Asian-Aust. J. Anim. Sci.
Vol. 23, No. 8 : 1028 - 1033
August 2010

www.ajas.info

Evaluation of Dietary Supplementation of Delta-aminolevulinic Acid and Chito-oligosaccharide on Production Performance, Egg Quality and Hematological Characteristics in Laying Hens

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ABSTRACT : The effects of delta-aminolevulinic acid (DALA) or chito-oligosaccharide (COS) in layer diets on production performance, egg quality and hematological characteristics were investigated in this 8-week trial. Two hundred and seventy 27-week-old (Hy-Line brown) layers were randomly assigned to 5 treatments with 9 replications per treatment. Dietary treatments were i) Control (basal diet); ii) DALA1 (basal diet+DALA 2 mg/kg); iii) DALA2 (basal diet+DALA 4 mg/kg); iv) COS1 (basal diet+COS 100 mg/kg) and v) COS2 (basal diet+COS 200 mg/kg). In this study, no significant difference was observed in feed intake and egg shell quality (eggshell breaking strength and egg shell thickness) among treatments. The inclusion of DALA increased egg production during the first 4 weeks. Egg weight was increased in both DALA (quadratic, $p<0.05$) and COS (linear, $p<0.05$) treatments compared with the control treatment. Laying hens fed the DALA treatment had an increased yolk color unit (quadratic, $p<0.05$) and haugh unit (linear, $p<0.05$) compared with the control group. Similarly, these characteristics were also affected by COS treatments, with both values being linearly increased ($p<0.05$) in COS treatments compared with the control treatment. Additionally, birds fed DALA treatments significantly increased (quadratic, $p<0.05$) the number of RBC, WBC and lymphocytes compared with the control treatment. Dietary DALA supplementation linearly increased ($p<0.05$) the serum iron concentration at the end of the 8th week. The inclusion of COS increased (linear, $p<0.05$) the concentration of RBC, WBC and lymphocytes compared with the control treatment. In conclusion, dietary DALA at the lower dosage (2 mg/kg) could exert better effects in laying hens than higher dosage (4 mg/kg). Birds fed DALA supplemented diet had an increased iron availability, egg weight, eggshell quality and immunity. Moreover, the inclusion of COS (200 mg/kg) can increase egg weight, eggshell quality and immunity in laying hens. Therefore, both the utilization of COS and DALA could be considered as a new strategy for optimizing egg quality and health condition of laying hens. (**Key Words :** Delta-aminolevulinic Acid, Chito-oligosaccharide, Performance, Egg Quality, Laying hen)

INTRODUCTION

Recently, the use of delta-Aminolevulinic acid (DALA) as a feed additive for livestock has received much interest for its possible utilization in the livestock feed industry. DALA is a precursor in porphyrin synthesis (Döring et al., 1998) and is a non-protein amino acid with a widespread distribution in living organisms. Mateo et al. (2006) suggested that DALA supplementation can increase RBC counts of weanling pigs. Our previous study also suggested dietary DALA supplementation at the level of 5 mg/kg can increase serum iron concentration and egg quality (Chen et al., 2008b). Therefore, investigation of dietary DALA may provide a new strategy for optimizing egg production and egg quality.

Chito-oligosaccharide (COS), which is easily obtained by chemical and enzymatic hydrolysis of poly-chitosan, was shown to reduce the establishment of pathogens in the intestine (Vishu Kumar et al., 2005) and to improve immune function (Okamoto et al., 2003). A previous study conducted by Huang et al. (2005) also suggested that dietary COS can increase nutrient digestibility and weight gain in broilers due to its antifungal and antimicrobial (Jeon et al., 2000) activities. Therefore, it can be postulated that, with its antimicrobial and antifungal function, COS supplementation can improve performance of laying hens. To the best of our knowledge, there has been no other research concerning the effect of COS in laying hens.

Therefore, the objective of the present study was to evaluate the effects of DALA or COS supplementation in

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Received December 25, 2009; Accepted March 15, 2010

layer diets on egg production, egg quality and hematological characteristics.

MATERIALS AND METHODS

Animal care and use

The protocols used for the current experiment were approved by the Animal Care and Use Committee of Dankook University.

Preparation of COS and DALA

Delta-amino acid and chito-oligosaccharide were supplied by EASY BIO System Inc (South Korea). Briefly, DALA was produced by *E. coli* containing the *R. capsulatus* heme A gene under the control of the constitutive promoter at a maximum concentration of 21 mM in the absence of levulinic acid, which is an inhibitor of DALA dehydratase. The chito-oligosaccharide used in this study was the natural product of chitin, chitosan and chitosan oligosaccharides that were produced by fermentation using live probiotics, including *Aspergillus oryzac*, *Bacillus subtillus, Saccharomyces cerevisiae* and *Lactobacillus acidophilus*. The fermentation product was comprised of: 18.6% crude protein, 14.5% crude fat, 10.7% crude fiber, 22.6% crude ash, 9.0% moisture, 4.3% calcium, 1.7% phosphorus, 4.0% chitin chitosan and 3.0% chitosan oligosaccharide.

Feeding regimen

Two hundred and seventy 27-week-old (Hy-line brown) layer chickens were used in this 8-week trial. All layers were randomly assigned to 5 treatments with 9 replicates per treatment. Each replicate consisted of 3 adjacent cages, with 2 hens per cage. The replicates were equally distributed into the upper and lower cages to minimize the effect of cage level. The hens were housed in a windowless laying house under a 17 h light: 7 h dark photo period at approximately 21°C. There was a 7-day adjustment period prior to the start of the experiment, during which the hens were provided with the control (CON) diet. All cages were equipped with nipple drinkers and a common trough feed. Feed and water were provided *ad libitum* throughout the experimental period.

Experimental design and diets

Dietary treatments were i) Control (basal diet); ii) DALA1 (basal diet+DALA 2 mg/kg); iii) DALA2 (basal diet+DALA 4 mg/kg); iv) COS1 (basal diet+COS 100 mg/kg) and v) COS2 (basal diet+COS 200 mg/kg). All diets used in the present study were formulated to meet or exceed the nutrient recommendations of the NRC (1994) based on corn, soybean and wheat. Treatment additives were included in the diet by replacing the same amount of corn and each treatment was made isolysinic and isocaloric.

Samples and measurements

Daily records of egg production and weekly records of feed consumption were kept throughout the experimental period. Egg production was expressed as an average hen-day production, which was calculated from the total number of eggs divided by the number of days and summarized on an average basis. A total of 36 saleable eggs (no shell defects, cracks or double-yolks) were randomly collected from each treatment at 17:00 h (4 eggs per replicate) on a weekly basis. The egg quality of the collected eggs was then determined at 20:00 h on the day of collection. Egg weight was measured using an egg multi tester (Touhoku Rhythm Co. Ltd., Tokyo, Japan). Eggshell breaking strength was evaluated using a model II egg shell force gauge (Robotmation Co., Ltd., Tokyo, Japan). A dial pipe gauge (Ozaki MFG. Co., Ltd., Tokyo, Japan) was used to measure egg shell thickness, which was determined based on the average thickness of the rounded end, pointed end, and the middle of the egg, excluding the inner membrane. Finally,

Table 1. Diet composition (as-fed basis)

Ingredients	%
Corn	22.00
Soybean meal (CP 46%)	22.00
Wheat	33.10
Grass meal	2.00
Rapeseed cake	4.00
Cornstarch	6.00
Rapeseed oil	2.50
Limestone	8.80
Tricalcium phosphate (P 18%)	1.70
Salt	0.30
DL-methionine (50%)	0.10
Vitamin-mineral premix[1]	0.50
Total	100.00
Chemical composition (%)[2]	
ME (kcal/kg)	2,775
CP	17.00
Lys	0.81
Met	0.36
Met+cys	0.67
Ca	3.70
Total P	0.61
Available P	0.37

[1] The premix provided per 1 kg of diet: vitamin A, 10,000 IU; vitamin D_3, 3,000 IU; vitamin E, 50 IU; vitamin K_3, 2 mg; vitamin B_1, 1 mg; vitamin B_2, 4 mg; vitamin B_6, 1.5 mg; vitamin B_{12}, 0.01 mg; Ca pantothenate, 8 mg; niacin, 25 mg; folic acid, 0.5 mg; choline chloride, 250 mg; manganese, 100 mg; zinc, 50 mg; iron, 50 mg; copper, 8 mg; iodine, 0.8 mg; selenium, 0.2 mg; cobalt, 0.2 mg.

[2] Calculated according to European Table (Janssen, 1989) as the sum of the ME content of components.

egg weight, egg yolk color and haugh unit were evaluated using an egg multi tester (Touhoku Rhythm Co. Lt., Tokyo, Japan).

At the beginning of the experiment, two birds per replicate were randomly selected and 5 ml of blood were collected from the left jugular vein using a sterilized needle. The samples were then transferred into a K_3EDTA vacuum tube (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA). At the end of the experiment, blood was collected from the same laying hens. The samples for serum analysis were then centrifuged at 3,000×*g* for 15 min, and aliquots were stored at -4°C until analyzed for serum total protein, albumin, hemoglobin (Hb), iron and total iron binding capacity (TIBC) using an automatic blood biochemistry analyzer (HITACHI 747, Hitachi, Tokyo, Japan). Red blood cell (RBC), white blood cell (WBC) and lymphocyte counts of the whole blood samples were determined using an automatic blood analyzer (ADVIA 120, Bayer, Tarrytown, NY, USA).

Statistical analysis

All data were evaluated by analysis of variance following the GLM procedure for a completely randomized design. Analyses were conducted using the SAS software program (SAS Institute, 1996), with pen serving as the experimental unit. For the blood profile data, the initial data was used as a covariate. Significant differences among the means of the treatment groups were determined by Duncan's multiple-range test. Variability in the data was expressed as standard error (S.E.) and a probability level of $p<0.05$ was considered as statistically significant. Orthogonal comparisons were made using polynomial regression to measure the linear and quadratic effects of increasing dietary concentrations of supplemental COS and DALA.

RESULTS

Egg production and egg weight

Through the entire experiment, no significant differences were observed in feed intake and egg shell quality (eggshell breaking strength and egg shell thickness) among treatments (Tables 2 and 3). The inclusion of DALA increased ($p<0.05$) egg production during the first 4 weeks. Egg weight was increased in both DALA (quadratic effect, $p<0.05$) and COS (linear effect, $p<0.05$) treatments compared with the control treatment. Laying hens fed DALA had an increased yolk color unit (quadratic effect, $p<0.05$) and haugh unit (linear effect, $p<0.05$) compared with the control treatment. Similarly, yolk color unit and haugh unit were also affected by COS treatments, with both values being linearly increased compared with the control treatment ($p<0.05$).

Hematological characteristics

Total protein, albumin, hemoglobin (Hb) and total iron binding capacity were not influenced by dietary treatment (Tables 4 and 5). The numbers of RBC, WBC and lymphocytes were increased (quadratic effect, $p<0.05$) by DALA treatments compared with the control treatment (Table 4). Dietary DALA supplementation linearly increased ($p<0.05$) the iron concentration at the end of the 8th week (Table 5). Moreover, the inclusion of COS linearly increased ($p<0.05$) the concentrations of RBC, WBC and lymphocytes compared with the control treatment.

DISCUSSION

Effect of DALA on laying hens

Previously, the administration of DALA was shown to influence the heme-iron status and immunity of livestock

Table 2. Effects of DALA and COS supplementation on egg production and egg weight in laying hens[1]

Dietary treatments	Feed intake (g)		Egg production (%)		Egg weight (g)		
	0 to 4 weeks	4 to 8 weeks	0 to 4 weeks	4 to 8 weeks	0 week	4 weeks	8 weeks
Control[1]	109	112	80.82	89.84	47.35	52.59	51.19
DALA1[1]	110	114	83.96	91.73	48.45	53.52	54.48
DALA2[1]	112	113	81.88	90.07	48.10	52.77	52.19
COS1[1]	109	111	82.76	89.49	47.63	52.66	54.32
COS2[1]	108	114	82.50	91.98	49.34	52.27	54.08
SE[2]	6.6	8.7	1.021	1.232	1.74	2.112	1.078
	---------- p-value ----------						
Linear (DALA)	0.268	0.577	0.215	0.213	0.633	0.125	0.128
Quadratic (DALA)	0.630	0.839	0.001	0.794	0.548	0.639	0.012
Linear (COS)	0.818	0.574	0.460	0.136	0.612	0.595	0.046
Quadratic (COS)	0.479	0.447	0.270	0.821	0.275	0.949	0.212

[1] Each mean represents 27 pens with 54 chicks each per treatment. Control = Basal diet; DALA1 = Basal diet with DALA 2 mg/kg; DALA2 = Basal diet with DALA 4 mg/kg; COS1 = Basal diet with COS 100 mg/kg; COS2 = Basal diet with COS 200 mg/kg.
[2] Pooled standard error.

Table 3. Effect of DALA and COS on yolk color unit, egg yolk index and Haugh unit in laying hens[1]

Dietary treatments	Eggshell breaking strength (kg/cm^2)			Egg shell thickness ($\times10^{-2}$, mm)			Yolk color unit			Haugh unit		
	0 week	4 weeks	8 weeks	0 week	4 weeks	8 weeks	0 week	4 weeks	8 weeks	0 week	4 weeks	8 weeks
Control[1]	4.11	4.54	4.98	33.6	32.8	35.2	8.20	8.99	8.80	94.3	87.8	88.4
DALA1[1]	3.71	4.38	4.60	32.6	32.9	35.1	8.18	9.19	9.27	90.7	92.1	89.7
DALA2[1]	4.44	4.52	4.76	33.9	32.3	34.9	8.52	9.06	9.01	93.1	94.5	90.2
COS1[1]	4.93	4.52	5.00	32.6	32.8	34.4	8.24	9.31	8.66	93.2	92.0	91.2
COS2[1]	4.59	4.55	4.91	33.6	33.2	35.1	8.36	8.73	9.28	92.9	96.2	89.6
SE[2]	0.482	0.193	0.171	1.01	1.41	1.46	0.142	0.253	0.242	2.51	1.41	1.62
	p-value											
Linear (DALA)	0.582	0.614	0.851	0.315	0.328	0.648	0.285	0.489	0.038	0.441	0.006	0.232
Quadratic (DALA)	0.356	0.496	0.682	0.368	0.821	0.158	0.364	0.357	<0.001	0.831	0.518	0.332
Linear (COS)	0.283	0.393	0.218	0.268	0.684	0.456	0.239	0.562	0.018	0.283	<0.0001	0.441
Quadratic (COS)	0.359	0.659	0.357	0.593	0.251	0.482	0.459	0.651	0.891	0.122	0.547	0.835

[1] Each mean represents 27 pens with 54 chicks each per treatment. Control = Basal diet; DALA1 = Basal diet with DALA 2 mg/kg; DALA2 = Basal diet with DALA 4 mg/kg; COS1 = Basal diet with COS 100 mg/kg; COS2 = Basal diet with COS 200 mg/kg.
[2] Pooled standard error.

Table 4. Effect of DALA and COS on RBC, WBC and lymphocyte concentrations in laying hens[1]

Dietary treatments	RBC ($\times10^6$, No/mm^3)		WBC ($\times10^5$, No/mm^3)		Lymphocyte (%)	
	0 week	8 weeks	0 week	8 weeks	0 week	8 weeks
Control[1]	1.91	1.71	3.43	3.48	74.4	60.5
DALA1[1]	2.00	2.02	3.54	3.85	78.6	76.2
DALA2[1]	2.08	1.95	3.70	3.45	83.6	73.6
COS1[1]	2.00	2.03	3.65	3.70	82.4	64.0
COS2[1]	2.07	2.01	3.63	3.67	81.6	79.3
SE[2]	0.072	0.112	0.221	0.262	3.15	5.25
	p-value					
Linear (DALA)	0.252	0.001	0.517	0.112	0.648	<0.001
Quadratic (DALA)	0.616	0.035	0.184	0.022	0.218	0.043
Linear (COS)	0.363	<0.0001	0.256	0.004	0.684	<0.0001
Quadratic (COS)	0.185	0.189	0.351	0.535	0.178	0.354

[1] Each mean represents 27 pens with 54 chicks each per treatment. Control = Basal diet; DALA1 = Basal diet with DALA 2 mg/kg; DALA2 = Basal diet with DALA 4 mg/kg; COS1, basal diet with COS 100 mg/kg; COS2 = Basal diet with COS 200 mg/kg.
[2] Pooled standard error.

Table 5. Effects of DALA and COS supplementation on Hb, total protein, albumin, iron and TIBC concentrations in laying hens[1]

Dietary treatments	Hb (g/dl)		Total protein (g/dl)		Albumin (g/dl)		Iron (μg/dl)		TIBC (μg/dl)[3]	
	0 week	8 weeks	0 week	8 weeks	0 week	8 weeks	0 week	8 weeks	0 week	8 weeks
Control[1]	8.90	7.93	5.26	6.12	1.92	2.32	634	743	719	802
DALA1[1]	9.18	8.52	4.88	5.96	1.98	2.38	624	819	596	785
DALA2[1]	8.78	9.26	4.98	5.52	2.28	2.34	637	864	625	814
COS1[1]	8.66	7.70	5.45	5.54	2.16	2.46	641	751	689	751
COS2[1]	8.56	6.40	5.18	5.82	2.08	2.34	647	770	607	741
SE[2]	0.523	0.882	0.289	0.341	0.161	0.082	49.1	48.5	44.1	48.1
	p-value									
Linear (DALA)	0.394	0.128	0.218	0.624	0.482	0.815	0.328	0.008	0.284	0.318
Quadratic (DALA)	0.196	0.364	0.158	0.538	0.648	0.696	0.654	0.254	0.365	0.842
Linear (COS)	0.433	0.228	0.184	0.184	0.268	0.185	0.481	0.128	0.182	0.764
Quadratic (COS)	0.589	0.535	0.354	0.539	0.147	0.352	0.325	0.292	0.482	0.419

[1] Each mean represents 27 pens with 54 chicks each per treatment. Control = Basal diet; DALA1 = Basal diet with DALA 2 mg/kg; DALA2 = Basal diet with DALA 4 mg/kg; COS1 = Basal diet with COS 100 mg/kg; COS2 = Basal diet with COS 200 mg/kg.
[2] Pooled standard error. [3] TIBC represents the total iron binding capacity.

due to its important role as a precursor of heme synthesis, which could increase heme-iron and heme-protein synthesis. Several recent studies also confirmed this effect. For example, Laftah et al. (2008) reported that DALA can enhance iron metabolism due to its effect on heme synthesis. Mateo et al. (2006) and Chen et al. (2008a) also suggested that DALA supplementation can increase RBC counts and the iron utilization of weanling pigs. Therefore, we hypothesized that investigation of dietary DALA may provide a new strategy for optimizing egg production and egg quality. Indeed in this study, dietary DALA administration at 2 or 4 mg/kg linearly increased serum iron concentration, which is in agreement with our previous study (Chen et al., 2008b) where we demonstrated that dietary DALA supplementation at 5, 10 and 15 mg/kg improved serum iron concentration. However, in that study the inclusion of DALA did not affect egg production, egg weight, yolk color unit and whole blood counts (RBC, WBC and lymphocytes). In contrast, all of the aforementioned criteria were quadratically increased in the present study. This may relate to the different dosages used in the two studies, indicating a lower dosage level employed in the current study may have had a greater effect than the higher dosage level used in our previous study. This is supported by Min et al. (2004), who suggested that dietary DALA supplementation at 2 mg/kg increased the hemoglobin concentration in weaning pigs, while Mateo et al. (2006) observed a decreased hemoglobin concentration when they supplemented nursing pig diets at 5 mg/kg DALA. Moreover, Chen et al. (2008b) also reported that serum iron concentration was more pronounced when 5 mg/kg DALA, rather than 10 to 15 mg/kg, was supplemented, which may strengthen the speculation that lower dosage level can exert a better effect on livestock than higher dosage levels. Furthermore, DALA supplementation increased the Haugh unit value at the end of the 4th week, indicating a higher storage profile was created by DALA supplementation. The reason for the increased effect is likely to be the increased iron availability induced by increasing DALA inclusion levels. However, the exact mechanism for this effect is unclear and further work is needed to investigate the means whereby this occurs.

Effects of oligosaccharides on laying hens

Recently, various oligosaccharides are being added to livestock feed as prebiotics to improve animal performance, enhance immune ability, and influence the gut microflora (White et al., 2002; Lemieux et al., 2003). Several studies have suggested that COS has antifungal (Hirano and Nagao, 1989) and antimicrobial (Jeon et al., 2000) activities. Du et al. (2001) reported that COS with degrees of polymerization between 3 and 8 enhanced immunity and growth performance in livestock. Thus, it was hypothesized that laying hen performance may be improved by COS supplementation. In the current study, although COS supplementation did not exert any effect on feed intake and egg production, it increased egg weight at the end of the study. The reason may be the increased nutrient digestibility as a result of antimicrobial and antifungal activities of COS. This assumption is supported by Li et al. (2007), who suggested that chito-oligosaccharide supplementation can increase nutrient digestibility in broilers. Moreover, studies using mannan-oligosaccharides (MOS) suggest that MOS supplementation improves egg production in broiler breeder hens (Stanley and Sefton, 1999; Berry and Lui, 2000). The reason for the improvement has been attributed to the ability of MOS to maintain gut health via adsorption of pathogenic bacteria. Therefore, we suggest that COS may operate in a similar fashion. However, since there is a scarcity of work in the literature on the effect of dietary COS in laying hens, it is difficult to speculate as to the exact mechanisms whereby COS exerts its effects.

Furthermore, measurement of blood concentration is routinely conducted to evaluate the response of animals to various prebiotics. In our study, the RBC and WBC counts and the lymphocyte numbers were linearly increased by COS supplementation, indicating that COS may have exerted beneficial effects on the immune system. This is in partial agreement with Okamoto et al. (2003), who suggested the COS supplementation may increase the lymphocyte concentration. Similarly, our previous studies (Wang et al., 2009; Zhou et al., 2009) showed that lymphocyte number and RBC count were linearly increased by COS supplementation in pigs and broilers, respectively. The discrepancy among these studies may possibly be due to the different hygienic conditions employed. Moreover, Chen et al. (2009) suggested that COS supplementation did not influence immunity under non-challenge conditions, but significantly increased WBC and lymphocyte counts when pigs were challenged with a lipopolysaccharide. Therefore, it is reasonable to speculate that COS supplementation increased immunity in the current study.

CONCLUSION

In the current study, dietary DALA supplementation at lower dosage (2 mg/kg) exerted a better effect on laying hens than higher dosage (4 mg/kg). Birds fed the DALA supplemented diet had an increased iron availability, egg production, egg weight, eggshell quality and immunity. Moreover, the inclusion of COS (200 mg/kg) increased egg weight, eggshell quality and immunity in laying hens. Therefore, both the utilization of COS and ALA could be considered as a new strategy for optimizing egg quality and

health condition of laying hens.

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