Revista Brasileira de Zootecnia
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ISSN 1806-9290
www.sbz.org.br

R. Bras. Zootec., 45(3):113-120, 2016

Growth performance, intestinal morphology, and meat quality in relation to alpha-lipoic acid associated with vitamin C and E in broiler chickens under tropical conditions

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ABSTRACT - This study was conducted to examine the effect of alpha-lipoic acid with vitamin C and E on growth performance, intestinal morphology, and meat quality in broiler chickens under tropical conditions. A total of 288 one-day-old male ROSS 308 chicks (40±0.1 g) were used in a completely randomized design and allotted to one of six dietary treatments to form sixe replicates per treatment (eight birds per cage). The six dietary treatments were: a corn-soybean meal-based diet (NC; no antimicrobial compounds added) with 8 ppm alpha-lipoic acid (ALA); 150 ppm vitamin C and 75 ppm vitamin E (E-75); E-75 plus ALA (E-75-ALA); 150 ppm vitamin C and 50 ppm vitamin E (E-50) plus ALA (E-50-ALA); and 150 ppm vitamin C and 25 ppm vitamin E (E-25) plus ALA (E-25-ALA). All dietary treatments were continuously provided in liquid form, dissolved in water. Birds were housed in a battery cage (n = 36), and were offered dietary treatments on an *ad libitum* basis. The ambient temperature was maintained at 32±1 ºC for the first three weeks and reduced gradually to 28 ºC by the end of the experiment (day 35) to induce moderate tropical condition. One bird per pen (n = 6), and another bird per pen (n = 6) were euthanized via cervical dislocation to obtain terminal ileum to measure villus height and crypt depth at day 21, and to harvest breast meat and drumsticks to evaluate meat quality traits at day 35, respectively. Dietary treatment E-75-ALA improved body weight and average daily gain compared with birds fed other dietary treatments from day 1 to day 35. Birds fed dietary treatment E-75-ALA and E-50-ALA had higher villus height than those fed the other dietary treatments at day 21. Dietary treatments E-75-ALA and E-50-ALA reduced thiobarbituric acid reactive substance (TBARS) in drumsticks compared with other dietary treatments, but only treatment E-75-ALA decreased TBARS in breast meat at day 35. Liquid form of antioxidant compounds such as E-75-ALA can improve growth performance, histology of terminal ileum, and meat quality traits in broiler chickens under moderate tropical condition for 35 days.

Key Words: broilers, heat stress, vitamin E, tropical condition

Introduction

Heat stress is known to be one of the most detrimental factors in overall poultry production (Lara and Rostagno, 2013). Although many studies have clearly elucidated the thermoneutral zone of broilers (18-22 ºC; Charles, 2002), it is not easy to maintain that temperature in a specific area or a period or the combination of both. Recent studies have demonstrated that heat stress has affected broiler productivity by reducing feed intake and efficiency, body weight, meat quality, and survivability (Sohail et al., 2012; Lu et al., 2007). Also, it has been shown that heat stress had immunosuppressant effects on birds and resulted in decreasing weights of lymphoid organs, total circulating antibodies, and phagocytic ability of macrophages (Quinteiro-Filho et al., 2012). Basically, birds attempt to maintain their thermal homeostasis from heat stress. During this process, the level of reactive oxygen species (ROS) is increased, the body enters a stage of oxidative stress, and heat shock proteins are released in response to stress, providing protection from ROS effects (Droge, 2002). Therefore, it can be assumed that antioxidant supplementation to the diet could alleviate impaired productivity of broilers in response to heat stress.

Recently, alpha-lipoic acid (ALA) has been used as a potent antioxidant agent, ranging from therapeutic application to dietary supplementation, widely distributed in foods and readily absorbed from the diet (Packer et al., 1995). Reed (1957) demonstrated the basic functions of ALA, including its antioxidant properties for the first time in leaves. Later, Packer et al. (1995) reported that ALA acted on dehydrogenase complexes as a co-factor. Also, ALA and its reduced form, dihydrolipoic acid (DHLA), had a function

Received August 24, 2015 and accepted December 3, 2015.
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http://dx.doi.org/10.1590/S1806-92902016000300005

as antioxidants in free-radical quenching, metal chelation, antioxidant recycling, and gene expressing (Packer et al., 1995). Likewise, ALA had the scavenging ability directly against the hydroxyl radical, the hypochlorous radical, and singlet oxygen, but not against the superoxide radical and the peroxyl radical (Suzuki et al., 1991; Scott et al., 1994; Kagan et al., 1992; Stevens et al., 1974). However, DHLA, known to rapidly convert into ALA, is capable of quenching the oxidants that cannot be scavenged by ALA (Kagan et al., 1992). In addition, ALA has shown antioxidant activity by chelating iron, copper, and other transition metals (Packer et al., 1995). Particularly, DHLA interacted with other antioxidants by regenerating cellular antioxidants, such as vitamin E, vitamin C, and glutathione from their radicals or inactive form (Biewenga et al., 1997). Consequently, it appears that DHLA is involved in indirect antioxidative activity.

Numerous studies have been conducted to evaluate the antioxidative effects of ALA on livestock. Bai et al. (2012) determined that ALA supplementation to the diet of sows during late-gestation and lactation improved the activity of antioxidant enzymes in the serum and improved the performance of sows and their nursing piglets. In broiler chickens, dietary ALA inhibited body fat deposition, decreased muscle glycolysis at early postmortem, and improved the water holding capacity, indicating that ALA supplementation could prevent the occurrence of PSE (pale, soft, exudative) meat (El-Senousey et al., 2013). Lipid peroxidation level, superoxide dismutase, and catalase enzyme activities and glutathione amounts in Japanese quail under heat stress conditions were ameliorated with the addition of ALA to the diet (Halıcı et al., 2012). However, few results have been reported regarding the synergic antioxidative effects of dietary ALA together with vitamins C and E in broilers (Sahin et al., 2003).

The objective of this study was, therefore, to evaluate the effects of antioxidants (ALA, vit. E, and vit. C) on growth performance, ileal morphology, and meat quality in broilers under chronic heat stress conditions. This study was also conducted with the purpose of elucidating the optimum levels of supplemented antioxidants in liquid form to the water supplied to broilers reared at a high ambient temperature.

Material and Methods

The practices and procedures for this experiment were reviewed and approved by the Chungnam National University Animal Ethics Committee (CNU-00521).

A 5-week experiment was conducted with 288 one-day-old male ROSS 308 broilers (40.0±0.1 g). Broilers were individually weighed and randomly assigned to one of six dietary treatments; each treatment had six replicates with eight birds per pen. All birds were raised in stainless steel battery cages of identical size (86 cm width × 57 cm length × 35 cm height) and provided with continuous lighting (24 h). The initial ambient temperature of the room was maintained at 32±1 °C for the first three weeks and decreased gradually to 28 °C, and 60 to 65% humidity was maintained using a room temperature control system by the end of the experiment (day 35). The basal corn-soybean meal diets were formulated to meet or slightly exceed the nutritional requirements of broilers during the starter (days 1-25) and grower (days 26-35) phases, according to NRC (1994) recommendations for broiler chickens (Table 1). The broilers were allowed *ad libitum* access to feed and fresh water. The six experimental treatments (i.e., liquid form) were given to broilers during the entire experimental period as follows: a corn-soybean meal-based diet (NC; no antimicrobial compounds added) with 8 ppm ALA; 150 ppm vitamin C and 75 ppm vitamin E (E-75); E-75 plus 8 ppm ALA (E-75-ALA); 150 ppm vitamin C and 50 ppm vitamin E (E-50) plus 8 ppm ALA (E-50-ALA); and 150 ppm vitamin C and 25 ppm vitamin E (E-25) plus 8 ppm ALA (E-25-ALA). Birds were monitored for mortality two times a day throughout the experiment.

Feed intake (FI) and body weight (BW) were recorded weekly per cage, and feed conversion ratio (FCR) was

Table 1 - Composition of the experimental diets

Item	Diet	
	1-3 weeks	3-5 weeks
Ingredient, %		
Corn	29.64	32.26
Wheat	30.00	30.00
Soybean meal	28.67	26.71
Canola meal	4.00	4.00
Vegetable oil	4.05	4.30
Salt	0.26	0.29
Limestone	1.02	1.51
Choline	0.12	0.10
Dicalcium phosphate	1.04	0.88
Vitamin/Mineral premix[1]	0.26	0.26
L-lysine	0.66	0.38
DL-methionine	0.29	0.30
Calculated analysis		
Crude protein, %	20	19
Metabolizable energy, kcal	3200	3200
Lysine, %	1.17	1.07
Methionine, %	0.56	0.53

[1] Provided the following nutrients (per kg of air-dry diet): vit. A - 12,000 IU; vit. D - 33,000 IU; vit. E - 15 mg; vit. K - 2 mg; thiamine - 2 mg; riboflavin - 6 mg; pyridoxine - 2 mg; calcium pantothenate - 0.03 mg; folic acid - 0.2 mg; niacin - 45 mg; biotin - 0.15 µg; calcium - 0.5%; Co - 0.5 mg (as cobalt sulfate); Cu - 10 mg (as copper sulfate); iodine - 0.9 mg (as potassium iodine); iron - 80 mg (as ferrous sulfate); Mn - 80 mg (as manganous oxide); Se - 0.2 mg (as sodium selenite); Zn - 80 mg (as zinc oxide).

calculated as FI divided by body weight gain (BWG) every week, for a 5-week period. At day 21, one bird per pen was selected to obtain ileum tissue to measure villus height and crypt depth. The birds were euthanized via cervical dislocation after a 12 h fast. Fragments of approximately 5 cm in length were obtained from the ileum, between Meckel's diverticulum and the anterior portion of the ileocecal junction. The excised fragments were immersed in a phosphate-buffered formalin solution. Two portions per sample were cut perpendicular to the longitudinal axis of the intestine and embedded in paraffin wax. Transverse sections were cut (3~5 μm). In the morphometric study, images were captured using a light microscope and a system that analyzes computerized images (Bio-Rad Microscience, UK). The height of 10 well oriented villi and their associated crypts were measured from each replicate per segment. The mean was obtained from these values. At the end of the experiment, one bird per pen was selected randomly. All birds fasted for 12 h were euthanized by exsanguination for carcass data. The carcasses were eviscerated manually and then immediately stored at −20 °C for subsequent analysis.

After the carcasses were thawed at room temperature, each breast and leg meat sample was obtained from each carcass, and then meat quality associated with antioxidative activity was analyzed.

The pH values of leg meat and breast meat were assessed. Briefly, 10 g of sample were homogenized for 30 s using a stomacher (400 Lab blender, Seward, England) in 100 mL distilled water; afterwards, the pH of the sample was measured using a pH meter (WTW pH 720, Germany).

To determine the cooking loss, each sample was cut into 2.5 cm thick slices that were then packaged in polyethylene bags and placed in an 80 °C water bath for 30 min. The samples were then removed and cooled at room temperature for 30 min, after which the cooking loss values were calculated based on the difference in the weight of the meat before and after cooking (Barbanti and Pasquini, 2005).

The thiobarbituric acid (TBA) values were measured according to the modified extraction method described by Witte et al. (1970). Briefly, 10 g of each sample, 15 mL of cold 10% perchloric acid, and 25 mL distilled water were added to this sample. After homogenizing the mixture at 10,000 rpm for 10 s in a homogenizer (AM-Series, Kaisha Ltd., Japan), the homogenate was filtered using qualitative filter paper no. 2. After adding and completely mixing 5 mL of the filtrate solution and 5 mL of 0.02 M TBA solution, the solution was allowed to stand for 16 h in a cool and dark place. The absorbance was measured at 529 nm suing a spectrophotometer (DU-650, Beckman, USA). 1,1,3,3,-Tetra 1,1,3,3,-Tetraethoxypropane (Sigma-Aldrich, St. Louis, MO, USA) was used as standard for TBA assay. Thiobarbituric acid values were expressed as milligrams of malonaldehyde (MA) per kilogram of sample (mg MA/kg), and the standard curve equation used was $y = 0.1975x + 0.0011$ ($r = 0.999$), in which y = absorbance for a given x, the TBA value.

The 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity of leg and breast meats was determined according to the modified method described by Blois (1958). Briefly, 1 mL of lemon balm ethanol extract and 1 mL of 0.2 mM DPPH were added to a test tube and mixed for 30 min at 37 °C, and the absorbance of the mixture was measured at 517 nm using a UV-spectrophotometer (Shimadzu UV-1601PC, Japan). At the same time, antioxidant activity was measured by the same method using ascorbic acid (Sigma-Aldrich, St. Louis, MO, USA), which is a natural antioxidant, as well as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), which are synthetic antioxidants, as a positive control group. The DPPH radical scavenging activity of each sample was calculated using the following formula: (absorbance of sample addition group/absorbance of the control group) × 100. Ascorbic acid, BHA, and BHT were used at 0.5 mg/mL. Sample concentration providing 50% inhibition (IC50) was calculated from the graph of inhibition percentage against sample concentration.

Data were analyzed univariately in a normal-linear model using the GLM procedure of SPSS (version 21.0, SPSS Inc., Chicago, Illinois, USA). The pen was the experimental unit for the growth performance (i.e., average daily gain [ADG], average daily feed intake [ADFI], and feed conversion ratio [FCR]). Initial BW was included in the model as a covariate for analyses of growth performance data. The individual chicken was considered the experimental unit for intestinal morphology and meat quality traits. Statistical significance was accepted at $P<0.05$. Pair-wise comparisons between means were made when appropriate using Fisher's protected LSD analysis when a significant treatment effect was observed.

Results

The results indicated the effects of antioxidant supplementation on broiler performance under moderate heat stress (Table 2). No significant difference appeared ($P>0.05$) in ADFI, FCR, or mortality among dietary treatments. However, birds fed E-75-ALA had higher ($P<0.05$) ADG than birds fed NC for 35 days (Table 2).

Birds fed E-75-ALA and E-50-ALA had improved ($P<0.05$) villus height on day 21. Nonetheless, birds fed

E-75-ALA had lower (P<0.05) crypt depth compared with negative control. Furthermore, birds fed E-75-ALA had higher (P<0.05) villus height:crypt depth ratio compared with NC, PC, and E-75 (Table 3).

No difference was found (P>0.05) in the pH values of leg or breast meat among the dietary treatments (Table 4). Birds fed E-75-ALA had higher (P<0.05) breast and leg meat cooking loss compared with NC (Table 4). Birds fed ALA combination had higher (P<0.05) DPPH radical scavenging activity of leg and breast meat among the dietary treatments. Similarly, birds fed E-75-ALA had lower (P<0.05) thiobarbituric acid reactive substances (TBARS) value in leg and breast meat compared with the negative control.

Table 2 - Effect of dietary antioxidant on growth performance and mortality rate of broiler chickens from 1 to 35 days of age

Item	NC	PC	E-75	E-75-ALA	E-50-ALA	E-25-ALA	SEM[1]	P-value
BW, g/bird								
Days 1-7	182.2	185.7	188.0	187.7	185.4	185.6	0.84	0.619
Days 8-14	481.4	482.9	486.2	498.7	494.4	490.9	3.94	0.369
Days 15-21	898.8	912.7	928.5	929.5	921.0	916.2	7.34	0.824
Days 22-28	1485.6	1528.7	1553.2	1595.6	1564.3	1553.4	10.52	0.083
Days 29-35	1912.7a	1964.6ab	1998.8ab	2038.2b	1994.6ab	1983.5ab	10.36	0.009
Days 1-35	992.1a	1014.9ab	1031.0ab	1050.0b	1031.9ab	1025.9ab	3.95	0.007
ADG, g/bird								
Days 1-7	20.3	20.8	21.1	21.1	20.8	20.8	0.11	0.291
Days 8-14	42.7	42.5	42.6	44.4	44.1	43.6	0.57	0.892
Days 15-21	59.6	61.4	63.2	61.5	60.9	60.8	1.34	0.989
Days 22-28	83.8	88.0	89.2	95.2	91.9	91.0	1.44	0.325
Days 29-35	61.0	62.3	63.7	63.2	61.5	61.4	2.11	0.999
Days 1-35	53.5a	55.0ab	56.0b	57.1b	55.8ab	55.5ab	0.34	0.051
ADFI, g/bird								
Days 1-7	22.2a	22.3a	23.7ab	23.8ab	24.3b	24.2b	0.20	0.178
Days 8-14	67.1	63.6	67.0	66.7	70.5	67.7	1.50	0.744
Days 15-21	82.0	77.1	78.3	79.8	72.4	79.8	1.48	0.562
Days 22-28	131.8ab	127.9ab	140.6b	128.4ab	120.4a	126.8ab	2.06	0.106
Days 29-35	110.8	111.6	110.7	117.5	109.1	110.9	2.58	0.975
Days 1-35	82.8	80.5	84.1	83.3	79.3	81.9	0.80	0.683
Feed conversion, g/g								
Days 1-7	1.10	1.07	1.12	1.13	1.17	1.16	0.01	0.255
Days 8-14	1.59	1.50	1.58	1.51	1.61	1.56	0.03	0.925
Days 15-21	1.39	1.26	1.26	1.31	1.20	1.37	0.04	0.705
Days 22-28	1.59b	1.46ab	1.59b	1.35a	1.32a	1.40ab	0.03	0.039
Days 29-35	1.93	1.97	1.74	1.95	1.92	1.88	0.10	0.993
Days 1-35	1.52	1.45	1.46	1.45	1.44	1.48	0.02	0.952
Mortality, n of birds								
Days 1-7	0.0	0.0	0.0	0.0	0.0	0.0	0.02	1.000
Days 8-14	0.2	0.2	0.2	0.0	0.0	0.0	0.00	0.709
Days 15-21	0.2	0.0	0.5	0.3	0.2	0.0	0.01	0.360
Days 22-28	0.0	0.0	0.0	0.2	0.0	0.0	0.03	0.573
Days 29-35	0.0	0.0	0.0	0.0	0.0	0.0	0.02	1.000
Days 1-35	0.1	0.0	0.1	0.1	0.0	0.0	0.00	0.544

BW - body weight; ADG - average daily gain; ADFI - average daily feed intake.
NC - negative control; PC - positive control (8 ppm alpha-lipoic acid); E-75 - 150 ppm vitamin C and 75 ppm vitamin E; E-75-ALA - E-75 plus ALA; E-50-ALA - 150 ppm vitamin C and 50 ppm vitamin E (E-50) plus ALA; E-25-ALA - 150 ppm vitamin C and 25 ppm vitamin E (E-25) plus ALA (E-25-ALA).
[1] Pooled standard error of the mean.
ab - means in the same row with different letters differ (P<0.05).

Table 3 - Effect of dietary antioxidant on villus height and crypt depth of broiler chickens on day 21

Item	NC	PC	E-75	E-75-ALA	E-50-ALA	E-25-ALA	SEM[1]	P-value
Villus height, µm	439.9a	463.6ab	475.0ab	517.1b	517.6b	478.8ab	5.17	0.002
Crypt depth, µm	140.2a	128.9ab	118.2abc	99.8c	110.2bc	113.0bc	2.10	0.001
Villus height:Crypt depth ratio	3.17d	3.63cd	4.03bcd	5.28a	4.78ab	4.30abc	0.087	0.001

NC - negative control; PC - positive control (8 ppm alpha-lipoic acid); E-75 - 150 ppm vitamin C and 75 ppm vitamin E; E-75-ALA - E-75 plus ALA; E-50-ALA - 150 ppm vitamin C and 50 ppm vitamin E (E-50) plus ALA; E-25-ALA - 150 ppm vitamin C and 25 ppm vitamin E (E-25) plus ALA (E-25-ALA).
[1] Pooled standard error of the mean.
abc - means in the same row with different letters differ (P<0.05).

Table 4 - Effect of dietary antioxidant on meat quality traits of broiler chickens on day 35

Item	NC	PC	E-75	E-75-ALA	E-50-ALA	E-25-ALA	SEM[1]	P-value
L-pH	6.61	6.60	6.59	6.53	6.66	6.57	0.132	0.231
B-pH	6.24	6.24	6.24	6.13	6.25	6.26	0.021	0.780
L-CL (%)	24.19a	25.30a	26.96ab	29.81b	27.38ab	27.48ab	0.50	0.014
B-CL (%)	21.93ab	20.34a	23.04bc	25.35c	23.69bc	23.02bc	0.41	0.001
L-DPPH (%)	57.01a	58.16a	57.28a	64.41b	65.26b	69.55c	0.91	0.001
B-DPPH (%)	63.43a	63.59a	64.83a	69.22b	69.04b	70.71b	0.68	0.001
L-TBARS (mg MA/kg)	0.60b	0.57ab	0.54ab	0.49a	0.49a	0.50ab	0.009	0.011
B-TBARS (mg MA/kg)	0.41c	0.38bc	0.34abc	0.29a	0.31ab	0.34abc	0.007	0.001

L - leg meat; B - breast meat; CL - cooking loss; DPPH - DPPH free radical scavenging activity; MA - malonaldehyde.
NC - negative control; PC - positive control (8 ppm alpha-lipoic acid); E-75 - 150 ppm vitamin C and 75 ppm vitamin E; E-75-ALA - E-75 plus ALA; E-50-ALA - 150 ppm vitamin C and 50 ppm vitamin E (E-50) plus ALA; E-25-ALA - 150 ppm vitamin C and 25 ppm vitamin E (E-25) plus ALA (E-25-ALA).
[1] Pooled standard error of the mean.
abc - means in the same row with different letters differ (P<0.05).

Discussion

In chicken rearing, heat stress has caused economic loss and concurrent welfare issues. The best way to avoid heat stress is maintaining the right ambient temperature, but it is costly to install a cooling system and keep that ambient temperature in cages, particularly in hot areas or in the summer season. Thus, many studies have focused on manipulating the diet to reduce the physiological and physical damages of broilers from heat stress. In the present study, antioxidants (vit. E, vit. C, and ALA) were added to drinking water, and the combination of antioxidants positively affected growth performance, intestinal morphology, and meat quality under moderate heat stress, suggesting that supplementation of dietary antioxidants could ameliorate the raising and productivity of broiler chickens. Sigel et al. (1995) reported that growth performance was decreased in birds when the ambient temperature rise was beyond the thermoneutral zone (i.e., over 32 ºC). In the present study, the birds fed a diet without any antioxidants showed decreased final BW and ADG compared with those fed a diet supplemented with vit. C, vit. E (75 ppm), and ALA (Table 2). This result was in agreement with previous reports that indicated decreased BW from heat stress, but that were compensated for by the addition of antioxidants such as ascorbic acid (Imik et al., 2012; Sahin et al., 2003). Our results suggested that antioxidants may enhance digestibility, resulting in increasing ADG and BW, although no significant difference was observed in ADFI (Table 2). Indeed, Wallis and Balnave (1984) reported that high environmental temperatures had detrimental effects on broilers by decreasing their digestibility of amino acids. Larbier et al. (1993) found that high temperature condition decreased the true digestibility of protein, which might deter the activity of protein digestive enzymes (trypsin, chymotrypsin, and amylase) under heat stress. These negative influences on digestibility were moderated through the supplementation of the antioxidant vitamins. Sahin and Kucuk (2001) demonstrated that the combination of 200 mg vit. C and 250 mg vit. E improved nutrient digestibility of dry matter, organic matter, crude protein, and ether extract in Japanese quails, which was also confirmed in broilers exposed to heat stress (Sahin et al., 2003). Nevertheless, we were not in the position to determine digestibility in the present study.

The present study could anticipate that supplementation of antioxidants increased the digestive capacity under heat stress, although there was no effect of antioxidants on feed intake (Tables 2 and 3). It is known that the gastrointestinal tract is sensitive to stressors (Suzuki, 1983; Burkholder et al., 2008). When broilers were exposed to feed withdrawal and heat stress, ileal morphology became aberrant, leading to increased attachment of *Salmonella Enteritidis* (Burkholder et al., 2008). Also, diminished feed intake due to heat stress affected the height of villi in broilers (Tarachai and Yamauchi, 2000). It is well known that the villus height: crypt depth ratio is the gut health index (Pluske, 1997). When the villus height:crypt depth ratio was low, the intestinal environment became more favorable to nutrition absorption than vice versa (Pluske, 1997). Caspary (1992) reported that increasing the villus height is considered a means to broaden the surface area, thereby improving the absorption of available nutrients. Thus, shortening villi can take a toll on the nutrient absorption by reducing the surface area. Proliferation of stem cells is present at the base of the crypt, which differentiates mostly from enterocytes. The enterocytes migrate up the villus and are extruded from the villus tip into the lumen (Imondi and Bird, 1966). In the course of the process, the enterocytes become mature and functional in terms of nutrient absorption and mucin secretion (Wright and Alison, 1984). In this regard, poor nutrient absorption may occur due to the decrease in villus height and increase in crypt depth. Similarly, Fan et al. (1997) reported that the villus height:crypt depth

ratio was closely associated with increased epithelial cell turnover. Also, Samanya and Yamauchi (2002) observed that longer villi were correlated with activated cell mitosis in chickens.

The morphometric analysis results in the present study showed that the supplementation of antioxidants increased villus height but decreased crypt depth in broilers reared under chronic heat stress. The birds supplemented with 150 ppm vit. C, 75 ppm or 50 ppm vit. E, and 8 ppm ALA had significantly higher villus height than negative control, whereas no significant difference occurred between positive and negative control groups (Table 3). Similar results were obtained for crypt depth and villus height: crypt depth ratio, except that supplementation of 150 ppm vit. C, 50 ppm vit. E, and 8 ppm ALA also provided a significantly higher villus height (Table 3). Based on these results, it seems that supplementation of 8 ppm ALA cannot ameliorate impaired intestinal morphology *per se*. In a sense, antioxidant activities of ALA can be expressed when it is supplemented together with vit. E and vit. C. According to Packer et al. (1995), it appears that ALA lacks biochemical capacity for scavenging the superoxide radical, hydrogen peroxide, and the peroxyl radical. Although ALA was ineffective against some oxidants, it has been widely known that ALA effectively interacts with other antioxidants by increasing the antioxidant activity of one another. For example, ALA and vit. C continuously recycled vit. E, which was predominantly used to protect membranes from lipid peroxidation as the major chain breaking antioxidant (Sies et al., 1994; Packer, 1992). Alpha-lipoic acid is also capable of recycling vit. E by regenerating vit. C (Biewenga et al., 1997). In addition, microsomal lipid peroxidation was inhibited by the reduced form of ALA, which is a dihydrolipoic acid in the presence of vit. E (Scholich et al., 1989). Alpha-lipoic acid inclusion *in vivo* increases the level of ubiquinol, which is the substance known to recycle vit. E under oxidative stress circumstances (Götz et al., 1994; Kagan et al., 1990). A study showed that 500 mg vit. E/kg did not have any effects on villus height, crypt depth, and villus height:crypt depth ratio (Murakami et al., 2007). Thus, vit. E may not act solely on the intestinal mucosa effectively. Turan and Mahmood (2007) suggested that the liberation of enterocytes from the villus tip cells due to apoptosis generated the large amount of free radicals in villus tip cells (Turan and Mahmood, 2007). In the present study, combination of antioxidants (vit. E, vit. C, and ALA) may effectively scavenge the generated free radicals caused by heat stress, consequently resulting in improved ileal morphology.

The detrimental influences in broilers by heat stress were found not only in their growth performance, but also meat quality. Heat stress induced alterations of muscle metabolism and membrane integrity, which could be associated with the meat quality (Lu et al., 2007; Zhang et al., 2012; Sanderock et al., 2001). The pH value of the meat is known to be an important physical factor in the postmortem stage. Because it indicates meat color, cooking loss, and meat sensory quality (Ahn and Maurer, 1990; Guignot et al., 1994). In this study, the pH value of both leg and breast meat was numerically lower in dietary vit. C, vit. E (75 ppm), and ALA than negative control (Table 4). We assume that antioxidants might have a positive effect on the pH value in breast and drumstick meat during storage. The 1,1-diphenyl-2-picrylhydrazyl free radical scavenging activity is a simple method to evaluate antioxidative activity (Yamaguchi, 1998; Arshad et al., 2011). In the present study, the diets including ALA showed significantly higher DPPH free radical scavenging activity than negative control (Table 4). These results suggest that breast and leg meat from the broilers that drank liquid antioxidants might have electron donors to neutralize free radicals, which is in agreement with results of a previous study (Jung et al., 2010). Many studies reported that chickens reared at a high temperatures had increased TBARS in plasma, organs, and carcass (Lin et al., 2000; Mahmoud and Edens, 2003; Gao et al., 2010). The supplementation of antioxidants prevented lipid oxidation in chicken meat that decreased TBARS value, which is a consequence of oxidant stress (Jung et al., 2010; Cortinas et al., 2005). The findings in the present study showed that both leg and breast meat samples from the broilers that drank the water supplemented with vit. C, vit. E (75 ppm), and ALA had lower TBARS values than the negative control group, suggesting that vit. E might improve lipid stability and reduce lipid oxidation in chicken meat (Cortinas et al., 2005). Gao et al. (2010) demonstrated that the high level of vit. E was effective for the performance of broilers by reducing oxidative stress.

Conclusions

Broilers that drink water supplemented with 150 ppm vitamin C, 75 ppm vitamin E, and 8 ppm alpha-lipoic acid reared under high ambient temperature have positive results in growth performance, ileal morphometry, and meat quality. Also, the optimal vitamin E level of 75 ppm when in combination with vitamin C at 150 ppm and alpha-lipoic acid at 8 ppm plays important roles in antioxidant activity, ameliorating heat stress. Birds under heat stress consume more water than food. Consequently, broiler chickens are expected to become less susceptible to heat stress by drinking water supplemented with antioxidants.

Acknowledgments

Financial support from Biogenoci Co. Ltd. is greatly appreciated. This paper was financially supported by the research fund of Chungnam National University. The authors thank Mr. Samiru Sudharaka Wickramasuriya and Mr. Cheol Woo Lee for the technical assistance.

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