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Lipid Stability and Antioxidant Profile of Microsomal Fraction of Broiler Meat Enriched with α-Lipoic Acid and α-Tocopherol Acetate

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ABSTRACT: The importance of the linkage between nutrition and health is a hot issue. Like other food-related sectors, the meat industry is undergoing foremost transformations, driven among other things by changes in consumer requirements. The present study was designed to evaluate the lipid stability and antioxidative potential of leg and breast microsomal fraction of broiler meat fed on ALA and ATA. For the first 3 weeks of growth, broilers were fed on feed supplemented with ATA (200 mg/kg of feed) and during the last 3 weeks broilers were fed on feed supplemented with ALA (25, 75, 150 mg/kg of feed) and a constant level of ATA (200 mg/kg of feed). The body weight of the carcass was measured after every week of growth until 6 weeks. Positive correlation between the antioxidant activity and the TPC was observed. Higher values of TBARS were detected in leg muscles than in breast muscles. HPLC data revealed ALA and ATA contents were higher in T_4 (leg, 5.55 ± 0.19 and 3.87 ± 0.15 μg/mg of protein; breast, 5.63 ± 0.20 and 2.03 ± 0.10 μg/mg of protein, respectively) and lowest in T_5 (ALA, leg, 1.40 ± 0.06 μg/mg of protein; breast, 1.54 ± 0.05 μg/mg of protein; ATA, leg, 1.25 ± 0.06 μg/mg of protein; breast, 0.63 ± 0.008 μg/mg of protein), in which the only oxidized oil was used. Oxidized oil in feed reduced weight gain and increased TBARS, whereas TPC, DPPH, ALA, and ATA values decreased in both leg and breast meat.

KEYWORDS: lipid stability, TBARS, α-lipoic acid, α-tocopherol acetate, antioxidant activity, broiler

INTRODUCTION

Poultry meat is particularly prone to oxidative deterioration due to its high concentration of polyunsaturated fatty acids.[1] It is generally accepted that lipid oxidation is the primary process by which quality loss of muscle occurs, apart from microbial spoilage.[2] Oxidation of lipid reactive oxygen species (ROS) generated in meat and organ tissues leads to serious health problems.[3] α-Lipoic acid (ALA) in energy metabolism is well-known as lipoamide, and it acts as cofactor in the dehydrogenase complexes that catalyze the oxidative decarboxylation of α-keto acids such as pyruvate and biologically functions in glucose metabolism to yield energy.[4] ALA has valuable potential in clinical interests as a cellular thiol-replenishing and redox-modulating agent.[5] ALA quenches the singlet oxygen and hydroxyl radicals and hypochlorous acid.[6] ALA has been reported to prevent lipid peroxidation in rats.[7]

Numerous classes of antioxidants are present in foods, and each antioxidant could exert a different defensive effect toward oxidation. Integrated antioxidant potential could be more important than the concentration of each single compound.[8] Free radical production and lipid peroxidation were significantly lowered in muscle microsomes from pigs fed an α-tocopherol-supplemented diet [9] and had a protective effect on lipid peroxidation in microsomes and mitochondrial membranes.[10,11] During refrigeration storage, the chop meat of pigs fed the α-tocopherol-supplemented diet was less prone to lipid oxidation.[12]

Zhang et al.[13] showed that dietary α-lipoic acid supplementation inhibits atherosclerotic lesion formation in rats, an inhibition that appears to be due to the antihypertriglyceridemic, antiobesity, and anti-inflammatory effects of α-lipoic acid. Experimentally, it was proved that the rich antioxidants-supplemented feed increased the antioxidant status of meat.[14]

α-Tocopherol acetate (ATA) has antioxidant benefits and is the most efficient chain-breaking, fat-soluble antioxidant in the tissues, and it is a concern that vitamin E supplements may have some adverse effects.[15,16] Unlike other fat-soluble vitamins, α-tocopherol is not accumulated to toxic levels. Rather, tissue levels are strongly regulated via increased hepatic metabolism and excretion, which could alter environmental toxins, metabolism of drugs, and other nutrients.[17] The present study was designed to determine the antioxidant profile, TBARS values, and deposition of α-lipoic acid and α-tocopherol acetate in the microsomal fraction of broiler meat.

MATERIALS AND METHODS

Reagents and Chemicals. α-Lipoic acid was purchased from Puritan's Pride USA, and α-tocopherol acetate was purchased from the Merck (Merck KGaA, Darmstadt, Germany). The reagents used for the present study, Folin−Ciocalteu (FC) reagent, gallic acid, hydrogen

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Table 1. Treatment Plan for Supplementation of Broiler Feed with α-Lipoic Acid and α-Tocopherol Acetate

Treatment no.	Treatment description
T_1	control
T_2	α-tocopherol acetate (200 mg) + α-lipoic acid (25 mg/kg feed)
T_3	α-tocopherol acetate (200 mg) + α-lipoic acid (75 mg/kg feed)
T_4	α-tocopherol acetate (200 mg) + α-lipoic acid (150 mg/kg feed)
T_5	oxidized oil
T_6	oxidized oil + α-tocopherol acetate (200 mg) + α-lipoic acid (150 mg/kg feed)

peroxide, ferrous sulfate, trichloroacetic acid, thiobarbituric acid, and hydrochloric acid, were purchased from Sigma-Aldrich, Tokyo, Japan.

Experimental Birds. One hundred and eighty broilers (1 day old), of Hubbard strain, were used for the present study. The birds in each experimental unit were kept in separate pens (4 ft × 3 ft × 1.5 ft), and the pens were disinfected by fumigation method. The temperature of the experimental room was maintained at 95 °F during the first week. It was then lowered by 5 °F until it reached 75 ± 2 °F. The birds were reared up to 6 weeks. For the first 3 weeks, the birds were fed feed supplemented with ATA (200 mg/kg of feed) and for last 3 weeks the feed was supplemented with ALA and ATA as indicated in Table 1. After 6 weeks, the slaughtering and sampling of red and white meat was done, and the meat was stored at −80 °C in a refrigerator (Sanyo, Japan).

Isolation of Microsomal Fraction of Meat. Six grams of meat sample was homogenized in potassium phosphate buffer, pH 7.4, by a homogenizer. A filtration process was performed to remove connective tissues from sample by using muslin cloth. The filtrate of each sample was centrifuged at 1000*g* for 10 min to remove the nuclear fraction. Supernatant was collected in a separate tube and then centrifuged to sediment mitochondria at 10000*g*. Supernatant (38 mL) was collected, 300 μL of 8 mM $CaCl_2$ was added to make a final concentration of 30 μL, and the mixture was centrifuged again at 10000*g*. The sediment (microsomes) was collected and stored for further analysis.

Total Phenolic Contents (TPC). The total phenolic contents were estimated by Folin−Ciocalteu method.[18] The microsomal fraction (100 μL) was mixed with 95% ethanol (500 μL), distilled water (2.5 mL), and 50% Folin−Ciocalteu reagent (250 μL). After 5 min, 5% Na_2CO_3 (500 μL) was added to the resultant mixture, which was vortexed and placed in the dark for 1 h. The absorbance of the sample was noted using a spectrophotometer (U-2001, model 121-0032, Hitachi, Tokyo, Japan) at 725 nm. Total phenolic contents were measured as gallic acid equivalent ranges from 0 to 450 μg/mL. Protein concentration of all the samples was equalized as 1 mg/mL by using bovine serum albumin as a standard for the calculation of TPC.

2,2′-Diphenyl-1-picrylhydrazyl (DPPH) Free Radical Scavenging Activity Assay. The antioxidant activity of the microsomal fractions was assessed by measuring their scavenging abilities to DPPH stable radicals. A sample of 125 μL was mixed with 0.0012 m DPPH solution followed by the addition of 95% MeOH up to a final volume of 4 mL. The absorbances of the resulting solution and the blank were recorded after 1 h at room temperature. The conversion the color of DPPH was read spectrophotometrically at 515 nm. Inhibition of free radicals by DPPH in percent (%) was calculated in according to the method of Bozin et al.:[19]

$$I\,(\%) = 100 \times (A_{\text{blank}} - A_{\text{sample}}/A_{\text{blank}})$$

Lipid Peroxidation Stability by Thiobarbituric Acid Reactive Substances (TBARS) Assay. The peroxidation stability of isolated membranes (microsomes) was determined by using the method described by Asghar et al.[12] The peroxidative reaction was initiated by adding 700 μL of $FeSO_4 \cdot 7H_2O$ (0.1 M) and 200 μL of H_2O_2 (0.1 M) in 7 mL of membrane suspension and placed in a water bath (37 °C). One milliliter of sample from the reaction flask was withdrawn at 30 min intervals for a period of 120 min and added to a volume of solution consisting of thiobarbituric acid (0.4%), trichloroacetic acid (10%), and hydrochloric acid (0.25 N). The mixture was heated in a boiling water bath for 15 min and then cooled. After centrifugation, the absorbance of the supernatant was determined at 532 nm, and the extent of membrane lipid peroxidation was calculated by using the following formula:

$$\text{nmol of malondialdehydes} = \frac{(\text{sample absorbance} - \text{blank}) \times \text{total sample vol}}{0.000156 \times 1000\ (\text{per mL})}$$

Estimation of α-Tocopherol Acetate in Leg and Breast Microsomes of Broiler. *Sample Preparation.* Sample was prepared according the method described by Asghar et al.[20] Microsomal sample (500 μL) was taken, and 1.5 mL of urea (6 M) was added to dissolve the meat tissue. For the stability of the microsomal membrane, 0.5 mL of ascorbic acid (5%) was added in the reaction mixture. Then 1 mL of urea (6 M) was also added. The tube was flushed with N_2, the screw caps were tightened, and the mixture was vortexed for 1−2 min to dissolve the sample. One milliliter of sodium dodecyl sulfate (SDS) 0.1 M solution was added and vortexed for 1 min to disintegrate the microsomal membrane. For the deproteination and freeing of ATA, 4 mL of ethyl alcohol containing 1% pyrogallol was incorporated in the mixture. After that, petroleum ether (10 mL) was added, and the mixture was centrifuged at 5000*g* for 5 min to facilitate the separation of phases. The solvent layer containing ATA was separated in the vial, and the pooled solvent was evaporated under nitrogen. α-Tocopherol acetate contents were dissolved in the mobile phase (100% methanol). The sample was filtered by using an anspec 0.45 μm microfilter, centrifuged at 5000*g* for 5 min so as to collect all of the filtrate, and then stored in the dark.

Mobile Phase Preparation. The mobile phase consists of methyl alcohol (HPLC grade); 100% methanol was prepared by filtering it through a typhlon filter assembly and then adjusted to HPLC.

Standard Preparation. The standard of ATA was prepared by using Sigma-Aldrich packed standard 1 mg/mL as stock solutions from which further dilutions were made in ranges of 10, 20, 50, and 100 μg/mL.

High-Performance Liquid Chromatography (HPLC) Isocratic system. α-Tocopherol acetate was separated and quantified using a Shimadzu (Japan) chromatographic system with 290 nm wavelength UV-Vis detector. The HPLC chromatograms were obtained by using a Shim-Pack CLC (C_{18}) column, 15 cm × 4.6 mm × 5 μm, System controller SCL-10 A, water pump LC-10 AT, and flow controller valve FCV-10 AL with a mobile phase of 100% methanol at a flow rate of 1 mL/min.

Estimation of α-Lipoic Acid in Leg and Breast Microsomes of Broiler. *Sample Preparation.* Sample was prepared according the method described by Satoh et al[21] with slight modifications. A 200 μL sample of microsomal fraction was taken from each treatment in the glass tubes, then homogenized with 2 mL of 20% metaphosphoric acid (w/v) on ice, and extracted with 3 mL of hexane containing 250 μL of isoproponal by vortexing for 30 min. The sample was centrifuged at 1500*g* and the upper hexane layer collected in a glass tube. This step was repeated twice and the hexane layer allowed to dry with N_2.

Table 2. Mean Values of Broiler Chick Body Weight Gain (Grams per Week)

week	T_1	T_2	T_3	T_4	T_5	T_6
1	146.36	150.40	153.80	145.03	144.76	146.80
2	445.13	366.16	369.86	355.06	342.73	347.56
3	678.46	689.40	670.83	695.76	676.40	669.76
4	1012.93	1006.37	1006.05	927.61	985.61	948.76
5	1383.36	1377.34	1392.49	1292.98	1329.26	1313.80
6	1830.60	1949.44	1912.00	1691.46	1782.19	1895.53
total means[a]	899.47 ab	923.18 a	917.50 a	906.87 ab	876.82 b	887.03 ab

[a] Means in the row having the same letter differ nonsignificantly.

Table 3. Total Phenolic Contents in Leg and Breast Microsomes of Broilers

	TPC on protein bases[a] (μg/mg of protein)	
treatment	leg microsomes	breast microsomes
T_1	168.5 ± 5.3 cd	158.2 ± 6.2 c
T_2	177.1 ± 4.8 bc	159.7 ± 5.6 bc
T_3	184.7 ± 5.7 b	169.0 ± 5.7 b
T_4	199.5 ± 5.8 a	186.5 ± 6.1 a
T_5	144.1 ± 4.6 e	132.5 ± 4.1 d
T_6	163.0 ± 6.8 d	160.4 ± 5.7 c

[a] Values are the mean ± SD of three independent determinations.

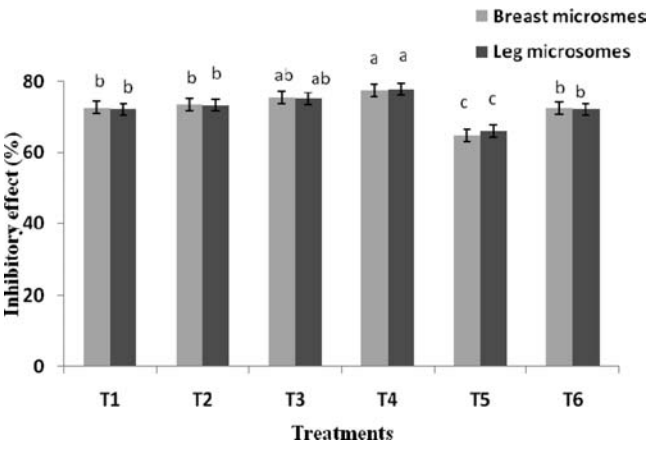

Figure 1. Free radical scavenging activity in broiler leg and breast microsomes.

Mobile Phase Preparation. The mobile phase was prepared by using acetonitrile and water (80:20(v/v)). The sample was eluted isocratically at a 1 mL/min flow rate.

Standard Preparation. The standards of ALA were prepared by dissolving ALA in *n*-hexane as a stock solution (25 mg/mL), and further dilutions were made (20, 30, and 40 μg/mL, respectively).

Conditions for High-Performance Liquid Chromatography (HPLC). α-Lipoic acid was separated by injecting 20 μL of sample and quantified by using a Shimadzu (Japan) chromatographic gradient system. The fluorescence detector (RF-10A×L) was operated at excitation and emission wavelengths of 343 and 423 nm, respectively, with a Shim-Pack CLC (C_{18}) column, 15 cm × 4.6 mm × 5 μm, system controller SCL-10A, water pump LC-10 AT, flow controller valve FCV-10AL, acetonitrile/water (80:20 v/v) mobile phase, and 1.0 mL/min flow rate.

Statistical Analysis. Growth data were subjected to two-way analysis of variance (ANOVA), whereas other parameters were statistically analyzed by one-way ANOVA using the least-squared difference test with the Statistic 8.1 program (Analytical Software).

RESULTS AND DISCUSSION

Broiler Growth. The result revealed a highly significant ($p < 0.01$) effect on weight gain of broilers among the treatments. Birds on T_2, containing a minimum level of ALA (25 mg), gained more weight (923.18 g), whereas birds on T_5, containing only oxidized oil, gained minimum weight (867.82 g) as compared to all other treatments, as shown in Table 2. It was clear that the treatment containing only oxidized oil had placed an adverse effect on the performance of broiler chicks. Similarly, significantly ($p < 0.05$) lower weight gain of broilers fed diets containing oxidized oil was observed, whereas antioxidant supplementation improved growth.[22] Similar findings were reported for ALA supplementation of a standard or high-fat diet fed to rats reflecting decreased body weight gain during 56 days.[23]

Total Phenolic Contents. Total phenolic contents showed their antioxidant activity in the subcellular membranes. The higher the TPC, the higher will be the free radical scavenging activity. The supplementation of feed with biological antioxidants (ALA and ATA) depicted the higher contents of phenols as the concentration of ALA increased with a constant level of ATA. As the concentration of ALA increased from 25 to 150 mg/kg feed in supplemented feed the TPC increased from 177.1 ± 4.8 to 199.5 ± 5.8 μg/mg of microsomal protein. T_4 showed the highest TPC as compared to control, which is evident from Table 3. α-Lipoic acid is water-soluble; therefore, the hydrophilic microsomes showed higher TPC.[18] Senevirathne et al.[18] reported that the hydrophilic fraction of *Ecklonia cavaon* had higher phenolic contents than the hydrophobic fractions. Total phenolic contents

Table 4. α-Tocopherol Acetate and α-Lipoic Acid Contents in Leg and Breast Microsomes of Broilers

	α-tocopherol acetate contents on protein bases[a] (μg/mg of protein)		α-lipoic acid contents on protein bases[a] (μg/mg of protein)	
treatment	leg microsomes	breast microsomes	leg microsomes	breast microsomes
T_1	1.69 ± 0.08 c	1.75 ± 0.06 c	1.53 ± 0.05 c	0.685 ± 0.01 c
T_2	3.59 ± 0.12 b	3.91 ± 0.14 b	1.68 ± 0.11 b	0.738 ± 0.03 b
T_3	5.07 ± 0.18 a	4.91 ± 0.18 a	1.79 ± 0.08 b	1.169 ± 0.05 b
T_4	5.55 ± 0.19 a	5.63 ± 0.20 a	3.87 ± 0.15 a	2.039 ± 0.10 a
T_5	1.40 ± 0.06 c	1.54 ± 0.05 c	1.25 ± 0.06 c	0.631 ± 0.008 c
T_6	3.18 ± 0.11 b	3.20 ± 0.13 b	2.31 ± 0.12 a	1.410 ± 0.04 a

[a] Values are the mean ± SD of three independent determinations.

were higher in treatment T_4, where the supplemented ALA was also high, and the TPC was in small quantity in treatment T_2, where the level of ALA was decreased. Therefore, the level of TPC increases in the increasing manner of ALA in feed. The TPC in the breast microsomes also exhibited the same pattern as in the leg microsomes. The treatment with oxidized oil (T_5), having the least amount of phenolic contents, and the treatment with a high level of antioxidant, T_4 (200 mg of ATA + 150 mg of ALA), had higher levels of phenolic contents (Table 3).

DPPH Free Radical Scavenging Activity. The DPPH radical is one of a few stable and commercially available organic nitrogen radicals bearing no similarity to the highly reactive and transient peroxyl radicals involved in various oxidative reactions in vivo.[24] DPPH free radical scavenging activity is one of those indicators that are important in determining the antioxidant potential of selected bioactive molecules. Figure 1 illustrates significant effects of treatments on the free radical scavenging activity of leg microsomes. The free radical scavenging activity of leg microsomes ranged from 66.03 ± 0.4 to 77.84 ± 1.5% inhibitions (Figure 1). Free radical scavenging activity is positively correlated with TPC: the higher the phenolic contents, the higher the percent inhibition in the leg microsomal fraction. The treatment with higher levels of ALA and ATA showed significant inhibition percentage as compared with the oxidized oil, which may be due to a high concentration of ROS compounds present in the sample. The DPPH assay as presented in meat displayed a significantly higher antioxidant activity (i.e., lower absorbance) in essential oil than did sage and control treatments.[25] Maximum percent inhibition in leg microsomes (77.84%) was recorded with T_4, whereas minimum inhibition (66.03%) was observed with T_5 (oxidized oil). The breast microsomes showed comparatively less percent inhibition than leg microsomes. The inhibition ranged from 64.86 to 77.50%. Breast microsomes had comparatively less TPC reported in our study, which strongly affect this activity. They have comparatively lower DPPH free radical scavenging activity than leg microsomes. The maximum percent inhibition (77.50%) was found in T_4, and minimum inhibition (64.86%) was shown in T_5 (Figure 1). DPPH free radical scavenging activity was calculated on the basis of microsomal protein.

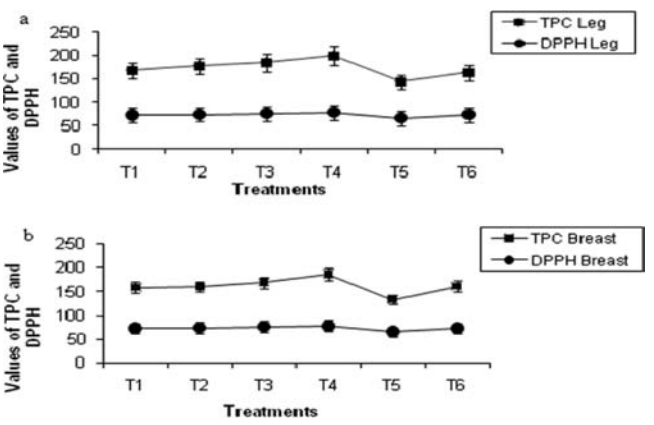

Figure 2. Comparison of antioxidant activities determined by TPC (μg/mg of protein) and DPPH (I%) in leg and breast microsomes.

Comparison of TPC and DPPH Free Radical Scavenging Activity. The comparison of TPC and DPPH free radical scavenging activity in breast microsomes is presented in Figure 2a. The same pattern exists between the TPC and the antioxidant activity DPPH. Both TPC and antioxidant activity were high in treatment T_4. Comparison of TPC and DPPH free radical scavenging activity in the leg microsomes is presented in Figure 2b. The same pattern exists between the TPC and DPPH. The correlation between the TPC and antioxidant activity was 0.9792 in leg microsomes and 0.9727 in breast microsomes.

Thiobarbituric Acid Assay. Lipid peroxidation is the measurement of MDA compound formed during autoxidation of lipids present in meat. Higher MDA compounds revealed higher amount of lipids, which deteriorate the meat. T_4 (200 mg ATA +150 mg ALA), which exhibited less MDA as compared with T_1 (control), and oxidized oil showed highest MDA formation in broiler leg microsomes. Treatments with supplemented antioxidants, ALA and ATA, depicted less peroxidation by producing lower amounts of MDA, which is evident from Figure 3. The trend is best explained by a polynomial curve. Our findings agreed with those of Lin et al.,[22] Asghar et al.,[26] Paniangvait et al.,[27] Nickander et al.,[28] and Morrissey et al.[29]

Breast microsomes also exhibited the same pattern as in the leg microsome shown in Figure 4. The highest amount of supplemented antioxidant showed higher lipid stability and hence proved that they kept the meat longer in better quality. Parazo et al.[30] and Ozkan et al.[7] reported that ALA is an antioxidant and that it prevents lipid peroxidation in rats.

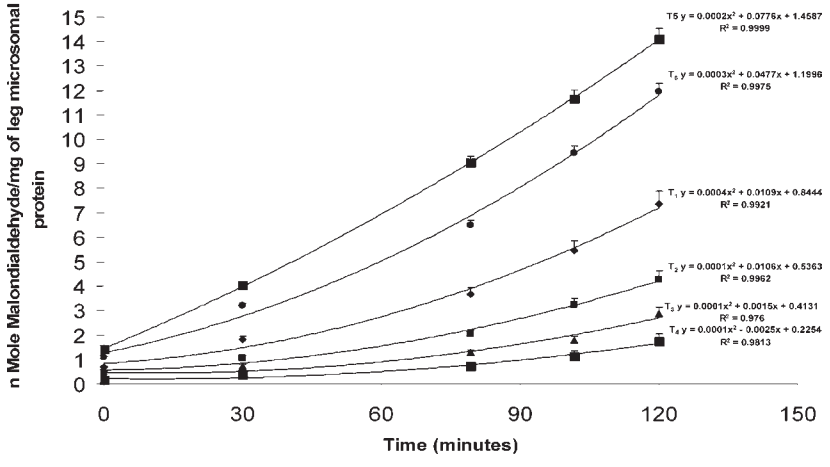

Figure 3. Ferrous sulfate/hydrogen peroxide-initiated autoxidation in leg microsomes of broilers.

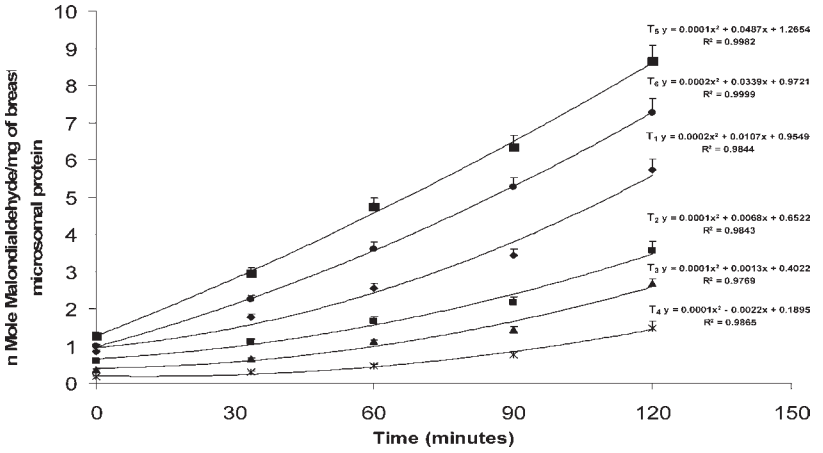

Figure 4. Ferrous sulfate/hydrogen peroxide-initiated autoxidation in breast microsomes of broiler.

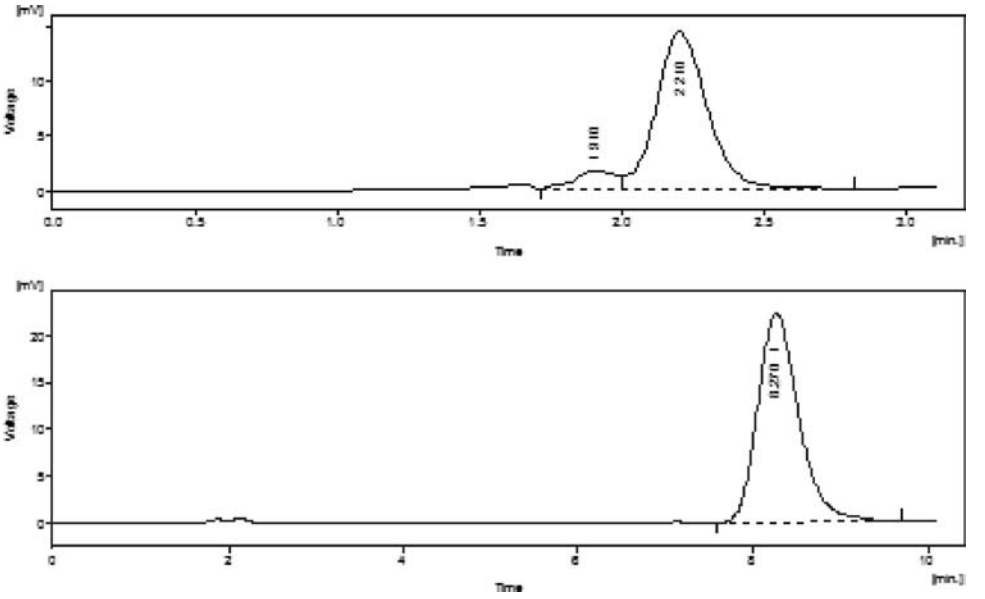

Figure 5. Chromatograms of α-tocopherol acetate and α-lipoic acid standards (10 μg/mL).

Oxidized oil showed the highest amount of MDA production in breast microsomes as compared with control. The MDA values found in leg meat were greater than those in breast meat, probably due to the greater fat content of the leg compared with the breast. Leg meat had a greater accumulation of linolenic acid compared with breast meat.[31]

The T_4 curve showed that the autoxidation process is slow, whereas in T_6 and T_5 the process is speedy as compared to T_4, T_3, and T_2. The trend of autoxidation is best explained by a polynomial curve. The calculation of malondialdehyde is based on protein present in the microsomes. The protein in microsomes was calculated by using the linear equation $Y = 0.0019X + 0.0035$ ($R^2 = 0.997$).

α-Tocopherol Acetate and α-Lipoic Acid Contents in Leg and Breast Microsomes. ATA is a biological antioxidant that protects membranes from oxidation. Supplementation of ATA in animal feed increases its deposition in tissues.[32] Asghar et al.[26] reported that free radical production and lipid peroxidation were significantly lowered in muscle and have protective effects on lipid peroxidation in the mitochondrial fraction of animals fed ATA-supplemented diet.[10,11] Figure 5 shows that the chromatographic conditions used in the present study allowed good separation of the individual component ATA. α-Tocopherol acetate content increased as the level of ALA increased in the feed of broilers, which is evident from Table 4. α-Tocopherol acetate contents also present in the control showed that it is also naturally present in the meat tissues. The ATA contents ranged from 1.40 ± 0.06 to 5.55 ± 0.19 μg/mg of microsomal protein in the leg microsomes, whereas in breast microsomes it ranged from 1.54 ± 0.05 to 5.63 ± 0.20 μg/mg of microsomal protein.

The ATA contents were highest in T_4 of both leg and breast microsomes, which have the highest amount of ALA with a constant level of ATA. Gradually the ALA concentration increases, and the ATA deposition also increases, which is evident from Table 4. Packer et al.[33] and Wen et al.[34] reported that ALA has a synergistic effect with α-tocopherol. α-Tocopherol acetate content in breast microsomes showed the same pattern as in the leg microsomes. The treatment with oxidized oil (T_5) reduced

the deposition of ATA (1.40 ± 0.06 μg/mg of microsomal protein) in leg microsomes and in breast microsomes (1.54 ± 0.05 μg/mg of microsomal protein) compared with all other treatments.

In treatments T_4 and T_6, the levels of ATA and ALA are the same but the deposition of ATA is small in T_6 due to the fact that treatment T_6 also contains oxidized oil, which inhibits the deposition of ATA.

α-Lipoic acid is an antioxidant present naturally in the tissues.[35] Our study showed that control (T_1) also had a small quantity of ALA. The ALA was deposited in the range from 1.25 ± 0.06 to 3.87 ± 0.15 μg/mg of microsomal protein in the leg microsomes, whereas in breast microsomes it ranged from 0.631 ± 0.008 to 2.039 ± 0.10 μg/mg of microsomal protein. The results showed that maximum ALA was deposited in T_4 (3.87 ± 0.15 μg/mg of microsomal protein in the leg microsomes and 2.039 ± 0.10 μg/mg of microsomal protein in the breast microsomes), as shown in Table 4. α-Lipoic acid and α-tocopherol acetate work synergistically with each other.[33,34] In treatments T_2, T_3, and T_4 the deposition rates of ALA increased, respectively, because the supplementation of ALA in the feed also increased, which is evident from Table 4. Treatment T_6, which contained the same quantity of ALA supplemented in feed as in treatment T_4, deposited a smaller quantity of ALA in T_6 than in T_4 because treatment T_6 contained the oxidized oil, which inhibited the deposition of ALA.

Hence, it is evident from Table 4 that as the concentration of ALA increased in the feed of animals, deposition in the biomembranes of the birds also increased. The chromatographic conditions used in the present study allowed good separation of the individual component ALA as shown in Figure 5. Our study was the first study in which ALA was supplemented in the feed of broilers.

α-Lipoic acid and α-tocopherol acetate significantly influenced the total phenolic contents and antioxidant activity of red and white meat microsomal fractions. It was concluded from the investigation that supplementation of poultry feed with α-lipoic acid and α-tocopherol acetate will extend the shelf stability of poultry meat due to their higher total phenolics and antioxidant status. Further studies are required for the development of antioxidants-enriched meat, which would benefit the meat industry.

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ABBREVIATIONS USED

MDA, malondialdehydes; ALA, α-lipoic acid; ATA, α-tocopherol acetate; TPC, total phenolic contents; TBARS, thiobarbituric acid reactive oxygen species; HPLC, high-performance liquid chromatography.

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