The effect of oregano and rosemary essential oils or alpha-tocopheryl acetate on performance and lipid oxidation of meat enriched with n-3 PUFA's in broilers

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Abstract

The study was conducted to compare the effects of two essential oils, fed individually or in combination, with alpha-tocopheryl acetate (alpha-TA) on performance parameters and lipid oxidation of broiler meat enriched with n-3 PUFAs. Seven hundred and twenty day-old Hubbard-JV unsexed broiler chicks were used. Treatments included; control: without antioxidant; alpha-TA200: with alpha-TA at 200 mg/kg; OO150: with oregano essential oil (OO) at 150 mg/kg; OO300: with OO at 300 mg/kg; RO150: with rosemary essential oil (RO) at 150 mg/kg; RO300: with RO at 300 mg/kg; OO75+RO75: both OO and RO at 75 mg/kg; OO150+RO150: both OO and RO at 150 mg/kg. The feeding program consisted of a starter diet to 21 d and a grower diet to the end of the trial at 42 d. Both feeds contained 15 g fish oil/kg as the source of n-3 PUFAs. Birds fed alpha-TA200 had significantly higher weight gains at 42 d than those fed OO150, OO75+RO75 and OO150+RO150. However, there were no differences in carcass yield, feed intake, feed conversion ratio or mortality between treatments. Neither essential oil exerted any growth-promoting effect on performance, and there was no interaction between them. Antioxidants retarded lipid oxidation as measured in TBARS, but the effect of each differed according to storage time (0, 3, 6, 9, 12 and 15 d) and meat type (breast or thigh). Alpha-TA200, RO300 and combined essential oils (EO) had higher antioxidant effects in breast meat, and OO150+RO150 in thigh meat, than the other treatments with antioxidant at zero time of storage. The ability of natural antioxidants to inhibit lipid oxidation to the end of the storage period was, in decreasing order, combinations of EOs > alpha-TA200 = OO300 = RO150 = RO300 > OO150 in breast meat; and combinations of EOs > RO at both levels > alpha-TA200 = OO150 = OO300 in thigh meat. Combinations of EOs had a greater effect than those fed individually or alpha-TA200 on inhibiting lipid oxidation, and protecting alpha-TA concentration in refrigerated meat enriched with n-PUFAs stored for 15 d. Thigh meat was more susceptible to lipid oxidation than breast meat. The combination of OO and RO, at 150 mg/kg, proved as effective as alpha-TA in retaining the sensory qualities of breast meat after 15 d storage, and was more effective than when these EOs were fed individually or at 300 mg/kg. There is a possible synergistic effect between oregano and rosemary essential oil in preventing lipid oxidation in stored meat enriched with n-3 PUFAs.

Keywords: Oregano, rosemary essential oils, herbs, performance, lipid oxidation, broiler meat, n-PUFAs
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Introduction

There is growing concern about the danger that antimicrobials in livestock diets might contribute to the growing list of antibiotic-resistant human pathogens (Madrid *et al*., 2003; Moser *et al*., 2003). The use of antibiotics has been severely curtailed in the European Union. As a consequence, new commercial additives of plant origin, considered to be natural products that consumers would accept, have been proposed to poultry producers. Essential oil or extracts from herbs and spices have received considerable attention as replacements for antibiotic growth promotants (Deschepper *et al*., 2003).

The antibacterial and anticoccidial effects of essential oils, or components from plant extracts, have received widespread attention and numerous reports exist in the literature. For example, Giannenas *et al*. (2003) determined that oregano essential oil exerted an anticoccidial effect against *E. Tenella* in broilers; Jamroz *et al*. (2003) found that plant extract (carvacrol, cinnamaldehyde and capsaicin) reduced the total *E. Coli* and *Clostridium perfringes* numbers in the intestines of broiler chickens; and Mitsch *et al*. (2004) reported that blends of essential oil components can control *Clostridium perfringens* colonization in the intestine and faeces of broiler chickens. Many studies have also been conducted on the effects of dietary essential oils or combinations thereof on the performance of poultry but with varying and conflicting results**.** While some reports (Hertrampf, 2001; Alçiçek *et al*., 2003) demonstrated that essential oils improved animal

performance, some researchers (Botsoglou *et al.*, 2003; Papageorgiou *et al.*, 2003) reported that these additives were not effective in this regard.

Essential oils derived from herbs and spices have also been studied for antioxidant properties in poultry meat (López-Bote *et al.*, 1998; Botsoglou *et al.*, 2002; Papageorgiou *et al.*, 2003). In recent years, consumers have become more concerned about functional foods such as eggs and meat enriched with n-3 polyunsaturated fatty acids (PUFAs), as they play an active role in the prevention and management of pathologies such as coronary heart disease, hypertension and type 2 diabetes (Simopoulos, 2000). Scientific studies have indicated that the n-3 PUFA concentration of poultry products may be increased by the addition of fish oil, flax seed, linseed oil and marine algae (López-Ferrer *et al.*, 1997; 1999; Basmacıoğlu *et al.*, 2003; Sirri *et al.*, 2003). However, an increase in the amount of n-3 PUFAs in foods, especially docosahexaenoic (DHA) and eicosapentaenoic (EPA), may confer greater susceptibility to lipid oxidation, and oxidative deterioration adversely affects the sensory quality of products, including odours or flavours during storage (Gonzalez-Esquerra & Leeson, 2000; 2001). Previous studies have indicated that lipid oxidation may be prevented by dietary plant extracts or essential oils in feeds for laying hens and muscovy ducks (Galobart *et al.*, 2001; Schiavone *et al.*, 2001). However, no information is available in the literature about the effect of essential oils on oxidative stability of broiler meat enriched with n-3 PUFAs. In addition, although essential oils were used either individually or as mixtures in many studies, the synergistic or antagonistic effects between essential oils have not been considered in the same study. Schiavone *et al.* (2001), who used rosemary and orange extracts at level of 400 mg/kg, suggested possible synergistic effects should be investigated between essential oils or extracts in further studies. The aims of this study were to investigate the individual and combined effects of two dietary essential oils, oregano and rosemary, on broiler performance, and to compare the efficacy of n-3 PUFAs and alpha-tocopheryl acetate (alpha-TA) on lipid oxidation and the sensory quality of breast and thigh meats.

Materials and Methods

Seven hundred and twenty unsexed day-old Hubbard-JV broiler chicks, obtained from a commercial hatchery, were weighed and randomly assigned to eight groups of 90 birds each. Each group was further subdivided into three sub-groups (replicates) of 30 birds. Each of the 24 sub-groups was housed in a floor pen (10 birds/m^2) equipped with wood shaving litter. The lighting regimen provided 24 h of light per day. Birds were given starter diet to 21 d and a grower diet thereafter, to 42 d. The experimental diet in mash form and water were provided *ad libitum* during the trial.

The control group were given a commercial basal diet supplemented with a premix containing 50 mg alpha-TA/kg. The ingredient and chemical composition of the diets is presented in Table 1. Two essential oils, derived from selected *Origanum onites* sp. and *Rosamarinus officinalis* L. growing wild in Turkey, were used in the study. Diets, iso-energetic and iso-nitrogenous, were formulated to meet the NRC (1994) requirements. The diets were supplemented with: no antioxidant (control), alpha-TA at 200 mg/kg feed (alpha-TA200), oregano essential oil at 150 mg/kg (OO150), oregano essential oil at 300 mg/kg (OO300), rosemary essential oil at 150 mg/kg (RO150), rosemary essential oil at 300 mg/kg (RO300), oregano essential oil at 75 mg/kg + rosemary essential oil at 75 mg/kg (OO75+RO75), and oregano essential oil at 150 mg/kg + rosemary essential oil at 150 mg/kg (OO150+RO150). All experimental diets contained fish oil (15 g/kg) to increase n-3 PUFA content of broiler meat. The fatty acid composition of fish oil and the basal diets used in the study, and breast and thigh meat obtained from birds fed diets enriched with n-3 PUFAs, are presented in Table 2.

Broilers were weighed individually at 0, 21 and 42 d, and feed intake per pen was recorded. Sex was determined at 42 d. Data were coded according to sex, and the effect of sex was included in the model. Feed conversion ratios were calculated by dividing feed intake by weight gain from 0 to 21, 21 to 42 and 0 to 42 d. Mortality was recorded daily and used to adjust the total number of birds to determine the total feed intake per bird. At 42 d, six males and six females were selected from each group (two males + two females/sub-group), weighed and slaughtered under commercial conditions. Carcasses were trimmed, and then breast and thigh muscle samples per sub-group were weighed separately and carcass yield was calculated. Breast and thigh meats were then vacuum-packaged and stored at –40 ^{o}C until required for analyses of fatty acids, lipid oxidation, alpha-Tocopherol (alpha-Toc) and sensory properties. Breast and thigh meat samples were thawed overnight at 4 ^{o}C. Meat samples were minced through a 4 mm die, wrapped in film, and stored at 4 ^{o}C for up to 15 d.

Table 1 The ingredient and chemical composition (g/kg) of the starter and grower diets

	Starter diet (0 to 21 d)	Grower diet (22 to 42 d)
Ingredients		
Maize	473.6	485.5
Wheat	100.0	100.0
Soyabean meal	231.5	127.9
Full-fat soyabean	135.0	225.0
Fish oil	15.0	15.0
Dicalcium phosphate	19.6	20.7
Limestone	11.7	11.9
Salt	2.4	3.1
Mineral premix*	1.0	1.0
Vitamin premix**	2.5	2.5
Anticoccidial	1.0	-
DL-methionine	4.0	4.0
L-Lysine HCl	2.7	3.4
Composition (analysed)		
Dry matter	898.0	896.8
Crude protein	219.6	197.6
Ether extract	62.4	77.6
Crude fibre	31.2	29.7
Crude ash	55.7	55.3
Starch	348.0	364.3
Sugar	36.3	30.0
Calcium	9.6	10.0
Total phosphorus	7.3	7.4
Calculated analysis		
Lysine	13.9	13.9
Methionine + Cystine	10.8	10.4
Metabolisable energy, MJ/kg	12.6	13.0

*Provides per kg of diet: Mn, 80 mg; Zn, 60 mg; Fe, 60 mg; Cu, 5 mg; Co, 0.2 mg; I, 1 mg; Se, 0.15 mg; choline chloride, 200 mg

**Provides per kg of diet: Vitamin A, 12 000 IU; vitamin D_3, 2 400 IU; vitamin E, 50 mg; vitamin K_3, 4 mg; vitamin B_1, 3 mg; vitamin B_2, 6 mg; niacin, 25 mg; calcium-D-pantothenate, 10 mg; vitamin B_6, 5 mg; vitamin B_{12}, 0.03 mg; D-biotin, 0.05 mg; folic acid, 1 mg

Lipid oxidation was determined at 0, 3, 6, 9, 12 and 15 d. A modification of the 2-thiobarbituric acid method (Ke *et al*., 1977) was used, and was expressed as 2-thiobarbituric acid reactive substances (TBARS) numbers (mg malondialdehyde (MDA)/kg sample). Ten grams of a sample were homogenized with distilled water in a blender and transferred to a Kjeldahl flask and distilled adding 2.5 mL, 4N HCl and 1 mL of Antifoam A for up to distillate aggregation. Thiobarbituric acid (TBA) reactant was added to 5 mL of distillate and incubated in a water bath for up to 30 min. The final solution and blank were measured spectrophotometrically at 538 nm. Absorbance value obtained, was multiplied by 7.8. Final value was mg malondialdehyde (MDA)/kg sample.

Total lipid concentration of the basal diets and broiler meats was analysed using a modification of the method of Bligh & Dyer (1959). The final lipid fraction was concentrated in a rotary evaporator at 50 °C under reduced pressure and then evaporated to dryness using nitrogen (N_2) atmosphere. The total lipid yield was quantified gravimetrically and all lipid determinations were done in triplicate. Total volatile matter; 5.0 g of broiler meat lipid was dried at 110 °C during 24 h and analyzed according to AOAC (1999).

Fatty acid methyl esters (FAMEs) of feed and broiler meat were determined on extracted lipids of feed and broiler meat by esterification reaction with 140 g/kg boron trifluoride (BF3)- methanol complex according to Tokuşoğlu & Ünal (2003). Extracted lipids from the samples were refluxed using 0.05 N NaOH with methanol and were then derivatised with Boron trifluoride (BF3)- methanol complex. This solution was then fractionated with saturated NaCl; the methyl ester (ME) phase was separated, and anhydrous sodium sulphate (Na_2SO_4) added to the final solution. Prior to injection into GC, the final extract was degassed for

up to 2 min to eliminate oxygen. One µL of extract was injected into the GC and fatty acid profiles were determined by gas-liquid chromatography [Perkin Elmer (Auto System) Gas Chrom, column:SGE (BP70X GC column) (60 m-capillary; 0.25 µm film thickness; 0.25-mm diameter WCOT fused-silica), detector: flame ionization detector (FID), column temp.: ramp 1:165 °C (increase with 1 °C/min) (2 min isotherm), ramp 2:225 °C (increase with 2 °C/min) (30 min isotherm), detector temp.: 250 °C, injector temp.: 250 °C, elution time: 35 min., inject. amount: 2 µL, split ratio:50:1, carrier gas: Helium (He), flow rate: 1.5 mL/min].

Table 2 Fatty acids composition of fish oil, basal diets and broiler breast and thigh meat

Fatty acids (% of FAs)	Fish oil	Diet (0 to 21d)	Diet (21 to 42 d)	Breast meat[1]	Thigh meat[1]
$C_{14:0}$	8.97	0.09	0.09	0.87 ± 0.01	0.91 ± 0.01
$C_{16:0}$	20.18	9.88	9.78	22.80 ± 0.05	22.77 ± 0.02
$C_{16:1}$	7.92	0.16	0.17	5.32 ± 0.07	5.26 ± 0.05
$C_{18:0}$	4.36	2.18	2.22	6.37 ± 0.05	6.56 ± 0.10
$C_{18:1}$	17.09	19.12	19.15	30.13 ± 0.01	30.28 ± 0.04
$C_{18:2n-6}$	1.91	53.10	56.78	12.84 ± 0.05	12.76 ± 0.09
$C_{18:3n-3}$	2.16	1.73	1.68	0.82 ± 0.01	0.88 ± 0.02
$C_{18:4n-3}$	2.21	-	-	-	-
$C_{20:4n-6}$	0.54	0.21	0.20	0.85 ± 0.06	0.82 ± 0.03
$C_{20:5n-3}$	9.86	2.00	2.00	1.22 ± 0.01	1.25 ± 0.01
$C_{22:5n-3}$	1.57	0.06	0.05	0.14 ± 0.03	0.13 ± 0.01
$C_{22:6n-3}$	17.92	8.89	9.00	4.39 ± 0.02	4.51 ± 0.06
Σ SFA	33.51	12.15	12.09	30.04 ± 0.11	30.24 ± 0.13
Σ MUFA	25.01	19.28	19.32	35.45 ± 0.08	35.54 ± 0.09
Σ PUFA	36.17	65.99	69.71	20.26 ± 0.18	20.35 ± 0.21
Σ n-3	33.72	12.68	12.73	6.57 ± 0.07	6.77 ± 0.10
Σ n-6	2.45	53.31	56.98	13.69 ± 0.11	13.58 ± 0.12
Σ n-6/n-3	0.07	4.20	4.48	2.08 ± 0.02	2.01 ± 0.01
Total lipid (g /100 g)	99.92	58.84	58.76	15.06 ± 0.27	16.72 ± 0.06

FAs: Fatty acids; SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid
[1]Mean values (n = 96) ± s.e.m.

Vitamin E (alpha-tocopherol) was determined on d 0, 3, 6, 9, 12 and 15, according to the modified method of Tokuşoğlu *et al*. (2003): 20 g of broiler meat was homogenized with 20 mL of chloroform/methanol (2:1 v/v) during 10 min at 0 °C. Homogenate was vortexed for up to 20 s and centrifuged at 4000 rpm for 10 min. Chloroform phase including extracted lipids was separated, and the remainder was homogenized three times with the above-mentioned procedure. Subsequent filtrates were collected and concentrated in a rotary evaporator at 25 °C and then evaporated to dryness using nitrogen (N_2) atmosphere. Dried eluate was suspended with 300 µL of chloroform/methanol (2:1 v/v) mixture and final extract was obtained. Anhydrous sodium sulphate (Na_2SO_4) was added to final extract and stored at -28 °C. Prior to injection into the HPLC, the final extract was degassed for up to 5 min and filtrated with 0.5 and 0.45 µm Acrodisk filters. Ten µL of extract was injected into the HPLC. High performance liquid chromatographic conditions were performed as suggested by Tokuşoğlu *et al*. (2003) for the alpha-Toc analysis [Equipment: Hewlett Packard (HP-1100 ChemStation Software); column: Hypersil-ODS column (250 x 4.6 mm, 5-µm, Phenomenex, CAL, USA); detector: UV Absorbance detector (296 nm.); mobile phase: n-hexane/ethyl acetate (90:10 v/v); sensitivity: 0.05 A.U.F.S; flow rate: 1.2 mL/min; column temperature: 25 °C; injected amount: 10 µL; analysis type: isocratic analysis].

Sensory qualities were obtained with 15 trained panellists, using two sensory scales. They measured taste, odour, aroma intensity, juiciness, tenderness and overall acceptability in breast meat samples at the start and end of storage, using the Descriptive Analysis Sensory Method of Williams & Damron (1998). An eight-point sensory scale for juiciness-aroma intensity-tenderness was applied, as was a six-point scale for taste-odour-overall acceptability (Table 3). A modified hedonic scoring scale was employed in which panellists evaluated two samples simultaneously. This was done to determine whether panellist could detect differences within and between treatments. Panellists were also requested to identify and compare any off-

flavours detected, such as rancid, bitter, metallic or other unique off-flavours. Panellists received a total of 2 h refresher training prior to performing these sensory evaluations. They were instructed to eat unsalted crackers and to drink water between each sample to clear their palate, and pause for 20 s between samples. Eight sessions were conducted per treatment and each was done in duplicate (n = 2).

Table 3 Description of the two scales used to test sensory qualities of breast meat

Scale	Juiciness-aroma intensity-tenderness	Taste-odour-overall acceptability
8	Extremely juicy/intense/tender	
7	Very juicy/intense/tender	
6	Moderately juicy/intense/tender	Excellent taste/pleasant odour/high overall acceptability
5	Slightly juicy/intense/tender	Good taste/standard odour/standard overall acceptability
4	Slightly dry/bland/tough	Slightly rancid taste/slightly rancid odour/acceptable limit
3	Moderately dry/bland/tough	Rancid-putrid taste/rancid odour/unacceptable limit
2	Very dry/bland/tough	Very rancid-putrid taste/rancid odour/unacceptable limit
1	Extremely dry/ bland/tough	Extremely rancid-putrid taste/ extremely rancid odour/certainly unacceptable limit

Standard proximate analysis techniques were used to determine the nutrient concentrations in the diets (Naumann & Bassler, 1993). Starch, sugar, total calcium and phosphorus were also analysed using the VDLUFA method (Naumann & Bassler, 1993). Metabolisable energy content of the diets was calculated based on chemical composition (Anonymous, 1991).

Data on body weight, body weight gain, feed intake, feed conversion ratio, carcass yield and mortality were analysed by JMP version 5.0 (SAS, 2002). The body weight, weight gain and carcass yield were analysed by a two-way analysis of variance, fixed effects model, including main effects of treatments (8 different) and sex (male-female), and interaction between the two factors. The results obtained for performance parameters were statistically analysed using Tukey's Multiple Range Test. Differences were considered to be significant based on the 0.05 level of probability. Regression analysis was conducted for performance parameters as follows:

Fitted terms: Constant+Oregano (OO)+Rosemary (RO)+ OO*RO.

The data for TBARS values, alpha-Toc and fatty acid concentrations were analysed by Statistica version 6.0 for Windows (STATISTICA, 1998) using the analysis of variance (ANOVA). In TBARS values and alpha-Toc concentration, fixed effects included main effects of treatments and storage time (six storage times), and interaction between the two factors. The results obtained for the lipid oxidation criteria were statistically analysed using Duncan's Multiple Range Test. Differences were considered to be significant, based on the 0.01 level of probability. Mean scores for sensory properties were calculated.

Results and Discussion

The effect of dietary supplementation of essential oils (EOs) and alpha-TA on body weight (BW), body weight gain (BWG) and carcass yield (CY) are presented in Table 4.

The dietary treatments had significant effects on BW and BWG ($P < 0.05$) but not on CY, nor were there any significant interactions between treatment and sex in these three variables. Except for birds fed RO150 and alpha-TA200, BW and BWG of birds fed diets with antioxidant did not differ ($P > 0.05$) from those on the control. RO150 decreased BW at 21 d and BWG in the period 0 to 21 d, and alpha-TA200 increased BWG in the period 21 to 42 d ($P < 0.05$). However, the negative effect of RO150 on BW disappeared at 42 d.

Table 4 The effect of including essential oils or alpha-tocopheryl acetate on body weight (BW), body weight gain (BWG) and carcass yield (CY)

	BW, g		BWG, g/d			CY, %
Treatments	21 d	42 d	0 to 21 d	21 to 42 d	0 to 42 d	
Control	746 ± 8.7^{ab}	2115 ± 21.2^{abc}	33.5 ± 0.42^{ab}	67.1 ± 0.87^{bcd}	50.3 ± 0.50^{abc}	73.4 ± 0.58
Alpha-TA200	753 ± 8.2^{a}	2231 ± 19.8^{a}	33.0 ± 0.39^{a}	70.4 ± 0.81^{a}	52.1 ± 0.47^{a}	75.0 ± 0.42
OO150	717 ± 8.8^{bc}	2120 ± 21.4^{abc}	32.1 ± 0.42^{bc}	66.8 ± 0.88^{bcd}	49.5 ± 0.51^{bc}	74.5 ± 0.36
OO300	744 ± 8.5^{ab}	2174 ± 20.6^{ab}	33.9 ± 0.40^{ab}	68.1 ± 0.84^{abcd}	50.8 ± 0.50^{ab}	74.4 ± 0.67
RO150	711 ± 8.5^{c}	2179 ± 20.5^{ab}	31.9 ± 0.40^{c}	69.9 ± 0.84^{ab}	50.9 ± 0.49^{ab}	74.5 ± 0.52
RO300	726± 8.2^{abc}	2182 ± 19.7^{ab}	32.6 ± 0.39^{abc}	69.3 ± 0.81^{abc}	51.0 ± 0.47^{ab}	74.0 ± 0.46
OO75+RO75	713 ± 8.6^{bc}	2088 ± 20.8^{c}	32.0 ± 0.41^{bc}	65.5 ± 0.85^{d}	48.7 ± 0.49^{c}	74.3 ± 0.50
OO150+RO150	726 ± 8.5^{abc}	2125 ± 20.5^{bc}	33.0 ± 0.40^{abc}	66.2 ± 0.84^{cd}	49.6 ± 0.49^{bc}	74.9 ± 0.66
P value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.46
Regression analysis			**Estimate ± s.e.$^{(t\ pr.)}$**			
Constant	-	-	32.6 ± 0.40 **	68.0 ± 0.85 **	50.3 ± 0.48 **	74.3 ± 0.33**
OO	-	-	0.02 ± 0.05	-0.05 ± 0.11	-0.07 ±0.13	0.00 ± 0.00
RO	-	-	-0.07 ± 0.05	0.01 ± 0.11	0.02 ± 0.13	-0.00 ± 0.00
OO*RO	-	-	0.00 ± 0.00	-0.00 ± 0.00	-0.00 ±0.00	0.00 ± 0.00
F pr.	-	-	0.09	0.22	0.38	0.75
R^2	-	-	0.60	0.20	0.14	0.01

^{a,b,c,d}means in the same column with common superscripts do not differ (P > 0.05)
**P < 0.01; Mean values ± s.e.m.

Birds fed alpha-TA200 had higher (P < 0.05) BW and BWG than those fed EOs (individual or combination) at a level of 150 mg/kg from 0 to 21 d. During the periods 21 to 42 and 0 to 42 d, BWG of birds fed the diet containing alpha-TA200 were higher (P < 0.05) than those of birds on diets containing oregano essential oil at 150 mg/kg (OO150) and both essential oil combinations at 150 and 300 mg/kg (OO75+RO75 and OO150+RO150). Regression analyses of the responses to the EOs (Table 4) indicate that there were no significant trends in BWG or CY as a result of the addition of EO at the doses used, nor was there a significant interaction between the EOs.

The effect of dietary supplementation with EOs and alpha-TA on feed intake (FI) and feed conversion ratio (FCR) from 0 to 21, 21 to 42 and 0 to 42 d, and mortality (MT) during the trial is presented in Table 5.

There were no differences (P > 0.05) in FI, FCR or MT between treatments. Regression analysis (Table 5) again proved that the addition of both essential oil types had no significant effect on food intake or FCR, and that there was no interaction between these EOs (P > 0.05).

Studies on the use of essential oils or oil combinations have yielded inconsistent results. Some researchers (Lee *et al*., 2003; Botsoglou *et al*., 2004), who used a mixture of essential oils, and Schiavone *et al*. (2001), Botsoglou *et al*. (2002) and Papageorgiou *et al*. (2003), who used rosemary and orange extracts at levels of 400 mg/kg in muscovy duck, oregano essential oils at levels of 100 and 200 mg/kg in turkeys, oregano essential oil at levels of 50 and 100 mg/kg in broiler, respectively, found that essential oils or oil combinations did not improve poultry performance. In contrast, Hertrampf (2001) noted that essential oil, isolated from oregano, supplementation to the drinking water (300 mL/1000 L) improved the performance of chickens, and Alçiçek *et al*. (2003) reported that a combination of essential oils improved chicken performance. Hernández *et al*. (2004), who used two plant extracts (essential oil extract from oregano, cinnamon, pepper, and Labiatae extract from sage, thyme and rosemary), reported that both plant extracts improved the digestibility of feeds for broilers, and that this improved the performance slightly. It is possible that the positive effect of plant extracts has been obtained only under unhygienic conditions (Giannenas *et al*., 2003) and this may explain the lack of response in the present study. Environmental conditions, the digestibility and composition of diets, varieties of herbs and species, and the region in which the plants are grown could all influence the results of such studies. In addition, Lee *et al*. (2003) reported that essential oil components, which are thymol and its isomer, carvacrol, have different effects on growth performance, and this may have an impact on the use of essential oils as growth enhancers.

Table 5 The effect of the inclusion of essential oils (EOs) and alpha-tocopherol acetate (alpha-TA) on food intake (FI) (as fed), feed conversion ratio (FCR) (g feed/g gain) and mortality (MT) (%) of broilers

	FI, g/bird			FCR, feed/g gain			MT, %
Treatments	0 to 21 d	21 to 42 d	0 to 42 d	0 to 21 d	21 to 42 d	0 to 42 d	0 to 42 d
Control	1085	2807	3893	1.56	2.00	1.86	5.56
alpha-TA200	1061	2861	3922	1.52	1.95	1.81	1.11
OO150	1006	2653	3658	1.52	1.90	1.78	5.56
OO300	1041	2742	3784	1.53	1.98	1.83	2.22
RO150	1068	2809	3877	1.62	1.91	1.82	1.11
RO300	1100	2735	3835	1.63	1.86	1.79	1.11
OO75+RO75	1004	2619	3624	1.51	1.91	1.78	2.22
OO150+RO150	1097	2726	3823	1.61	1.97	1.85	0.00
s.e.m.	38.52	53.14	83.30	0.06	0.05	0.05	1.57
				P value			
Treatment	0.49	0.08	0.18	0.64	0.46	0.86	0.16
Regression analysis				**Estimate ± s.e.$^{(t\ pr.)}$**			
Constant	1051±24.0**	2792±39.4**	3843±58.5**	1.54±0.03**	1.96±0.03**	1.82±0.03**	3.60±1.04**
OO	-0.10±0.15	-0.36±0.25	-0.46±0.37	-0.00±0.00	-0.00±0.00	-0.00±0.00	-0.00±0.00
RO	0.14±0.15	-0.17±0.25	-0.04±0.37	0.00±0.00	-0.00±0.00	-0.00±0.00	-0.01±0.01
OO*RO	0.00±0.00	-0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	-0.00±0.00
F pr.	0.46	0.44	0.60	0.18	0.46	0.67	0.21
R^2	0.12	0.12	0.09	0.21	0.12	0.07	0.20

Mean values in columns do not differ (P > 0.05); **P < 0.01

The total n-3 PUFA concentration of breast and thigh meat was 65.7% and 67.7%, respectively. Levels of EPA and DHA were 12.2% and 43.9% in breast meat and 12.5% and 45.1% in thigh meat, respectively. Previous studies concerning the possibility of enriching the n-3 PUFAs in poultry products have shown fish oil to be the most effective for this purpose (Hargis & Van Elswyk, 1993; Leskanich & Noble, 1997; López-Ferrer *et al*., 1999; Schiavone *et al*., 2001). The effect of dietary treatments on the lipid oxidation (TBARS values) of breast and thigh meat enriched with n-3 PUFAs at zero time and at the end of storage is given in Table 6.

Table 6 The effect of treatments on the lipid oxidation of breast and thigh meat (TBARS, mg MDA/kg meat[1])*

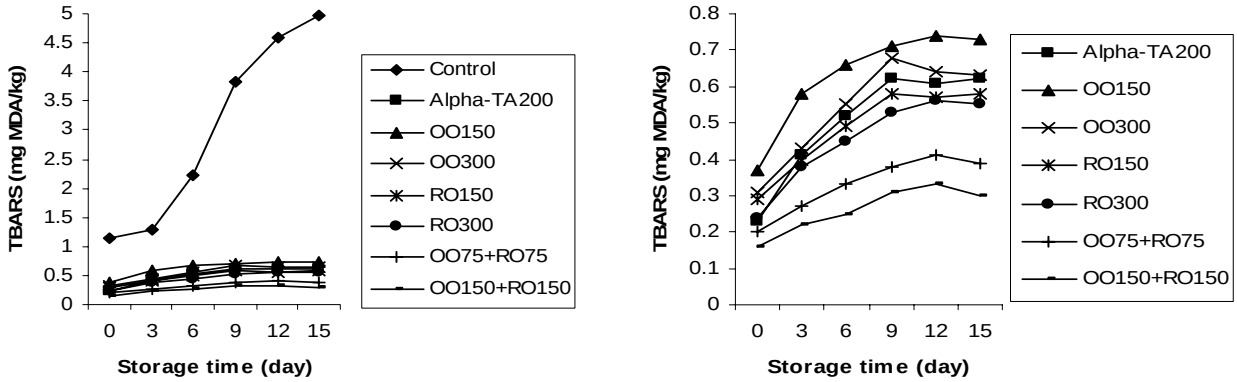
	Breast meat		Thigh meat	
Treatments	**Day 0**	**Day 15**	**Day 0**	**Day 15**
Control	1.13^{a} ± 0.04	4.97^{a} ± 0.09	1.25^{a} ± 0.0.6	4.99^{a} ± 0.05
Alpha-TA200	0.23^{d} ± 0.02	0.62^{c} ± 0.05	0.29^{c} ± 0.02	0.67^{b} ± 0.02
OO150	0.37^{b} ± 0.05	0.73^{b} ± 0.04	0.44^{b} ± 0.03	0.75^{b} ± 0.09
OO300	0.31^{c} ± 0.07	0.63^{c} ± 0.02	0.38^{b} ± 0.05	0.65^{b} ± 0.05
RO150	0.29^{c} ± 0.03	0.58^{c} ± 0.01	0.35^{b} ± 0.02	0.60^{c} ± 0.04
RO300	0.24^{d} ± 0.05	0.55^{c} ± 0.05	0.30^{c} ± 0.04	0.55^{c} ± 0.02
OO75+RO75	0.20^{d} ± 0.01	0.39^{d} ± 0.03	0.27^{c} ± 0.05	0.41^{d} ± 0.05
OO150+RO150	0.16^{d} ± 0.01	0.30^{d} ± 0.02	0.20^{d} ± 0.02	0.31^{e} ± 0.01
Effects	**P value**			
Treatment (T)	0.0001		0.0001	
Storage time (ST)	0.0001		0.0001	
T*ST	0.0001		0.0001	

$^{a-e}$ Means within columns with different superscript differ at P < 0.01; [1]Mean values (n = 12) ± s.e.m.

* TBARS: 2-thiobarbituric acid reactive substances numbers; MDA: mg malondialdehyde

Breast and thigh meat samples from all treatments enriched with antioxidant had lower ($P < 0.01$) TBARS values than those of the control during storage (Figure 1-A,B). This result agrees with the reports of López *et al*. (1998), who used rosemary, sage and alpha-TA, and of Schiavone *et al.* (2001), who used rosemary, peel of orange extracts and alpha-TA. The TBARS values for all treatments increased up to nine days of storage, but the highest rises in TBARS values over time were noted in the control breast and thigh meat samples.

The effect of treatment on TBARS values of meat samples differed depending on storage time (0, 3, 6, 9, 12 and 15 d) and meat type (breast or thigh). In breast meat, alpha-TA200, RO300 and treatments in which RO and OO were combined had higher ($P < 0.01$) antioxidant effects than the other treatments with antioxidant during the initial storage period (Table 6) and after 15 d of storage ($P < 0.01$). This is well illustrated in Figure 1. Whilst breast meats from alpha-TA200 supplemented treatment had lower ($P < 0.01$) TBARS values than those from OO150 at all storage time points, there were no differences among alpha-T200 and OO300 except for zero and nine days of storage (Table 6). Breast meat samples from treatment with RO used at 300 mg/kg contained similar TBARS values to those from alpha-TA200, except after six and nine d of storage.



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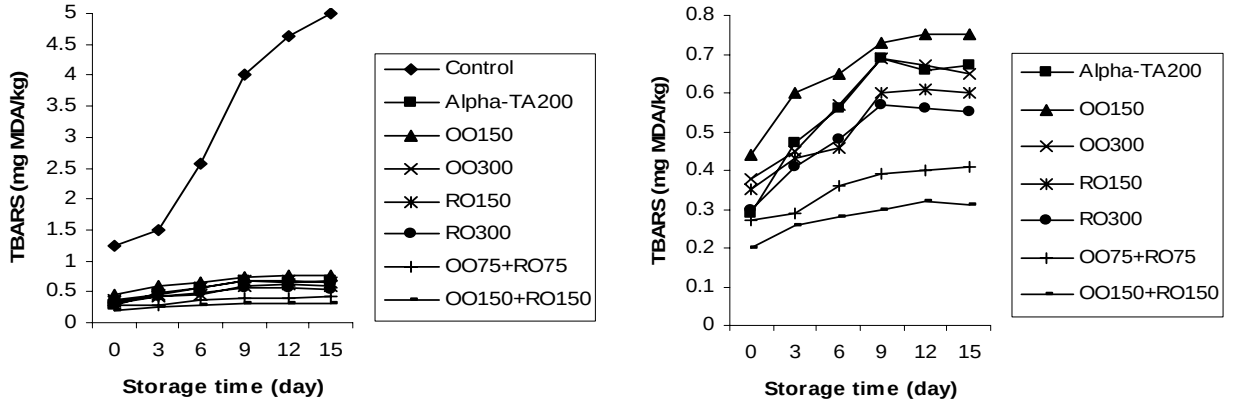

B

Figure 1 Effects of essential oils (EOs) and alpha-tocopheryl acetate (alpha-TA) on lipid oxidation (TBARS values) in breast meat (A) and thigh meat (B) enriched with n-3 polyunsaturated fatty acids (PUFAs) during 15 d of refrigerated storage

In thigh meat, the combined OO150+RO150 treatment was more effective than the other treatments on oxidative stability during storage (Table 6 and Figure 1). Whilst alpha-TA200, RO300 and OO75+RO75 treatments showed similar antioxidant activity at zero time of storage, RO and treatments in which RO and OO were combined had lower ($P < 0.01$) TBARS values than alpha-TA200 treatment at 3, 6, 9, 12 and 15 d. Alpha-TA200 demonstrated similar lipid stability as OO at both levels at 6, 9, 12 and 15 d of storage.

Rosemary essential oil had higher antioxidant activity than oregano essential oil in preventing lipid oxidation at all storage times for both types of meat (Figures 1 and 2). Some researchers found that the *in vitro* antioxidant activity of rosemary was higher than oregano (Martinez-Tome *et al*., 2001), whereas other researchers determined that oregano or thyme has a higher activity on lipid oxidation (Lee & Shibamoto, 2002). Thigh meat samples from treatments enriched with RO contained lower ($P < 0.01$) TBARS values than those from alpha-TA200 during storage, except for the initial storage, but the superior effect of RO compared with alpha-TA200 appeared after only 6 and 9 d of storage in breast meat. In contrast, Schiavone *et al*. (2001) observed that the antioxidant effect of rosemary extract at 400 mg/kg was lower than supplemented alpha-TA in breast meat of muscovy duck. Alpha-TA200 had higher ($P < 0.01$) antioxidant activity than OO150 in breast meat during storage. However, this effect was not evident in thigh meat samples except for days zero and 3 of storage. When OO was used at the higher concentration, no significant differences were evident between alpha-TA200 and OO in both types of meat from the beginning of storage. Botsoglou *et al*. (2002) found that dietary alpha-TA supplementation at level of 200 mg/kg displayed greater antioxidant activity than oregano oil (50 and 100 mg/kg). However, these researchers determined that dietary oregano oil at level of 200 mg/kg showed an equivalent effect to dietary alpha-TA at 200 mg/kg in their further study (Botsoglou *et al*., 2003).

At the higher dose of essential oils, TBARS values were numerically lower at all storage times in both meat samples. However, the effect of dose on TBARS values differed statistically according to essential oil type, whether these were used individually or in combination, and storage time (Table 6). Some studies have shown that oregano essential oil at 200 mg/kg displayed greater antioxidant activity than at 100 mg/kg feed in chicken and turkey breast and thigh meat samples (Botsoglou *et al*., 2003; Papageorgiou *et al*., 2003).

In another study, rosemary extracts at 500 and 1000 mg/kg did not show differences in lipid stability in n-3 fatty acid enriched eggs (Galobart *et al*., 2001).

Mean alpha-Toc concentration in breast and thigh meats is presented in Table 7. There was a significant increase in alpha-Toc concentration in both meats as a result of supplementing the diet with alpha-TA at 200 mg/kg (Figure 2-A,B).

Table 7 The effect of treatments on alpha-Toc concentration of breast and thigh meats (mg/100 g meat[1])

	Breast meat		Thigh meat	
Treatments	**Day 0**	**Day 15**	**Day 0**	**Day 15**
Control	$0.25^{c} \pm 0.01$	$0.01^{d} \pm 0.00$	$0.10^{c} \pm 0.02$	$0.01^{d} \pm 0.00$
Alpha-TA200	$1.42^{a} \pm 0.06$	$1.32^{a} \pm 0.00$	$1.48^{a} \pm 0.03$	$1.09^{a} \pm 0.02$
OO150	$0.24^{cd} \pm 0.03$	$0.01^{d} \pm 0.00$	$0.11^{b} \pm 0.01$	$0.01^{d} \pm 0.00$
OO300	$0.25^{c} \pm 0.04$	$0.01^{d} \pm 0.01$	$0.11^{b} \pm 0.02$	$0.01^{d} \pm 0.00$
RO150	$0.26^{bc} \pm 0.02$	$0.05^{c} \pm 0.01$	$0.10^{c} \pm 0.01$	$0.04^{cd} \pm 0.02$
RO300	$0.27^{b} \pm 0.05$	$0.06^{c} \pm 0.00$	$0.11^{b} \pm 0.02$	$0.05^{c} \pm 0.01$
OO75+RO75	$0.26^{bc} \pm 0.10$	$0.07^{bc} \pm 0.01$	$0.11^{b} \pm 0.05$	$0.05^{c} \pm 0.03$
OO150+RO150	$0.26^{bc} \pm 0.03$	$0.08^{b} \pm 0.01$	$0.11^{b} \pm 0.02$	$0.07^{b} \pm 0.00$
Effects	**P value**			
Treatment (T)	0.0001		0.0001	
Storage time (ST)	0.0001		0.0001	
T*ST	0.0001		0.0001	

[a-d] means within columns with different superscript differ at $P < 0.01$; [1]Mean values ($n = 12$) ± s.e.m.

Breast meat samples from RO300 had higher alpha-Toc concentration than those from the other treatments with EOs at zero storage time. After 15 d of storage, RO used at both levels and in combination with OO increased alpha-Toc concentration of breast meat ($P < 0.01$). However, all treatments with EOs resulted in higher alpha-Toc concentration in thigh meat compared with the control before storage commenced. However, the superior effect of these treatments on alpha-Toc concentration disappeared after

15 d of storage, except for RO300 and treatments in which RO and OO were combined. Oregano essential oil did not affect alpha-Toc concentration in thigh meat samples compared with the control, except at the initiation of storage. In contrast, Botsoglou *et al*. (2003) found significantly higher alpha-Toc concentrations in frozen breast meats from turkeys fed oregano oil compared with controls. The combination of EOs was superior to the use of individual EOs and alpha-TA200 on TBARS values of breast and thigh meats from the onset of storage. No published article could be found in which a combination of RO with OO had been used for this purpose.

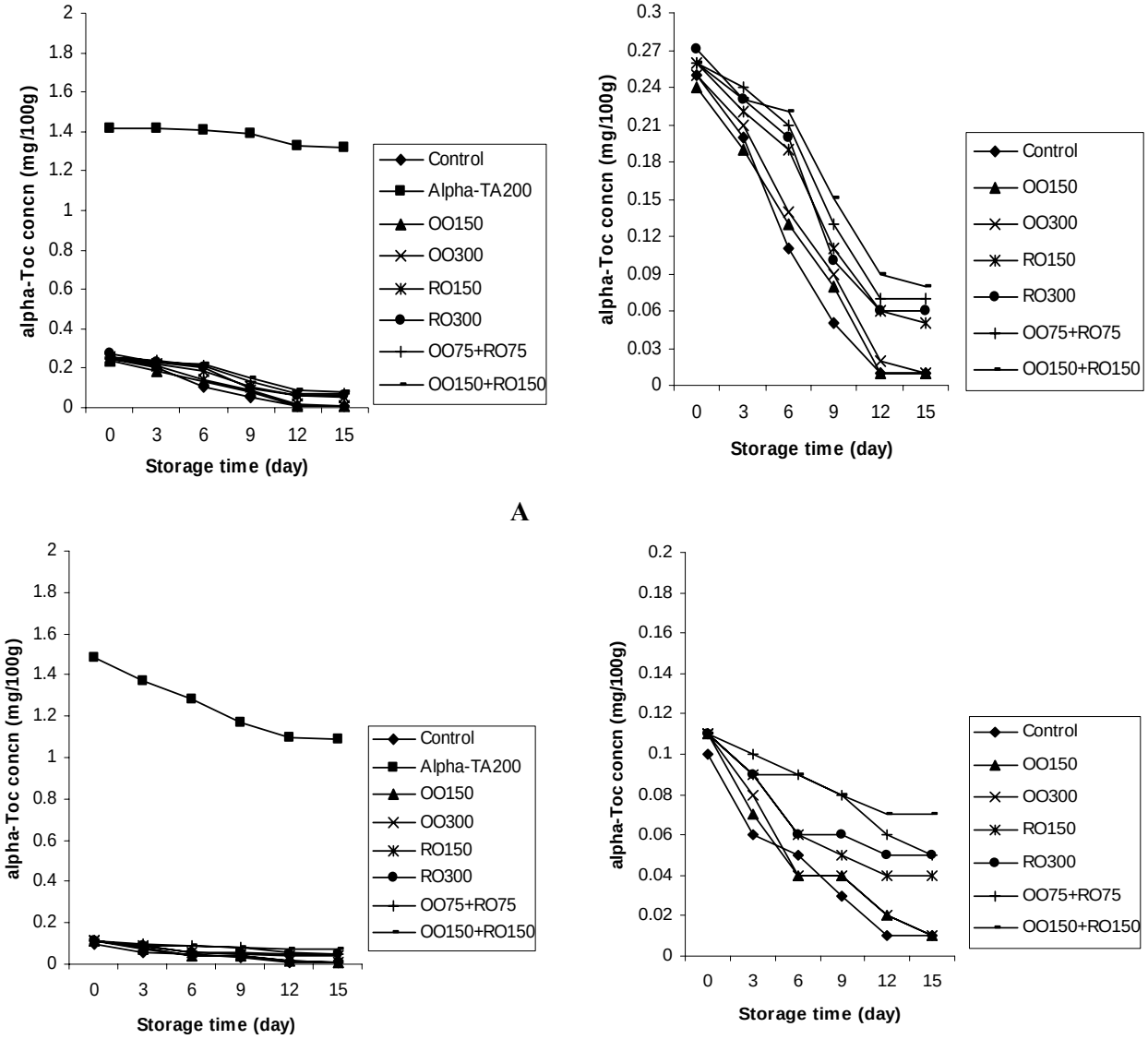

Figure 2 Effect of essential oils (EOs) and alpha-Tocopheryl acetate on alpha-Tocopherol (alpha-Toc) concentration (mg/100 g) in breast meat (A) and thigh meat (B) enriched with n-3 PUFAs during 15 d of refrigerated storage

Rosemary essential oil was more effective than oregano essential oil on alpha-Toc retention in meat samples. There was no effect of the level of supplementation of EOs on alpha-Toc concentration in the present study. Mean alpha-Toc concentration in meat samples from all treatments decreased with length of storage. In contrast to our result, Botsoglou *et al*. (2003) reported a positive correlation with supplementation level using oregano essential oil.

Treatments in which EOs were combined resulted in higher alpha-Toc concentrations in both breast and thigh than all other treatments. These treatment combinations proved to have antioxidant properties, thereby reducing the reliance on alpha-Toc as the antioxidant (Figures 1 and 2).

Thigh meat appeared to be more susceptible on day 0 to oxidation than breast meat (higher TBARS, Table 6), and this may be attributed to the higher total lipid (16.72 *vs*. 15.06 g/100g) and n-3 PUFAs (6.77% *vs*. 6.57%) contents in thigh meat. These results are in agreement with López-Ferrer *et al*. (1997) who reported that n-3 PUFAs are deposited in higher concentration in thigh meat than in breast meat when the n-3 PUFA content in the feed is increased. In contrast, Kralik *et al*. (2003) reported that n-3 PUFAs such as EPA and DHA are deposited in greater amounts in lipid of breast muscle than in thigh lipid, and total n-3 PUFAs were higher in breast meat than in thigh meat (6.02% *vs*. 3.71%). Some researchers have suggested that the lower oxidative stability of thigh meat is related to a higher absolute content of PUFAs with more than two double bonds in the fat (Jensen *et al*., 1995; Botsoglou *et al*., 2004). Ajuyah *et al.* (1993) reported that oxidative changes are more extensive in dark than in white meat due to the higher content in dark meat of lipids, phospholipids and PUFAs.

Thigh meat samples had lower alpha-Toc concentrations that did breast meat samples, in contrast to the reports by Papageorgiou *et al*. (2003) and Botsoglou *et al*. (2004). The lower alpha-Toc concentration of thigh meat measured in this study resulted in a lower oxidative stability in this meat. The results of the sensory analysis performed to evaluate the effect of treatment on breast meat quality after 0 and 15d of storage are given in Table 8.

Table 8 Effect of treatment on the sensory qualities of breast meat samples at sampling (0 d) and after 15 d of storage

Treatments	Taste		Odour		Juiciness		Aroma intensity		Tenderness		Overall acceptability	
	0 d	15 d	0 d	15 d	0 d	15 d	0 d	15 d	0 d	15 d	0 d	15 d
Control	5.51	1.89	5.42	1.27	4.58	6.87	5.13	2.88	6.59	5.87	5.76	2.37
Alpha-TA200	5.48	5.09	5.39	4.98	4.22	4.92	5.09	4.79	6.77	6.18	5.84	5.19
OO150	3.94	2.33	4.56	4.02	4.87	5.13	7.43	5.72	7.23	6.89	4.75	3.72
OO300	2.47	2.08	3.77	3.31	5.56	5.84	7.97	6.67	7.52	7.06	3.93	2.88
RO150	3.13	2.21	3.95	3.52	4.82	5.22	7.56	4.67	7.08	6.73	4.36	4.03
RO300	2.38	2.13	2.84	2.03	5.05	5.45	7.68	6.48	7.31	6.59	3.72	3.13
OO75+RO75	5.89	5.17	5.97	5.07	4.73	5.11	6.18	5.17	6.91	6.24	5.95	5.01
OO150+RO150	4.13	3.97	4.09	3.42	5.19	5.38	7.03	6.82	7.38	6.76	4.69	3.98
Pooled s.e.m.	0.06	0.05	0.03	0.02	0.09	0.06	0.02	0.03	0.05	0.02	0.03	0.04

Taste-odour-overall acceptability: Scored an a 1-6 scale, 6 = excellent taste/pleasant odour/high overall acceptability; 5 = good taste/standard odour/standard overall acceptability; 4 = slightly rancid taste/slightly rancid odour/acceptable limit; 3 = rancid-putrid taste/rancid odour/unacceptable limit; 2 = very rancid-putrid taste/rancid odour/unacceptable limit; 1 = extremely rancid-putrid taste/extremely rancid odour/certainly unacceptable limit

Juiciness-aroma intensity-tenderness: Scored on a 1-8 scale, 8 = extremely juicy/intense/tender; 7 = very juicy /intense/tender; 6 = moderately juicy/intense/tender; 5 = slightly juicy/intense/tender; 4 = slightly dry/bland/tough; 3 = moderately dry/bland/tough; 2 = very dry/bland/tough; 1 = extremely dry/bland/tough

Prior to conducting the descriptive sensory test, breast meat samples were assessed for fishy flavour, and it was determined that all treatments fortified with 15 g fish oil/kg feed did not exhibit a fishy flavour in the final product. In various studies at higher levels than that used in this study, unacceptable odours or flavours have been detected in meat of broilers fed fish oil (Edwards & May, 1965; López-Ferrer *et al*., 1997). Hargis & Van Elswyk (1993) reported that fish oils at concentrations greater than 10 to 20 g/kg in diets resulted in organoleptic problems in the final product.

Treatments with all antioxidants other than alpha-TA200 and OO75+RO75 caused deterioration in taste and odour at zero time of storage. As the dietary essential oil concentration increased, the deterioration was considerable. In spite of taste and odour deterioration in meats enriched with n-3 PUFAs, EO treatments did not reduce juiciness, aroma intensity or tenderness. The overall acceptability of breast meat samples from the control treatment were similar to those of alpha-TA200 and OO75+RO75 treatments at the start of the storage period, and these treatments were significantly better than other antioxidant treatments. However, the

deterioration in the acceptability of breast meat on the control treatment was considerably higher than that of alpha-TA200 and OO75+RO75 treatments by the end of the storage period (2.37 *vs*. 5.19 and 5.01). The deterioration in the sensory qualities of broiler meat enriched with n-3 PUFAs may be prevented by the addition of dietary alpha-TA and a combination of essential oils at a level of 150 mg/kg (OO75+RO75) during refrigerated storage.

Conclusions

The beneficial effects of essential oils on broiler performance reported in the literature were not evident in this study. However, the increased lipid oxidation resulting from the incorporation into the feed of n-3 PUFAs was prevented by the dietary essential oils, oregano and rosemary. A combination of these essential oils had a greater effect than when they were used individually, or where alpha-TA was used, in preventing lipid oxidation of broiler meat enriched with n-3 PUFAs. The combination of these essential oils, at 150 mg/kg, proved as effective as alpha-TA in retaining the sensory qualities of breast meat after 15 d storage, and was more effective than when fed at 300 mg/kg. We conclude that there is a possible synergistic effect between oregano and rosemary essential oils in reducing the rate of lipid oxidation during storage.

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