Effects of Incremental Levels of α-Tocopherol Acetate on Performance, Nutrient Digestibility and Meat Quality of Commercial Broilers

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ABSTRACT : This experiment was conducted to explore the efficacy of α-tocopherol acetate (AT), a commercial supplement containing tocopherols, in commercial broilers. Three hundred and thirty Ross broiler chicks (4-d old) were randomly distributed and allotted to five dietary treatments for six weeks. Each treatment comprised 3 pens as replicates containing 22 chicks per pen. The five dietary treatments were: 0 mg/kg AT (negative control); 10 mg/kg AT; 50 mg/kg AT; 100 mg/kg AT; and 200 mg/kg AT; respectively, supplied totally by the supplement under study. Supplementation of AT improved weight gain significantly ($p<0.05$), with higher values in the 100 and 200 mg/kg AT fed group than the control during all phases of study, but feed intake remained unaffected. The nutrient digestibility studies conducted after 15 and 35 days of experimental feeding showed significantly higher digestibility of CP, ether extract and gross energy, in these two groups over the control diet. Carcass traits like dressing percentage and the color of the breast meat did not differ significantly due to treatments, but significantly ($p = 0.0152$) lower abdominal fat percent was noted in the 200 mg/kg fed group. Higher ($p = 0.0003$) tibia bone strength was noticed in groups fed diets above 50 mg/kg AT because of higher bone mineral content. The serum levels of tocopherols were not influenced but the muscle tocopherols content showed a positive linear trend with the dietary levels supplemented. The thio-barbituric acid reactive substances (TBARS) level in meat also suggested that supplemental AT has a protective role in rancidity. Overall, it could be concluded that AT supplementation at higher levels was found beneficial for growth and increased chicken meat quality. ***(Asian-Aust. J. Anim. Sci. 2006. Vol 19, No. 2 : 203-208)***

Key Words : α-Tocopherol, Broiler, Meat, Vitamin, Bone

INTRODUCTION

Supplementation of fat-soluble vitamins over and above the NRC (1994) recommendations is a general trend commonly followed these days in commercial vitamin premixes (Coehlo, 2000). Plenty of research work is available showing a higher dietary allowance of tocopherols above 10 mg/kg level suggested by NRC (1994) has beneficial effects with chicks (Mazija et al., 1992; McIlroy et al., 1993; Haq et al., 1996) such as enhanced immune competence, especially under commercial production conditions. It is a well-established fact that supplementation with tocopherols has some protective role under stressing conditions (Chae et al., 2005). It has also been proved that tocopherols play a role in growth, immunity and in the protection of biological systems against oxidative damage in live animals as well as in meat and meat products (Tengerdy et al., 1984; Jacobsen et al., 1995). These antioxidant functions are important because the oxidation of muscle lipids after slaughter can adversely affect the flavor and nutritive value of fresh, frozen and cooked meat and meat products (Morrissey et al., 1998).

Moreover, tocopherols in the form of tocopheryl acetate are more prone to oxidation due to temperature, moisture and humidity, which all cumulatively work to reduce the vitamin potency. Hence, antioxidants such as ethoxyquin are purposefully added in diets to protect the formation of peroxides in feeds (Scott and Stoewsand, 1961) and may decrease the amount of α-tocopheryl quinone in tissues (Dudin and Dvinskaya, 1984). The tocopherol requirements as suggested by NRC (1994), are not adequate to meet the requirements of fast growing birds and are not sufficient to maintain the storage stability of meat and meat products (Lohakare et al., 2005). The present study was conducted to investigate the effect of incremental levels of α-tocopherol acetate (AT) on performance, nutrient digestibility and chicken meat quality in broilers during the summer season in Korea.

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MATERIALS AND METHODS

Design, animals and sample preparation

For a six-week feeding trial, a total of 330 broiler chicks (Ross, 4-day old, average 56 g body weight) were allotted to one of five dietary treatments. Each treatment comprised 3 pens as replicates containing 22 chicks each. The dietary tocopherols level as AT used were 0, 10, 50, 100 and 200 mg/kg, respectively. The 4-day old chicks were raised with their respective diets in a room with rice hull as litter material. For the initial three days, the chicks were raised with a commercial starter diet before allocation to experimental treatments.

Basal diets (mash) were formulated and analyzed values contain 22.4% and 20.26% crude protein for the starter (4-21 d of age) and the finisher (4-6 weeks) diets, respectively

Table 1. Composition of basal diets

	Starter (4-21 days)	Finisher (22-42 days)
Ingredients (%)		
Corn	56.06	59.90
Soybean meal (44% CP)	22.44	20.76
Corn gluten meal	7.00	8.00
Fish meal	6.16	3.00
Animal fat	6.00	5.70
Tri-calcium phosphate	0.92	1.12
Limestone	0.59	0.87
Vitamin premix[1]	0.10	0.10
Trace mineral premix[2]	0.20	0.20
Salt	0.25	0.25
L-lysine HCl	-	0.03
DL-methionine (50%)	0.20	-
Choline chloride (25%)	0.08	0.07
Total	100.00	100.00
Chemical composition (%)		
ME (MJ/kg)	13.39	13.39
Crude protein	22.04	20.26
Calcium	0.90	0.90
Av. Phosphorus	0.40	0.35
Lysine	1.14	1.00
Methionine	0.53	0.40
Methionine+cystine	0.90	0.75

[1] Supplied per kg diet: 9,000 IU retinol acetate, 1,800 IU cholecalciferol, 1 mg thiamin, 10 mg riboflavin, 4 mg pyridoxine, 0.02 mg cyanocobalamine, 1 mg menadione, 12 mg pantothenic acid, 30 mg niacin, 0.03 mg biotin, 0.5 mg folic acid, 3 mg ethoxyquin.

[2] Supplied per kg diet: 80 mg Fe, 80 mg Cu, 100 mg Zn, 120 mg Mn, 2 mg I, 0.1 mg Co, 0.2 mg Se.

(Table 1). Adding AT in the vitamin premix, which was subsequently mixed in the diet, made the required levels of tocopherols per group. The basal vitamin premix did not contain AT. The AT used was supplied by Roche Vitamins Korea Ltd., Seoul, Korea. The temperature during first week was 34±1°C and was gradually reduced to 26±1°C by 21 days of age, after which the chicks were maintained at room temperature, as it was summer season (July-August). Pen size was 1.5×1.2 meters. Chicks had *ad libitum* access to feed and water.

At the end of the experiment, nine chicks per treatment were sacrificed (3 chicks per pen). Carcass, abdominal fat and breast meat percentages were measured. Blood and breast meat samples were taken to analyze tocopherol content and meat color. The serum was separated by centrifuging the blood at 3,000 rpm for 20 minutes at 1°C and stored at -20°C until analysis. The right tibia was separated and stored at -20°C and then used for bone breaking strength using a Universal testing machine (Model SFM-20, United Calibration Corporation, USA) and the force required was measured as kg/mm^2. The left tibia was removed to determine its chemical composition on dry fat free bones as described by AOAC (1990).

For a digestibility trial, thirty chicks (6 birds/ treatment; 2 birds representative of each pen) were allocated in individual cage (one bird/cage) to collect fecal samples. Starter and finisher diets containing 0.25% chromic oxide as an indigestible marker were fed on day 15 and 35 of feeding, respectively. Fecal samples were taken from each bird on the fourth day after feeding the marked diets as like our reported studies (Lohakare et al., 2005). Feces were dried in forced-air drying oven at 60°C for 3 days and stored until chemical analysis.

Analyses

Body weight gain and feed intake were recorded at 21 and 42 days of age. Proximate analysis of samples was made according to the methods of AOAC (1990). Gross energy, tocopherols and chromium were measured with an adiabatic bomb calorimeter (Model 1241, Parr Instrument Co. Molin, IL.), HPLC (Waters, Model 486, USA) and spectrophotometer (Jasco Co. Model V-550, Japan), respectively.

Chicken meat color was measured with a color difference meter (Yasuda Seiko Co., CR310, Minolta, Japan). Bone mineralisation was studied in terms of bone breaking strength of tibia, and ash, calcium and phosphorus content. For this, the tibia was freed of soft tissues. The dried (100°C, 3 h) bone samples were defatted in petroleum ether for 48 h. The right tibia was separated and stored at -20°C and then used for bone breaking strength using a Universal testing machine and the force required was measured as kg/mm^2. The left tibia was used for determining its chemical composition on dry fat free bones as described by AOAC (1990). The breast meat was stored at 1°C for 10 days after slaughter to determine thiobarbituric reactive substances (TBARS) as milligrams of malonaldehyde (mg/kg) by the method of Sinnhuber and Yu (1977). The tocopherols content in the breast meat was also measured by HPLC.

Statistical analysis

Statistical analysis was conducted by using the GLM procedure of SAS software (1991) using a completely randomized design. The treatments were the main effects. Data was subjected to one-way analysis of variance using GLM procedure of SAS. Linear and quadratic polynomials were evaluated for increasing AT levels. When significant interactions were found, comparisons among means were made by Duncan's multiple range tests and significance was accepted at $p<0.05$.

RESULT AND DISCUSSION

Performance and nutrient digestibility

The supplemental effect of AT on the performance of broilers was recorded at day 21st and 42nd and the data is

Table 2. Effect of α-tocopherol acetate on growth performance of broilers

Treatments	Control	α-Tocopherol acetate				SE[1]	Linear	Quadratic
	0	10	50	100	200			
Starter (4-21 d)								
Weight gain (g)	466[d]	510[c]	527[bc]	548[ab]	560[a]	9.54	0.00	NS[2]
Feed intake (g)	985	947	985	1,016	1,051	16.58	NS	NS
Feed:gain	2.11[a]	1.86[b]	1.87[b]	1.85[b]	1.88[b]	0.04	0.04	NS
Finisher (22-42 d)								
Weight gain (g)	920[b]	993[ab]	1,011[ab]	1,047[a]	1,054[a]	18.88	0.02	NS
Feed intake (g)	2,469	2,351	2,433	2,525	2,692	111.13	NS	NS
Feed:gain	2.67	2.37	2.40	2.41	2.56	0.10	NS	NS
Overall (4-42 d)								
Weight gain (g)	1,386[c]	1,503[b]	1,538[ab]	1,595[ab]	1,614[a]	25.11	0.00	NS
Feed intake (g)	3,454	3,380	3,402	3,510	3,669	102.29	NS	NS
Feed:gain	2.49	2.25	2.22	2.20	2.28	0.07	NS	NS

[a, b, c, d] Values with different superscripts in the same row differ significantly ($p<0.05$).
[1] Pooled standard error. [2] Not significant.

Table 3. Effect of α-tocopherol acetate on nutrient digestibility (%) in broilers

Treatment	Control	α-Tocopherol acetate				SE[1]	Linear	Quadratic
	0	10	50	100	200			
Starter (4-21 d)								
Crude protein	72.14[c]	72.18[c]	72.54[b]	73.17[a]	73.20[a]	0.13	0.00	NS[2]
Ether extract	73.13[b]	73.23[ab]	73.39[a]	73.34[a]	73.40[a]	0.04	0.01	NS
Gross energy	70.19[d]	71.30[c]	73.28[b]	73.42[b]	74.13[a]	0.40	0.00	0.00
Finisher (22-42 d)								
Crude protein	71.15[b]	71.16[b]	71.42[b]	73.25[a]	73.24[a]	0.26	0.00	0.00
Ether extract	72.75[c]	72.98[bc]	73.11[b]	73.87[a]	74.05[a]	0.14	0.00	0.02
Gross energy	71.23[c]	71.38[c]	71.40[c]	73.37[b]	73.79[a]	0.29	0.00	0.00

[a, b, c, d] Values with different superscripts in the same row differ significantly ($p<0.05$).
[1] Pooled standard error. [2] Not significant.

presented in Table 2. Significantly ($p<0.05$) higher weight gains were noted with increasing levels of dietary AT; especially at higher levels of supplementation than the control group, at all three phases (starter, finisher and overall) of the study. Neither linear nor quadratic effects were noticed with respect to feed intake at all levels. The feed:gain ratio was significantly ($p<0.05$) improved in supplemented diets only during the starter phase. The present findings were contradictory to the reports by Frigg et al. (1990) in broilers fed a diet containing 200 mg/kg tocopherols that showed the highest means body weight and the best feed conversion, but the effects were not significant as compared with the control. Franchini et al. (1988) and Rice and Kennedy (1988) reported that benefits in performance from high dietary concentration of tocopherols would be observed in the presence of free radical attack on the immune system. Since we have conducted experiment in the summer season, the summer stress might have triggered increased production of lipid free radicals and hence better growth was found in the supplemented groups. This could also be confirmed from the TBARS values in breast meat, which showed lower values in AT supplemented diets at higher doses in the present study. Guo et al. (2001), fed 0, 5, 10, 50 and 100 mg/kg tocopherols to broiler chicks and did not find any effect on feed intake at 0-3 weeks measurement, but tocopherols supplementation tended to improve growth and feed utilization. However, there was no significant correlation between performance and dietary levels supplemented. They did not find any effect during the 0-6 weeks period. The present study contradicts the findings of Sheehy et al. (1991), in which dietary levels of 5, 25, 65 or 180 mg/ kg tocopherols did not affect chick growth. Severe growth depression and poorer feed to gain ratio occurred where no tocopherols was supplemented. No significant effect on feed intake and feed conversion ratio was found due to dietary treatments during finisher and overall phase of the study. Bartov and Frigg (1992) supplied 100 or 150 mg of tocopherols per kg of diet to a basal diet containing 24 mg/kg tocopherols, and found no influence on feed intake, body weight and feed conversion.

The nutrient digestibility studies (Table 3) on the experimental feeds noted a linear ($p<0.05$) trend with respect to digestibility of crude protein, ether extract and gross energy, during both phases. The significantly ($p<0.05$) higher values were noted in the 100 and 200 mg/kg fed groups as compared with the control. Except for gross energy digestibility at both intervals, the digestibility of

Table 4. Effect of α-tocopherol acetate on carcass traits and meat color

Treatment	Control	α-Tocopherol acetate				SE[1]	Linear	Quadratic
	0	10	50	100	200			
Carcass traits (%)								
Dress percent	73.90	73.02	73.89	75.64	75.96	0.64	NS[2]	NS
Breast meat	10.96	13.46	11.03	12.99	12.92	0.36	0.09	0.07
Abdominal fat	2.45[a]	2.23[ab]	2.25 [ab]	2.14 [ab]	2.03[b]	0.05	0.01	NS
Meat color								
L*	58.79	58.43	58.86	58.86	58.99	0.21	NS	NS
a*	10.22	10.45	10.59	10.50	10.66	0.09	NS	NS
b*	7.79	7.39	7.14	7.12	7.14	0.23	NS	NS

[a, b] Values with different superscripts in the same row differ significantly ($p<0.05$).
[1] Pooled standard error. [2] Not significant.

Table 5. Effect of α-tocopherol acetate on bone resistance and composition of tibia bone

Treatment	Control	α-Tocopherol acetate				SE[1]	Linear	Quadratic
	0	10	50	100	200			
Bone resistance (kg)	20.31[b]	22.51[b]	26.68[a]	27.54[a]	28.62[a]	0.97	0.00	NS[2]
Bone composition (%)								
Dry matter	80.23	74.75	79.47	81.51	76.41	0.77	NS	NS
Crude ash	53.75	52.25	53.79	54.82	53.42	1.04	NS	NS
Calcium	15.05	16.07	17.79	17.67	17.90	0.51	NS	NS
Phosphorous	6.54	5.83	6.89	6.52	6.70	0.27	NS	NS

[a, b] Values with different superscripts in the same row differ significantly ($p<0.05$).
[1] Pooled standard error. [2] Not significant.

crude protein and fat were statistically similar in 100 and 200 mg/kg fed birds. The higher nutrient digestibilities improved the weight gains in these groups. The digestibility of all nutrients studied was significantly reduced where no AT was supplemented, which culminated into lower weight gain. The gross energy digestibility was also reported higher in 10 mg/kg and 20 mg/kg tocopherols supplemented diets, when compared with the non-supplemented group, in our earlier studies conducted during the starter and finisher phase in broilers (Lohakare et al., 2004).

Carcass characteristics and meat color

Carcass traits revealed no effect of dietary AT on dressing percent, although numerically higher values were noted for the supplemented diets at 100 and 200 mg/kg levels as shown in Table 4. The breast meat percentage tended to improve by AT supplementation but could not achieve a significant level. The abdominal fat percent was significantly ($p = 0.0152$) reduced due to higher dietary AT content (200 mg/kg) group, which could be beneficial with respect to consumer's preference. The meat color when compared with the standard values did not reveal any particular trend. In chicken meat colors, 'a' value for redness and the 'b' value for yellowness remain unchanged due to dietary AT supplementation. The color stability of meat could be attributed to a tocopherols-mediated reduction in lipid and myoglobin oxidation following supplementation of tocopherols, but it remained obscure why the meat quality was not affected in the non-supplemented group. Faustman et al. (1989) recorded the color stability of beef and attributed it to a tocopherol-mediated reduction in lipid and myoglobin oxidation following dietary tocopherol supplementation.

Bone resistance and composition

The tibia breaking strength was linearly ($p<0.05$) increased by increasing the AT levels in the diet. This suggests a possible role of tocopherols in strengthening the bone. Bone resistance was lowest where no AT were supplemented. Few studies have been conducted with respect to supplementation of AT on bone strength and composition. The studies by Xu et al. (1994) indicated that supplemental tocopherols enhanced bone formation of chicks and showed the possibility that this vitamin affects bone metabolism The composition of the bone was not affected significantly (Table 5) in our study due to different dietary treatments, although the calcium content of bone tended to be higher ($p = 0.0598$) in AT supplemented diets at higher levels and this may be the reason for increasing the bone strength in these groups due to increased bone mineralisation. The calcium content in the left femur bone of rat was increased by supplementing tocopherols at 90 mg/kg body weight/ day (Norazlina et al., 1999), and they postulated that tocopherol supplementation induced calcium deposition in bones but it inhibited deposition of other minerals that also contribute to bone mineral density such as magnesium, phosphate and zinc. The reason that bone calcium content tended to improve in the AT supplemented groups is unclear to us hence further studies are recommended in this area.

Table 6. Effect of α-tocopherol acetate on serum, muscle tocopherol content and thio-barbituric acid reactive substances (TBARS) in breast meat after storage

Treatments	Control	α-Tocopherol acetate				SE[1]	Linear	Quadratic
	0	10	50	100	200			
Serum (μg/mL)	1.60	1.87	1.92	1.98	2.00	0.07	NS[2]	NS
Muscle (ppm)	0.17[d]	0.24[c]	0.26b[c]	0.27[ab]	0.28[a]	0.01	0.00	0.01
TBARS (mg/kg)								
0 d	0.32	0.31	0.30	0.27	0.25	0.02	NS	NS
5 d	1.68[a]	1.57[a]	1.54[a]	0.96[b]	1.12[b]	0.08	0.00	NS
10 d	2.72[a]	2.64[a]	2.61[a]	1.62[b]	1.44[c]	0.15	0.00	0.00

[a, b, c, d] Values with different superscripts in the same row differ significantly ($p<0.05$).
[1] Pooled standard error. [2] Not significant.

Serum, muscle tocopherol content and effect on TBARS in breast meat

The serum levels of tocopherols were not influenced due to dietary supplementation, although its levels in breast muscles were in agreement with the levels of AT supplemented in the diet. Tocopherol content in muscles revealed significantly ($p = 0.0001$) higher levels as the level of supplementation increased in the diet (Table 6). Both linear and quadratic effects were recorded with the levels of AT supplemented in the diets and its levels in muscle. The breast meat was ground and stored at 1°C for 10 days and the thio-barbituric acid reactive substance levels (TBARS) were measured at 0, 5 and 10 d after storage. These values were significantly ($p>0.05$) lower in the AT supplemented diets after 5 and 10 days of storage in 100 and 200 mg/kg added diets. These results suggest that tocopherols improved the lipid stability of meat during storage and were in agreement with that reported by Guo et al. (2001). There exists an inverse correlation between the muscle thio-barbituric acid reactive substances and tocopherol content in the muscle. The retention of tocopherols in the muscle might have prevented the meat from oxidative damage during storage. The antioxidant functions of tocopherols persist after slaughter and delayed the onset of oxidation reactions in meat and meat products (DeVore et al., 1983). This might be the reason for improving the keeping quality of meat as was also noticed by observing the tocopherol content in the muscles. There also exists possibility that supplementation of tocopherols at higher levels have benefited birds, which resulted in the improved weight gain because of stronger anti-peroxidation capacity of tissues with higher retention of tocopherol in muscles as recorded. The present findings were in accordance with that recorded by Monahan et al. (1992), Guidera et al. (1997), Mitsumoto et al. (1998) and Lauridsen et al. (1999), where tocopherols supplementation of diets of meat producing animals effectively elevates muscle tocopherols levels and lowers the susceptibility of muscle and ultimately products to lipid oxidation and the onset of flavor defects.

The VE requirement as suggested by NRC (1994), 10 mg/kg feed, at times may not meet the requirements of the bird and is not sufficient to maintain the storage stability of the meat and meat products. This study concluded that AT supplementation at higher levels was found beneficial for the growth of broilers and increased the chicken meat quality.

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