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Effect of *Sargassum sp.* and Vitamin E on Stability of Fish Oil Enriched Meat in Broiler Chickens

Research Article

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ABSTRACT

The present study was aimed to assess the influence of brown marine algae and vitamin E (VE) (160 ppm α-tocopheryl acetate) on stability of chicken meat enriched with fish oil. In this study, 360 d-old broilers (Arbor Acres Plus) were randomly assigned to 6 treatment groups with 4 replicates of 15 birds in each. Experimental groups consisted of corn-soybean basal diet (C), corn-soybean basal diet with fish oil (F), corn-soybean basal diet supplemented vitamin E (E), corn-soybean basal diet supplemented fish oil with vitamin E (FE), fish oil with 5% dried marine alga (F 5% A) and fish oil with 10% dried marine alga (F 10% A). Fish oil and vitamin E were supplemented at 2 last weeks of trial. Chickens were slaughtered in d 42 and meat samples were stored at 4 °C. Malondialdehyde (MDA) was measured at 0, 3 and 6 days after slaughter. Results showed that fish oil enhanced meat lipids per oxidation and with passage of time after slaughter this increment was greater. Treatments using vitamin E showed lower levels of MDA during the storage and increased meat stability in fish oil enriched meat. Marine algae in F 10% A increased meat stability in thigh and breast muscles in 3 and 6 days after slaughter (P<0.05). Fatty acid profile showed an enhancement in omega-3 fatty acids accumulation in thigh and breast samples, significantly (P<0.05) with fish oil, but vitamin E had no significant effect on this profile. Inclusion 5% and 10% marine algae and fish oil together led to increasing omega-3 fatty acids in thigh muscle. Five percent and 10% marine alga decreased serum cholesterol and triglyceride levels significantly (P<0.05). In conclusion, administration of diet supplemented with vitamin E and marine alga improved stability of broiler's meat enriched with omega-3 fatty acids.

KEY WORDS broiler, fish oil, marine alga, meat stability, vitamin E.

INTRODUCTION

Fish oil has high amount of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) therefore, its addition to animals' feed is more efficient than vegetable oil sources in enrichment of long-chain ω-3 polyunsaturated fatty acids (PUFA) in meat. However, the chicken's meat enriched with PUFA is prone to more rancidity during refrigeration. It has been shown that lipid oxidation is one of the primary mechanisms of quality deterioration in foods, especially in meat products (Kanner, 1994). In muscle and fat tissue, oxidation continues postmortem and affects the stability of meat. Therefore, vitamin E supplementation is thought to be an effective measure to prevent lipid peroxidation in tissues enriched with ω-3 fatty acids (Cho and Choi, 1994). The antioxidant function of vitamin E in poultry meat prevents the formation of secondary oxidation products (Grau *et al.* 2001a; Grau *et al.* 2001b). Previous researchers have

shown that vitamin E supplementation led to lower levels of lipo-oxidation compounds, such as 2-heptanone and 1-penten-3-ol in lamb meat fed with ω-3 sources (Rivas-Cañedo *et al.* 2013). Many natural antioxidants have already been isolated from different kinds of plants such as oilseeds, cereal crop, vegetables leaves, roots, spices and herbs (Shon *et al.* 2003). However, natural antioxidants are not limited to terrestrial sources. Some of the marine alga is considered to be a rich source of antioxidants (Lim *et al.* 2002). Generally, marine algae contain high concentrations of the basic nutrients like high biological value proteins, minerals, vitamins and polyunsaturated fatty acids, particularly α-linoleic acid, and also their extracts have shown strong antioxidant properties and it has led to attract scientists attention (Yuan and Walsh, 2006). Cox *et al.* (2013) reported that using of seaweed (*Himanthalia elongata*) extract could significantly show antioxidant activity due to its specifics ingredient including phenolic content and a DPPH (2,2-diphenyl-1-picrylhydrazyl). In other study Jimenez-Escrig *et al.* (2001) reported antioxidant activity of marine alga and indicated strong antioxidative activity of *Fucus vesiculosus* brown algae. Some active antioxidant compounds from brown alga were identified as phylophenols and carotenoids (Yan *et al.* 1999).

The objective of this study was to evaluate the effect of dietary vitamin E and marine algae on broiler chicken performance and meat stability when dietary enriched with fish oil.

MATERIALS AND METHODS

Test product
Brown marine alga (*Sargassum sp.*) was obtained from Chabahar coastal areas of Persian Golf, Iran. Approximate composition of this seaweed is shown in Table 1. In this study *Sargassum sp.* was used as a source of antioxidant in the chicken diets for the first time.

Birds and diets
A total of 360, one-d-old broiler chicks (Arbor Acres Plus) with an initial average body weight (BW) of 35.30 g were obtained from a commercial hatchery. At first day the chicks were weighed individually and distributed by BW into 6 treatments with 4 replicates of each treatment. Room temperature was kept at 32 ˚C during the first week of the trial and then reduced gradually to 23 ˚C. The broilers were raised in floor pens with a 23 h/day light program. Diets in mash form and water were available *ad libitum* for 42 d. All diets were formulated according to NRC (1994) requirements for broiler chickens. At the end of the experimental period, birds were weighed and feed consumption was recorded for feed efficiency conversion.

Ingredients of diets are shown in Table 2. Experimental diets were as follows: 1) control corn soybean diet (C); 2) fish oil (F); 3) vitamin E (E); 4) fish oil with vitamin E (FE); 5) fish oil with 5% marine alga (F 5% A) and 6) fish oil with 10% dried marine alga (F 10%A). Fish oil (3%) and vitamin E (160 IU) were supplemented in 2 last weeks.

Table 1 Approximate nutrient composition of Seaweed (*Sargassum sp.*), DM

Item	Amount
Dry matter, %	94.8
Gross energy	12.37 kj/kg
Crude fat, %	6.5
Crude protein, %	10.59
Fiber, %	12.8
Ash, %	34.57
Calcium mg/kg	1.62
Phosphor mg/kg	0.24

Samples collection
At end of the experiment, one bird per pen was randomly selected and slaughtered (4 birds of each treatment). Then, thigh and breast muscles were collected. Samples were stored for 0, 3 and 6 days at 4 ˚C in refrigerator to measure MDA production. Thigh muscle and breast muscle were stored at -20 ˚C for fatty acids analysis. At 42 days of age blood samples (1.5 mL) were obtained via wing vein and serum samples were collected. Serum was isolated by centrifugation at $3000 \times g$ for 15 min and stored at -20 ˚C until used for analysis. The abdominal fat was removed and weighed.

Chemical analysis
For both diet and seaweed, dry matter, crude protein, crude fat, crude fiber and ash were analyzed according to the methods of the association of official analytical chemists AOAC (2000).

Calcium and phosphorous were measured by atomic absorption spectrophotometry. The extent of lipid oxidation was determined by measuring the TBA-reacting substances at 0, 3 and 6 d of storage, therefore, two grams of dark meat was weighed for analysis. Values of tiobarbitoric acid (TBA) were measured through a third derivative spectrophotometry method after acid aqueous extraction and were expressed as micrograms of malondialdehyde (MDA) per gram of muscle using the procedure described by Grau *et al.* (2001a). Fatty acid (FA) extraction of the samples was performed by Metcalf *et al.* (1996) method. The fatty acid content was determined using a gas chromatograph (UNICAM 4600, UK) equipped with a BPX70 fused silica capillary column (30 m×0.22 mm i.d.) with a 0.25 μm film thickness of stationary phase. Temperatures were: column 280 ˚C, injector 260 ˚C and detector 280 ˚C. Helium was used as carrier gas and sample volume injected was 0.2 μL.

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Table 2 Ingredients and nutrient composition of experimental diets

Ingredients (%)	Starter			Grower			Finisher			
	C	F 5% A	F 10% A	C	F 5% A	F 10% A	C[*]	F[*]	F 5% A	F 10% A
Maize	63.8	59.75	55.5	71.20	66.69	62.64	72.11	68.2	64.5	60.5
Soybean meal	32.01	31.57	31.32	24.75	24.92	24.5	22.5	24.05	23.5	23.7
Marine algae	0	5	10	0	5	10	0	0	5	10
Corn oil	0	0	0	0	0	0	1.28	0	0	0
Fish oil	0	0	0	0	0	0	0	3	3	3
Dicalcium phosphate	1.7	1.75	2	1.70	1.7	1.65	1.73	1.7	1.65	1.7
Limestone powder	1.41	1.2	0.45	1.2	0.65	0.38	1.2	1.2	0.6	0
Salt	0.35	0	0	0.32	0	0	0.35	0.4	0	0
Methionine	0.23	0.23	0.23	0.33	0.33	0.33	0.33	0.33	0.25	0.33
Vitamin-mineral premix[**]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Sand	0	0	0	0	0	0	0	0.62	0	0.27
Analyzed content										
Metabolizable energy (kj.kg)	12.14	12.14	12.14	12.56	12.56	12.56	12.98	12.98	12.98	12.98
Crude fiber (CF)	3.5	3.8	4	3.5	3.8	4	3.5	3.5	3.8	4
DM (%)	88	88	88	88	88	88	88	88	88	88
Crude fat (%)	3.2	3.2	3.2	3.32	3.32	3.32	4	4	4	4
Crude protein (%)	21	21	21	18.5	18.5	18.5	17.5	17.5	17.5	17.5

[*] With addition 160 IU vitamin E to C and F treatment, resulted E and FE treatment.
[**] Each kilogram of premix contain of: vitamin A: 3600000 IU; vitamin D_3: 800000 IU; vitamin E: 7200 IU; vitamin K_3: 800 mg; vitamin B_1: 720 mg; vitamin B_2: 2640 mg; vitamin B_3: 4000 mg; vitamin B_5: 12000 mg; vitamin B_6: 1200 mg; vitamin B_9: 400 mg; vitamin B_{12}: 6 mg; Biotin: 40 mg; Choline chloride: 200000 mg; Mn: 40000 mg; Fe: 20000 mg; Zn: 36000 mg; Cu: 4000 mg; I: 400 mg and Se: 80 mg.

Total serum cholesterol (TC) and triglycerides (TG) were measured by the standard enzymatic method (Pars Azmoon kit, Pars Azmoon Inc., Tehran, Iran).

Statistical analysis

Data were analyzed by one-way ANOVA using the general linear models (GLM) procedure for factorial analysis of SAS software. Significant differences among individual group means were determined with Duncan's multiple range test option of the GLM procedure of SAS software (SAS, 1999).

RESULTS AND DISCUSSION

Performance parameters

The results of performance are shown in Tables 3 and 4. Body weight of broilers in groups contain marine algae were decreased on the end of grower and finisher periods (P<0.05). Body weight gain and feed intake were lower in diets supplemented with 5% and 10% *Sargassum sp*. No significant difference was observed among C, E, F and FE treatments in finisher period.

It means that fish oil and vitamin E alone or together doesn't have any effect on body weight and feed consumption, however, F treatment showed lowest feed consumption.

In the entire period, the lowest feed consumption was related to F 10% A. In this study, C, F, E and FE treatments had the highest feed conversion ratio (FCR); however, there were no significant differences between these treatments.

Chickens assigned to FE treatment showed highest FCR and F treatment showed lowest FCR.

Chemical analysis

As shown in Tables 5 and 6 fish oil supplementation in diet increased ω-3 fatty acids content, especially long chain FA compared to the control group. 5% and 10% marine algae increased accumulation of ω-3 fatty acid as compared to F treatment in thigh muscle. Marine algae didn't affect C16:0, C18:1 and C18:2 fatty acids content in thigh and breast muscles. E treatment as compared with C caused lower C18:2 content in thigh sample, significantly. F 5% A and F 10% A as compared with F showed a significant increase in linolenic acid as one of ω-3 PUFA in thigh muscle, but did not affect breast muscle. Fish oil added to diet could increase EPA and DHA in thigh and breast muscles significantly. There was no significant difference in saturated fatty acids (SFA) profile in vitamin E supplemented diets. However, some increment was observed in SFA in thigh and breast muscle. The use of marine alga led to increasing of SFA in thigh muscle.

Meat stability

Fish oil dietary supplementation decreased the stability of meat against lipid per-oxidation. The levels of MDA in thigh and breast muscles are summarized in Table 7. E treatment showed the lowest MDA in both samples in 0, 3 and 6 days after slaughtering. Differences between the control group and treatment groups in day zero were not significant.

Table 3 Body weight (BW), body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) of broilers (14 and 28 d)

Treatments	BW (g)			Daily BWG (g)		BWG (g)		Daily FI (g)		FI (g)		FCR	
	0	2[1]	4[2]	2[1]	4[2]	2[1]	4[2]	2[1]	4[2]	2[1]	4[2]	2[1]	4[2]
C	45	412	1192	26.21	55.71	367[a]	780[a]	32.5	90.64	455[a]	1269[a]	1.24[b]	1.62[b]
F 5% A	45	365	1023	22.85	47	320[b]	658[b]	30.17	78	421[b]	1092[b]	1.31[a]	1.66[b]
F 10% A	45	345	955	21.42	43.57	300[b]	610[b]	29.28	74.42	410[b]	1042[b]	1.36[a]	1.70[a]
SEM		11	17	0.29	0.3	29	74	1.2	2.9	15	49	0.012	0.0027
P-value		0.025	0.0001	0.015	0.003	0.012	0.028	0.041	0.01	0.011	0.0001	0.028	0.0001

[1] Second week.
[2] Fourth weeks.
SEM: standard error of the means.
C: control corn soybean diet; 5% A: diet plus 5% marine alga and 10% A: diet plus 10% marine alga.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Table 4 Body weight (BW), body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) of broiler chickens (finisher period)

Treatments	BW (g)	Daily BWG (g)	BWG (g)	daily FI (g)	FI (g)	FCR
C	2202	48.09	1010[a]	110.21	2325[a]	2.3[b]
F	2260	50.8	1068[a]	109.33	2296[a]	2.15[b]
E	2184	47.23	992[a]	112.33	2359[a]	2.37[b]
FE	2200	48	1008[a]	114	2400[a]	2.38[b]
F 5% A	1681	31.33	658[b]	84.8	1781[b]	2.71[a]
F 10% A	1548	28.23	593[b]	83.95	1763[b]	2.98[a]
SEM	85	1.1	41	12.2	95	0.012
P-value	0.0001	0.015	0.02	0.02	0.018	0.011

SEM: standard error of the means.
C: control corn soybean diet; F: fish oil; E: vitamin E; FE: fish oil with vitamin E; F 5% A: fish oil with 5% marine alga and F 10% A: fish oil with 10% dried marine alga.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Table 5 Fatty acids analysis in thigh muscle (%)

Treatment	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	EPA	DHA	ω-3	n-6	SFA	USFA	PUFA	MUFA	n-6/ω-3	USFA/SFA
C	26.80[a]	8.20[b]	6.50[ab]	41.70	12.47[a]	0.30[c]	0.10[c]	0.25[b]	0.65[c]	12.47[a]	3.30[a]	63.02[ab]	13.40[a]	25.02	19.18[a]	1.90
F	25.60[b]	9.20[ab]	3.90[c]	42.10	11.78[ab]	0.50[b]	0.70[a]	0.50[b]	1.7[b]	11.78[ab]	29.50[b]	64.78[a]	13.30[a]	25.7	6.93[bc]	2.20
E	27.20[a]	10.30[a]	5.70[ab]	41.40	9.67[b]	0.30[c]	0.20[c]	0.26[b]	0.76[c]	9.67[b]	32.90[ab]	62.13[b]	10.60[b]	25.9	12.72[b]	1.90
FE	25.80[b]	10.70[a]	5.60[b]	40.80	12.20[a]	0.60[ab]	0.47[b]	0.41[b]	1.48[b]	12.2[a]	31.40[b]	65.18[a]	13.70[a]	25.83	8.24[b]	2.10
F 5% A	27.50[a]	9.90[ab]	5.70[ab]	40.80	9.60[b]	0.70[a]	0.70[a]	0.97[a]	2.37[a]	9.63[b]	33.20[a]	62.70[b]	12.10[ab]	25.3	4.06[c]	1.90
F 10% A	24.70[c]	10.00[ab]	6.90[a]	42.30	11.71[ab]	0.70[a]	0.77[a]	0.72[a]	2.19[ab]	11.71[ab]	31.60[ab]	66.20[a]	13.90[a]	26.19	5.34[c]	2.10
SEM	0.32	0.22	0.23	0.21	0.39	0.05	0.03	0.06	0.11	0.31	0.47	0.38	0.39	0.17	0.3	0.08
P-value	0.027	0.034	0.022	0.053	0.011	0.017	0.024	0.036	0.013	0.032	0.041	0.033	0.024	0.08	0.021	0.054

SEM: standard error of the means.
C: control corn soybean diet; F: fish oil; E: vitamin E; FE: fish oil with vitamin E; F 5% A: fish oil with 5% marine alga and F 10% A: fish oil with 10% dried marine alga.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; SFA: saturated fatty acids; USFA: unsaturated fatty acids; PUFA: polyunsaturated fatty acids and MUFA: monounsaturated fatty acids.

Table 6 Fatty acids analysis in breast muscle (%)

Treatment	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	EPA	DHA	ω-3	n-6	SFA	USFA	PUFA	MUFA	N-6/ω-3	USFA/SFA
C	26.30	8.71	9.11[a]	41.39[b]	10.13[b]	0.17[b]	0.001[b]	0.00[b]	0.17[b]	10.13[b]	35.40[a]	60.40[b]	10.31[c]	25.05[b]	60.59[b]	1.71
F	24.90	8.79	5.60[c]	44.60[a]	12.87[a]	0.35[a]	0.50[a]	0.55[a]	1.47[a]	12.87[a]	30.50[c]	67.70[a]	14.30[a]	26.71[ab]	8.70[c]	2.22
E	25.80	8.90	7.49[b]	44.80[a]	10.26[b]	0.11[b]	0.00[b]	0.00[b]	0.11[b]	10.26[b]	33.30[abc]	64.20[ab]	10.30[c]	26.90[a]	93.27[a]	1.93
FE	25.10	9.01	6.75[bc]	43.87[ab]	12.63[a]	0.40[a]	0.80[a]	0.65[a]	1.87[a]	12.63[a]	31.80[bc]	67.30[a]	14.50[a]	26.40[ab]	6.75[c]	2.12
F 5% A	27.80	9.70	6.08[c]	42.36[b]	10.67[b]	0.42[a]	0.50[a]	0.75[a]	1.70[a]	10.67[ab]	33.90[ab]	64.50[ab]	12.40[b]	26.04[ab]	6.28[c]	1.90
F 10% A	25.20	9.80	5.10[c]	44.17[a]	11.97[ab]	0.37[a]	0.70[a]	0.66[a]	1.75[a]	11.97[a]	30.30[c]	67.70[a]	13.70[ab]	27.01[a]	6.48[c]	2.23
SEM	0.35	0.23	0.30	0.35	0.28	0.30	0.075	0.073	0.17	0.28	0.49	0.66	0.40	0.23	3.23	0.066
P-value	0.070	0.12	0.013	0.023	0.032	0.029	0.038	0.0001	0.0001	0.02	0.03	0.026	0.031	0.028	0.023	0.12

SEM: standard error of the means.
C: control corn soybean diet; F: fish oil; E: vitamin E; FE: fish oil with vitamin E; F 5% A: fish oil with 5% marine alga and F 10% A: fish oil with 10% dried marine alga.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; SFA: saturated fatty acids; USFA: unsaturated fatty acids; PUFA: polyunsaturated fatty acids and MUFA: monounsaturated fatty acids.

In fact, adding seaweed to the diet did not affect MDA levels in d-0. In general, with passage of time after slaughter, the increment of MDA production was greater in all treatments and this process shows more increase in F and F 5% A treatments.

Groups E and FE had the lowest MDA in the thigh and breast after 3 and 6 days, which indicates the effect of vitamin E.

Cholesterol and triglyceride in blood serum

Diets supplemented with 5% and 10% algae showed significantly lower cholesterol and triglyceride in blood serum at the end of grower and finisher periods (Table 8).

Performance

The performance of chickens was affected by dietary marine algae.

Table 7 Meat stability in thigh and breast muscles (μg MDA/g sample)

Treatment	Thigh			Breast		
	Day 0	Day 3	Day 6	Day 0	Day 3	Day 6
C	4.00[ab]	11.35[b]	24.98[b]	4.13[a]	11.18[b]	32.7[b]
F	4.13[ab]	16.55[a]	51.6[a]	4.13[a]	16.93[a]	58.1[a]
E	1.50[c]	3.00[d]	6.38[d]	1.38[b]	5.00[c]	7.98[d]
FE	3.00[b]	6.05[cd]	14.5[cd]	2.13[b]	9.75[b]	19.5[c]
F 5% A	5.00[a]	18.25[a]	53.9[a]	4.38[a]	17.48[a]	55.6[a]
F 10% A	3.63[ab]	6.5[c]	15.45[c]	3.88[a]	7.6[bc]	21.53[ca]
SEM	0.43	1.02	3.14	0.29	0.97	3.38
P-value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

SEM: standard error of the means.
C: control corn soybean diet; F: fish oil; E: vitamin E; FE: fish oil with vitamin E; F 5% A: fish oil with 5% marine alga and F 10% A: fish oil with 10% dried marine alga.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).

There are few references in the literature in relation to the supplementation of marine algae in chicken feed, e.g., Ginzberg *et al.* (2000) and Carrillo *et al.*(2008) reported that egg production, feed conversion ratio, egg weight and egg shell thickness were not affected by use of marine algae in diet. Ginzberg *et al.* (2000) showed significant decrease in feed consumption of chickens fed with algal biomass as compared to the control group. This reduction in feed consumption can relate to the high content of fiber (polysaccharide) in the algal biomass. But there is no difference in feed consumption with algae supplementation to hens diet (Carrillo *et al.* 2008).

Addition of marine alga led to increasing of FCR in total period. Alga contains high concentrations of phenolic compounds, which reduce feed intake, N retention, N utilisation and digestion (De Lange, 2000). Also, seaweeds contain chelating minerals that caused anion-cation imbalance and depression in growth. High content of alginate in marine algae can decrease daily body weight (Gardiner *et al.* 2008).

In a current study Kang *et al.* (2013) showed that using of dietary Chlorella, a genus of single-cell green algae, had no significant differences among the treatments for feed intake or feed conversion of broiler chickens during the whole experimental period, but the BW gain was significantly higher (P<0.05) in Chlorella-supplemented groups compared with the control group.

Diet enriched with fish oil did not affect on feed consumption, weight gain and FCR. These data are agreed with results of Korver *et al.* (1998). In agreement with our study, the performance of the hens and egg weights were not affected either by the type of lipid supplement or by the vitamin E level. In contrast to our results Farhoomand and Checaniazer (2009) showed that fish oil supplementation in the diets significantly affected (P<0.01) all the performance parameters measured, with the exception of feed intake in the broiler chicks. Other scientists reported that vitamin E cannot significantly affect performance of broilers (Guetchom *et al.* 2012).

In addition to its activities as an antioxidant, VE is involved in other cellular processes, such as immune function, metabolic processes and many other cell-signaling pathways (Brigelius-Flohé and Traber, 1999). Guo *et al.* (2001) reported that supplement of vitamin E in diet decreased BW significantly, but feed intake and mortality were not affected, however, there is trend to improving BW and FCR. This may be caused by low peroxidation in organs and tissues, such as liver and muscles.

Table 8 Cholesterol and triglyceride in blood serum (mg/100 mL)

Treatment	Cholesterol	Triglyceride
C	168[a]	88[a]
F	139[a]	102[a]
E	161[a]	93[a]
FE	144[a]	89[a]
F 5% A	80[b]	56[b]
F 10% A	40[b]	77[b]
SEM	3.27	1.1
P-value	0.028	0.035

SEM: standard error of the means.
C: control corn soybean diet; F: fish oil; E: vitamin E; FE: fish oil with vitamin E; F 5% A: fish oil with 5% marine alga and F 10% A: fish oil with 10% dried marine alga.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Fatty acids composition
In agreement with our results, Ginzberg *et al.* (2000) showed that C16:0 and C18:1 fatty acids content in thigh and breast muscles were not affected by algae supplementation, and C18:2 was not affected, as reported by Grigorova *et al.* (2006). Dietary supplementation of *Sargassum sp.* increases the content of DHA in poultry meat and this is consistent with what was observed in a previous study by Carrillo *et al.* (2008). In a study that was conducted in order to compare the effect of fresh fish oil and algal sources on the oxidation stability of the poultry meat and polyunsaturated fatty acid, Rymer *et al.* (2010), reported that the algal diets had a higher concentration of C18:3 ω-3, compared with the fresh fish oil diet and had a higher concentration of C18:2n-6 as well. In present study vitamin E did not affect ω-3 fatty acids accumulation in thigh and breast muscles.

Barja *et al*. (1996) also showed that a high dose of vitamin E is not more effective than an intermediate one for optimal protection against lipid peroxidation and this can led to lower PUFA in tissues. But, Cherian *et al*. (1996) observed a significant increase in the content of 20:5 ω-3 and 22:6 ω-3 fatty acids in the yolk, adipose tissue, and white meat of chickens as a result of vitamin E supplementation. Surai and Sparks (2000) suggested that vitamin E supplemented in diets enriched with fish oil increased DHA accumulation in most tissues. In other studies, adding different levels of α-tocopherol in diet did not show significant difference on yolk fatty acids, however, this supplementation when compared with control group could reduce linolenic, EPA, DPA, DHA and ω-3 fatty acids (Galobart *et al*. 2001). A high dose of α-tocopherol can act as prooxidant and also can disorder fatty acids intestinal absorption. Similar to our results about thigh muscle, Carrillo *et al*. (2008) reported that marine algae could increase saturated fatty acids (SFA) in hen's eggs.

Meat stability

Along with increasing polyunsaturated fatty acids in the diet, we saw an increase in chicken's meat's MDA that led to oxidative deterioration during storage. Adding fish oil to diet decreased oxidative stability in poultry meat. Surai and Sparks (2000) showed that diet enriched with fish oil increased oxidation index in nearly all tissues of cockerels, because TBARS accumulation resulted in lipid peroxidative increase. The oxidative stability of poultry meat depends on vitamin E content in meat phospholipids that is dependent on the vitamin E added to the diet which directly influence the lipid oxidation stability of the meat and processed meat products (Wen *et al*. 1997). Narciso-Gaytán *et al*. (2011) have shown that the high supplementation of vitamin E (200 mg/kg) was effective in inhibiting the development of lipid oxidation than what commercial level used (42 mg/kg), especially in thigh meat. Thus, it could be concluded that the higher deposition of α-tocopherol in the muscle tissues, caused by the high supplemental level of vitamin E, had higher antioxidant activity in the meat. As reported in previous studies, Rahmani *et al*. (2008) showed that diets supplemented with high levels of vitamin E significantly reduced MDA in the thigh and breast meats at different storage times.

Avila-Ramos *et al*. (2012) have shown that vitamin E supplementation had significant effect on the lipid oxidation stability of cooked chicken breast meat having used dietary oregano essential oil and also reported that the antioxidant effect of vitamin E was higher with a higher supplementation level. Addition of 10% marine algae to diet also led to significant decrease in MDA. Not enough significant difference was seen between FE and F 10% A.

This can be due to the antioxidant property of algae. No significant differences were observed between the samples of thigh and breast meat stability in three days. Results in this study showed dietary marine algae and vitamin E can delay lipid peroxidation in breast and thigh muscles of chickens.

Similar studies have reported that use of grape pomace in chicken's diet (Goni *et al*. 2007) and supplementation of tea powder in broiler diet (Eid *et al*. 2003) could delay lipid peroxidation.

In another study Xiao *et al*. (2011) compared the antioxidant activity of the vitamin E and an algae-based antioxidant. The results of their experiment indicated that the biological roles of VE, including its activity as an antioxidant, can be greatly mimicked at the transcriptional level by algae-based antioxidant, and they suggest a synergistic relationship between VE and algae-based antioxidant in the broiler diets.

In other point of view it has been shown that the genes altered by VE that were related to immune function, lipid metabolism, carbohydrate metabolism, drug metabolism, vitamin and mineral metabolism may play key roles related to the activities of VE supplementation. In a study by Xiao *et al*. (2011) on pathway analysis of the genes alteration by VE supplementation, it was detected that gene networks are associated with cells cycling, morphology, cellular assembly and organization, nervous system development, and skeletal - muscular development and function. This indicates extensive effect of VE supplementation at the transcriptional level.

Serum parameters

In current study marine algae reduced blood cholesterol. This might be due to its sterols and polysaccharides content (alginic acid, fucoidan, cellulose, xylose, glucouronic acid) (Jimenez-Escrig and Cambrodon, 1999). Phenolic acids also play an important role in liver cholesterol catabolism (Yugarani *et al*. 1992), because it inhibits intestinal absorption of fat (Ikeda *et al*. 1992).

The researchers reported reduction in cholesterol levels in rats following feeding with seaweed (Dvir *et al*. 2000). In another study, red algae reduced cholesterol in eggs (Ginzberg *et al*. 2000). Fiber in diet can decrease cholesterol level due to bile acids and lipids adsorption. Addition of fish oil and vitamin E has no effect on serum cholesterol, so there was no difference between F, E, FE and C groups. Supplementation of diets with 5 and 10% alga reduced triglyceride levels in grower and finisher periods, but vitamin E and fish oil did not affect this parameter. In agreement with our results, Surai and Sparks (2000) showed that fish oil and vitamin E had no effect on serum triglyceride level significantly.

CONCLUSION

In conclusion, fish oil supplementation in diet increased ω-3 fatty acids, especially long chain fatty acids in broiler's meat. Supplementation of *Sargassum sp.* also significantly increased accumulation of ω-3 fatty acid in chicken's meat and helped meat stability during the storage time. Addition of vitamin E in broiler diet increased meat stability and decreased MDA after 3 and 6 days of the storage.

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