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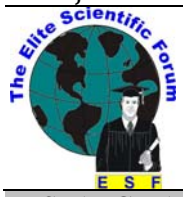

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RESEARCH ARTICLE

Antioxidant Potential and Storage Stability of Broiler Leg Meat Fed on Extruded Flaxseed and α- tocopherol Acetate

Rashida Parveen[1,*], Muhammad Issa Khan[1], Faqir Muhammad Anjum[1] and Munir Ahmad Sheikh[2]
[1]National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakistan
[2]Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad, Pakistan

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***Corresponding Author:**
rashidaparveen73@yahoo.com

ABSTRACT

Dietary supplementation of antioxidants is an active approach to boost up the oxidative stability of muscle tissues leading towards healthier and functional meat production for human consumption. Present study was planned to assess the effect of extruded flaxseed (EFS) and α-tocopherol acetate (ATA) supplementation in broiler feed on oxidative stability of broiler leg meat. One-day-old broiler chicks (Cobb 550) were randomly divided in to 8 treatment groups in triplicate having 4 birds in each replicate. Broiler feed was supplemented with EFS at level of 100, 150 and 200g/kg alone and in combination with ATA (200mg/kg). Birds were slaughtered after six weeks and their leg meat was subjected to quantification of antioxidants and storage stability. The oxidative stability of leg meat was significantly enhanced by supplementation of EFS and ATA. Higher antioxidant potential was for feed supplemented with higher level of EFS (200g/kg) in combination with ATA (200mg/kg). Thiobarbituric acid reactive substances (TBARS) assay indicated the production of malondialdehydes in different treatments and significantly lower ($P<0.05$) values were observed in groups fed on high level of EFS supplemented with ATA. Conclusively, dietary supplementation of EFS along with ATA improved antioxidant capacity and stability of broiler leg meat.

INTRODUCTION

Lipid oxidation of leg meat muscles is an important parameter with respect to its potential to affect the human health in poultry meat consumers. It has been demonstrated that stability of broiler leg meat can be altered by changing their diet through antioxidants. The notable strategies for diminishing lipid oxidation of meat utilize diets containing antioxidants. Currently, the interest in natural antioxidants has increased because they are considered to be safer than the synthetic antioxidants and have greater application potential for consumers' acceptability, palatability, stability and shelf-life of meat products (Naveena et al., 2008). It is instigated that addition of flax dietary fiber extract rich in viscous dietary fibers significantly increased fat excretion and lowered total and LDL-cholesterol. Flaxseed is the richest plant source of the ω-3 polyunsaturated fatty acids (PUFA) α-linolenic acid (ALA; C18:3n-3). Flaxseed oil mainly originated as triacylglycerol (98%) with lower contents of phospholipids (0.9 %) and free fatty acids (0.1%) (Mueller et al., 2010). The concentration of α-linolenic acid (ω-3 PUFA), a potent anti-carcinogenic compound in flaxseed oil ranges approximately from 40–60%. Other bioactive components, such as linoleic acid and oleic acid are also present, each at 15% level (Williams et al., 2008). The α- tocopherol acetate (ATA) is a lipid soluble antioxidant (Saladino et al., 2008) and known as acceptable supplement. It is the most efficient chain-breaking, fat soluble antioxidant in tissues of animals. It has 15 times more powerful antioxidant potential among the different isomers of vitamin E. It has appreciable influence on rabbit meat quality because it participates in increasing the functionality of the meat as it increases ATA content in meat (Bielanski and Kowalska, 2008; Selim et al., 2008). More interestingly, it is also involved in enhancing the oxidative stability of the meat muscles during refrigeration (Zsedely et al., 2008). As an outcome,

awareness of consumer about the health benefits of ω-3 PUFA is increasing their demand for food products enriched with ω-3 PUFA (Chekani et al., 2008). Keeping in view, this study was conducted to examine the effect of dietary extruded flaxseed EFS and ATA on anti-oxidative potential and lipid stability of broiler leg meat.

MATERIALS AND METHODS

Preparation of Experimental Feeds and rearing of Birds

Raw flaxseed was extruded using single screw extruder, Extru-tech E325 (Extru-tech, Sabetha, Kansas) and conditions for extrusion of flaxseed were; barrel speed (80-120rpm), final head temperature (80-120°C) and feeder speed (12-16rpm) as described by Wu et al. (2008). The EFS was added to broiler feed at level of100, 150 and 200 g/kg alone or in combination with 200 mg/kg ATA on addition basis in the broiler feed. One-day-old broiler chicks of "Cobb-550" strain (n=96) were purchased from Jadeed Chicks Private Limited, Faisalabad, Pakistan. The chicks were randomly divided into 8 groups from T_0 to T_7 having 3 replicates in each group and 4 birds in each replicate. T_0 was fed on control feed, T_1 on ATA 200mg/kg, T_2 on 100g EFS/kg of feed, T_3 on 150g EFS/kg of feed, T_4 on 200g EFS/kg feed, T_5 on 100EFS+ATA200mg/kg, T_6 on 150EFS+200mgATA/kg and T_7 on 200gEFS+200mg ATA/kg of feed. Birds were reared under standard management conditions and fed on basal diets for first 3 weeks followed by experimental diets till the age of 42 days. The weight of the birds was recorded on weekly basis. At the end of experimental period birds from each replicate were randomly picked, slaughtered by following the Halal Islamic ethical Guidelines. Leg meat samples were separated and frozen at -20°C till further analysis.

Assessment of anti-oxidant status of leg meat

The antioxidant activity of leg meat was assessed by measuring their free radical scavenging capabilities to DPPH stable radicals (Brand-Williams et al., 1995). Meat sample (125 µL) was mixed with 0.0012 M DPPH solution through 95% MeOH addition to gain final volume of 4 mL. The absorbance of the resulting solution and the blank was recorded after 30 min at room temperature. The disappearance of DPPH was read by using spectrophotometer (Hitachi, Japan) at 515 nm. Inhibition of free radicals by DPPH in percent (%) was calculated by using the following formula:

Inhibition (%) = [100×{A blank –A sample}/ A blank]

TBARS of broiler meat sample was determined by measuring mg of malondialdehyde per kg of meat following the method described by Asghar et al. (1989).The peroxidative reaction was initiated by adding ferrous sulphate and hydrogen peroxide to the membrane suspension held in a water bath at 37 °C. One milliliter sample prepared in buffer solution (0.1 MKCl; 0.05 M NaOH; 0.13 M lactic acid) with pH 5.3–5.4 was withdrawn at 30 min intervals for a period of 120 min and added to a same volume of solution of thiobarbituricacid (0.4%)+trichloroacetic acid (10%)+ hydrochloric acid (0.25 N). The mixture was heated in water bath for 15 min and then cooled. After centrifugation, the absorbance of the supernatant was determined at 532 nm. The extent of membrane lipid peroxidation in terms of malondialdehyde production was calculated by using the formula as follows:

MDA level= [OD 532×100/1.56]×TV/ d wt×1000]

Where,

OD= Optical density, TV=total volume of extract (ml), Wt=weight of tissues

Peroxide value (POV) and β -carotene antioxidant activity bleaching test (BCB)

Primary rate of oxidation in leg meat samples (peroxide value) was determined by following the method of PORIM (1995) and results were expressed in miliequivalent (mEq) of active oxygen per kilogram of sample. Antioxidant activity was also determined with slight modification of β-carotene bleaching method described by (Pratt et al., 1980).

Quantification of α-tocopherol Acetate by using HPLC

The α-tocopherol was quantified by using the methodology of Asghar et al. (1990) with minor modifications. Briefly, meat tissue (1 gm) was mixed with 1.5 mL of urea (6M) in a centrifuge tube. Then meat tissues were disintegrated by adding 1 ml of sodium dodecyl sulphate (SDS) solution (0.1M)in a tube. Ethyl alcohol (4 mL) containing 1% pyragallol was added in the tube to deproteinate the ATA. The separation phase was facilitated by adding 10 mL of petroleum ether in tube and vortexed for 2 minute. Then tube was centrifuged at 2000 rpm for 5 minutes and upper solvent layer was transferred in a vial. For the solubilization of ATA in mobile phase, EtOH (500 µL) was added in tube and vortexed for 1 minute, at 45 °C. The sample was filtered through anspec H-1056 micro filter. The standard of ATA was prepared by adding l µg of ATA in l mL of methanol. A Shimadzu (Kyoto, Japan) HPLC system consisting of two LC-10AD pumps, an SCL 10A system controller, manual injection syringe and a degasser (DGU-12A) was used. For reversed-phase chromatography, the analytical column was a 5-um particle size Shim-Pack CLC (C18) column, 15cm x 4.6 mm x 5 µm; Shinwa Chemicals, Kyoto, Japan) The columns were maintained at 25 °C by a CTO-10A column oven (Shimadzu). The effluent was monitored by SPD-10AVvp UV-Visible detector equipped with an 8µL flow cell. The UV-Vis detector was set at 290 nm. A linear gradient elution from methanol (100%) was adopted. The rate of flow of mobile phase was adjusted as 1.0 mL/minute. The

length of peak areas was calculated using V-station chromatography software (GL Science, Tokyo, Japan).

Statistical analysis

The data thus obtained was subjected to statistical analysis using the software package (Statistic 8.1). One way analysis of variance (ANOVA) and least significance difference (LSD) tests was used to estimate the level of significance between mean values of different treatments.

RESULTS

Total phenolic contents

The results regarding the total phenolic contents (TPC) are depicted in Table 1. Findings of the current investigation revealed that TPC values in samples of leg meat varied significantly among treatments. However, non-significant variations were observed for trial conducted during different years. TPC values for leg meat varied from 0.475 (T_0) to 1.206 mg/GAE (T_7). The highest TPC value (1.206 mg/GAE) was recorded in meat of broilers chicks fed on feed supplemented with 20% extruded flaxseed and 200 mg/g ATA (T_7); whereas, lowest value (0.475 mg/GAE) was noted in control group (T_0). It is evident from results that TPC of leg meat increased with EFS supplementation, however, highest values were observed for meat obtained from birds fed on feed supplemented with EFS in combination with ATA.

Thiobarbituric acid reactive substances (TBARS)

The lesser production of TBARS depicts the oxidative stability of meat and meat products. Results of present study revealed that TBARS of broiler leg meat varied significantly from 0.172 to 0.244 mg of MDA/kg for broilers fed on feed supplanted with EFS alone and in combination with ATA (Table 2).The lowest TBARS of leg meat (0.172 mg of MDA/kg) was recorded for T_7followed by T_6(0.178 mg of MDA/kg meat) while highest TBARS were observed in meat of broiler fed on control feed (0.244 mg of MDA/ kg meat).

Free radical scavenging Assay (DPPH)

Supplementation of feed with EFS and ATA imparted significant effect on DPPH of broiler meat samples (Table 3). The results showed that DPPH of leg meat varied significantly as a function of EFS and ATA supplementation in broiler feed. The lowest DPPH value (61.03%) of leg meat was noticed in T_0 (control) while highest inhibition (77.52%) was found in broilers meat muscles fed on diet containing EFS and ATA (T_7). On whole, inhibition capacity increased linearly with increase in EFS supplementation.

Ferric Reducing Antioxidant Power (FRAP) in leg meat

The results regarding ferric reducing antioxidant power in broiler leg meat are shown in Table 4. The FRAP value significantly varied with supplementation of EFS and ATA in broiler feed. Data revealed that the minimum FRAP value (1.555 µ mole /Fe+2/g of meat) was shown by T_0 (control) while maximum value was observed in T_7 (1.831, µ mole /Fe+2/g of meat). The ferric reducing antioxidant power in broiler leg meat increased significantly with the increase in level of EFS supplementation.

Table 1: Total phenolic contents mg GAE/100gmeat in leg

Treatment	TPC (mg GAE/100g meat)		
	Leg meat		
	Year 1	Year 2	Means
T_0	0.476± 0.001	0.473± 0.0011	0.475h± 0.028^{h}
T_1	0.556± 0.002	0.557± 0.002	0.556g± 0.017^{g}
T_2	0.584± 0.002	0.580± 0.002	0.582f± 0.018^{f}
T_3	0.693± 0.002	0.683± 0.002	0.688e± 0.117^{a}
T_4	0.732± 0.001	0.733± 0.001	0.733d± 0.005^{e}
T_5	0.757± 0.002	0.760± 0.001	0.758c± 0.05^{c}
T_6	1.027± 0.002	1.027± 0.015	1.027b± 0.053^{b}
T_7	1.205± 0.001	1.207± 0.015	1.206a± 0.023^{d}
Mean	0.754± 0.015^{b}	0.753± 0.048^{a}	

Values are represented as mean ± SD of n= 3 Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05) T_0 =Control, T_1 = α-tocopherol acetate 200mg /kg of feed, T_2 = 10% extruded flaxseed/kg of feed, T_3 = 15% extruded flaxseed/kg of feed, T_4 = 20% extruded flaxseed /kg of feed, T_5 = 10% extruded flaxseed + α-tocopherol acetate 200 mg/kg of feed,T_6 = 15% extruded flaxseed+ α-tocopherol acetate 200 mg/kg of feed, T_7 = 20 % extruded flaxseed + α-tocopherol acetate 200 mg /kg of feed

Table 2: Thiobarbituric acid reactive substances (mg of MDA/kg) in leg meat tissues

Treatment	Leg meat		
	Year 1	Year 2	Means
T_0	0.247±0.001	0.245± 0.001	0.244±0.007 ^{a}
T_1	0.237± 0.009	0.240± 0.002	0.241± 0.001^{a}
T_2	0.193± 0.0015	0.190± 0.0017	0.191±0.015^{b}
T_3	0.186± 0.015	0.186± 0.012	0.186±0.001^{c}
T_4	0.178± 0.007	0.178± 0.001	0.178±0.002^{d}
T_5	0.180 0.009±	0.179± 0.013	0.180±0.002^{d}
T_6	0.179± 0.006	0.178± 0.015	0.178±0.001 ^{d}
T_7	0.173 ±0.001	0.170± 0.001	0.172±0.007^{e}
Mean	0.197±0.003^{b}	0.196±0.005^{a}	

Values are represented as mean ± SD of n= 3 Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05 T_0 =Control, T_1 = α-tocopherol acetate 200mg /kg of feed, T_2 = 10% extruded flaxseed/kg of feed, T_3 = 15% extruded flaxseed/kg of feed, T_4 = 20% extruded flaxseed /kg of feed, T_5 = 10% extruded flaxseed + α-tocopherol acetate 200 mg/kg of feed,T_6 = 15% extruded flaxseed+ α-tocopherol acetate 200 mg/kg of feed, T_7 = 20 % extruded flaxseed + α-tocopherol acetate 200 mg /kg of feed

Peroxide values in leg meat muscles

Results showed that peroxide values varied expressively from 0.138 to 0.663 mEq O_2/kg as a function of EFS and ATA supplementation (Table 5). The highest peroxide values were stated in broilers leg

Table 3: Free radical scavenging activity (DPPH %) in broiler leg meat

Treatment	DPPH scavenging activity (%)		
	Leg meat		
	Year 1	Year 2	Means
T_0	60.36± 0.89	61.69±0.88	61.03g±0.99[g]
T_1	64.71± 0.90	65.01±1.15	64.86f±0.75[g]
T_2	66.05± 0.56	66.38±0.87	66.21f±1.12[ef]
T_3	68.69± 0.88	68.73±0.86	68.71e±1.16[de]
T_4	70.70± 0.90	71.02±1.17	70.86d±1.06[cd]
T_5	73.68± 0.88	74.37±1.22	74.02c±1.11[bc]
T_6	75.72± 0.89	76.46±1.12	76.09b±1.14[ab]
T_7	77.37± 0.88	77.68±0.89	77.52a±1.36[a]
Mean	69.66± 1.17[a]	70.17± 1.16[b]	

Values are represented as mean ± SD of n= 3 Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05) T_0 =Control, T_1 = α-tocopherol acetate 200mg /kg of feed, T_2 = 10% extruded flaxseed/kg of feed, T_3 = 15% extruded flaxseed/kg of feed, T_4 = 20% extruded flaxseed /kg of feed, T_5 = 10% extruded flaxseed + α-tocopherol acetate 200 mg/kg of feed,T_6 = 15% extruded flaxseed+ α-tocopherol acetate 200 mg/kg of feed, T_7 = 20 % extruded flaxseed + α-tocopherol acetate 200 mg /kg of feed.

Table 4: Ferric reducing antioxidant power (µmole of /Fe^{+2}/g meat) of broiler leg meat

Treatment	Leg meat		
	Year 1	Year 2	Means
T_0	1.547±0.88	1.563±0.46	1.555± 0.06 [g]
T_1	1.579±1.17	1.587±0.89	1.583± 0.01 [f]
T_2	1.643±0.89	1.653±0.76	1.648± 0.009 [e]
T_3	1.713± 0.56	1.723±0.54	1.718± 0.012 [d]
T_4	1.737± 0.88	1.740±0.69	1.738± 0.01 [c]
T_5	1.773±1.15	1.777±0.58	1.775± 0.006 [b]
T_6	1.828±1.12	1.833±0.47	1.831±0.01 [a]
T_7	1.591±0.97	1.600±0.90	1.831±0.007 [f]
Mean	1.676±0.85[a]	1.685±0.93[b]	

Values are represented as mean ± SD of n= 3 Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). T_0 =Control, T_1 = α-tocopherol acetate 200mg /kg of feed, T_2 = 10% extruded flaxseed/kg of feed, T_3 = 15% extruded flaxseed/kg of feed, T_4 = 20% extruded flaxseed /kg of feed, T_5 = 10% extruded flaxseed + α-tocopherol acetate 200 mg/kg of feed,T_6 = 15% extruded flaxseed+ α-tocopherol acetate 200 mg/kg of feed, T_7 = 20 % extruded flaxseed + α-tocopherol acetate 200 mg /kg of feed.

meat fed on control feed (T_0); whereas, lowest values were found in broilers leg meat fed on diet containing higher level of EFS in combination with ATA(T_7).

β-carotene in leg muscles

Findings of the present study showed that β-carotene concentration changed considerably as a function of EFS and ATA supplementation in feed (Table 6). The β-carotene contents differed in leg muscles from 0.138µg/g (T_5) to 0.663 µg/g (T_7). Higher concentration of β-carotene was observed in meat of broilers fed on higher level of EFS in combination with ATA.

Table 5: Peroxide values (mEq O_2/kg) of broiler leg meat

Treatment	Leg meat		
	Year 1	Year 2	Means
T_0	0.600±0.23	0.727±0.35	0.663a± 0.002[h]
T_1	0.660±0.10	0.407±0.33	0.533a±0.017[g]
T_2	0.223±0.009	0.210±0.24	0.217b±0.018[f]
T_3	0.190±0.24	0.200±0.22	0.195b±0.005[a]
T_4	0.187±0.22	0.200±0.014	0.193b±0.117[d]
T_5	0.163±0.37	0.150±0.10	0.157b±0.028[e]
T_6	0.167±0.48	0.153±0.26	0.160b±0.09[c]
T_7	0.113±0.08	0.163±0.38	0.138b±0.07[b]
Mean	0.288±0.59[b]	0.276±0.48[a]	

Values are represented as mean ± SD of n= 3 Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05) T_0 =Control, T_1 = α-tocopherol acetate 200mg /kg of feed, T_2 = 10% extruded flaxseed/kg of feed, T_3 = 15% extruded flaxseed/kg of feed, T_4 = 20% extruded flaxseed /kg of feed, T_5 = 10% extruded flaxseed + α-tocopherol acetate 200 mg/kg of feed,T_6 = 15% extruded flaxseed+ α-tocopherol acetate 200 mg/kg of feed, T_7 = 20 % extruded flaxseed + α-tocopherol acetate 200 mg /kg of feed.

The α -Tocopherol Acetate content in leg meat

The findings regarding ATA content of broiler leg meat are shown in Table 7. It is clear from data that ATA content varied significantly from 20.09 to 73.17 mg/g in broiler leg meat fed on EFS and ATA supplemented feed. The mean values showed that lowest ATA contents (20.09 mg/g) were observed in T_0 (control) while the highest value (73.17 mg/g meat) was observed for meat of broiler fed on 20% EFS with ATA supplemented feed.

DISCUSSION

The broiler leg meat is claimed to have larger proportion of fat due to increased fat deposition in this part and thus having high rate of lipid oxidation. Lipid oxidation is considered to be the major source of deterioration of meat quality and reduced shelf life. Findings of the current study revealed that dietary incorporation of flaxseed and ATA impart beneficial impacts on the oxidative stability of the broiler meat. Total phenolic contents were seen to be gradually improved in leg meat with the increase in EFS level alone or in combination with ATA. The results of present study are in line to those of Imran et al. (2015). The results of present study are also in lines with Yong et al. (2013) who studied the effect of wild grape supplementation at different levels (0%, 0.25%, or 0.5%)in broiler feed and recorded higher total phenolic content in the leg meat of broilers fed on wild grape as compared to the control. Similarly, Serpen et al. (2012) indicated that broilers fed on α-tocopherol supplemented feed resulted in higher antiradical power due to the accumulation of phenolic compounds in muscles. Likewise, Sacchetti et al. (2008) also showed

Table 6β -carotene concentration µg/g of broiler leg meat

Treatment	Leg meat		
	Year 1	Year 2	Means
T_0	0.187±0.56	0.200±0.78	0.193±0.03[b]
T_1	0.163±0.24	0.150±0.85	0.157± 0.041[b]
T_2	0.167±0.89	0.153±0.78	0.160± 0.011[b]
T_3	0.190±0.78	0.200±0.66	0.195± 0.002[b]
T_4	0.223±0.47	0.210±0.42	0.217± 0.34[b]
T_5	0.113±0.59	0.163±0.49	0.138± 0.42[b]
T_6	0.660±0.68	0.407±0.59	0.533± 0.10[a]
T_7	0.600±0.89	0.727±0.69	0.663± 0.28[a]
Mean	0.288± 0.001[b]	0.276±0.049[a]	

Values are represented as mean ± SD of n= 3 Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05)T_0 =Control, T_1 = α-tocopherol acetate 200mg /kg of feed, T_2 = 10% extruded flaxseed/kg of feed, T_3 = 15% extruded flaxseed/kg of feed, T_4 = 20% extruded flaxseed /kg of feed, T_5 = 10% extruded flaxseed + α-tocopherol acetate 200 mg/kg of feed,T_6 = 15% extruded flaxseed+ α-tocopherol acetate 200 mg/kg of feed, T_7 = 20 % extruded flaxseed + α-tocopherol acetate 200 mg /kg of feed.

Table 7: α -Tocopherol content (mg/g) in the broiler leg meat

Treatment	Leg meat		
	Year 1	Year 2	Means
T_0	20.09±1.40	20.09±1.23	20.09± 03.7[h]
T_1	32.07±1.86	33.07±1.18	32.57± 07.5[g]
T_2	47.02±1.15	48.00±1.10	47.51± 01.5[f]
T_3	50.49±1.44	51.33±0.99	50.91±1 .91[e]
T_4	52.20±1.45	53.00±1.25	52.60± 1.58[d]
T_5	66.40±1.45	67.39±1.37	66.90± 2.95[c]
T_6	70.05±1.73	70.87±1.56	70.46± 1.92[b]
T_7	73.67±1.76	72.67±1.70	73.17± 2.01[a]
Mean	51.50±1.03 [a]	52.05±1.15 [b]	

Values are represented as mean ± SD of n= 3 Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05)T_0 =Control, T_1 = α-tocopherol acetate 200mg /kg of feed, T_2 = 10% extruded flaxseed/kg of feed, T_3 = 15% extruded flaxseed/kg of feed, T_4 = 20% extruded flaxseed /kg of feed, T_5 = 10% extruded flaxseed + α-tocopherol acetate 200 mg/kg of feed,T_6 = 15% extruded flaxseed+ α-tocopherol acetate 200 mg/kg of feed, T_7 = 20 % extruded flaxseed + α-tocopherol acetate 200 mg /kg of feed.

that feed containing exogenous antioxidants like vitamin C & E, ubiquinols and polyphenols enhanced total phenolic contents of poultry meat when birds were fed on antioxidant enriched feed. Similar results are reported by Basmacioglu et al. (2003).

The lipid peroxidation is one of the major mechanisms of quality deterioration in meat. It takes place when hydroxyl radicals attack at fatty acid side chains of membrane phospholipids. Malondialdehyde (MDA) is a product of lipid peroxidation and TBARS which serve as oxidative damage index. The change in quality is attributed due to deterioration in flavor, color, texture and nutritive value of the meat due to the production of toxic compounds. It is obvious from the results that MDA varied significantly with different levels of feed supplemented with flaxseed and ATA. Ragni et al. (2014) also explained that supplementation of EFS reduced the rate of oxidation.

Haak et al. (2009) explained that α-tocopherol alone or in combination with rosemary & green tea extracts retards lipid oxidation in meat. Likewise, Jang et al. (2010) also found that supplementing of poultry diet with quercetin at level of 200 ppm/kg feed substantially reduced free radical formation and reactive oxygen species. These results are also in line with the findings of Parveen et al. (2013) who supplemented vitamin E with alpha lipoic acid in broilers and found decreased formation of free radicals in supplemented feed fed broiler meat compared to control.

One of the most widely used tests for oxidative rancidity is determination of peroxide value. Peroxide value in chicken meat is a measure of the concentration of peroxides and hydro peroxides formed in the initial stages of lipid oxidation (Khan et al., 2015). Results regarding β-carotene contents are in accordance with Alina et al. (2012) who studied the effect of red palm mid fraction addition in sausages and recorded momentous variation in β-carotene contents. The α–tocopherol is one of the major lipid soluble antioxidants belonging to tocopherol family which cannot be synthesized by the animal cells (Saladino et al., 2008). The α-tocopherol protects the membrane from oxidation. The level of vitamin E in the muscles depends on its dietary level, supplementation period, the fiber type distribution and metabolic characteristic. The absorption of vitamin E in broilers is variable with an average of 42% (Barroeta, 2007). The results are in positions of kastoe (2013) who defined flaxseed as a source of natural antioxidants and reported that its supplementation increased the tocopherol contents. Furthermore, Cortinas et al. (2006) verified that vitamin E is influenced by the type of the muscle fibers; vitamin E level in thigh meat is 1.8 to 2 fold higher than in breast meat. The present investigation showed that antioxidant concentration was higher in the treatments where the combination of ATA and EFS supplementation in the feed of broilers was used as compared to other treatments. It can be concluded from results of study that EFS supplementation in combination with ATA significantly increased the antioxidant potential and oxidative stability of broiler leg meat.

Authors' contributions

RP carried out the trials of the broiler birds, conducted data analysis and drafted the manuscript. MIK helped in planning the experimental design and helped out during the research work. FMA and MAS provided technical assistance regarding research and data interpretation. All authors read and approved the final manuscript.

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