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Effects of dietary delta-aminolevulinic acid and vitamin C on growth performance, immune organ weight and ferrum status in broiler chicks

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ABSTRACT

A total of 480 one-day-old male broilers (Arbor Acre) were used to test the effects of dietary δ-aminolevulinic acid (ALA) and vitamin C (VC) supplementation on performance, blood characteristics, ferrum (Fe) status and immune organ weight. There were 6 treatments with 3 ALA (0, 5 and 10 kg^{-1}) and 2 VC (0 and 500 mg kg^{-1}) levels according to a 2×3 factorial arrangement. The experimental diets were fed to chicks for 5 weeks. Growth performance was not affected by ALA or VC supplementation in any experimental period. The interaction between ALA (10 mg kg^{-1}) and VC significantly increased the serum hemoglobin and Fe concentrations in serum, liver and breast meat ($P<0.05$). Supplementation with ALA (10 mg kg^{-1}) significantly increased the RBC concentration at the end of the experiment ($P<0.05$). The weights of liver, spleen, thymus and bursa of Fabricius were unaffected by dietary treatments. The effect of VC was shown to increase the L* value of breast meat in broilers. In conclusion, these results suggest that providing broilers with diets that contained ALA and VC can improve their Fe status without adversely affecting their growth performance, immune related blood profiles and organ weights.

1. Introduction

It is well known that Fe is an essential mineral that plays a critical role within cells that associate with oxygen utilization, enzymatic systems and overall cell function throughout the body. The absorption of Fe from the diet is thus determined more by meal composition than by the amount of Fe present in the diet. There are two forms of Fe found in the diet, non-heme Fe and heme Fe, with heme Fe being more efficient to be absorbed and utilized than non-heme Fe (Hallberg et al., 1989; Morris, 1987). Actually, approximately two-thirds of the Fe in the body is found in hemoglobin, while smaller amounts of Fe are found in myoglobin that helps supply oxygen to muscle and in enzymes that assist biochemical reactions in cells (Oates and West, 2006). The biosynthesis of heme initially occurs in the mitochondrion and involves the condensation of glycine and succinyl CoA to form δ-aminolevulinic acid (ALA). This reaction, catalyzed by ALA synthase, is the rate-limiting reaction of the heme biosynthesis process. This biosynthesis pathway is also controlled by the heme concentration, which exerts negative feedback on the activity of ALA synthase. Recently, it has been suggested that the addition of exogenous ALA may overcome the limitation of ALA synthase during heme synthesis, thereby causing more heme to be available in the body for utilization (Mateo et al., 2006; Wang et al., 2009).

Based on the mechanism proposed above, several studies have been conducted to determine if ALA supplementation exerts beneficial effects on livestock. The results of studies conducted in weanling pigs demonstrated that dietary 0.05% ALA supplementation could improve Fe, hemoglobin, and lymphocyte concentration (Min et al., 2004) and red blood cell counts (Mateo et al., 2006). Chen et al. (2008a) reported that 10 mg kg^{-1} ALA can improve DM, N digestibility, the levels of hemoglobin, and WBC counts (Chen et al., 2008a) of blood.

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However, the optimal levels of ALA being supplemented in other animals such as poultry are also of interest. The result of a previous study indicated that feeding ALA (5, 10, and 15 mg kg^{-1}) to broilers exerted few effects in broilers (Chen et al., 2008b). In addition, the key role of ascorbic acid (vitamin C) for the absorption and utilization of dietary non-heme Fe is generally accepted (Hallberg et al., 1989; Hartiti et al., 1995). However, no study has been conducted so far to determine whether vitamin C (VC) can exert a positive effect on utilization of heme-iron (ALA). Therefore in the current study, we evaluate the effects of combined treatment with ALA and VC on performance, immune organ weight and Fe status in broilers.

2. Materials and methods

2.1. Experimental design and broiler husbandry

All procedures used in this study were approved by the Animal Care and Use Committee of Dankook University. A total of 480 one-day-old male Arbor Acre broilers were obtained from a commercial hatchery (Yang Ji Company, Cheonan, Choongnam, Korea). The broilers were weighed and randomly placed into 24 battery cages (1 m × 1 m) with 20 broilers in each cage, giving 4 replicate cages per dietary treatment. The average initial body weight was 43.0 ± 5.0 g (SD) and this experiment was conducted for 35 days. The experimental design consisted of 6 dietary treatments resulting from 3 ALA addition levels (0, 5 and 10 mg kg^{-1}) and 2 VC levels (0 and 500 mg kg^{-1}). A 2-phase feeding program was used: a starter diet from 1 to 14 days and a finisher diet from 15 to 35 days. All broilers were fed maize-soybean meal-based diets that were formulated to meet or exceed the National Research Council (1994) nutrient recommendations (Table 1). The ALA (EASY BIO System, Inc., Korea) was produced by recombinant *Escherichia coli* containing the *Rhodobacter capsulatus* hemA gene. The ALA was separated by HPLC to supply a final product with a purity of 90%. The VC was supplied by Yuhan Co., Ltd (Korea) with the concentration over 91%. All the additives were added in the feed for 6 batches respectively.

Table 1
Diet composition (as-fed basis).

Ingredient, g kg^{-1}	0–2 weeks (starter)	3–5 weeks (finisher)
Maize	556.7	632.1
Soybean meal (CP 48%)	282.5	246.1
Maize gluten meal (CP 60%)	65.0	35.0
Soybean oil	55.0	48.9
Tricalcium phosphate	24.6	22.9
Limestone	8.9	7.5
Salt	2.0	2.0
DL-methionine	0.7	0.7
L-lysine-HCl	0.6	0.8
Vitamin premix [1]	2.0	2.0
Trace mineral premix [2]	2.0	2.0
Analysis composition		
ME (MJ kg^{-1})	13.02	12.82
Crude protein	218.0	189.0
Lys	11.2	10.4
Ca	10.3	9.1
Available P	4.4	3.1
Met + Cys	9.8	9.8
Fe, mg kg^{-1}	71	68

[1] Provided per kg of premix: 15,000 IU vitamin A, 3750 IU vitamin D_3, 37.5 mg vitamin E, 2.55 mg vitamin K_3, 3 mg vitamin B_1, 7.5 mg vitamin B_2, 4.5 mg vitamin B_6, 24 μg vitamin B_{12}, 51 mg niacin, 1.5 mg folic acid, 126 mg biotin, and 13.5 mg pantothenic acid.

[2] Provided per kg of diet: 37.5 mg Zn (as $ZnSO_4$), 137.5 mg of Mn (MnO_2), 37.5 mg of Fe (as $FeSO4 \cdot 7H_2O$), 3.75 mg of Cu (as $CuSO_4 \cdot 5H_2O$), 0.83 mg of I (as KI), 0.23 mg of Se (as $Na_2SeO_3 \cdot 5H_2O$), and 1408 mg of choline.

Broilers were housed in stainless steel battery cages with concrete floors covered with clean rice bran or hulls. All cages were in the same room and the temperature was kept at approximately 33 °C during the first 3 days and then reduced by 3 °C every week until a temperature of 24 °C was reached. Artificial light was provided 24 h/day via fluorescent lights. All diets were fed in mash form and feed and water were provided *ad libitum* throughout the experimental period.

2.2. Sampling and measurements

The body weight (BW) and feed consumption were measured per cage at the beginning of the experiment, at day 14, and at the end of the experiment. This information was then used to determine the body weight gain (BWG) and feed intake (FI) per cage as well as the feed conversion ratio (FCR). At the end of the experiment, 12 chicks (3 chicks/cage) were randomly selected from each treatment and blood samples were then collected from the wing vein using a sterilized syringe. After collection, half of the sample was transferred into either a vacuum (clot activator with gel) or K_3EDTA vacuum tube (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ) immediately and then stored in a refrigerator at −4 °C until further analysis. The remainder of the sample was centrifuged at 3000 × *g* for 15 min to separate the serum. The Fe and total Fe binding capacity (TIBC), hemoglobin, total protein and albumin concentrations in the serum were then determined using an automatic biochemistry analyzer (HITACHI 747, Hitachi, Tokyo, Japan). In addition, the white blood cell (WBC), red blood cell (RBC), lymphocyte and hematocrit (HCT) levels were analyzed using an automatic blood analyzer (ADVIA 120, Bayer, NY, USA).

At day 35, 12 broilers (3 chicks/cage) were randomly selected from each treatment group and then were slaughtered by cervical dislocation and the liver, spleen, thymus and bursa of Fabricius were then removed and cleaned from digesta. Organ weight was expressed as a percentage of BW.

The breast meat Hunter L* (lightness), a* (redness), and b* (yellowness) values were measured using a Minolta CR410 chromameter (Konica Minolta Sensing Inc., Osaka, Japan). Duplicate pH values for each sample were measured using a pH meter (Fisher Scientific, Pittsburgh, PA).

2.3. Statistical analysis

The data were analyzed by ANOVA as a 2 × 3 factorial arrangement of treatments, using a GLM procedure of SAS (SAS Institute, 1996). The cage served as the experimental unit during the feeding period, whereas individual bird was considered to be the experimental unit for other parameters. The final model included main effects of ALA and VC, as well as the interaction between ALA and VC. When significant ($P<0.05$) interactions were observed, the means were