Effect of different levels of dietary vitamin E (DL-α-tocopherol acetate) on the occurrence of various degrees of white striping on broiler breast fillets

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ABSTRACT White striping could be a potential reason for the rejection of raw breast fillets in the market. The condition is characterized grossly by the white striations occurring on the fillets showing myopathic changes on microscopic examination. Early research has shown similar lesions in the case of nutritional muscular dystrophy, which is a condition caused mainly by the deficiency of vitamin E in the diet. The present study was intended to evaluate the effect of different levels of dietary vitamin E (DL-α-tocopherol acetate) on the incidence of normal, moderate, and severe degrees of white striping, by modern description, on broiler breast fillets. Basal diets adequate for starter (0 to 18 d), grower (19 to 32 d), and finisher (33 to 49 d) age periods supplemented with 15, 50, 100, 200, and 400 IU of vitamin E/kg of feed were used in the study. Each of the 5 diet treatments were fed to 8 pens (53 birds each) of male broilers from a commercial strain. At 49 d, 5 birds were randomly selected from each pen (n = 40 birds/diet treatment) and were processed. Live weight, ready-to-cook weight, weight of the fillets, wings, tenders, legs, and the racks were obtained. The fillets were scored for the 3 degrees of white striping. There were no significant differences among the diet treatments with respect to the weight and carcass yield parameters. Furthermore, the diet treatments did not show any significant effect on the occurrence of normal, moderate, and severe degrees of white striping. However, fillet weight was the only parameter that had a significant effect on the occurrence of white striping. Higher degrees of white striping were seen associated with heavier fillets, which is in accordance with previous studies. Different levels of vitamin E levels used in the present study did not show any significant effect on the occurrence of 3 degrees of white striping. These results suggest that dietary vitamin E level is not associated with the modern condition of white striping in broiler breast meat.

Key words: vitamin E, tocopherol, white striping, broiler breast, muscular dystrophy

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INTRODUCTION

Vitamin E is a fat-soluble vitamin that is of plant origin. It is important for the health of humans and animals because of its effect on the integrity and optimal function of various organ systems. Most important property of vitamin E is that it can act as a lipid-soluble antioxidant. It acts synergistically with selenium-dependent glutathione peroxidase, catalase, and superoxide dismutase to remove the intermediates formed during free radical chain reaction (Herrera and Barbas, 2001). Thus, it helps to prevent the propagation of free radicals and avoid damage to DNA, proteins (including enzymes), and the polyunsaturated fatty acids present on cell membrane (Mézes et al., 1997). Also, vitamin E has immunoregulatory effect, which could be through the modulation of cyclooxygenase and lipoxygenase pathways resulting in control on the synthesis of substances such as prostaglandins and leukotrienes (Blumberg, 1994; Leshchinsky and Klasing, 2001).

According to NRC (1994), the dietary requirement of vitamin E for broilers is 10 IU/kg of feed. However, establishment of vitamin E requirements in isolation is difficult because of its interrelationships with various other conditions. Hidiroglou et al. (1992) suggested that the requirement of vitamin E may be influenced by various factors affecting the variability of vitamin E in feedstuffs and the physiological status of the bird. Some of these factors are the amount, type, and degree of oxidation of fat present in the diet, presence of other dietary antioxidants such as selenium, iron, copper, sulfur-containing amino acids, and so on, and harvesting, drying, or storage conditions of feeds that results in destruction of vitamin E. It is also necessary to consider the possible genetic differences in requirements,

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variations in absorbability of vitamin E, destruction of vitamin E in the gastrointestinal tract, variation in the quantity of vitamin E transferred from breeder hen to chick, increased requirements due to diseases, stress, or increased metabolic demands (Hidiroglou et al., 1992).

Clinical manifestations of the deficiency of vitamin E may depend upon the species of the animal or bird, age, and also the organs affected. The deficiency of vitamin E, along with associated nutrients such as selenium and sulfur-containing amino acids, can mainly result in pathological conditions such as encephalomalacia, exudative diathesis, nutritional muscular dystrophy (**NMD**) in chicks, ducks, and turkeys (Klasing, 2008). Nutritional muscular dystrophy is characterized grossly by white striations in the breast muscle in the direction of the muscle fibers and sometimes also in the leg muscles (Dam et al., 1952; Scott et al., 1955; Machlin and Shalkop, 1956; Machlin and Pearson, 1956; Nesheim et al., 1959; Ferguson et al., 1964; Bunyan et al., 1967; Netke et al., 1969; Klasing, 2008). There will be wide linear areas of degeneration involving several adjacent muscle fasiculi, which contribute to the white-colored bands seen grossly. The microscopic examination of the lesions showed hyaline, waxy, or Zenker's degeneration accompanied with fragmentation, hyalinization, loss of striation, multiplication of cells, infiltration by heterophil cells, clumping of fibers into eosinophilic masses, and in more severe cases with calcification as well (Dam et al., 1952; Machlin and Shalkop, 1956).

Recently, a condition known as white striping, which has a negative effect on consumer acceptance of broiler breast fillets (Kuttappan et al., 2012b), is gaining the attention of broiler producers. Gross and microscopic lesions associated with white striping are similar to those of NMD previously described (Kuttappan et al., 2009, 2011). However, the occurrence of white striping is seen in birds that are fed with an adequate amount of vitamin E in their diets. Perhaps the fast-growing birds may need greater amounts of vitamin E in their muscles for normal muscle growth. Various studies showed that an increase in the dietary level of vitamin E could result in an increased amount of the vitamin E in muscles (Sheldon, 1984; Sheehy et al., 1991; De Winne and Dirinck, 1996; Lauridsen et al., 1997). Therefore, the present study is intended to compare the effect of different levels of dietary vitamin E on the occurrence of 3 degrees of white striping in broiler breast fillets.

MATERIALS AND METHODS

Experimental Design

The present study included 5 different diet treatments with 15, 50, 100, 200, and 400 IU/kg of vitamin E levels. The basal diet was formulated in such a way as to meet typical industry nutrient standards (Agri-Stats, Fort Wayne, IN). The composition of the basal diet for the starter (1 to 18 d), grower (19 to 32 d), and finisher (33 to 49 d) age periods of birds were as given in the Tables 1 and 2. Diet also contained phytase (equivalent of 0.10% nPP and 0.10% Ca) and was fortified with complete vitamin (without vitamin E) and mineral mixture. The basal diet was then supplemented with different levels (15, 50, 100, 200, and 400 mg/kg) of DL-α-tocopherol acetate as a source of vitamin E. Each of the 5 diet treatments were assigned to male broilers of a commercial strain in a completely randomized design. There were 8 replicates, with 53 birds per replicate. The birds were maintained on litter floor pens (1.5 × 3 m) with ad libitum access to feed and water. At 49 d, 5 birds were randomly selected from each pen and were processed using commercial style methods.

Processing of the Birds

Feed was withdrawn 8 h before processing, and the birds were given free access to water. All the birds were weighed and processed in the commercial inline system at the University of Arkansas Processing Pilot Plant on the same day. At the plant, the birds were hung on a shackle line, electrically stunned (11 V, 11 mA, 11s), manually severed the left carotid artery and jugular vein, bled out (1.5 min), soft scalded (53.8°C, 2 min), and picked using inline commercial defeathering equipment. The carcasses were then eviscerated, rinsed, prechilled at 12°C for 15 min, and chilled for 45 min at 1°C in immersion chilling tanks. Each carcass was weighed separately before deboning, and the ready-to-cook (**RTC**) weight was obtained. After deboning, weight of the breast fillets, wings, tenders, legs, and racks were obtained for each carcass. The yield of carcass parts were calculated and reported both as the percentage of live and RTC weights. All the fillets (pectoralis major) were scored to estimate the frequency of 3 degrees [normal (**NORM**), moderate (**MOD**), and severe (**SEV**)] of white striping as described by Kuttappan et al. (2012b).

Statistical Analysis

Live weight, RTC weight, weight of the carcass parts, and the yield (both based on live weight and RTC weight) data were analyzed using GLM and least squares means were calculated and separated with Tukey's honestly significant difference test at $P < 0.05$ (JMP version 9.0, SAS Institute Inc., Cary, NC). The parameters associated with the occurrence of 3 degrees of white striping were determined using multinomial stepwise logistic regression (PASW Statistics 18, SPSS Inc., Chicago, IL). During the above analysis, the MOD and SEV degrees of white striping were considered as the dependent variables with reference to the NORM category. The independent variables were the diet treatments, mean-centered (to avoid multicollinearity) covariates (live weight, RTC weight, weight of the carcass parts and the yields, both based on live weight and RTC weight), and their 2- and 3-way interactions. The results from the analysis were reported as the in-

Table 1. Composition (% as fed) and the calculated nutrient content of basal diets

Item	Starter (1 to 18 d)	Grower (19 to 32 d)	Finisher (33 to 49 d)
Ingredient			
Yellow corn	57.822	62.528	68.0500
Poultry oil	1.553	2.297	2.622
Meat and bone meal	4.00	3.00	2.500
Soybean meal	34.84	30.265	24.923
Limestone	0.421	0.500	0.577
Dicalcium phosphate	0.238	0.281	0.230
Sodium chloride	0.373	0.407	0.430
MHA-84[1]	0.369	0.332	0.287
L-Threonine	0.014	0.014	0.017
L-Lysine HCl	0.101	0.117	0.113
Vitamin premix[2]	0.050	0.040	0.032
Trace mineral mix[3]	0.100	0.100	0.100
Coban 90[4]	0.050	0.050	0.050
DSM[5] phytase	0.019	0.019	0.019
Selenium 0.06% premix	0.050	0.050	0.050
Total	100.000	100.000	100.000
Nutrient content			
CP, %	22.75	20.48	18.14
Calcium,[6] %	0.91	0.83	0.77
Total P, %	0.63	0.57	0.51
Available P,[6] %	0.46	0.41	0.37
Methionine, %	0.64	0.58	0.52
Lysine, %	1.32	1.19	1.03
Tryptophan, %	0.27	0.24	0.21
Threonine, %	0.89	0.80	0.71
Arginine, %	1.53	1.36	1.17
Sodium, %	0.20	0.21	0.21
TSAA, %	1.00	0.91	0.82
ME, kcal/kg	3,027.20	3,106.40	3,170.20

[1]Methionine hydroxyl analog calcium salt (Novus International, St. Louis, MO).

[2]See Table 2 for details.

[3]Provides per kilogram of diet: Mn (from $MnSO_4{\cdot}H_2O$), 100 mg; Zn (from $ZnSO_4{\cdot}7H_2O$), 100 mg; Fe (from $FeSO_4{\cdot}7H_2O$), 50 mg; Cu (from $CuSO_4{\cdot}5H_2O$), 10 mg; I [from $Ca(IO_3)2{\cdot}H_2O$], 1.0 mg.

[4]Elanco Animal Health division of Eli Lilly & Co., Indianapolis, IN.

[5]DSM Nutritional Products, Parsippany, NJ.

[6]Assumes 0.10% equivalency from phytase addition.

dependent variable(s) included in the model that has/have a significant ($P < 0.05$) effect on the occurrence of white striping. Odds ratio, 95% CI, and the respective P-values for the independent variable(s) included in the model were obtained. Additionally, to evaluate the pattern of occurrence of 3 degrees of white striping with respect to the factors included in the final model, the data were grouped into equal-sized bins using interactive binning procedure with cut off set at 10th percentile (JMP 9.0, SAS Institute Inc.). The mean estimated probabilities were then calculated and plotted to the respective bins.

RESULTS AND DISCUSSION

Weights and yields of broilers fed varying levels of vitamin E are shown in Table 3. In the present study, there were no significant differences ($P > 0.05$) in any of these weights or yield parameters with respect to the level of dietary vitamin E. This finding was in

Table 2. Composition of vitamin premix (units/kg of feed)

Item	Starter (1 to 18 d)	Grower (19 to 32 d)	Finisher (33 to 49 d)
Vitamin A, IU	9,300	7,440	5,952
Vitamin D, IU	3,110	2,488	1,990
Vitamin K, mg	1.9	1.5	1.2
Niacin, mg	48.2	38.6	30.8
Pantothenic, mg	13.4	10.7	8.6
Riboflavin, mg	8.7	7.0	5.6
Thiamine, mg	2.1	1.7	1.3
Pyridoxine, mg	2.9	2.3	1.9
Folic, mg	0.955	0.764	0.611
Biotin, mg	0.101	0.081	0.065
Vitamin B_{12}, mg	0.0145	0.012	0.009

agreement with previous studies conducted where BW were not affected by feeding different levels of dietary α-tocopherol (Sheehy et al., 1991; Bartov and Frigg, 1992). Meanwhile, Chae et al. (2006) reported that 100 and 200 mg of α-tocopherol acetate/kg of feed resulted in significant improvement in weight gain compared with the negative control group. However, they did not observe any significant differences in the dressing and the breast meat percentages. Gao et al. (2010) reported that higher levels of α-tocopherol acetate (200 IU/kg of feed) could alleviate the oxidative stress produced by dexamethasone injection and can improve the growth performance in broiler chickens. Rice and Kennedy (1988) suggested that the advantage of higher levels of dietary vitamin E will be witnessed mainly when the group is exposed to some kind of stressor. The absence of differences in the growth performance among the 5 dietary treatments in this study indicates that all the birds might have been exposed to stress below the threshold level to get a beneficiary effect from higher levels of dietary vitamin E on growth performance and yield.

The distributions of 3 degrees of white striping in the diet treatment groups are shown in Table 4. Some variation was seen in the frequency, but no consistent trend was observed. The majority (66 to 86%) of birds in every treatment exhibited MOD striping. Furthermore, there was no evidence to indicate that higher levels of dietary vitamin E would decrease the incidence of white striping. This was supported by the findings from multinomial logistic regression analysis. The results from the logistic regression revealed that among the different variables (diet treatments, live weight, RTC weight, weight of the carcass parts and the yield, and their 2- and 3-way interactions), only fillet weight was significantly ($P < 0.05$) associated with the occurrence of 3 degrees of white striping (Table 5). It was determined based on the OR of fillet weight in relation to the occurrence of 3 degrees of white striping. Odds ratio (**OR**) indicates the increased or decreased chance of occurrence of a dependent category (MOD and SEV degrees of white striping) as a result of a unit increase in the continuous variable (fillet weight in g). An OR > 1 indicates an increased chance, whereas an OR < 1 indicates lesser chance of occurrence. The results from the present study showed that both MOD and SEV had an OR greater than 1, which indicates that with reference to NORM there was a higher chance of occurrence of MOD ($P = 0.004$) and SEV ($P < 0.001$) fillets as the fillet weight increased. Therefore, MOD and SEV degrees of white striping are seen associated with heavier fillets. The finding was in accordance with results from previous studies (Bauermeister et al., 2009; Kuttappan et al., 2009).

The pattern of occurrence of 3 degrees of white striping with respect to the fillet weight is shown in Figure 1. The graph clearly indicates that fillet weight does have an effect. As the fillet weight increased, there was a reduction in the probability of occurrence of NORM fillets, whereas that of SEV fillets increased. The MOD fillets showed a peak where the lines of NORM and SEV crossed, and it decreased toward either side where there was an increased probability for either the NORM or the SEV fillets. However, the present study showed a higher percentage (85.45%) of fillets with white striping (either the MOD or SEV), whereas the percentage reported by Kuttappan et al. (2009) was 55.75%. Here, the chance for individual variations in scoring is less because the same people did the scoring for both of these studies. Interestingly, the birds used in the pres-

Table 3. Mean weights[1] and yields[1] of broilers fed various levels of vitamin E

Parameter	Level of vitamin E in diet (IU/kg) 15	50	100	200	400	Pooled SEM
Weight (g)						
Live	3,517	3,561	3,545	3,627	3,595	36.35
RTC[2]	2,782	2,863	2,827	2,866	2,851	33.98
Wings	286	287	289	291	298	3.37
Breast	659	679	688	691	696	13.01
Tenders	139	147	144	146	148	2.30
Legs	863	900	870	899	894	10.40
Rack	800	816	810	806	810	10.87
Yield (% of live weight)						
RTC	79.3	80.3	80.0	79.0	79.3	0.42
Wings	8.2	8.1	8.2	8.0	8.2	0.08
Breast	18.8	19.0	19.4	19.0	19.3	0.28
Tenders	4.0	4.1	4.1	4.0	4.1	0.06
Leg	24.7	25.2	24.6	24.7	24.6	0.24
Rack	22.8	22.9	22.9	22.3	22.4	0.22
Yield (% of RTC weight)						
Wings	10.3	10.1	10.3	10.2	10.3	0.10
Breast	23.6	23.7	24.2	24.1	24.2	0.32
Tenders	5.0	5.2	5.1	5.1	5.2	0.07
Legs	31.2	31.3	30.8	31.3	31.2	0.26
Rack	28.8	28.5	28.6	28.3	28.2	0.24

[1]No significant differences noted among treatments ($P > 0.05$).

[2]RTC = ready-to-cook carcass.

Table 4. Frequency of occurrence of 3 degrees of white striping in 5 diet treatments

Dietary vitamin E level	Degree of white striping[1] NORM		MOD		SEV	
	Count	%	Count	%	Count	%
15 IU/kg (n = 35)	8	22.86	27	77.14	0	0
50 IU/kg (n = 33)	7	21.21	22	66.67	4	12.12
100 IU/kg (n = 36)	3	8.33	32	88.89	1	2.78
200 IU/kg (n = 38)	6	15.79	29	76.32	3	7.89
400 IU/kg (n = 38)	2	5.26	33	86.84	3	7.89

[1]Breast fillets with normal or no striping (NORM), moderate (MOD), or severe (SEV) degree of white striping.

ent study had a higher mean RTC weight (2,856.26 g) at a younger age (49 d) than those used in the previous study (RTC weight 2,440.01 g; 59 to 63 d of age), which could have ensued in a higher incidence of white striping due to increased growth rate. In fact, Kuttappan et al. (2012a) reported that increased growth rate resulted in increased incidence of higher degrees of white striping. Further studies are warranted to determine the incidence of the condition in the poultry meat industry.

These results suggest that there could be differences in the etiology of NMD and the recently observed condition of white striping. Even though the main gross lesion associated with white striping seems to be similar to NMD, the pattern of the lesion may be different. White striping shows clear white lines on the surface of normal-colored fillets, whereas NMD lesions are seen as coalescing white areas and the fillets are pale in color. Another important aspect is that the poultry diet low in vitamin E may also be associated with other conditions such as encephalomalacia and exudative diathesis (Klasing, 2008). However, such pathological conditions have not been observed in flocks showing the occurrence of white striping. So far, occurrence of higher degrees of white striping can be seen even in birds fed with adequate amounts of dietary vitamin E. In addition, the occurrence of NORM, MOD, and SEV lesions of white striping can be seen in flocks grown on the same diet and the more severe cases are associated with heavier and older birds (Bauermeister et al., 2009; Kuttappan et al., 2009). These results imply that the occurrence of white striping may not be directly associated with a dietary deficiency of vitamin E or the associated nutrients. However, future studies should assess other dietary and management factors associated with flocks exhibiting white striping in breast fillets. Based on the studies conducted so far, white striping occurs due to muscle damage or myopathic changes mainly associated with higher growth rate in broiler birds (Kuttappan et al., 2009, 2012a). However, the lack of effect due to increased dietary vitamin E does not necessarily confirm that vitamin E is ineffective. There may be conditions that reduce or prevent the amount of active vitamin E reaching the breast muscle. Previous research has suggested that muscle fiber growth in rapidly growing birds may have outgrown their supporting systems (Wilson et al., 1990), especially decreased capillary density, which could result in reduced supply of nutrients (such as vitamin E) and oxygen as well as the slower removal of lactic acid from the muscles (Hoving-Bolink et al., 2000). This might have led to the growth-associated myopathy seen in white striping (Kuttappan et al., 2009, 2012a). Even though the increased dietary vitamin E can increase the level of vitamin E in muscle, it will mainly depend on vascularity to the muscle tissue (Sheldon, 1984; De Winne and Dirinck, 1996). Further studies are needed to evaluate the capillary density in relation to the amount of vitamin E in the breast muscles in fast-growing broilers. The results from the present study suggest that even if there is reduction in the vitamin E reaching the fast-growing muscles, we may not be able to compensate it with increasing dietary vitamin E. Furthermore, a detailed histological study on the tissue changes occurring in meat with white striping is needed to get valuable information about the etiology of the condition.

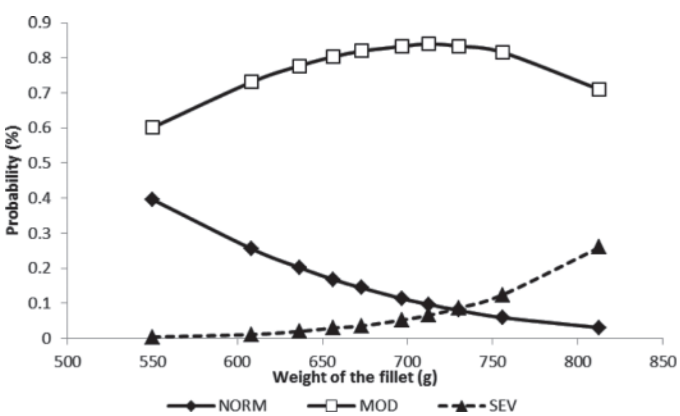

Figure 1. Probability of occurrence of white striping with respect to the weight of the butterfly breast fillets. NORM = normal fillets/no visual white striping; MOD = moderate degree of white striping; SEV = severe degree of white striping. Values were pooled across the diet treatments.

In conclusion, though white striping has some similarities to NMD caused by the deficiency of dietary vitamin E, the increments of dietary vitamin E (15, 50, 100, 200, and 400 IU/kg of fed) used in the study did not result in any significant association with respect to the occurrence of white striping in broiler breast fillets. This implies that, in contrast to NMD, an increased level of dietary vitamin E cannot prevent the occur-

Table 5. Odds ratio (OR)[1], 95% CI, and the probability level of the variable associated with the occurrence of moderate (MOD) and severe (SEV) degrees of white striping

	MOD			SEV		
Parameter	OR	95% CI	*P*-value	OR	95% CI	*P*-value
Fillet weight	1.011	1.003 to 1.018	0.004	1.026	1.013 to 1.039	<0.001

[1]Odds ratio determined with the NORM (no striping) category as the reference.

rence of white striping. Meanwhile, the occurrence of white striping is significantly related to the weight of the fillets compared with other carcass weight and yield parameters evaluated in the study.

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