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Wheat Germ Oil and α-Lipoic Acid Predominantly Improve the Lipid Profile of Broiler Meat

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Supporting Information

ABSTRACT: In response to recent assertions that synthetic antioxidants may have the potential to cause toxic effects and to consumers' increased attention to consuming natural products, the poultry industry has been seeking sources of natural antioxidants, alone or in combination with synthetic antioxidants that are currently being used by the industry. The present study was conducted to determine the effect of α-lipoic acid, α-tocopherol, and wheat germ oil on the status of antioxidant enzymes, fatty acid profile, and serum biochemical profile of broiler blood. One-day-old (180) broiler birds were fed six different feeds varying in their antioxidant content: no addition (T_1), natural α-tocopherol (wheat germ oil, T_2), synthetic α-tocopherol (T_3), α-lipoic acid (T_4), α-lipoic acid together with natural α-tocopherol (T_5), and α-lipoic acid together with synthetic α-tocopherol (T_6). The composition of saturated and unsaturated fatty acids in the breast and leg meat was positively influenced by the different dietary supplements. The content of fatty acid was significantly greater in broilers receiving T_2 both in breast (23.92%) and in leg (25.82%) meat, whereas lower fatty acid levels was found in broilers receiving diets containing T_6 in the breast (19.57%) and leg (21.30%) meat. Serum total cholesterol (113.42 mg/dL) and triglycerides (52.29 mg/dL) were lowest in the group given natural α-tocopherol and α-lipoic acid. Wheat germ oil containing natural α-tocopherol alone or with α-lipoic acid was more effective than synthetic α-tocopherol in raising levels of antioxidant enzymes superoxide dismutase, catalase, and glutathione reductase while lowering plasma total cholesterol, low-density lipoprotein, and triglycerides and raising high-density lipoprotein and plasma protein significantly. It was concluded that the combination of wheat germ oil and α-lipoic acid is helpful in improving the lipid profile of broilers.

KEYWORDS: *α-lipoic acid, α-tocopherol, antioxidant enzymes, fatty acid profile, wheat germ oil*

INTRODUCTION

Poultry meat is often preferred over other meats due to the nutritional quality of its oil, rich in polyunsaturated fatty acids (PUFA). However, poultry meat is more prone to oxidative rancidity also due to a high level of PUFA.[1] Lipid oxidation in poultry meat is one of the primary causes of deterioration in meat quality, apart from microbial spoilage.[2] Because the nutritional value of meat products is related to PUFA, lipid oxidation is also an important issue, leading to loss of PUFA as well as cholesterol oxidation. By creating positive changes in lipid composition of the muscle cell membrane, oxidative stability can be enhanced. PUFA are more sensitive to oxidation than saturated fatty acid (SFA) and can act as pro-oxidants. However, PUFA are more beneficial for health than SFA. Addition of natural and synthetic antioxidants to animal diets may both improve the fatty acid profile and have positive effects on the shelf life of meat.[3]

α-Lipoic acid (α-LA) and its reduced form, dihydrolipoic acid (DHLA), have received widespread attention as antioxidants. For example, dietary supplementation of α-LA improves metabolism of carbohydrates, decreases blood pressure, and controls histopathological and biochemical degradation.[9] "Reactive oxygen species" (ROS) are a group of oxygen-containing species such as hydroxyl radical, superoxide anion, and hydrogen peroxide. These species are considered to provoke cardiovascular disease, cancer, and many other chronic disorders of aging. The benefits of α-LA are considered due to the ROS-scavenging ability of α-LA.[4] α-LA is helpful in quenching hydroxyl radicals, singlet oxygen, and hypochlorus acid,[5] whereas DHLA scavenges hypochlorus acid, hydroxyl radicals, peroxyl radicals, and superoxide anion radicals.[6] α-LA is also known as lipoamide, as it acts as a cofactor in the dehydrogenase complex that catalyzes the oxidative decarboxylation of α-keto acids such as pyruvate during glucose metabolism to yield energy.[7] Also, α-LA has an important role in cellular thiol replenishment and as a redox-modulating agent.[8] Presently, α-LA is used to treat complications associated with diabetes in Western countries.[9] The mechanism is thought to be reduction of the disulfide group of the lipoic acid to its dithiol form, DHLA. Thus, α-LA as a food supplement could have diverse biological benefits.[4]

The main sources of vitamin E include wheat germ, sunflower, corn, and soybean oils. The different forms of vitamin E are four tocopherols and four tocotrienols. α-Tocopherol is the most abundant form and possesses the highest biological activity and is best absorbed by the gastrointestinal tract in humans. The absorption of vitamin E

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happens mainly through the lymphatic system, the molecules being transported to the liver inside triglyceride-rich chylomicrons.[2] α-Tocopherol is the major isoform secreted by the liver within very-low-density lipoproteins (VLDL). It is transported back to the liver inside low-density lipoproteins (LDL), which are recognized and removed from the plasma by LDL-specific receptors.[10]

Wheat germ oil is a rich source of both tocopherols and tocotrienols. Wheat germ contains lipids (10−15%), protein (26−35%), sugar (17%), fiber (1.5−4.5%), and minerals (4%). It also contains significant quantities of bioactive compounds including tocopherols (1300−2700 mg/kg), phytosterols (24−50 mg/kg), policosanols (10 mg/kg), carotenoids (4−38 mg/kg), thiamin (15−23 mg/kg), and riboflavin (6−10 g/kg).[11] Wheat germ oil is a source of easily available vitamin E, which could act as an inhibitor of oxidative processes in chicken muscle. It protects cells against the effects of free radicals, which could otherwise contribute to the development of chronic disease and deterioration of chicken meat.[12]

Antioxidant enzymes, including catalase (Cat), glutathione peroxidase (GPx), and superoxide dismutase (SOD), are manufactured and controlled within the cell, which requires dietary supplies of their component nutrients.[13] Cells and organisms can be sheltered from the destructive effect of superoxide anion by SOD, a key antioxidant defense enzyme. To eliminate the damaging effects of ROS, the SOD enzyme works in concert with GPx and Cat. The main aim of the study was to evaluate the effect of wheat germ oil and α-lipoic acid on the fatty acid profile and serum biochemical profile of broilers.

MATERIALS AND METHODS

Procurement of Raw Material and Experimental Design. Wheat germ was collected from Sunny Flour Mills, Lahore, Pakistan. α-LA and synthetic α-tocopherol were purchased from Shaanxi Sciphar Hi-Tech Industry Co, Ltd., China, and from Merck (Merck KGaA, Darmstadt, Germany), respectively. All of the reagents and chemicals for this research were purchased from Sigma-Aldrich (Germany) and Merck (Germany). One-day-old chicks (50 ± 5 g body weight) were purchased from Jadeed Chicks Private Limited, Faisalabad, Pakistan. Fine sawdust and Bromo-Sept (for disinfection of the pens) were procured from the local market of Faisalabad. Two trials were conducted for this study with 180 birds purchased for each trial. The first trial was conducted during 2010−2011, and the second trial was conducted during the session 2011−2012. Chicks were weighed individually and then randomized into 18 experimental units each consisting of 10 birds. The broilers were divided into six groups with three replicates in each group. The birds were fed the control diet during the first 2 weeks and then the experimental diet started. After 6 weeks, the broilers were slaughtered according to the Halal ethical guidelines. Blood samples were also collected from the jugular vein and stored in heparinized tubes in the refrigerator. Breast and leg meat samples were also collected for fatty acid analysis. The composition of basal diet is given in Table 1, and details of the feed supplementation plan are given in Table 2.

Homogenization. Meat samples (5g) were placed in capped 50 mL polypropylene tubes following homogenization in phosphate buffer (pH 7.4) and glycerol (20%) using an ice−water bath during homogenization. The tubes were at 4 °C on ice-cold water during homogenization. Connective tissues were removed using muslin cloth prior to storage at −20 °C.

Analysis of Fat Content in Broiler Meat. The fat content was determined according to the method described by the AOAC.[14] The dried broiler meat (1 g) prepared according to the method described for the analysis of moisture content was homogenized and then was extracted with *n*-hexane using a Soxhlet extractor. The extract was placed in a previously tarred beaker, and the solvent was evaporated at 45 °C in an oven. The sample was cooled, and the crude fat was weighed and its percentage calculated using the following equation:

$$\text{fat content (\%)} = \frac{\text{wt of broiler meat} - \text{wt of defatted broiler meat}}{\text{wt of broiler meat}} \times 100$$

Table 1. Composition of Basal Feed

ingredient	quantity (g/kg)
corn	39
rice broken	2.07
rice polishing	5.60
cottonseed meal	2.20
canola meal	2.0
corn gluten 60%	2.30
sunflower meal	12.40
soybean meal	15.0
fish meal	6.60
L-lysine	0.15
DL-methionine	0.08
dicalcium phosphate	1.20
limestone	0.90
premix	0.50
nutrient composition (calculated)	
metabolized energy (kcal/kg)	2900
crude protein (%)	21.03
lysine (%)	1.1
methionine (%)	0.52

Table 2. Animal Feed Supplements[a]

	supplementation per kg feed
T_1	control
T_2	wheat germ oil (200 mg of natural α-tocopherol)
T_3	synthetic α-tocopherol (200 mg)
T_4	α-lipoic acid (150 mg)
T_5	α-lipoic acid (150 mg) + wheat germ oil (200 mg of natural α-tocopherol)
T_6	α-lipoic acid (150 mg) + synthetic α-tocopherol (200 mg)

[a]Natural α-tocopherol was used as whole wheat germ oil, quantified for α-tocopherol.

Muscle Fatty Acid Profile. The total fatty acids were extracted from breast and leg muscle samples using the method of Folch et al.[15] that uses an antioxidant to prevent oxidation during sample preparation. Breast and leg meat samples were homogenized in 40 mL of chloroform/methanol [2:1 (v/v)]. The mixture containing the extracted fatty acids was filtered through no. 1 Whatman paper (Whatman International Ltd., Maidstone, UK). The paper was then washed with 10 mL of chloroform/methanol [2:1 v/v)]. Normal saline solution (12 mL) was added to facilitate phase separation, and the mixture was shaken vigorously for 1 min and then left to stand for 4 h. After 4 h, the upper phase was discarded and the lower phase was evaporated by vacuum rotary evaporation at 70 °C. The total lipid extracts were then rediluted in 5 mL of fresh chloroform/methanol [2:1 (v/v)] and immediately transferred to a capped methylation tube. Transmethylation of the extracted fat to fatty acid methyl esters (FAME) was carried out using KOH in methanol and 14% methanolic boron triflouride (BF_3).[16] An internal standard, heneicosanoic acid (C21:0), was added to each sample prior to transmethylation to determine the individual fatty acid contents within the meat samples. Methyl esters were quantified by GC (Agilent Technologies 6890 N). One microliter was injected into the chromatograph, equipped with an autosampler, a split/splitless injector, and a flame ionization detector (FID). The injector temperature was 250 °C and the detector temperature, 300 °C. The column temperature program runs were

initiated at 100 °C for 2 min, warmed to 170 °C at 10 °C/min, held for 2 min, warmed to 220 °C at 7.5 °C/min, and then again held for 20 min to facilitate optimal separation. Results are presented as percentage of total fatty acids.

Antioxidant Enzymes. *Superoxide Dismutase Assay.* SOD activity was determined by an NADH oxidation method described by Paoletti and Mocali[17] with some modification. The reaction mixture (180 μL) contained triethanolamine−diethanolamine-HCl buffer (75 mM) at pH 7.4, NADH (0.28 mM), EDTA−$MnC1_2$ (2.3 and 1.17 mM, respectively), mercaptoethanol (0.9 mM), and muscle extract (20 μL). Absorbance at 340 nm was determined every 7 min for a total of 28 min using a plate reader. One unit of SOD activity was defined as the amount of extract required to inhibit the rate of NADH oxidation by the control by 50%.

Catalase Assay. Cat activity was determined by measuring the decrease in the H_2O_2 concentrations spectrophotometrically at 240 nm and 22 °C, according to the method described by Aebi[18] with some modifications. The reaction mixture (200 μL) contained muscle extract (20 μL) and H_2O_2−phosphate buffer solution (180 μL), which was prepared by diluting H_2O_2 (30%) with 0.05 M phosphate buffer (pH 7.0) until the absorbance at 240 nm was 0.50. An extinction coefficient of 0.024 mM^{-1} cm^{-1} was used to calculate H_2O_2 concentrations. This value has been adjusted for the 0.55 cm path length using a plate reader. A unit of Cat activity was defined as the amount of extract needed to decompose 1 μmol of H_2O_2 per minute at 22 °C.

Glutathione Reductase (GR) Assay. GR activity was determined by measuring the decrease in absorbance at 340 nm according to the method described by Goldberg and Spooner[19] with some modifications. The reaction mixture consisted of 160 μL of sodium phosphate (0.12 M), 10 μL of EDTA (15 mM), 10 μL of oxidized glutathione (65 mM), and muscle extract (10 μL). After mixing and waiting 5 min, 10 μL of NADPH (4.5 mM) was added, and the absorbance at 340 nm was monitored using a plate reader over 5 min. An extinction coefficient of 3.421 mM^{-1} cm^{-1} was used for calculation of the NADPH concentration.

Serum Analysis. Total cholesterol concentrations were estimated according to the method of Stockbridge et al.[20] Distilled water, standard solution, or plasma samples (20 μL of each) were taken into labeled test tubes. The absorbance at 505 nm of samples or standard against a water blank was recorded using a CESIL spectrophotometer (CE7200, UK). High-density lipoprotein concentrations in blood were determined using a HDL-cholesterol kit based on the method of Assmann.[21] To 200 μL of serum was added 500 μL of diluted reagent, and the mixture was allowed to stand for 10 min at room temperature. The final absorbances of sample, standard, and blank were recorded at a wavelength of 505 nm. Low-density lipoprotein concentration in blood was determined as described by McNamara et al.[22] Blood triglyceride concentration was estimated by the liquid triglyceride method described by Annoni et al.[23] Ten microliters of standard and sample was taken into test tubes along with one blank. Test tubes were incubated for 5 min at 37 °C, and absorbance was recorded at a wavelength of 540 nm. Total protein concentration was determined according to the Biuret method described by Josephson and Gyllensward.[24] The absorbance was recorded at 540 nm. Blood albumin concentration was estimated by the Bromcresol Green (BCG) method described by Webster.[25] Bromcresol green reagent (3 mL) was taken in three test tubes labeled as blank, standard, and test sample. Distilled water (0.01 mL), standard, and sample were added to the respective test tubes. The absorbance of the standard and test sample was recorded against the reagent blank at a wavelength of 630 nm.

Statistical Analysis. Data were analyzed by using two-way ANOVA for the antioxidant enzymes and the serum biochemical profile using the software package Statistic 8.1 (Analytical Software, Tallahassee, FL, USA) by following the principles outlined by Steel et al.[26] When differences were found, means were compared by the Duncan multiple-range test.

RESULTS AND DISCUSSION

Fat Contents in the Breast and Leg Meat. The supplementation of α-lipoic acid in feed of broilers decreased the fat content considerably in both breast and leg meat compared with the control, whereas the addition of wheat germ oil in the broiler feed increased significantly the fat contents. The rate of accession of fat content in the treatment in which wheat germ oil fed to broilers was higher (19.3−22.6%) with respect to control in the breast and leg meat, whereas the rate of reduction of fat content was 30.43−31.70% in the breast and leg meat, respectively (Figure 1). The higher level of α-lipoic

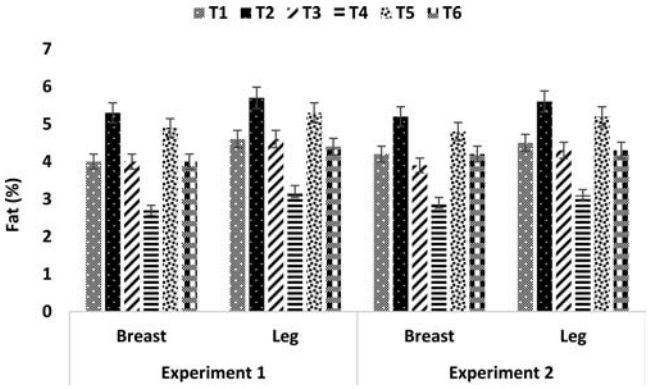

Figure 1. Fat content of broiler breast and leg of both experiments.

acid reportedly increased the nonesterified fatty acids of plasma and reduced the plasma triacylglycerol concentrations at the age of 6 weeks in broiler birds.[27] It is also reported that α-lipoic acid possesses the activity to reduce the fat associated with atherosclerosis,[28] suggesting that α-lipoic acid reduces the total fat content in the broiler meat.

Fatty Acid Profile. The composition of fatty acids in the breast meat of birds fed control feed supplemented with natural α-tocopherol, synthetic α-tocopherol, or their combinations is presented in Table 3. Results indicate that the composition of saturated and unsaturated fatty acids in the breast meat was affected significantly by the different feeds. The profiles of several fatty acids, including palmitoleic acid (C16:0), margaric acid (C17:0), and margaroleic acid (C17:1), did not differ significantly among treatments. However, palmitic acid (C16:0) content was significantly greater in the breast meat from broilers fed natural α-tocopherol, as were stearic and linoleic acids. In a repeat study, oleic acid was also elevated (Supporting Information Supplementary Table 1).

The effect of wheat germ oil was to raise PUFA and SFA levels by a small amount (23.9 vs 21.1% of total and 30.9 vs 37.3, respectively; Table 3). However, saturated fatty acids were unchanged by other dietary treatments. Polyunsaturated fatty acids, including oleic, linoleic, and linolenic acids, were significantly elevated in the breast meat of broilers fed natural α-tocopherol and α-LA plus natural α-tocopherol. However, oleic acid levels were not elevated in the breast meat of broilers fed α-LA plus synthetic α-tocopherol. During a repeat experiment (year 2011−2012), the stearic acid in the breast meat of broilers fed T_3, T_4, and T_6 did not differ from control, although T_2 and T_5 showed a 20% increase in the content of this fatty acid (Supporting InformationSupplementary Table 1).

The results of the present study showed that more fatty acids were found in the breast meat of broilers fed the wheat germ oil supplemented feed. This may be in part due to the high

Table 3. Fatty Acid Composition and Profile in Broiler Breast Meat during Experiment 1[a]

	fatty acids (%) in breast meat[b]									fatty acid profile (%) in breast meat[c]					
treatment[d]	$^{1}C_{16:0}$	$^{2}C_{16:1}$	$^{3}C_{17:0}$	$^{4}C_{17:1}$	$^{5}C_{18:0}$	$^{6}C_{18:1}$	$^{7}C_{18:2}$	$^{8}C_{18:3}$	$^{9}C_{18:4}$	SFA	MUFA	PUFA	UFA	SFA/UFA	PUFA/SFA
T_1	18.1bc	1.9a	0.3a	0.1a	12.4bc	29.7ab	15.1ab	0.4bc	5.4a	30.9bc	31.8ab	21.1b	52.9bc	0.5a	0.6a
T_2	21.3a	1.9a	0.3a	0.2a	15.6a	31.1a	17.2a	1.2a	5.5a	37.3a	33.2a	23.9a	57.1a	0.6a	0.6a
T_3	17.7bc	1.9a	0.3a	0.1a	12.2bc	29.1ab	14.8bc	0.4bc	5.4a	30.2bc	31.2bc	20.7bc	52.0c	0.5a	0.6a
T_4	17.3c	1.8a	0.3a	0.1a	12.0c	29.7ab	14.2bc	0.4bc	5.2a	29.6c	31.8bc	19.9 cd	51.7 cd	0.5a	0.6a
T_5	20.3ab	1.9a	0.3a	0.1a	13.7ab	31.0a	17.0a	1.2ab	5.4a	34.4b	33.1a	23.7a	56.8ab	0.6a	0.6a
T_6	17.4c	1.9a	0.2a	0.1a	11.8c	28.7b	14.0c	0.4c	5.1a	29.5c	30.8c	19.5d	50.3d	0.5a	0.6a

[a]Values with different letters in the column are significantly different from one another ($p > 0.05$). [b]Values shown are mean of three individual measurements, variance <10%. [1]Palmitic; [2]palmitoleic; [3] margaric; [4] margaroleic; [5]stearic; [6]oleic; [7]linoleic; [8]linolenic; [9]arachidonic. [c]SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = poly unsaturated fatty acids, UFA = unsaturated fatty acids, SFA/UFA = ratio of saturated fatty acids and unsaturated fatty acids, PUFA/SFA = ratio of poly unsaturated fatty acids and saturated fatty acids [d]T_1 = control; T_2 = natural α-tocopherol 200 mg/kg feed; T_3 = synthetic α-tocopherol 200 mg/kg feed; T_4 = α-lipoic acid 150 mg/kg feed; T_5 = α-lipoic acid 150 mg + natural α-tocopherol 200 mg/kg feed; T_6 = α-lipoic acid 150 mg + synthetic α-tocopherol 200 mg/kg feed.

Table 4. Fatty Acid Composition and Profile in the Leg Meat of Broilers during Experiment 1[a]

	fatty acids (%) in leg meat[b]									fatty acid profile (%) in leg meat[c]					
treatment[d]	$^{1}C_{16:0}$	$^{2}C_{16:1}$	$^{3}C_{17:0}$	$^{4}C_{17:1}$	$^{5}C_{18:0}$	$^{6}C_{18:1}$	$^{7}C_{18:2}$	$^{8}C_{18:3}$	$^{9}C_{18:4}$	SFA	MUFA	PUFA	UFA	SFA/UFA	PUFA/SFA
T_1	19.6bc	1.9a	0.2a	0.07a	13.1bc	31.2bc	15.9ab	0.4bc	5.8a	32.9b	33.3b	22.2b	55.6b	0.5a	0.6a
T_2	21.8a	2.0a	0.1a	0.06a	16.9a	33.2a	18.3a	1.2a	5.8a	38.9a	35.3a	25.5a	60.8a	0.6a	0.6a
T_3	19.1bc	1.9a	0.1a	0.07a	13.0bc	31.0bc	15.7ab	0.4bc	5.8a	32.3bc	33.0bc	22.0b	55.1bc	0.5a	0.6a
T_4	18.4c	1.9a	0.1a	0.05a	12.4c	30.8c	15.7ab	0.4bc	5.8a	31.1c	32.7c	21.9bc	54.7bc	0.5a	0.7a
T_5	21.4ab	2a	0.1a	0.06a	15.4ab	33.1ab	18.3a	1.1ab	5.8a	37.1a	35.1a	25.3a	60.5a	0.6a	0.6a
T_6	18.0c	1.9a	0.1a	0.05a	12.2c	30.1c	15.1c	0.4c	5.7a	30.5c	32.1c	21.3c	53.4c	0.5a	0.7a

[a]Values with different letters in the column are significantly different from one another ($p > 0.05$). [b]Values shown are mean of three individual measurements, variance <10%. [1]Palmitic; [2]palmitoleic; [3] margaric; [4]margaroleic; [5]stearic; [6]oleic; [7]linoleic; [8]linolenic; [9]arachidonic. [c]SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, UFA = unsaturated fatty acids, SFA/UFA = ratio of saturated fatty acids and unsaturated fatty acids, PUFA/SFA = ratio of polyunsaturated fatty acids and saturated fatty acids. [d]T_1 = control; T_2= natural α-tocopherol 200 mg/kg feed; T_3= synthetic α-tocopherol 200 mg/kg feed; T_4= α-lipoic acid 150 mg/kg feed; T_5= α-lipoic acid 150 mg + natural α-tocopherol 200 mg/kg feed; T_6= α-lipoic acid 150 mg + synthetic α-tocopherol 200 mg/kg feed.

Table 5. Superoxide Dismutase (SOD), Glutathione Reductase (GR), and Catalase (Cat)[a]

	SOD (U/mL)			GR (U/mg protein)			Cat (U/g protein)		
treatment	2010	2011	mean	2010	2011	mean	2010	2011	mean
T_1	156.0 ± 5.1	147.5 ± 4.6	151.7 ± 5.3e	38.1 ± 1.2	40.1 ± 1.3	39.1 ± 1.2e	124.9 ± 4.3	120.1 ± 4.7	122.5 ± 4.9d
T_2	178.2 ± 6.2	183.0 ± 5.8	180.6 ± 7.4c	42.3 ± 1.4	41.1 ± 1.2	41.7 ± 1.4c	134.9 ± 5.7	137.3 ± 4.9	136.1 ± 5.4b
T_3	169.0 ± 5.2	166.3 ± 6.3	167.6 ± 5.8d	39.2 ± 1.3	40.4 ± 1.4	39.8 ± 1.5e	129.8 ± 5.2	125.1 ± 5.7	127.5 ± 5.2cd
T_4	172.6 ± 6.1	168.8 ± 4.7	170.7 ± 6.3d	40.7 ± 1.5	40.9 ± 1.3	40.8 ± 1.4d	130.6 ± 4.7	127.7 ± 5.1	129.1 ± 4.9c
T_5	197.9 ± 7.2	201.4 ± 8.4	199.6 ± 7.9a	45.0 ± 1.6	47.4 ± 1.5	46.2 ± 1.6a	140.6 ± 5.8	146.5 ± 6.2	143.6 ± 6.1a
T_6	185.3 ± 6.2	192.6 ± 5.7	188.9 ± 7.5b	43.1 ± 1.4	42.7 ± 1.4	42.9 ± 1.4b	136.9 ± 5.4	141.3 ± 5.4	139.1 ± 5.7ab
mean	176.5 ± 7.4	176.6 ± 7.1		41.4 ± 1.2	42.1 ± 1.3		133.0 ± 5.2	133.0 ± 5.5	

[a]Means sharing similar letters in a column are statistically nonsignificant ($P > 0.05$). Values are means of three individual measurements.

concentration of saturated and unsaturated fatty acids present in the wheat germ oil. The lowest fatty acid content was found in the breast meat of broilers receiving synthetic α-tocopherol and α-LA, possibly in part due to the fact that α-LA is reported to have a lipid-lowering effect.[29] The findings of many scientists indicate that n-3 PUFA-enriched diets increase the deposition of these fatty acids in muscles.[30] Wheat germ oil is considered a good source of n-3 PUFA and thus may increase the content of n-3 PUFAs in the meat. In the present study, wheat germ oil contributed to a significant increase in the total content of unsaturated fatty acids (UFAs) in lipids of breast meat, mostly due to an increase in the levels of monounsaturated C_{18} fatty acid (oleic). A previous report found that PUFA-enriched diets modified and increased these fatty acids in the breast muscle of chickens at 42 days of age, which supports the results obtained in the present study.[31] The composition of fatty acids in the leg meat of broilers fed these different diets showed that the compositions of saturated and unsaturated fatty acids in the broiler leg meat were affected similarly (Table 4). The fatty acid content present in leg meat was a little higher, compared to the breast meat of the broilers. As with leg meat, fatty acid levels of palmitoleic acid, margaric acid, and margaroleic acid did not vary with the feed treatments. However, palmitic acid was significantly greater in the leg meat of broilers fed natural α-tocopherol (wheat germ oil), with or without added α-LA (Table 4).

Arachidonic acid content in the leg meat was unchanged by these different dietary treatments. Polyunsaturated fatty acids, including oleic, linoleic, and linolenic acid, were also greater in the leg meat of broilers fed natural α-tocopherol with and

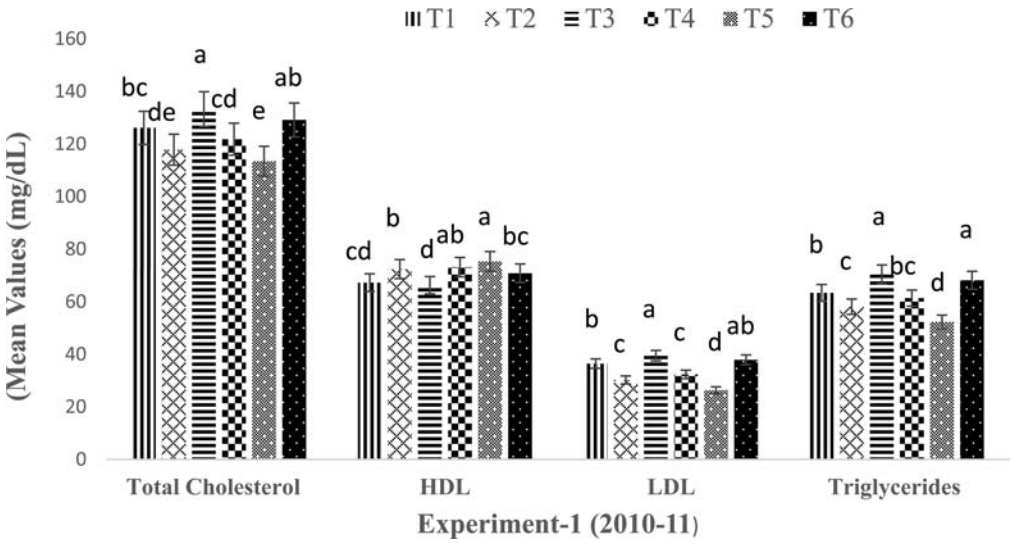

Figure 2. Total cholesterol, HDL, LDL, and triglycerides of serum of broilers fed different dietary supplements during experiment 1.

without α-LA (Table 4). However, oleic acid was lower in the leg meat of broilers fed α-LA and synthetic α-tocopherol during a repeat experiment (Supporting Information Supplementary Table 2). Stearic acid was increased in the leg meat from broilers fed T_2 and T_5 but not in broilers from other dietary treatments (Table 4).

The results of the present study show that greater fatty acid content was found in both breast and leg meat from broilers fed wheat germ oil, which contained both saturated and unsaturated fatty acids. The lowest levels of fatty acids were found in meat from broilers fed synthetic α-tocopherol and α-LA, which is consistent with the fact that α-LA is reporting to have a lipid lowering effect.[28] Both SFA and PUFA concentrations were greater in the broilers fed wheat germ oil, which is a good source of these fatty acids. Total UFA and PUFA contents of wheat germ oil are about 81 and 64%, respectively.[32] The wheat germ oil was added to the diet at 120 g/kg feed (chosen to provide 200 mg α-tocopherol/kg feed).

Antioxidant Enzymes. Serum SOD, which works in conjunction with catalase and peroxidases to diminish the harmful effects of ROS, varied significantly within the dietary treatments, from 156.0 to 197.9 ± 5.1 U/mL and from 147.5 to 201.4 ± 8.4 U/mL during the experimental years 2010−2011 and 2011−2012, respectively (Table 5). The birds fed wheat germ oil plus α-LA exhibited the greatest levels (197.9 ± 5.1 U/mL) followed by synthetic α-tocopherol plus α-LA (185.3 ± 6.2 U/mL) and wheat germ oil alone (178.2 ± 6.2 U/mL). However, all dietary supplements increased serum SOD in both years. The results show that antioxidants increase SOD activity in broilers. This is in agreement with a previous paper showing increased SOD activity in broilers fed α-tocopherol (60 mg/kg).[33] α-LA may indirectly influence the activity of SOD, thereby preventing the deleterious effect of superoxide radicals formed. Rodent studies showing α-LA increased SOD activity support the present study, in which dietary α-LA increased SOD activity in the broilers.[34,35]

Glutathione is the major antioxidant present in the body, acting as a substrate and cofactor in many metabolic reactions and also regulating the intracellular redox status.[36] It is actively involved in quenching free radicals through many reactions including conjugation of electrophiles by glutathione transferase and reducing glutathione reductase after quenching ROS.[37] Glutathione reductase of broiler serum varied <20%, although all dietary supplements caused a significant increase (Table 5). The birds receiving wheat germ oil plus α-LA showed the greatest GR levels followed by those on synthetic α-tocopherol plus α-LA and wheat germ oil alone. Broiler serum Cat was also increased by 15−20% in all dietary supplement treatments. (Table 5). The greatest catalase activity (140.6 U/g protein) was observed in birds fed wheat germ oil plus α-LA followed by synthetic α-tocopherol plus α-LA and wheat germ oil as for SOD.

GR and Cat activities were also found to be higher in the antioxidant treatments compared to the control. Previous studies reported that activities of Cat and glutathione levels were independently influenced by concentrations of α-LA and α-tocopherol.[38] They found that GSH and Cat levels were increased in diets containing 60 mg/kg α-LA. Similar findings showed that diets supplemented with 40 ppm of α-LA increased the total glutathione pool.[39] Also, supplementation with α-LA reduced oxidative stress in horses, as measured by glutathione and antioxidant enzyme levels in blood.[40] Increasing α-tocopherol in the diet (50 mg/kg) increased glutathione and Cat levels, compared to 10 mg/kg α-tocopherol. Similar findings were reported of α-tocopherol (50 IU/kg) supplemented to laying hens increasing serum glutathione and Cat.[41] Some of the changes observed in tissue antioxidant enzyme activities may have arisen from the individual and synergistic effects of antioxidant vitamins, fatty acids, phytosterols, and phenolic compounds found in the structure of wheat germ oil, rather than α-tocopherol alone.

Serum Cholesterol. Total cholesterol level in broiler serum varied between 113.4 and 133.2 mg/dL (Figure 2). A repeat experiment showed similar values (Supporting Information-Supplementary Figure 2). The greatest total cholesterol value (133.2 mg/dL) was observed in birds fed synthetic α-tocopherol followed by synthetic α-tocopherol plus α-LA. The birds receiving wheat germ oil plus α-LA showed the lowest total cholesterol, 10% below values in broilers receiving no supplements. This finding agrees with others who reported that wheat germ oil caused a reduction in total cholesterol.[42] In the current study, wheat germ oil reduced the total cholesterol level, but the combination of α-LA and wheat germ oil gave the greatest reduction in total cholesterol. The linoleic acid present

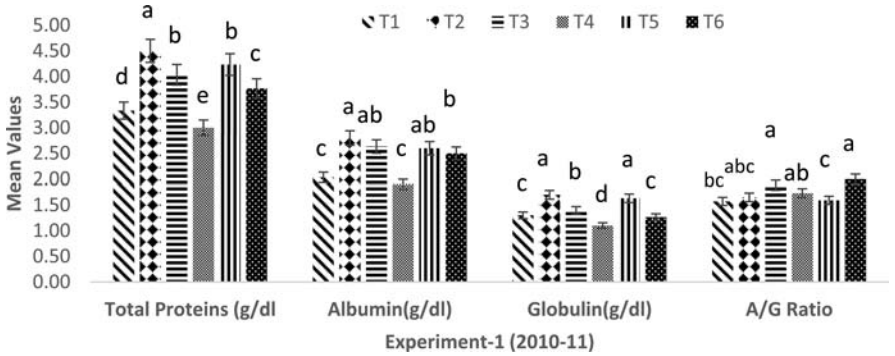

Figure 3. Total protein, albumin, globulin, and A/G ratio of serum of broilers fed different dietary supplements during experiment 1.

in the wheat germ oil may aid in the elimination of cholesterol.[43,44] Alternatively, phytosterols present in the wheat germ oil may help in the reduction of the cholesterol level. Phytosterols intrinsic to wheat germ are biologically active and have a prominent role in reducing cholesterol absorption.[45] Plant sterols/stanols inhibit cholesterol absorption, possibly by competitively inhibiting its incorporation into the mixed micelles in the small intestine. Daily consumption of 1−2 g of plant sterols or stanols was shown to cause a 10−20% reduction in low-density lipoprotein cholesterol (LDL cholesterol).[46]

The results of the current study are in consistent with others who reported that total cholesterol levels were lowered by incorporating α-LA (4.2 mg/body weight) into the diet of New Zealand white rabbits.[47] α-LA possesses both lipid-lowering and antiatherosclerotic properties, due to both lowering total cholesterol and lowering LDL levels as well as reduction in atherolesion formation in diet-induced hypercholesterolemic rabbits.[29] The concentrations of total cholesterol in sera increased significantly with dietary α-tocopherol concentration, irrespective of the source of oil (Figure 2). The serum concentrations of total cholesterol have previously been reported to increase progressively and significantly with each increment in dietary α-tocopherol.[48,49]

High-density lipoprotein (HDL) in broiler serum for different dietary treatments is given in Figure 2, showing a reproducible pattern of elevation with wheat germ oil and α-LA and lowering with synthetic α-tocopherol. Serum LDL exhibited a reversal of this pattern, being elevated in broilers fed synthetic α-tocopherol and lowest in broilers given wheat germ oil plus α-LA (Figure 2). A similar trend was found when the study was repeated (Supporting InformationSupplementary Figure 2). It was previously reported that the administration of wheat germ oil caused a reduction in LDL levels when rats were orally fed 200 mg/kg/day of wheat germ oil.[42] In the current study, not only did treatment with wheat germ oil reduce LDL levels but the combination of α-LA and wheat germ oil gave the maximum reduction in LDL levels. The concentration of cholesterol in serum increased significantly with dietary α-tocopherol concentrations (Figure 2). Although the mechanism of α-LA on serum cholesterol and LDL is unknown, it probably involves lipoprotein lipase (LPL) activity or hepatic cholesterol metabolism.[29,50] The LPL activity and HDL level are increased in cholesterol-fed New Zealand white rabbits after administration of NO-1886 (ibrolipim), a lipoprotein lipase activator.[51] Possibly α-LA initiates LDL receptor synthesis in the liver, which in turn could increase the uptake of cholesterol back into the hepatic system and increase synthesis of apoprotein A to reverse cholesterol transport.[52]

Serum Triglycerides. Serum triglycerides varied among treatments. Serum triglycerides for different dietary treatments ranged from 52.2 to 70.4 mg/dL (Figure 2), with a very similar range in a repeat experiment (Supporting Information Supplementary Figure 2). Broilers receiving synthetic α-tocopherol exhibited the highest triglyceride value (70.4 mg/dL), followed by synthetic α-tocopherol plus α-LA. Past work suggests that serum triglycerides increase with dietary α-tocopherol concentration, irrespective of the source of oil.[48,49] In contrast, α-LA caused a drop in triglycerides, and the wheat germ oil plus α-LA exhibited the lowest triglyceride value (52.2 mg/dL). The results of the present study agree with previous findings that triglycerides were lowest in a group in which α-LA was fed to male rats.[53] The administration of wheat germ oil has been reported to cause a reduction in serum triglyceride levels, which agrees with the findings of the present study.[42] Absorption of α-LA occurs both in the intestine and at the blood−brain barrier. It exerts hypoglycemic and hypotriglyceridemic effects in chickens in addition to several other therapeutic effects.[54] Our data support the idea that wheat germ oil, with or without α-LA, lowers serum triglyceride and cholesterol.

Serum Proteins. Major serum proteins can be divided into albumin and globulin. Most plasma cholesterol circulates as HDL (α-2 globulin fraction) and LDL (β-globulin fraction) complexes.[55] These lipoproteins became the principal cholesterol transport and about 40−44% of the total serum proteins. The total protein and albumin of broiler serum varied across dietary treatments (Figure 3). The greatest protein (4.5 g/dL) was observed in broilers fed wheat germ oil, followed by broilers receiving wheat germ oil plus α-LA (4.2 g/dL). Similar values were found when the experiment was repeated (Supporting Information Supplementary Figure 3). The albumin content of broiler serum from broilers fed different dietary treatments was found to range from 1.9 to 2.8 g/dL (Figure 3). The greatest albumin level (2.8 g/dL) was in broilers fed wheat germ oil. The broilers fed α-LA yielded the least (1.9 g/dL) albumin level. Similar values were found in a repeat experiment (Supporting Information Supplementary Figure 3). The results of the present study support earlier findings that in the serum concentration of total protein, albumin and globulin increased with increasing concentrations of dietary α-tocopherol.[48]

The increased serum concentrations of these proteins could be due to the improved digestion and absorption of dietary nutrients in the presence of higher concentrations of dietary α-

tocopherol. Although an increase in plasma proteins in chickens receiving dietary α-tocopherol was previously reported,[49] there are no reports of the impact of wheat germ oil, which we show here was superior to α-tocopherol.

Serum globulin of broiler serum also varied among dietary treatments with a range from 1.1 to 1.7 g/dL (Figure 3) with results repeating in a second study (Supporting Information-Supplementary Figure 3). The highest globulin level was found in birds fed wheat germ oil, followed by wheat germ oil plus α-LA and synthetic α-tocopherol. Serum albumin and globulin levels in broilers fed α-LA were lower than those of the control group. The A/G ratio in serum from different treatment groups is given in Figure 2, showing that the A/G ratio varied from 1.5 to 2.0; this finding was repeated in our second study (Supporting Information Supplementary Figure 3). The highest A/G ratio (2.0) was found in broilers fed synthetic α-tocopherol plus α-LA followed by synthetic α-tocopherol alone, with the lowest (1.5) A/G ratio in broilers fed control diet. However, these findings were not consistent in a second study in which wheat germ oil caused a ratio lower than control. However, in both studies the serum globulin concentration increased with α-tocopherol, confirming previous studies[48,49]

α-Lipoic acid, α-tocopherol, and wheat germ oil all influenced the fatty acid composition as well as the serum cholesterol level in broilers. Wheat germ oil, a natural source of α-tocopherol, was studied here in the feed of broilers for the first time and showed excellent results, alone and in combination with α-LA, in lowering the serum LDL and enhancing the serum HDL level in the broilers. The contents of SFA and PUFA were greater in the group fed wheat germ oil, whereas α-LA reduced the fatty acid content, in confirmation of its known effect. In a nutshell, the supplementation of wheat germ oil alone or in combination with α-LA had beneficial consequences for the human diet, because poultry meat exhibited improved fatty acid and cholesterol profiles. Further studies are needed on the consumption of this kind of functional meat to evaluate the lipid profile of the consumer.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

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