Dietary oregano (*Origanum vulgare* L.) aqueous extract improves oxidative stability and consumer acceptance of meat enriched with CLA and n-3 PUFA in broilers.

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ABSTRACT The effect of a dietary oregano aqueous extract on meat fatty acid profile, quality, and consumer acceptance in chickens fed a diet rich in polyunsaturated fatty acids (PUFA) was evaluated in 3 consecutive trials.

For each trial, 171 day-old Ross 308 chicks were randomly divided in replicates of 19 birds each and assigned to one of 3 experimental diets: 1) basal control diet, 2) basal diet supplemented with 0.2 g/kg of oregano aqueous extract, and 3) basal diet supplemented with 150 ppm of vitamin E (as positive control). To better analyze the antioxidant activity of both oregano and vitamin E, all the experimental diets were enriched with a fatty acid supplement consisting in a mixture of PUFA at the same dose (1.16 %) in both starter and finisher feeds. Oregano supplementation positively influenced ($P < 0.05$) broiler live performance. No differences were observed in physicochemical or proximal composition or in total fatty acid composition of breast meat. Dietary oregano influenced meat composition in terms of total phenolic content, antioxidant capacity, and thiobarbituric acid-reactive substances, improving meat resistance to oxidation, compared to both other groups. During consumer tests, meat from the 3 dietary groups obtained the same liking score in a blind session. Under informed condition, consumer perception was positively influenced by labeling for all the considered attributes. Furthermore, consumers showed a higher expectation for meat derived from chickens fed the oregano extract when compared to that deriving from the other 2 groups.

Results obtained in the present study allow stating that using oregano aqueous extract in diets enriched with PUFA can represent a valid solution to increase live weight of chickens, improve resistance to oxidation of meat, and positively influence consumer perception of poultry meat.

Key words: fatty acids, antioxidants, meat quality, consumer's choice, phytogenics

2018 Poultry Science 0:1–12
http://dx.doi.org/10.3382/ps/pex452

INTRODUCTION

The decrease of the omega 6 (n-6)/omega 3 (n-3) polyunsaturated fatty acid (**PUFA**) ratio in human diet is recognized as one of the challenges of modern agriculture (González-Ortiz et al., 2013; Salem and Eggersdorfer, 2015). It is well established that modern Western diets contain excessive amounts of n-6 PUFA, which can promote many diseases, including inflammatory and autoimmune disorders, cancer, and cardiovascular pathologies (Simopoulos, 2016). Low concentrations of n-3 PUFA in blood have been correlated also with poor cognitive performance and behavior in children (Montgomery et al., 2013).

Conjugated linoleic acids (**CLA**) are recognized to reduce cardiovascular diseases, positively influence body composition and bone health, and reduce risk of diabetes and cardiovascular diseases (Bhattacharya et al., 2006).

To enhance human consumption of these bioactive fatty acids (**FA**), several enriched foods, mostly animal products, are being produced either by adding PUFA directly during the finisher phase of rearing or by modifying animal diets. The poultry industry can be one of the most convenient sectors to reach this objective, considering that consumption of poultry meat is predicted to be 50 kg per capita worldwide in 2050 (Kearney, 2010). Many studies have already investigated the possibility to enrich chicken diet with different PUFA sources (Gonzalez-Esquerra and Leeson,

Received March 30, 2017.
Accepted January 9, 2018.
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2001; Lopez-Ferrer et al., 2001; Betti et al., 2009). It has been shown that the inclusion of fish or vegetable oils in high concentration in poultry diets can exert some negative effects, such as a compromised oxidative balance in live animals and a higher oxidative susceptibility of the derived meat (González-Ortiz et al., 2013). In addition, oxidation can negatively affect meat healthfulness, creating toxic compounds such as malondialdehyde (**MDA**) and cholesterol oxidation products (Wood et al., 2004). Moreover, fish and vegetable sources of PUFA can modify the organoleptic quality of meat (Betti et al., 2009), causing the recording of lower ratings when subjected to evaluation by the final consumer. The alternative could be the use of other feed additives rich in PUFA (Kalogeropoulos et al., 2010). To improve meat FA composition and to avoid off-flavors, the supplementation of rations with a FA supplement rich in CLA and docosahexaenoic acid (**DHA**) was successfully tested in a series of studies (Branciari et al., 2016; Panda et al., 2016)

To avoid, or defer oxidation both in feed and meat, synthetic antioxidants such as butylated hydroxyanisole (**BHA**) and butylated hydroxytoluene (**BHT**) have been widely used. Nowadays, the finding of several side effects of BHA and BHT (Silva and Lidon, 2016) and the increased consumer concern about chemical residues in animal products have turned the attention of researchers to different classes of natural antioxidants.

Vitamin E represents the major antioxidant in cell membranes, able to interrupt lipid oxidation by scavenging radicals (Harsini et al., 2012). Compared to the other liposoluble vitamins, α-tocopherol does not accumulate to toxic levels in the body. Studies (Lu et al., 2014; Habibian et al., 2015) reported that meat derived from chickens fed with high doses of vitamin E presented lower susceptibility to lipid oxidation. Considering the positive effects on some meat quality parameters, such as drip loss and color stability, the inclusion of vitamin E could also be responsible for higher liking scores by the consumers.

In the last yr, a new class of antioxidant has been widely studied. Phytogenic feed additives are "plant derived products used in animal feeding to improve performance of agricultural livestock" (Windisch et al., 2008). Among the plants studied, oregano (*Origanum vulgare* L.) seems to be one of the most promising. It is able to exhibit antioxidant and antibacterial properties (Calleja et al., 2015; Rodriguez-Garcia et al., 2015) and increase antioxidant capacity in both chickens (Zeng et al., 2015) and their derived meat and meat products (Al-Hijazeen et al., 2016). The majority of the studies on poultry is focused on the inclusion of essential oils in the diet. To meet the growing attention concerning environmental matters, aqueous extract methods are being developed through a process of bio-liquefaction based on enzyme bio-catalysis, resulting in solvent-free and thus environmentally friendly products. The aqueous extract obtained contains all the bioactive compounds of the plant (phytocomplexes) instead of the solvent-extract oily fraction typical of the essential oils (EO). Nevertheless, aqueous extracts are still rarely used if compared to the essential oil.

The newest research, focused on the role of plant extract in poultry nutrition, showed that oregano aqueous extract (**OAE**) can improve broiler performance and immune function and contribute to a balanced gut microflora. (Franciosini et al. 2016, Scocco et al. 2016). To the best of our knowledge, the effects of dietary OAE on the nutritional quality of poultry meat have not yet been investigated.

In view of this, the aim of this study was to determine the effect of a dietary OAE, compared to a high level of α-tocopherol, on broiler performance and FA profile, quality, and sensory parameters of meat from chickens fed a diet rich in CLA and DHA.

MATERIALS AND METHODS

Animals and Experimental Design

In 3 trials performed in 3 consecutive yr, 171 day-old Ross 308 chicks were randomly divided into replicates of 19 birds each and assigned to one of 3 experimental diets (3 replicates per treatment) and raised for 42 d according to Aviagen® (2014) recommendations. Feeds were formulated according to NRC (1994). Feed formulation and chemical composition of the basal diet (starter and finisher) can be found in Table 1. The dietary treatments were: 1) basal control diet (C), 2) basal diet supplemented with 0.2 g/kg of OAE (O), and 3) basal diet supplemented with 150 ppm of vitamin E (E) as positive control. To better analyze the antioxidant activity of both OAE and vitamin E, the basal diets were enriched with a PUFA supplement at the same dose (1.16 %) in both starter and finisher feeds. OAE composition can be found in Franciosini et al. (2016) and Scocco et al. (2016).

All the procedures were conducted according to European recommendations (Directive 2007/43/EU), which set welfare standards for keeping chickens for meat production and to the Guide for the Care and Use of Agricultural Animals in Research and Teaching (McGlone, 2010). Experimental protocol was approved by the Council of the Department of Veterinary Medicine, University of Perugia.

For each experiment, at d 1, 21, and at the end of the trial (d 42), all the birds were weighed, and feed intake was evaluated for the calculation of average daily gain and overall feed conversion ratio.

At d 42 of each trial, 10 broilers from each replicate were slaughtered in a local slaughterhouse and meat samples were collected for further analyses.

Physicochemical Analysis of Feed and Meat

Samples of the feeds (n=54; 3 samples each wk of the 3 42-day-long trials) were collected weekly during the

Table 1. Ingredients and chemical composition of the basal diets

	Starter	Grower—Finisher
Ingredients (kg/100 kg)		
Maize	54.00	59.00
Wheat middlings	6.00	6.50
Corn gluten	1.15	1.00
Soybean meal, 46% CP	31.99	24.99
Extruded soybean	–	3.00
Soybean oil	2.00	1.50
Calcium carbonate	1.00	0.50
Dicalcium phosphate	1.50	1.25
Sodium chloride	0.35	0.30
Vitamin and mineral mix[1]	0.50	0.50
Lysine	0.15	0.10
Methionine	0.20	0.20
Fatty acid supplement[2]	1.16	1.16
Composition (g/100 g)		
Dry matter	90.21	89.82
Crude protein	21.44	19.82
Crude fat	4.59	5.07
NDF	10.20	11.83
ADF	2.19	2.18
Lignin (s.a.)	0.49	0.63
Crude ash	5.99	5.62
Starch	41.85	42.39
Total calcium	1.20	1.20
Total phosphorus	0.70	0.60
Available phosphorus	0.52	0.44
Lysine	1.20	1.00
Methionine + Cystine	0.88	0.83
Tryptophan	0.23	0.21
ME (Mcal/Kg)	3.03	3.09

CP: crude protein; NDF: neutral detergent fibre; ADF: acid detergent fibre ME: metabolizable energy.

[1]Supplied per kilogram of diet: vitamin A, 12,500 I.U. (retinol); vitamin D3, 3,000 I.U.; vitamin E, 50 mg (tocopheryl acetate); vitamin K3, 2 mg; thiamine, 2 mg; riboflavin, 4 mg; pyridoxine, 1 mg; cyanocobalamin, 0.015 mg; pantothenic acid 15 mg; folic acid, 50 mg; biotin, 10 mg; cholinechloride, 60; iodine, 3 mg; selenium, 20 mg; iron, 3 mg; manganese, 12, mg; copper, 1,5 mg; zinc, 5 mg.

[2]Supplied per kilogram of diet: C18:2 9c,11t, 2.5 g; C18:2 10t, 12c, 2.5 g; C20:3, 0.02 g; C20:5, 0.02 g; C22:6. 0.63 g; others, 0.36 g.

trials, and the chemical composition of the samples was analysed. The composition of the basal diets (starter and finisher) is indicated in Table 1. The dry matter was evaluated using AOAC method 934.01 (AOAC, 2000). The crude protein, crude fat, and ash were determined according to AOAC procedures 976.06, 920.39 and 942.05, respectively (AOAC, 1990). The methods of Van Soest et al. (1991) were used for the analyses of the neutral detergent fiber, acid detergent fiber, and lignin (sa). Sodium sulphite, but not amylase, was used in the neutral detergent fiber procedure. Both the neutral detergent fiber and acid detergent fiber are expressed inclusive of ash. The calcium and phosphorous concentrations were determined following AOAC method 985.35 (Julshamn et al., 1998) and AOAC method 964.06 (AOAC, 1996), respectively.

Samples of *Pectoralis major* muscle of 10 animals for each replicate in all 3 trials were analyzed for chemical composition according to the Association of Analytical Chemists methods (AOAC, 2000). The moisture content was obtained by oven drying meat samples (125°C for 2 h) (method 950.46). The fat content was gravimetrically determined using ether solvent extraction (method 960.30). The nitrogen content was determined using the Kjeldahl method (method 992.15). The protein content was obtained multiplying the total Kjeldahl nitrogen with a coefficient factor of 6.25. The ash content was obtained using a muffle furnace at 600°C (method 923.03). The TBARS (2-thioBarbituric acid reactive substances) value was determined according to Ranucci et al. (2015), and the results were expressed as mg malonaldehyde (MDA)kg^{-1}.

Analysis of Total Phenolic Content In Feed and Meat

Feed and meat samples were extracted using the method described by Branciari et al. (2015a) with some modification. Briefly, 1 g of sample was homogenized with 20 mL of ethanol 80% (w/v), and the homogenate was vortex mixed and centrifuged for 30 min at 6,000 rpm at 35°C. For evaluating the phenolic content using the Folin–Ciocalteu method (Singleton et al., 1999), 20 μL of the supernatant were transferred into a tube containing 1.58 mL of H_2O_2, and 100 μL of Folin–Ciocalteu phenol reagent (Sigma-Aldrich, St. Louis, MO) were added and mixed. Afterwards, 20% (w/v) of Na_2CO_3 solution (300 μL) was added and mixed. The solution was immediately transferred to an incubator and left at 40°C for 30 minutes. The absorbance of the sample was measured at 765 nm using an Ultrospec 2100 pro UV/visible spectrometer (Amersham Pharmacia Biotech, Buckinghamshire, UK).

For the quantitative determination of total phenolic content, a gallic acid (Sigma-Aldrich, St. Louis, MO) standard calibration curve ($y = 0.0011x + 0.023$; $R^2 = 0.9998$), corresponding to a concentration range of 0.05 to 0.75 mg/mL, was used. The total phenolic concentration (**TPC**) concentration was expressed as mg gallic acid equivalents (**GAE**) per gram.

Antioxidant Capacity of Feed and Meat

The antioxidant capacity of feed (10 samples for each treatment in triplicate) and meat was determined using the oxygen radical absorbance capacity method ($\mathbf{ORAC_{FL}}$) based on the fluorescence decay rate of a probe in the presence of a radical oxygen species (**ROO**) compared with that of a reference standard, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Sigma-Aldrich, Steinheim, Germany). The extraction was performed on 2 g of meat or feed sample according to Prior et al. (2003).

The $ORAC_{FL}$ assays were carried out on a FLUOstar OPTIMA microplate fluorescence reader (BMG LABTECH, Offenburg, Germany) at an excitation wavelength of 485 nm and an emission wavelength of 520 nm. The procedure was based on the method of Zulueta et al. (2009) with slight modifications. Briefly, 2,20-azobis (2-methylpropionamide) dihydrochloride (**AAPH**; Sigma-Aldrich) was used as a peroxylradical

generator, Trolox was used as a reference antioxidant standard, and fluorescein was used as a fluorescent probe. A 100 μL volume of diluted sample, blank or Trolox calibration solution (10 to 80 μmol), was mixed with 1 mL of fluorescein (80 nM); then, 200 μL of each mixture were placed in a well of the microplate. The microplate was placed in the reader and preincubated for 15 min at 37°C. To each well, 60 μL of AAPH were automatically added to initiate the reaction. The fluorescence was measured every 1.9 minutes. All the reaction mixtures were prepared in duplicate, and at least 3 independent assays were performed for each sample. The final $ORAC_{FL}$ values were calculated by using a linear regression equation (Y = a + bX) to describe the relationship between the Trolox concentration (Y) and the net area under the FL decay curve (X). Linear regression was used in the range of 10 to 80 lM Trolox. The data are expressed as micromoles of Trolox equivalents (**TE**) per gram of sample (μmol TE g^{-1}) by applying the following formula:

$$Orac\,(\mu molTE) = \frac{Ctrolox\,(AUCSample - AUCBlank)\,k}{(AUCTrolox - AUCBlank)}$$

Where: C*trolox* is the concentration of Trolox, k is the sample dilution factor, and AUC is the area below the fluorescence decay curve of the sample, the blank, and Trolox, respectively, calculated by applying the following formula (Ou et al., 2001) in a Microsoft Excel spreadsheet (Microsoft, Washington, DC):

$$AUC = 0.5 + f1/f0 + ...fi/f0$$

Where: *f*1 is the initial fluorescence reading at t = 0 min, and *fi* is the fluorescence reading at time i. The net AUC for each sample was obtained by subtracting the AUC of the corresponding blank from that of the sample.

Fatty Acids Analysis of Meat

An aliquot (30 g) of the *P. major* muscle from 10 chickens per replicate belonging to the 3 dietary treatments in the 3 trials was homogenized in chloroform-methanol (1:2, v/v) in order to extract the lipid fraction. Total lipids were isolated as described by Bligh and Dyer (1959).

Fatty acid methyl esters (**FAME**) were obtained from total lipids through alkaline transmethylation (Suter et al. 1997). The qualitative analysis of FAME was carried out using a Focus gas chromatograph (Thermo Electron Corporation, West Palm Beach, FL) equipped with a CP-Sil88 fused silica capillary column (100 m × 0.25 mm i.d., film thickness 0.2 μm, Chrompack, Middelburg, The Netherlands) and a quadrupole mass detector (FocusDSQ). The carrier gas was helium at a flow rate of 1.6 mL/min; the oven temperature program started from 160°C, was raised to 240°C at a rate of 4°C/min, and remained at 240°C for 10 minutes. The injector temperature was 260°C. The sample was injected into a split/splitless system. The ion source temperature of the mass detector was set at 260°C. The mass spectrum was acquired using Xcalibur Data System ver. 1.4. Peaks were identified by comparison with known standards and using the NIST mass spectral database. The quantitative analysis of FAME was performed by means of gas chromatography using a CP-9002 apparatus (Chrompack, Middelburg, The Netherlands) equipped with a flame ionization detector (**FID**) and the same column and operative conditions reported above. The temperature of the detector was set at 260°C. A Supelco (Bellefonte, PA) standard solution containing a mixture of 37 FAME was used for identification of peaks and for the calculation of correction factor of the individual FA peak areas. FA compositions (wt %) were calculated by the corrected peak area normalization method. The concentrations of FA in mg/100 g of meat were measured against nonadecanoic acid methyl ester (C19:0) as an internal standard.

α-Tocopherol Analysis

The lipid extraction for the determination of the tocopherol fraction was performed according to (Hewavitharana et al., 2004) with slight modification. A representative portion of raw chicken breast (1 g) was placed in 50 mL of absolute ethanol, and the mixture was homogenized for 30 seconds. Subsequently, 5 mL of distilled water were added, and the content was homogenized for 15 seconds. Then 4 mL of hexane were added, and the sample was homogenized for further 15 seconds. The tube was capped and centrifuged at 2,500 rpm (1750 g; t = 10 min; T = 20 °C). After separation of 2 phases, the upper phase (hexane) containing the lipids was vacuum dried in a rotary evaporator and used for Ultra Performance Liquid Chromatography (UPLC) analysis.

UPLC analysis was carried out using ACQUITY UPLC H-Class (Milford, MA), an isocratic flow consisting of a mixture of hexane/2-propanol/glacial acetic acid (99.5 : 0.5 : 0.1; v/v). The column was an Ascentis® Express HILIC (2.7 nm 150 mm x 2.1 mm SUPELCO, Bellefonte, PA). The auto-sampler and the column were maintained at 30°C.

The detector was a fluorimeter (FLR ACQUITY UPLC) at an excitation wavelength of 290 nm and an emission wavelength of 330 nm. The flow rate was 0.3 mL/min, and the volume of injection was 1 μL. For the quantitative analysis of α-tocopherol, a calibration curve was obtained by injecting standard solution at10 different concentration (0.305, 0.612, 1.25, 2.5, 5, 10, 20, 40, 80, and 160 mg/L). The coefficient of the determination of the calibration curve was higher than 0.9774.

Meat Quality Measurements

The pH was measured on *P. major* muscle after 45 min and 24 h postmortem using a penetrating electrode connected to a portable pH-meter (Mod SG2, Mettler Toledo AG, Schwerzenbach, Switzerland). The color measurements were performed 24 h after slaughter on *P. major* muscle following a 30-minute bloom period at the refrigeration temperature. Color coordinates (CIE L*a*b* color system, 1976) were determined using a Minolta Chromameter CR400 (Minolta, Osaka, Japan—light source of D65 calibrated against a standard white tile). The results were expressed as lightness (L*), redness (a*), and yellowness (b*). The hue value ($\tan^{-1}$ b*/a*) and saturation index, or chroma [$(a^{*2} + b^{*2})^{1/2}$], also were calculated.

Drip loss and cooking loss were performed on *P. major* muscle as described by Honikel (1998). For drip loss determination, meat samples were held in a plastic box on a grid parallel to the fiber direction and then stored at 4°C for 24 hours. For cooking loss determination, meat samples were held in plastic bags, then cooked in a water-bath at 80°C for 1 h and finally cooled under running tap water for 30 minutes. Samples were weighed before and after the test, and losses were calculated as 100 x (initial weight-final weight)/initial weight.

The Warner-Bratzler (WB) shear force measurement also was performed according to the Honikel (1998) method. Three cylindrical cores (Ø 1.25 cm), which were cut parallel to the LL muscle fibers, were obtained from cooking loss samples and tested for the shear force value using a WB shear device fitted to an INSTRON universal texting machine (INSTRON model 1011, INSTRON Instrument, Norwood, MA; 50 kg loading range, shearing velocity 100 mm/min). The peak force, which was expressed in Newtons, was recorded and then converted to kg/cm^2.

Sensory Analysis

A series of consumer tests was performed at the Department of Veterinary Medicine, University of Perugia. Consumers (mainly represented by students and staff members) were asked to complete a questionnaire that included information regarding their age, sex, and frequency of meat consumption (Branciari et al., 2012). Vacuum-packed breasts (frozen 24 h after slaughter and maintained at -20°C for 3 mo) were thawed for 24 h at +4°C. The assessors tasted samples of the *P. major* muscle, which was placed on steel trays covered with aluminium foil and oven cooked at 180°C (10% relative humidity) for approximately 25 min to an internal temperature of 71.1°C, which was measured using a thermometer with a handheld probe (TES-1300, TES Electrical Electronic Co., Taipei, Taiwan). Breasts were cooked with salt and spices. The cooked breast was cut into 2 × 2 × 2 cm pieces and kept warm until the slices were served.

The consumer tests were performed in 3 sessions under different conditions (blind, expected, and informed), one wk apart, as reported by Branciari et al. (2016). For each session, 100 regular poultry meat consumers (aged 20 to 60, 50 females and 50 males) were used (regular consumers were those who had a consumption frequency of at least once a wk). A practicing session was performed before the test to allow consumers to become familiar with the use of a 9-point hedonic scale (from 1, "dislike extremely" to 9, "like extremely"). In the first session, one sample/group was monadically served on white plastic plates identified by 3 random digit codes. Consumers received no information (blind experimental condition) and were asked to rate sensory attributes using the 9-point hedonic scale for juiciness, texture, taste, and overall liking. In the second session, the participants were asked to assess on the same hedonic scale their liking expectation from chicken meat (expectation test) when given the following information regarding the animal diet: 1) meat from a chicken fed a standard diet; 2) meat from a chicken fed a standard diet enriched with oregano (aqueous extract), a natural active compound with many potential health benefits, and 3) meat from a chicken fed a standard diet enriched with vitamin E, an antioxidant already used in feed industries. In the third session, the participants rated the samples in the informed condition, similar to the procedures followed in the blind test, except these samples were accompanied by label information regarding the animal feeding system used in the expectation test.

Statistical Analysis

Data were reported as least square means and SEM. Homogeneity of variance was confirmed, and the comparison between means was done by ANOVA (SAS, 2001). The model included dietary treatment (control, OAE, and vitamin E), yr of experiment (3 trials in 3 consecutive yr), and the replicate (3 replicates/treatment). The Tukey test was used for comparison of the means among different dietary treatments, and significance was accepted at a probability of 0.05 ($P < 0.05$), according to the MSD (minimum significant differences) test. Tendencies were discussed for $0.5<P<1.0$.

RESULTS AND DISCUSSION

Growth Performance

Growth performance data of chickens are reported in Table 2. Results obtained in the trials showed the positive effects of the oregano-supplemented diet on animal performance. Live weight values were higher in the O group at both 21 and 42 d compared to the C group and at 21 d compared to the E group. Average daily gain (**ADG**) values in the first period (1 to 21 d) showed no differences, even if a tendency for the O group to show higher values was observed. In the second period (21

Table 2. Growth performance of chickens

	Live weight Days			Average daily gain		Overall feed conversion ratio
	1	21	42	1 to 21	21 to 42	
C	42.93	457.95^{B}	1789.90^{B}	20.757	61.56^{B}	1.82
E	43.21	451.32^{B}	1832.49^{AB}	20.381	67.38^{AB}	1.75
O	43.28	491.41^{A}	1949.67^{A}	20.843	70.59^{A}	1.71
SEM	0.193	7.605	40.029	0.456	2.033	0.049
p	0.406	0.007	0.0034	0.058	0.0036	0.078

C, chickens fed a basal control diet; E, chickens fed a basal diet supplemented with 150 ppm of vitamin E; O, chickens fed a basal diet supplemented with 0.2 g/kg of oregano aqueous extract.
^{A,B}Within a row, means without a common superscript differ ($P < 0.05$).

Table 3. Results of physical-chemical and proximal composition analyses on breast meat

	C	E	O	SEM	p
pH 45'	6.28	6.24	6.25	0.042	0.794
pH 24h	5.82	5.76	5.70	0.036	0.073
L* 24h	51.41	50.23	52.69	0.831	0.127
a* 24h	0.43	0.80	0.50	0.183	0.324
b* 24h	4.35	4.60	4.49	0.325	0.864
Drip %	2.78	2.76	2.83	0.207	0.966
Cooking loss %	19.17	17.22	19.94	0.145	0.413
Shear (kg/cm^2)	3.55	3.77	3.76	0.207	0.694
Moisture (g/100 g)	74.63	74.60	74.59	0.218	0.987
Protein (g/100 g)	22.82	22.80	22.80	0.102	0.980
Lipid (g/100 g)	1.39	1.46	1.47	0.172	0.942
Ashes (g/100 g)	1.14	1.14	1.13	0.025	0.931

Results are given as mean values of 90 samples (n=90, 10 samples x 3 replicates x 3 dietary treatments) for each of the 3 trials.
C, meat from chickens fed a basal control diet; E, meat from chickens fed a basal diet supplemented with 150 ppm of vitamin E; O, meat from chickens fed a basal diet supplemented with 0.2 g/kg of oregano aqueous extract.

to 42 d), the O group registered higher ADG compared to the C group. Overall feed conversion ratio was not different among groups.

Results obtained are in line with other studies conducted by the same team (Franciosini et al., 2016; Scocco et al., 2016) in which OAE-supplemented diets were able to increase the performance of broilers. Other studies have already reported the beneficial effects deriving from the administration of phytogenic feed additives derived from *Origanum vulgare* (Hernandez et al., 2004; Hashemipour et al. 2013). However, aqueous extracts have been poorly investigated, and comparisons among results are difficult to make due to the differences in chemical composition of the feed additives.

Meat Quality Measurements and Proximate Composition

The results of the meat quality traits and proximate composition performed on *P. major* muscle are reported in Table 3. Proximate composition of muscle was not affected by diet. This result was in agreement with other authors who found that the supplementation with an antioxidant herb medicinal extract did not modify the proximate composition of breast meat (Jang et al., 2008). Similar results for pH and WHC was reported by Young at al. (2003) on breast meat of female chickens (Ross 208) fed with 3% Turkish oregano (*Origanum onites*) or supplemented with 200 ppm of α-tocopherol and 1,000 ppm of ascorbic acid. Literature data concerning the relationship between dietary antioxidants and meat L* values are almost contradictory, as a mix of oregano and garlic oil dietary supplementation in broilers significantly decreased the L* value of the meat, but these results were not confirmed when only oregano was used (Young et al., 2003, Kirkpinar et al., 2014). No difference in the pH, L*, or drip loss values were found in breast meat of broiler chickens fed a diet supplemented with 20 IU D-α-tocopherol or DL-α-tocopherol acetate for 42 d (Cheng et al., 2016) or in breast meat of turkey fed different doses of D-α-tocopherol or DL-α-tocopherol acetate (Rey et al., 2015). The other color parameters considered are generally related to myoblobin oxygenation/oxidation (Faustman et al., 2010) or meat pigmentation due to the feed ingredients (Rajput et al., 2014). The observed limited changes in muscle a* values, contrary to what might be expected in meat when antioxidants are used, could be due to the limited time of storage before the color determination. In meat of other species, the difference in the a* value between control and animals fed with antioxidants increases only during the storage of the samples (Ranucci et al., 2015; Branciari et al., 2015b). Furthermore, in *P. major* muscle, the main muscle fibers are α-white fibers, which contain a lower amount of iron when compared to the α-red or β-red type fibers (Wood et al., 2004; Branciari et al., 2009) and for this reason are less susceptible to oxidation. No differences were detected by other authors after feeding poultry with D-α-tocopherol- or oregano-enriched diets (Young et al., 2003; Cheng et al., 2016). Meat shear force values and proximal composition were not different among the groups.

Antioxidant Capacity and Oxidative Stability in Feed and Meat

The TPC and $ORAC_{FL}$ measured in feed are presented in Table 4, whereas TPC, $ORAC_{FL}$,

Table 4. Total phenolic content (TPC) and antioxidant capacity ($ORAC_{FL}$) in feeds

$ORAC_{FL)}$	C	E	O	SEM	p
TPC (mg GA g^{-1})	0.90^{B}	0.86^{B}	1.14^{A}	0,0418	<0.001
$ORAC_{FL}$ (μmol TE g^{-1})	28.17^{B}	30.27^{AB}	33.64^{A}	0.905	0.01

Results are given as mean values of 54 samples (3 samples each wk of 3 42-day-long trials).

C, basal control diet; E, basal diet supplemented with 150 ppm of vitamin E; O, basal diet supplemented with 0.2 g/kg of oregano aqueous extract.

^{A,B}Within a row, means without a common superscript differ ($P < 0.05$).

α-tocopherol, and TBARS of meat samples are reported in Table 5.

The TPC value of the O group was always higher ($P < 0.001$) than that of C and E groups in both feed and meat. The higher value of polyphenols detected in O meat was a consequence of the higher presence of polyphenols in the animal diet (Table 4). Dietary supplementation has been proved to be a strategy to introduce phenolic compounds in meat (Rupasinghe et al., 2010; Ranucci et al., 2015; Forte et al., 2017).

As expected, α-tocopherol dietary supplementation led to an increase of α-tocopherol levels in muscle. In the O group, the amount of α-tocopherol was not different from the control, in agreement with the results reported by Young et al. (2003). Nonetheless, the amount of muscle α-tocopherol in O and E groups was not significantly different, as polyphenols exert a protection toward the oxidation of α-tocopherol (Terramoccia et al., 2013).

Animals fed with vitamin E or oregano showed lower values of TBARS in muscle. Several authors found lower TBARS in broiler muscle after α-tocopherol supplementation (Young et al., 2003; Giannenas et al., 2005), and decreased lipid oxidation in chicken muscle following supplementation with antioxidants originating from plants, e.g., tea catechins (100 to 300 mg/kg) (Tang et al., 2000) and rosemary-sage extracts (500 mg/kg) (Lopez-Bote et al., 1998) has been widely demonstrated. Dietary supplementation has been proved to be a strategy to introduce a natural antioxidant into phospholipid membranes where it may effectively inhibit the oxidative reactions at their localized sites (Lauridsen et al., 1997). In particular, oregano contains phenolic antioxidants that react with lipid and hydroxyl radicals and convert them into stable products (Yanishlieva-Maslarova and Heinonen, 2001). It is known that tocopherol is not incorporated directly into the membrane where lipid oxidation is initiated. Higher concentrations of α-tocopherol are found in mitochondria and microsomes that may provide greater protection against lipid oxidation, which may affect the stability of the entire muscle cell and subsequently affect meat quality factors (Lauridsen et al., 1997).

The results obtained for the $ORAC_{FL}$ determinations in feed and meat samples are reported in Table 4 and Table 5. As for the feed, differences were recorded only between C and O diets. The same trend was confirmed for the meat samples, where an increase in the antioxidant activity, compared to control, was found in the O group. Oregano has been demonstrated to possess high antioxidant activity due to the high content of polyphenols, such as protocatechinic acid and its phenyl glucoside, caffeic acid, rosmarinic acid, and a phenolic derived of rosmarinic acid (Cervato et al., 2000). Rosmarinic acid exhibits the highest antioxidant activity among all the compounds detected in the aqueous extract (Branciari et al., 2015b). Tocopherol homologues also were found in the dichloroethane extract of oregano (Cervato et al., 2000). Nonetheless, α-tocopherol addition to diets does not enhance antioxidant acidity in meat as reported by other authors (Gatelier et al. 2004, Descalzo and Sancho, 2008). A small effect of vitamin E was noted by Renerre et al. (1999) only in thigh muscle. As vitamin E is a free radical chain-breaking antioxidant, it is likely that would protect different antioxidant defense systems present, which are not of an enzymatic nature (Renerre et al., 1999).

Fatty Acids Profile

The FA composition (wt % of total FA) of breast meat samples deriving from chicken fed with different diets is reported in Table 6.

Twenty-two FA were identified in all investigated samples. No differences in FA percentage composition were observed among the samples deriving from the 3 dietary treatments. The portion of the saturated FA (**SFA**) was the most abundant in all samples. It accounted for about 39 to 40 % of the total FA. The level

Table 5. Total phenolic content (TPC), antioxidant capacity ($ORAC_{FL}$), α-tocopherol content, and thiobarbituric acid-reactive substances (TBARS) in breast meat

	C	E	O	SEM	p
TPC (mg GAE g^{-1})	0.31^{B}	0.32^{B}	0.39^{A}	0.0116	<0.001
$ORAC_{FL}$ (μmol TE g^{-1})	18.02^{B}	18.76^{A}	21.43^{A}	0.584	<0.001
α -tocopherol (μg g^{-1} of lipid)	111.23^{B}	189.80^{A}	137.77^{AB}	16.836	0.042
TBARS (mg MDA kg^{-1})	0.32^{B}	0.17^{A}	0.21^{A}	0.024	0.002

Results are given as mean values of 90 samples (n=90, 10 samples x 3 replicates x 3 dietary treatments) for each of the 3 trials.

C, meat from chickens fed a basal control diet; E, meat from chickens fed a basal diet supplemented with 150 ppm of vitamin E; O, meat from chickens fed a basal diet supplemented with 0.2 g/kg of oregano aqueous extract.

^{A,B}Within a row, means without a common superscript differ ($P < 0.05$).

Table 6. Total fatty acid composition (weight % of total fatty acids) of breast meat

	C	E	O	SEM	p
Fatty acids (%)					
C14:0	0.91	0.78	0.75	0.053	0.053
C16:0	26.53	24.88	26.36	4.185	0.289
C17:0	0.15	0.11	0.15	0.007	0.556
C18:0	12.57	13.17	12.78	1.164	0.536
C20:0	0.19	0.11	0.15	0.007	0.209
Σ SFA	**40.34**	**39.05**	**40.20**	**3.746**	**0.375**
C16:1	2.56	1.75	1.95	0.563	0.111
C16:1isomer	0.39	0.32	0.30	0.016	0.417
C17:1	0.05	0.06	0.06	0.003	0.993
C18:1	24.20	24.09	24.67	3.405	0.834
C18:1t	0.38	0.35	0.48	0.087	0.726
C20:1	0.30	0.33	0.41	0.036	0.566
Σ MUFA	**27.88**	**26.90**	**27.86**	**4.527**	**0.596**
C18:2 n-6	21.05	22.29	21.20	2.357	0.244
C18:3 n-3	1.49	1.30	1.27	0.122	0.427
C18:2 Δ9c, 11 t (CLA)	1.19	1.33	1.36	0.063	0.370
C18:2 Δ10t,12c (CLA)	0.65	0.78	0.87	0.035	0.125
C20:2	0.53	0.66	0.58	0.027	0.312
C20:3 n-6	0.82	0.82	0.56	0.224	0.351
C20:4	3.28	3.67	3.03	1.462	0.615
C20:5 n-3	0.38	0.42	0.54	0.027	0.359
C22:4	0.69	0.74	0.57	0.081	0.575
C22:5 n-3	0.72	0.75	0.70	0.048	0.911
C22:6 n-3	0.98	1.28	1.28	0.506	0.635
Σ PUFA	**31.78**	**34.05**	**31.94**	**9.403**	**0.292**
Σ n-3	3.57	3.76	3.76	0.696	0.882
Σ n-6	21.86	21.86	21.77	1.726	0.111
n-3/n-6	0.16	0.16	0.17	0.001	0.760
Σ CLA	1.84	2.11	2.23	0.176	0.221
AI	0.65	0.61	0.64	0.004	0.400
TI	0.38	0.35	0.38	0.001	0.235

Results are given as mean values of 90 samples (n=90, 10 samples x 3 replicates x 3 dietary treatments) for each of the 3 trials.

C, meat from chickens fed a basal control diet; E, meat from chickens fed a basal diet supplemented with 150 ppm of vitamin E; O, meat from chickens fed a basal diet supplemented with 0.2 g/kg of oregano aqueous extract.

Cm: nΔx: m=number of carbon atoms, n= number of double bonds, x=position of double bonds.

SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; AI, atherogenic index; TI, thrombogenic index. The atherogenic index (AI) was calculated according to Chilliard et al. (2003) as follows: (C12:0 + 4 × C14:0 + C16:0)/(MUFA + PUFA); the thrombogenic index (TI) was calculated in accordance with Ulbricht and Southgate (1991) using the formula: (C14:0 + C16:0 + C18:0)/(0.5 × MUFA + 0.5 × n-6 PUFA + 3 × n-3 PUFA + n-3/n-6 PUFA).

Table 7. CLA isomers and PUFA n-3 content (mg/100 g meat) in chicken breast meat

	C	E	O	SEM	p
C18:2Δ*9c, 11 t (CLA)*	15.05	15.59	15.91	13.752	0.939
C18:2Δ*10t,12c (CLA)*	7.85	9.21	9.64	6.609	0.545
C18:3 n-3	15.03	15.09	14.33	16.968	0.968
C20:5 n-3	5.06	3.91	5.11	1.273	0.321
C22:5 n-3	9.87	9.11	8.54	3.91	0.569
C22:6 n-3	15.25	20.35	16.07	9.890	0.059

Results are given as mean values of 90 samples (n=90, 10 samples x 3 replicates x 3 dietary treatments) for each of the 3 trials.

C, meat from chickens fed a basal control diet; E, meat from chickens fed a basal diet supplemented with 150 ppm of vitamin E; O, meat from chickens fed a basal diet supplemented with 0.2 g/kg of oregano aqueous extract.

of monounsaturated FA (**MUFA**) was comparable to that of PUFA, which represented about one-third of total FA. The most abundant FA were palmitic (C16:0), oleic (C18:1), and linoleic (C18:2 n-6), showing percentages higher than 20%. As a result, it can be concluded that the dietary supplementation with vitamin E (150 mg/kg feed) or OAE (150 mg/kg feed) did not affect the FA composition of breast meat.

Concerning the vitamin E supplementation, its effect on the meat fatty acid composition remains controversial. Our results partially agree with previous studies. Bolukbasi et al. (2006) found that the addition of vitamin E (100 and 200 mg/Kg) to broiler diets did not affect the FA composition of breast meat. On the contrary, Li et al. (2009) showed that a dietary supplementation with vitamin E (200 mg/kg feed) led to lower SFA and greater PUFA proportions in chicken breast meat compared to control and 10 mg/kg vitamin E treatments. Furthermore, Zdanowska-Sasiadek et al. (2016) recorded an increase of n-3 PUFA, especially C22:6 n-3, and a decrease in the n-3/n-6 PUFA ratio in chicken breast meat following dietary supplementation with vitamin E (200 mg/kg).

Regarding the oregano dietary supplementation, despite the intensive investigation about performance and meat oxidative stability in chickens, little information is available concerning its effects on meat FA profile. Our results agree with previous works. Giannenas et al. (2016) found that the supplementation of broiler diets with oregano essential oil, laurel essential oil, and attapulgite did not influence the FA profile of breast meat. Similarly, Hashemipour et al. (2013) reported that a dietary supplementation with carvacrol and thymol, the main components of oregano essential oil, did not alter the FA profile of chicken breast meat.

Taking into account all data sets, it is possible to conclude that in the present experimental conditions, the inclusion of vitamin E or OAE in CLA and PUFA n-3 enriched diets was not able to improve the stability of PUFA, and, consequently, a selective increase of these bioactive FA in breast meat samples was not revealed. Similarly, in a previous work (Pacetti et al., 2014), the addition of rosemary and/or OAE (2 g/Kg) into a CLA-enriched diet was not able to affect FA composition or CLA accumulation in pork meat from the *Longissimus lomborum* muscle.

However, the use of feed enriched with CLA (C18:2 9c,11t; C18:2 10t, 12c) and PUFA n-3 (C20:5 n-3 and C22:6 n-3) (Table 1) leads to chicken breast meat with an interesting nutritional quality. Taking into account the strong interest toward the role of poultry meat as a functional food, it is noteworthy to examine the absolute amounts (mg/100 g of meat) of bioactive FA in samples obtained by our feeding strategies (Table 7). The total PUFA n-3 amounts revealed in broiler meat accounted for about 40 mg/100 g breast meat. The CLA fraction of all samples was composed by C18:2 9c,11t; C18:2 10t, 12c isomers. The transfer of dietary CLA to broiler meat allowed an enrichment

Table 8. Blind, informed and expected liking scores for chicken meat

			ATTRIBUTES			
			Appearance	Texture	Taste	Overall liking
BLIND	C		5.64	5.97	5.78	5.91
	E		5.36	5.71	5.37	5.51
	O		5.84	5.59	5.79	5.97
		SEM	0.140	0.112	0.136	0.142
		P-value	0.185	0.403	0.228	0.220
INFORMED	C		5.62^{B}	5.54^{B}	5.47^{B}	5.54^{C}
	E		6.09^{B}	6.00^{AB}	5.89^{B}	6.14^{B}
	O		6.55^{A}	6.45^{A}	6.68^{A}	6.82^{A}
		SEM	0.267	0.262	0.355	0.368
		P-value	<0.001	<0.01	<0.001	<0.001
EXPECTED	C		–	–	–	5.95^{C}
	E		–	–	–	6.64^{B}
	O		–	–	–	7.14^{A}
		SEM	–	–	–	0.343
		P-value	–	–	–	<0.001

Results are the mean values of 100 consumers for each test (blind, informed, and expected) performed on meat samples deriving from each of the 3 trials.

C, meat from chickens fed a basal control diet; E, meat from chickens fed a basal diet supplemented with 150 ppm of vitamin E; O, meat from chickens fed a basal diet supplemented with 0.2 g/kg of oregano aqueous extract.

D: diet.

[A,B]Within a column within the test, means without a common superscript differ ($P < 0.05$).

accounting for about 21 to 26 mg/100 g, according to Sirri et al. (2003).

According to Meyer et al. (2003) the recommended intakes of long chain n-3 PUFA (Σ 20:5n-3, 22:5n-3, and 22:6n-3) range from 0.16 to 1.6 g/d, whereas a number of clinical studies suggest that beneficial effects can be observed with dietary intakes of 3.4 to 6.8 g/d of an isomeric mixture of CLA (Benjamin et al., 2015).

Sensory Analyses

The results of consumer tests performed under blind and informed conditions as well as the effect of consumer expectation for chicken meat are reported in Table 8. The 3 groups received the same scores in the blind test. This result was not confirmed when the sample was accompanied by a label (informed condition). Consumers were positively affected by the information, giving a higher score to the O samples for all attributes compared to the control, and a higher score for taste and overall liking than the vitamin E sample. Furthermore, consumers showed a higher expectation for the O samples, followed by E samples and finally by the C samples. Consumer choice can be influenced by product information, and different studies on several meat species have shown how the effect of the label modifies sensory perception and hedonic expectations (Branciari et al., 2014; Ranucci et al., 2015; Branciari et al., 2016). Although the dietary supplementation with α-tocopheryl acetate effectively controlled lipid oxidation, no differences in consumer acceptability scores were found in the present study, in agreement with the data reported by Bou et al.(2004) and Ruiz et al. (2001). Similar results were found by Blum et al. (1992) in chicken and by Bartov et al. (1983) in turkey, who found that vitamin E addition in animal diets did not improve meat sensory quality. Probably in the present experiment, the time of storage was too short for oxidative processes to decrease meat sensorial quality. Indeed, Poste et al. (1996) found that 4 d of storage were necessary for cooked poultry meat to show high rancidity scores.

CONCLUSIONS

The experimented dietary treatments, consisting of CLA- and PUFA n-3-enriched diets, supplemented with either OAE (0.2 g/kg fed diet) or vitamin E (150 ppm feed), were able to obtain functional poultry meat rich with bioactive FA, such as CLA and PUFA n-3.

However, despite the well-known and testified antioxidant activity of oregano and vitamin E, in the present experimental condition, the dietary supplementation with these antioxidants was not able to provide a selective increase of CLA or PUFA in chicken breast samples.

Results obtained in terms of performance, meat quality, and sensory properties allow stating that the use of OAE can represent a valid solution to improve live weight of chickens and resistance to oxidation of meat and increase consumer acceptance and perception. Moreover, the use of this phytogenic feed additive could help producers in meeting the increased consumers' demand for a more environmentally friendly product, a reduction of the use of synthetic antioxidants, and a functional food.

ACKNOWLEDGMENTS

Support for this work was provided by the Ministry of Economic Development, Italy (Project MI01_00148-Industria 2015- Bando Nuove Tecnologie per il Made in Italy).

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