0.06	0.08
vitamin premix[2]	0.20	0.20
trace mineral premix[3]	0.20	0.20
Chemical composition[4]		
ME, MJ/kg	12.97	12.76
crude protein, %	22.00	19.00
lysine, %	1.10	1.00
Ca, %	1.00	0.90
P, %	0.80	0.75

[1] starter diets, provided during 0-3 weeks; finisher diets, provided during 4-5 weeks
[2] provided per kg of diet, IU: vit. A 15,000, vit. D_3 3,750 ; mg: vit. E 37.5, vit. K_3 2.55, vit. B_1 3, vit. B_2 7.5, vit. B_6 4.5, vit. B_{12} 24, niacin 51, folic acid 1.5, biotin 126, pantothenic acid 13.5
[3] provided per kg of diet, mg: Zn 37.5, Mn 37.5, Fe 37.5, Cu 3.75, I 0.83, S 62.5 and Se 0.23
[4] calculated values

Sampling and measurements

On days 1, 7, 21 and 35, the broilers were weighed by pen and feed consumption was recorded during each feeding period. The feed conversion ratio (feed/gain) was also calculated.

At the end of the experiment, 12 broilers were randomly selected from each treatment (2 birds per cage) and blood samples were collected from the wing vein into a sterile syringe. After collection, half of the sample was transferred immediately into either a vacuum (clot activator with gel) or K_3EDTA vacuum tube (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ) and stored at -4°C. Samples for serum analysis were then centrifuged at 3,000 *g* for 15 min and serum was separated. Total iron binding capacity (TIBC), and the serum

concentrations of iron, haemoglobin, total protein, and albumin were determined using an automatic biochemistry analyser (HITACHI 747, Japan). Blood cell counts (WBC, RBC and lymphocytes) were analysed using an automatic blood analyser (ADVIA 120, Bayer, NY).

After blood collection, the same broilers were weighed individually and killed by cervical dislocation. The liver, spleen and bursa of fabricius were removed and weighed. Organ size was expressed as a percentage of body weight.

Statistical analysis

All of the data in this experiment were analysed by the general linear model procedure of SAS (1996). An individual cage was considered the experimental unit for growth performance, blood and organ weight data. In addition, orthogonal comparisons were conducted to measure the linear and quadratic effects for increasing dietary concentrations of supplemental ALA. Statements of statistical significance were based on $P<0.05$, while $P<0.10$ was considered indicative of a tendency.

RESULTS

The effects of dietary supplementation of ALA on growth performance in broilers are presented in Table 2. In none of the growth periods (days 0-7, 8-21, and 22-35), were weight gain, feed intake and feed/gain affected by administration of different experimental diets. Similarly, growth performance was also not influenced by ALA supplementation throughout the feeding period.

Table 3 shows the effect of dietary ALA supplementation on blood characteristics in broilers. At the end of the experiment, blood cell counts (WBC, RBC and lymphocyte) in whole blood were not influenced by ALA supplementation. Serum total protein and albumin concentrations were also not affected by addition of ALA to the diets. The haemoglobin concentration tended to increase as the ALA supplementation level increased (linear effect; $P<0.10$). In contrast to our previous hypothesis, iron-related serum criteria, including iron concentration and TIBC, were not influenced by ALA supplementation.

The effects of supplementation of ALA on immune organ weight are presented in Table 4. Liver weight was not affected by ALA supplementation. However, spleen weight was increased in ALA-supplemented treatments compared with CON treatment (linear effect; $P<0.05$). Similarly, bursa of fabricius weight was also increased with increased ALA supplementation levels (linear effect; $P<0.05$).

Table 2. Effect of δ-aminolevulinic acid (ALA) supplementation on growth performance in broilers[1]

Item	CON[2]	ALA1[2]	ALA2[2]	ALA3[2]	SE[3]
0-7 days					
weight gain, g	81	84	85	83	4
feed intake, g	112	123	119	118	5
feed/gain	1.38	1.46	1.40	1.42	0.06
8-21 days					
weight gain, g	513	506	522	519	16
feed intake, g	788	805	799	793	9
feed/gain	1.54	1.59	1.53	1.53	0.05
22-35 days					
weight gain, g	804	814	843	831	30
feed intake, g	1487	1483	1529	1502	35
feed/gain	1.85	1.82	1.81	1.81	0.06
0-35 days					
weight gain, g	1398	1404	1450	1433	26
feed intake, g	2387	2411	2447	2413	37
feed/gain	1.71	1.72	1.69	1.68	0.03

[1] two hundred eighty broiler with an average body weight of 39.08 g

[2] abbreviations: CON, basal diet; ALA1, basal diet with ALA 5 mg/kg; ALA2, basal diet with ALA 10 mg/kg; ALA3, basal diet with ALA 15 mg/kg; ALA, δ-aminolevulinic acid

[3] pooled standard error

Table 3. Effect of δ-aminolevulinic acid (ALA) supplementation on blood characteristics in broilers

Item	CON[1]	ALA1[1]	ALA2[1]	ALA3[1]	SE[2]
RBC, $\times 10^6$/mm^3	2.04	2.20	2.12	2.16	0.06
WBC, $\times 10^4$/mm^3	41.5	44.9	43.2	44.6	1.6
Lymphocyte, %	88.7	90.8	86.3	86.6	2.8
Total protein, g/dl	3.10	3.08	2.98	3.23	0.09
Albumin, g/dl	1.42	1.43	1.38	1.50	0.05
Haemoglobin, g/dl[3]	8.85	9.17	9.49	9.67	0.24
Iron, µg/dl	111	112	105	120	12
TIBC, µg/dl[4]	165	191	176	198	16

[1] abbreviations: CON, basal diet; ALA1, basal diet with ALA 5 mg/kg; ALA2, basal diet with ALA 10 mg/kg; ALA3, basal diet with ALA 15 mg/kg; ALA, δ-aminolevulinic acid

[2] pooled standard error

[3] linear effect; $P<0.10$

[4] TIBC, total iron binding capacity

Table 4. Effect of δ-aminolevulinic acid (ALA) supplementation on immune organ weight in broilers

g/100 g BW	CON[1]	ALA1[1]	ALA2[1]	ALA3[1]	SE[2]
Liver	2.23	2.33	2.18	2.25	0.09
Spleen[3]	0.097	0.122	0.138	0.122	0.013
Bursa of fabricius[3]	0.190	0.227	0.270	0.294	0.024

[1] abbreviations: CON, basal diet; ALA1, basal diet with ALA 5 mg/kg; ALA2, basal diet with ALA 10 mg/kg; ALA3, basal diet with ALA 15 mg/kg; ALA, δ-aminolevulinic acid

[2] pooled standard error

[3] linear effect; $P<0.05$

DISCUSSION

Haeme synthesis starts with the condensation of glycine and succinyl-CoA to form ALA. ALA synthase catalyses this reaction and is considered the rate-limiting enzyme for the entire haeme synthesis pathway. It has been clearly demonstrated that haeme has a feedback effect on ALA synthase (Ponka, 1997). Therefore, when the haeme concentration increases, the feedback regulation will suppresses ALA synthase activity and limit haeme synthesis. It is, therefore, expected that additional ALA may sustain haeme synthesis even under conditions of high haeme concentrations and low ALA synthase activity. Subsequently, improved haeme biosynthesis may increase the concentration of such haeme proteins as haemoglobin or WBC, as well improve as iron status.

Previous ALA feeding trials in weanling pig models indicated that growth performance was not affected by ALA supplementation, which is in agreement with the current study (Mateo et al., 2006; Chen et al., 2008). An iron depletion-repletion study in chickens using an Fe-deficient casein-dextrose diet provided clear evidence that dietary iron concentrations were associated with growth performance (Boling et al., 1998). This response, however, was based on an iron deficient diet. In the current study, the basal diet was formulated to meet the nutrient requirements of broilers. Therefore, it seemed that the basal diet supported a normal growth rate of broilers and further improvement of iron status could hardly influence growth performance. Similarly as in this experiment, the study conducted by Vahl and van Klooster (1987) used a conventional maize-soyabean diet including 107 mg iron/kg and further supplemented with graded dosages of iron. They found that weight gain increased to a plateau with 20 and 60 mg/kg of iron added in diet, whereas further addition (180, 540 or 1620 mg/kg) of iron depressed growth performance. Our current study aimed to improve iron status through better iron utilization, not supplementation, therefore, such a negative effect would not have appeared. In addition, Biehl et al. (1997) suggested that growth performance was not as sensitive as haemoglobin so it is not an iron-

response criterion; this might be another reason for the lack of growth response observed in the current study.

In contrast with our results, an earlier study conducted by Mateo et al. (2006) suggested that supplementation of 0.05% ALA in nursing pig diets increased RBC concentration whereas the haemoglobin concentration was not affected. Another feeding trial in pigs conducted by Min et al. (2004) indicated that both RBC and serum haemoglobin concentrations were increased by 0.2% ALA supplementation. In that study, several other criteria also improved, e.g., serum total protein, iron concentration, as well as WBC and lymphocyte concentrations. According to a recent study, Chen et al. (2008) also suggested that dietary ALA supplementation (10 mg/kg) can improve RBC and serum iron levels in weanling pigs. To our knowledge, the current study is the first report of ALA supplementation in broiler diets. In fact, the study of ALA utilization in livestock is a new concept and few studies have been reported yet. The discrepancy between the present study and previous pig experiments also indicates that the effect of ALA in farm animals may be species related.

Another possible reason for the lack of dietary ALA effects in broilers can be interpreted in terms of the sensitivity of the analysed blood parameters. Underwood and Suttle (1999) described that the physiological influences of iron deficiency are firstly iron depletion in organs (such as the liver, kidney and spleen), secondly, decreases in plasma iron, haemoglobin, haematocrit and myoglobin levels, then finally, dysfunction and disease. Because a commercial broiler diet was used as the basal diet in this experiment, serious iron deficiency was not expected to develop. Therefore, further studies should be conducted to investigate whether ALA supplementation can influence iron status when chickens are subjected to iron depletion or overload situations.

Measurement of immune organ weights is a common method for evaluation of immune status in chickens (Heckert et al., 2002). Such related organs include bursa of fabricius, liver and spleen, etc. Good development of these organs is crucial for optimal immune response. For instance, the bursa of fabricius has been suggested to be the primary site of immunoglobulin synthesis (Glick, 1977). The spleen is also considered an essential lymphoid organ that plays an important role in cell-mediated immunity, e.g., its function in the development of suppressor T cells (Welles and Battisto, 1978). In the current study, both the bursa of fabricius and spleen weight were significantly increased in the ALA treatment, which to some extent indicated that broilers in this treatment presented better immunity than in the other ones. Similarly, Chen et al. (2008) also reported that ALA supplementation at the level of 10 mg/kg had a beneficial effect on the immune response of weanling pigs during an inflammatory challenge.

The mechanisms of ALA supplementation influencing the immunity of chickens may be ascribed to several different pathways. First, ALA supplementation may

increase the haeme concentration. Haeme has been reported to play important catalytic and regulatory roles in all cells (Zhu et al., 2002). Lyoumi et al. (1999) also suggested that haeme can boost the expression of positive acute-phase reactants in rats during an inflammatory challenge, which indicated that haeme can positively affect the immune function of animals. Similarly, Arruda et al. (2005) suggested haeme can be considered a proinflammatory molecule, which may play a central role during the onset and/or persistence of inflammation. Secondly, immunity may affected by improved iron status. Haeme is considered to be the major functional form of iron (Atamna, 2004). Increased haeme concentrations may subsequently improve iron utilization because haeme iron is utilized much better than non-haeme iron (Morris, 1987). Kochanowski and Sherman (1985) noted that iron nutrition can affect development of cell-mediated immunity. In addition, other functions such as iron involvement in proper enzymatic function of immune cells and regulation of cytokine production were also reviewed by Beard (2001). Therefore, the increased immune organ development observed in the current study may probably result in improved immunity of broilers.

CONCLUSIONS

The results of this study indicate that ALA supplementation does not significantly affect growth performance and blood profiles, whereas it can improve immune organ development. The related mechanism of ALA's effect may probably be ascribed to its influence on haeme or iron utilization by animals. More studies are required to further determine the efficiency and exact mode of action of ALA when used in livestock.

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