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Effect of Alpha-lipoic Acid and Vitamin E in Diet on the Performance, Antioxidation and Immune Response in Broiler Chicken

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Abstract: An experiment was conducted to study the effects of alpha-lipoic acid at graded levels (0, 20, 40, 60, 80 mg/kg), Vit-E (10, 50 mg/kg) and their combination in a 5 x 2 factorial design on performance, anti-oxidation and immune response of broilers. 400 day-old male broilers were divided into 10 treatment groups, each with 8 replications (5 broilers in each) and fed one of the test diets till 6 weeks of age. Supplementation of Lipoic acid in broiler diet at 80 mg/kg gave significantly ($p<0.05$) higher BWG than control. The humoral immune response to NDV at 28 days age significantly ($p<0.05$) increased with lipoic acid at 20 and 80 mg/kg plus Vit-E 50 mg/kg. At 42 days age, HI titers were significantly ($p<0.05$) higher in all diets supplemented with lipoic acid than control. Cell mediated immunity was significantly ($p<0.05$) higher on diets that contained lipoic acid at 60 and 80 mg/kg than control. Similarly, Vit-E at 50 mg/kg also had significantly ($p<0.05$) higher CMI than Vit-E at 10 mg/kg. Supplementation of lipoic acid and vitamin E influenced the activities of lipid peroxidase. Inclusion of lipoic acid in all dietary treatment groups increased glutathione peroxidase levels. RBC catalase levels significantly ($p<0.05$) increased on diets that contained lipoic acid at 60 mg/kg. It is concluded that alpha - lipoic acid at 80 mg/kg and Vitamin E at 50 mg/kg may be supplemented to the broiler diet for improved growth, antioxidant status and better immune responses.

Key words: Alpha-Lipoic Acid (ALA), vitamin E, growth, antioxidation, immunity, broilers

INTRODUCTION

In modern poultry production, the broilers are selectively bred to reach the market weight early. As a consequence of faster growth rate, birds are under physiological and immunological stress that makes them more sensitive to infectious diseases (Gregar, 2006). Besides, poultry feed is more and more supplemented with fats from vegetable and animal sources to reach the higher body weight. Use of higher level of vegetable fat rich in unsaturated fatty acids in the feed increases the oxidative stress in broilers, which is characterized by elevated lipid peroxidation or reactive oxygen species (Salvati *et al*., 1993; Gokce *et al*., 2000). When the accumulation of these reactive oxygen species exceeds the cellular antioxidant capacity in tissues/body fluids they may damage biological macro molecules leading to cellular injury and dysfunction (Mates *et al*., 1999). This results in reduced immune potential in birds leading to greater disease susceptibility and sub-optimal production.

Dietary modifications are among the most preferred and practical ways to ameliorate oxidative stress through certain anti stress compounds. Alpha-lipoic acid is described as universal antioxidant because it can scavenge a wide range of free radicals, directly and indirectly regenerate antioxidants, chelate a wide variety of metals that are associated with increased production of free radicals and inhibit gene over-expression (Packer *et al*., 1995). Lipoic acid is both fat and water-soluble, which promotes rapid passive transport across cellular membranes. In the interior of the cell, the oxidized form of LA, dihydrolipoic acid, regenerates vitamin E, vitamin C and glutathione peroxidase (Biewenga *et al*., 1997). Additionally, LA functions as a cofactor in several mitochondrial multi enzyme complexes involved in energy production (Marangon *et al*., 1999).

Vitamin E is a potent biological antioxidant. It prevents oxidation of unsaturated lipid materials within the cells, thus, protecting the cell membrane from oxidative damage. Secondly, it enhances humoral and cell mediated immune responses (Heinzerling *et al*., 1974). Recent studies support the combined use of lipoic acid and vitamin E, when used together their antioxidant capabilities are improved as lipoic acid recycles vitamin E and decreases oxidative stress and protects immune cells. Therefore, the present study was conducted to determine the effects of using Alpha-lipoic acid individually and in combination with vitamin E on the performance, immune responses and antioxidant status of broilers.

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MATERIALS AND METHODS

Birds and diets: A total of four hundred one day-old commercial male broiler chicks (Ven Cobb) were divided at random in to 10 dietary groups, each with 8 replications and each consisting of 5 chicks and were maintained in battery brooders from day old to upto 6 weeks. Corn-soya based control diet was prepared. The nine experimental diets were prepared by supplementing various levels of lipoic acid (20, 40, 60 and 80 mg/kg diet), Vitamin E (10 and 50 mg/kg) and their combination to the control diet. The composition of the experimental diets, both broiler starter (0-4 weeks) and broiler finisher (5-6weeks) were presented in Table 1. Feed and water are provided *ad libitum*. Chicks were vaccinated against New castle disease on 7th and 28th day of age and with IBD Intermediate Georgia strain vaccine on14th day of age.

Measurements: Performance of broilers in terms of body weight gain was recorded on individual bird basis and feed intake replicate-wise at weekly intervals. The Feed Conversion Ratio (FCR) was calculated as feed intake per unit bodyweight gain at weekly intervals.

Antioxidant evaluation: At fifth week of age, blood samples were collected from six birds from each dietary treatment and transferred in to centrifuge tubes containing citrate buffer (citrate 0.8 g/100 ml) (1.5 ml/10 ml blood) for erythrocyte separation and enzyme estimation. Blood samples were centrifuged at 2000 rpm for 15 min at 4°C to separate buffy coat and erythrocyte pellet. The erythrocytes were harvested and washed thrice with phosphate buffer saline (P^H 7.4). The packed RBC obtained was mixed with an equal volume of phosphate buffer saline and then lysed with distilled water (erythrocyte: distilled water 1: 20), the same procedure was followed for estimation of lipid peroxidase (Placer *et al*., 1966) and Red Blood Cell Catalase (RBCC) (Bergmeyer, 1983). Enzyme activities were expressed as nmol MDA (malondialdehyde)/mg protein and units per 9 of haemoglobulin, respectively. The haemolysate was also analyzed for the activities of glutathione peroxidase (GSH-PX) (Paglia and Valentine, 1967) which was expressed as units/ml.

Table 1: Composition of basal diet (g/kg)

Ingredient name	Starter (0-4 weeks)	Finisher (5-6 weeks)
Maize	552.37	627.88
Soyabean meal	392.57	310.76
Oil (Veg)	17.42	26.81
Salt	3.00	4.50
Dicalcium phosphate	18.00	16.75
Shell grit	10.20	7.18
Saw dust	0.30	0.29
Trace mineral mixture[1]	1.20	1.20
Vitamin premix[2]	0.50	0.50
Choline chloride, 50%	1.00	1.00
L-Lysine HCl	0.64	0.59
DL-methionine	2.30	2.04
Coccidiostat	0.50	0.50
Nutrient composition (calculated)		
ME (Kcal/kg)	2900	3050
Crude protein (%)	22	19
Lysine (%)	1.2	1.0
Methionine (%)	0.54	0.48

[1]Trace mineral provided per kg diet: Manganese 120 mg, Zinc 80 mg, Iron 25 mg, copper 10 mg, Iodine 1 mg and selenium 0.1 mg. [2]vitamin premix provided per kg diet: vitamin A 20000IU, vitamin D_3 3000IU, Vitamin E 10 mg, vitamin K 2 mg, riboflavin 25 mg, vitamin B_1 1 mg, B_6 2 mg, B_{12} 40 mcg, Niacin 15 mg

Table 2: Effect of Alpha-Lipoic Acid (ALA) and vitamin E on the performance of broilers (1-6 Wk of age)

Vitamin E	Lipoic acid	Body weight gain (g)	Feed intake (g)	FCR
10	0	2181	3661	1.76
10	20	2107	3654	1.73
10	40	2118	3675	1.73
10	60	2119	3734	1.76
10	80	2142	3643	1.70
50	0	2068	3584	1.73
50	20	2106	3675	1.74
50	40	2108	3609	1.71
50	60	2141	3633	1.69
50	80	2237	3781	1.68
Main effects of vitamin E				
10		2114	3673	1.74^{b}
50		2132	3656	1.71^{a}
Main effects of lipoic acid				
	0	2074^{b}	3623	1.75
	20	2106^{ab}	3664	1.74
	40	2113^{ab}	3642	1.72
	60	2130^{ab}	3683	1.72
	80	2190^{a}	3712	1.69
SEM		11.77	23.21	0.008

Means in a column with no common superscript differ significantly ($p<0.05$)

Immunological assessment: On 43rd day of age, six birds from each dietary treatment were injected intra dermally in the right wattle with 100 µg of Phytohaemaglutinin-P (PHA-P) in 0.1 ml of normal saline solution to measure the cellular immune response by Cutaneous Basophilic Hyper Sensitivity (CBH) test (Edelman *et al.*, 1986). The thickness of wattle was measured using micrometer before inoculation and 24 h post inoculation and CBH response was calculated using the formula:

$$\frac{\text{Post injection skin thickness}}{\text{Pre-injection thickness}} \times 100$$

Blood samples were collected from six birds individually from each dietary group at 28th and 42nd day of age and antibodies specific for Newcastle Disease Virus (NDV) were detected in sera of chicks by Heamagglutination Inhibition (HI) test and were expressed as $\log_2$ titers (Allan *et al.*, 1978).

Statistical analysis: The data were analyzed using General Linear Model procedure of Statistical Package for Social Sciences (SPSS) 10th version and comparison of means was done using Duncan's multiple range test (1955) and significance was considered at $p<0.05$.

RESULTS AND DISCUSSION

Growth performance: The results of present study indicated that supplementation of ALA at 20, 40 and 60 mg/kg resulted in numerically increased body weight gains than control and statistically comparable with control. However, chicks fed on supplemental ALA at 80 mg/kg exhibited significant ($p<0.05$) beneficial effect for body weight gain compared with those fed a diet not supplemented with ALA. This finding is compatible with previous report that chicken benefited from dietary supplementation of ALA and gained weight faster than control (Diaz-Cruz *et al.*, 2003). On the contrary, Hamano *et al.* (1999) observed that supplemental ALA had no effect on the final body weights, no significant interaction between LA and chicken age and ALA had no preventable effect on corticosterone-induced negative growth performance. There was no difference in final body weight gains between chicks supplemented with Vit E at 10 or 50 mg/kg. Similar findings were observed by Nameghi *et al.* (2007) with 50 IU/kg. Alpha-Lipoicacid and Vitamin E interaction did not influence the final body weights of broilers.

The cumulative feed intake of broilers on diet that contained graded levels of lipoic acid and Vitamin E and their combination was not affected. Similar findings were observed by Akapinar *et al.* (2008), who reported that feed intake was not significantly affected by exposure to stress or ALA in rats Maddock *et al.* (2003) reported that weaned pigs fed on diets with ALA had no effect on average feed intake. Kim *et al.* (2004) reported that ALA decreased the AMPK activity in the hypothalamus and caused decrease in food intake in rats. The diets containing various levels of lipoic acid, vitamin E and their combination did not influence feed conversion ratio of broilers. Similar findings were reported by Maddock *et al.* (2003) in pigs.

Immune response

Humoral immune response: The humoral immunity evaluated in terms of response to NDV at the age of 28 days showed that HI titers on the diets containing combination of ALA at 20 or 80 mg/kg plus Vitamin E at 50 mg/kg were significantly higher than control diet (Table 3). But, at 42 days of age HI titers were not affected significantly by the combination of ALA and vitamin E. However, HI titers were significantly higher in all diets supplemented with ALA than control. Similar findings were also observed in beef cattle by Schmidt *et al.* (2006), who found a rapid recovery from infectious bovine rhino tracheitis virus challenge in cattle fed rations supplemented with 32 mg/kg body weight of lipoic acid. Ohmori *et al.* (1986) found that ALA significantly restored the suppressed antibody response to sheep erythrocytes (SRBC) in cyclophosphamide-injected mice. HI titers were significantly higher on diets that contained vitamin E at 50 mg/kg compared to vitamin E at 10 mg/kg. This concur with the reports of Nameghi *et al.* (2007), Swain *et al.* (2000) and Raza *et al.* (1997), who reported increased humoral immune response to NDV with Vit E supplementation.

Cell mediated immunity: Cutaneous Basophilic Hypersensitivity (CBH) response was significantly higher in diets containing ALA at 60 and 80 mg/kg compared to those fed diet not supplemented with LA (Table 3). Ohmori *et al.* (1986) found that LA does not stimulate the proliferation of lymphocytes non specifically, but would augment the antibody response by enhancing the activity of a specific clone of helper T cells. Packer *et al.* (1997) described that Alpha-lipoic acid regulates aspects of the immune system and in particular, T-lymphocytes. They have discovered that ALA boosts glutathione levels in T cells (glutathione is absolutely essential for the functioning of the immune system). The higher CBH response on diets containing vitamin E at 50mg/kg compared to control was probably due to immunomodulating effect of Vit E on cellular immunity in broilers. Similar observations were reported by Wen-jie *et al.* (1996) at 80 mg/kg (Lymphocyte transformation), Raza *et al.* (1997) at 300 IU/kg (delayed hyper sensitivity index) and Nameghi *et al.* (2007) at 75 IU/kg. Pletsityi

Table 3: Effect of Alpha-Lipoic Acid (ALA) and Vitamin E on Immune response to ND

		NDV		
Vitamin E (mg/kg)	ALA (mg/kg)	28 days	42 days	CBH Response
10	0	3.83^{cd}	117.96	117.96
10	20	3.66^{d}	125.15	125.15
10	40	4.83^{abcd}	143.80	143.80
10	60	4.83^{abcd}	137.73	137.73
10	80	5.00^{cd}	150.75	150.75
50	0	4.16^{ab}	136.93	136.93
50	20	6.00^{a}	148.9	148.9
50	40	4.5^{bcd}	142.34	142.34
50	60	4.66^{bcd}	167.52	167.52
50	80	5.50^{ab}	153.02	153.02
Main effects of vitamin E				
10		4.43^{y}	6.36	135.08^{y}
50		4.96^{x}	6.33	149.74^{x}
Main effects of lipoic acid				
	0	4.00^{b}	5.25^{b}	127.44^{b}
	20	4.83^{a}	6.50^{a}	137.03^{ab}
	40	4.66^{ab}	6.25^{a}	143.07^{ab}
	60	4.75^{ab}	6.83^{a}	152.62^{a}
	80	5.25^{a}	6.91^{a}	151.89^{a}
SEM		0.120	0.102	2.43

Means in a column with no common superscript differ significantly ($p<0.05$)

(1997) reported that Vit E has immuno stimulatory and immuno modulatory properties which stimulate the production of interleukin-A and thereby activating proliferation of both T and B lymphocytes. Most of these functions of Vit E are mediated via action of potent antioxidant. We hypothesize that ALA and Vit E may have different effects on cellular free radical antioxidant balance, which results in activation states of immune cells. However, there was no significant interaction between ALA and vitamin E levels on CBH response.

Oxidative parameters: Malonyl Dialdehyde (MDA) concentration in erythrocytes was measured as a biomarker of lipid peroxidation and oxidative stress. The graded levels of lipoic acid and vitamin E and their interactions significantly reduced the MDA levels. The diet containing vitamin E at 50 mg/kg had significantly reduced MDA levels than vitamin E at 10 mg/kg. It appears that MDA concentrations are inversely proportionate to the vitamin E content in the diet. Additionally lipoic acid plays a role in the regeneration of vitamin E from its radical form and thus recycling the vitamin E and utilizing the same for scavenging radical products from lipid peroxidation. Sahin *et al.* (2001) reported that supplemental Vit E from 62.5-500 mg/kg linearly decreased MDA concentration. Gonzalez-Perez *et al.* (2001) reported that Alpha-Lipoic Acid (LA) and vitamin E showed synergistic effects against lipid peroxidation by oxidant radicals in several pathological conditions in rats. In this study, it has clearly indicated the interaction of ALA and Vit E. Moderate additions of combination of Vit E (50 mg/kg) and ALA reduced lipid peroxidation more than the higher additions.

Additionally, the present study provides experimental data showing that GSH-Px and CAT activities decreased in the control birds. Therefore, in line with results of earlier reports, it could be suggested that the elevated lipid peroxidation in stress could be resulted from the decreased activities of antioxidant enzymes (Zaidi *et al.*, 2003; Zaidi and Banu, 2004). Activities of catalase and glutathione peroxidase were independently influenced by concentrations of lipoic acid and vitamin E but there were no interaction effects. GSH-Px levels were significantly increased in the diet containing ALA at 60 mg/kg. RBC catalase levels were significantly higher in diets containing LA at 60 mg/kg. Similar findings were also observed by Diaz-Cruz *et al.* (2003), who reported that diets supplemented with 40 parts/106 of ALA lowered thiobarbituric acid reactive substances and hydroxyl radicals in the liver and increased total glutathione pool. Williams *et al.* (2001) reported that supplementation of horses with lipoic acid reduced oxidative stress in treated animals as measured by glutathione and anti-oxidant enzymes presence in the blood. Vit E at 50 mg/ kg significantly ($p<0.05$) increased GSH-Px and Catalase levels compared to vit E at 10 mg/kg. Similar findings were observed by Harset Yerdibi and Gulhan Turkay (2008) who reported that vitamin E (50 IU/kg) supplemented to laying hens during heat stress had reduced lipid peroxidation and increased erythrocyte GSH-Px and catalase levels. Hence changes in the enzymatic antioxidants in erythrocytes indicated

Table 4: Effect of Alpha-Lipoic Acid (ALA) and Vitamin E on activities of certain enzymes involved in cell oxidation

		Lipid peroxidation	Glutathione peroxidase	RBC Catalase
Vitamin E (mg/kg)	ALA (mg/kg)	nmolMDA/mg protein	Units/ml	CAT/g Hb
10	0	1.76[a]	217.78	235.55
10	20	1.29[b]	256.38	272.06
10	40	1.23[b]	299.58	255.14
10	60	1.22[b]	315.26	293.05
10	80	1.07[c]	292.64	269.01
50	0	1.31[b]	260.02	298.74
50	20	1.21[b]	328.94	312.35
50	40	1.32[b]	293.04	277.37
50	60	1.09[c]	367.49	360.51
50	80	1.22[b]	299.11	312.36
Main effect of Vit E				
10		1.31[y]	276.33[y]	264.96[y]
50		1.23[x]	309.72[x]	312.27[x]
Main effect of lipoic acid				
	0	1.53[c]	238.90[c]	267.15[b]
	20	1.25[b]	292.66[b]	292.21[ab]
	40	1.27[b]	296.31[b]	266.25[b]
	60	1.16[a]	341.37[a]	326.78[a]
	80	1.14[a]	295.88[b]	290.68[ab]
SEM		0.13	5.51	6.57

Means in a column with no common superscript differ significantly ($p<0.05$)

that birds experienced less stress after supplementation of LA and Vitamin E compared to control group. It is concluded that Lipoic acid at 80 mg/kg and Vitamin E at 50 mg/kg may be supplemented to the broiler diet for improved growth, antioxidant status and better immune responses.

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