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

Claudio Forte[*], Raffaella Branciari*, Deborah Pacetti[†], Dino Miraglia[*], David Ranucci[*], Gabriele Acuti[*], Michele Balzano[†1], Natale G. Frega[†], Massimo Trabalza-Marinucci[*]

[*]Department of Veterinary Medicine, University of Perugia, S. Costanzo 4, 06126 Perugia, Italy

[†]Department of Agricultural, Food and Environmental Sciences, Marche Polytechnic University, Via Brecce Bianche, 60131 Ancona, Italy

[1]Corresponding author: m.balzano@univpm.it, tel 0039 071 2204317.
Department of Agricultural, Food and Environmental Sciences, Marche Polytechnic University

ABSTRACT

Three consecutive trials were performed to determine the effect of dietary oregano aqueous extract on meat fatty acid profile, meat quality and sensorial properties, compared to high level of α-tocopherol, in chickens fed a diet rich in polyunsaturated fatty acids (PUFA).

For each trial, one hundred and seventy-one day old Ross 308 chicks per trial were randomly divided in replicates of 19 birds each and assigned to one of three 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. 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 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 physical-chemical and proximal composition or in total fatty acid composition of breast meat. Dietary oregano influenced meat composition in terms of total phenolics content, antioxidant capacity and thiobarbituric acid-reactive substances, improving meat resistance to oxidation, compared to both other groups. During consumer tests, meat from the three dietary groups obtained the same liking score in blind session. Under informed condition, consumers perception was 'positively influenced by labeling for all the considered attributes. Furthermore, consumers showed an higher expectation for meat derived from chickens fed oregano then meat deriving from the other two groups.

Results obtained in the present study allow to state that using *Origanum vulgare* aqueous extract in diet enriched with PUFA can represent valid solution to increase live weight of chickens, improving resistance to oxidation of meat and positively influence consumer perception of poultry meat.

Key Words: fatty acids, antioxidants, meat quality, consumer's choice, phytogenics**.**

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 the modern agriculture (González-Ortiz et al., 2013; Salem and Eggersdorfer, 2015). It is well established that the modern Western diets contain excessive amounts of n-6 PUFA which can promote many diseases, including inflammatory and autoimmune disorders, cancer, cardiovascular pathology (Simopoulos, 2002). Low concentration of n-3 PUFA in blood has been correlated also with poor cognitive performance and behavior in children (Montgomery et al., 2013).

Other fatty acids (FA) sources can exert beneficial effects deriving from their consumption. Conjugated linoleic acids (CLA) are recognized to reduce cardiovascular diseases, to positively influence body composition and bone health and to reduce risk of diabetes and cardiovascular disease (Bhattacharya et al., 2006).

To enhance human consumption of these bioactive fatty acids several enriched food, mostly animal products, are being produced both adding directly PUFA at the end of the productive cycle or modifying animal diets. Poultry industry can be one of the most convenient sector to reach this objective, considering that consumption of poultry meat is predicted to be 50 kg per capita in 2050 (Kearney, 2010). Many studies had already investigated the possibility to enrich chicken diet with different PUFA sources (Betti et al., 2009; Lopez-Ferrer et al., 2001; Gonzalez-Esquerra and Leeson, 2001). It has been shown that the inclusion of fish or vegetable oils in high concentration in poultry diets can exert some negative effects such has compromise the oxidative balance in live animals and, consequently, the oxidative susceptibility of derived meat (González-Ortiz et al., 2013). In addition, oxidation can negatively affect meat healthfulness creating toxic compounds like 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 production of other feed additives rich in PUFA (Kalogeropoulos et al., 2010). To improve meat fatty

68 acid composition and to avoid off-flavours, the supplementation of rations with a FA supplement rich
69 in CLA and docosahexaenoic acid (DHA), was tested.
70 Anyhow, the issue of high livels of lipid oxidation in a feed rich in PUFA has to be considered.
71 To avoid, or at least defer oxidation both in feed and meat, synthetic antioxidants such as butylated
72 hydroxyanisole (**BHA**) and butylated hydroxytoluene (**BHT**) were widely used. Nowadays, the
73 findings of several side effects of these compounds (Goodman et al., 1990) and the increased
74 consumers concern about chemical residues in animal products, tourned the attention of the
75 researchers to different classes of natural antioxidants.
76 Vitamin E represents the major antioxidant in cell membrane, able to interrupt the lipid oxidation
77 acting as radical scavenging (Harsini et al., 2012). Compared to the other liposoluble vitamins, α-
78 tocopherol does not undergo processes of accumulation of toxic levels in the body. Studies (Lu et al.,
79 2014; Habibian et al., 2015) reported that meat derived from chickens fed with high doses of vitamin
80 E presented lower susceptibility to lipid oxidation. Acting on different meat quality parameters, such
81 as drip loss or color stability (Morrissey et al., 1994), the inclusion of vitamin E could also bring to
82 the achievement of higher liking scores by the consumers.
83 In the last years, a new class of antioxidant has been widely studied. Phytogenic feed additives are
84 "plant derived products used in animal feeding to improve performance of agricultural livestock"
85 (Windisch et al., 2008). Among the plants studied, oregano (*Origanum vulgare* L.) seems to be one
86 of the most promising. It is able to exhibit antioxidant and antibacterial properties (Rodriguez-Garcia
87 et al., 2015; Calleja et al., 2015) and increase antioxidant capacity in both chickens (Zeng et al., 2015)
88 and their derived meat and meat products (Al-Hijazeen et al., 2016). The majority of the studies on
89 poultry is focused on the inclusion of essential oils (**EO**) in the diet. To meet the growing attention
90 concerning environmental matters, aqueous extracts (**AE**) are being developed through a process of
91 bio-liquefaction based on enzyme bio-catalysis, resulting solvent-free and thus environmentally
92 friendly. AE obtained contains all the bioactive compounds of the plant (phytocomplexes) instead of
93 the solvent-extract oily fraction typical of the EO. Nevertheless, AE is still rarely used if compared

to the EO.

Newest research focused on the role of plant extract in poultry nutrition, shown that oregano aqueous extract (OAE) could 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 oregano AE on the nutrional quality of poultry meat have not yet been exploited.

In view of this, the aim of this study was to determine the effect of dietary oregano AE on performance, meat fatty acid profile, meat quality and sensorial properties, compared to high level of α-tocopherol, in chickens fed a diet rich in CLA and DHA.

MATERIALS AND METHODS

Animals and Experimental Design

In three consecutive trials one hundred and seventy-one day old Ross 308 chicks were randomly divided in replicates of 19 birds each and assigned to one of three experimental diets (3 replicates per treatment) and raised for 42 days according to Aviagen® (2012) 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 oregano AE (**O**) and 3) basal diet supplemented with 150 ppm of vitamin E (**E**). To better analyze the antioxidant activity of both oregano AE and vitamin E, the basal diets was enriched with a fatty acid supplement consisting in a mixture PUFA 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 Council Regulation (EC) No. 1804/1999 and Italian directives on animal welfare for experimental and other scientific purposes (DL 01/27/1992, n. 116). Experimental protocol was approved by the Council of the Department of Veterinary Medicine, University of Perugia.

For each experiment, at day 1, 21 and at the end of the trial (day 42) all the birds were weighed and feed intake was evaluated for the calculation of average daily gain and overall feed conversion ratio. At day 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 were collected weekly during the trial 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 fibre (NDF), acid detergent fibre (ADF) and lignin (sa). Sodium sulphite, but not amylase, was used in the NDF procedure. Both the NDF and ADF 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 *P. major* muscle of 10 animals for each replicate in all the 3 trials were analysed 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

145 Feed and meat samples were extracted using the method described by Branciari et al. (2015a) with
146 same modification. Briefly, 1 g of sample was homogenized with 20 mL of ethanol 80% (w/v), the
147 homogenate was vortexed and centrifuged for 30 min 6000 rpm at 35 °C. For evaluating the phenolic
148 content using the Folin-Ciocalteu method (Singleton et al., 1999) 20 µL of the surnatant were
149 transferred into tube containing 1.58 mL of H_2O_2, 100 µL of Folin-Ciocalteu phenol reagent (Sigma-
150 Aldrich, St. Louis, MO, USA) was added and mixed. Afterwards 20% (w/v) of Na_2CO_3 solution (300
151 µL) was added and mixed. The solution was immediately transferred to an incubator and left at 40°C
152 for 30 min. The absorbance of the sample was measured at 765 nm using an Ultrospec 2100 pro
153 UV/visible spectrometer (Amersham Pharmacia Biotech, Buckinghamshire, UK).
154 For the quantitative determination of total phenolic content, a gallic acid (Sigma-Aldrich, St. Louis,
155 MO, USA) standard calibration curve ($y= 0.0011x + 0.023 R^2 = 0.9998$), corresponding to a
156 concentration range of 0.05-0.75 mg/mL was used. The total phenolic concentration (**TPC**)
157 concentration was expressed as mg gallic acid equivalents (**GAE**) per g.
158
159 ***Antioxidant capacity of Feed and Meat***
160 The antioxidant capacity of feed (ten samples for each treatment in triplicate) and meat was
161 determined using the oxygen radical absorbance capacity method (**$ORAC_{FL}$**) based on the
162 fluorescence decay rate of a probe in the presence of a radical oxygen species (**ROO**) compared with
163 that of a reference standard, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman- 2-carboxylic acid,
164 Sigma-Aldrich, Steinheim, Germany). The extraction was performed on 2 g of meat or feed sample
165 according to Prior et al. (2003).
166 The $ORAC_{FL}$ assays were carried out on a FLUOstar OPTIMA microplate fluorescence reader (BMG
167 LABTECH, Offenburg, Germany) at an excitation wavelength of 485 nm and an emission
168 wavelength of 520 nm. The procedure was based on the method of Zulueta et al. (2009) with slight
169 modifications. Briefly, 2,20-azobis (2-methylpropionamide) dihydrochloride (AAPH; Sigma-
170 Aldrich) was used as a peroxyl radical 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 (10–80 µmol) was mixed with 1 mL of fluorescein (80 nM); then, 200 µL of each mixture was 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 was automatically added to initiate the reaction. The fluorescence was measured every 1.9 min. All the reaction mixtures were prepared in duplicate, and at least three 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 10-80 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, USA)

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

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 three 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 (**FAMEs**) were obtained from total lipids through alkaline transmethylation (Suter et al. 1997). The qualitative analysis of FAMEs was carried out using a Focus gas

chromatograph (Thermo Electron Corporation, West Palm Beach, FL, USA) 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, 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 FAMEs 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, USA) standard solution containing a mixture of 37 FAMEs was used for identification of peaks and for the calculation of correction factor of the individual fatty acid peak areas. Fatty acid compositions (wt %) were calculated by the corrected peak area normalization method. The concentrations of fatty acids in mg/100g 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 (1g) was placed in 50 mL of absolute ethanol, the mixture was homogenized for 30 s. Subsequently, 5 mL of distilled water were added and the content was homogenized for 15 s. Then 4 mL of hexane were added and the sample was homogenized for further 15 s. The tube was capped and centrifuged at 2500 rpm (1750 g; t = 10 min; T = 20 °C). After separation of two phases, the upper phase (hexane) containing the lipids was vacuum dried in a rotary evaporator and used for UPLC analysis.

UPLC analysis was carried out using ACQUITY UPLC H-Class (Milford, MA, USA), 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 a Ascentis® Express HILIC (2.7 nm 150 mm x 2.1 mm SUPELCO, Bellefonte, PA, USA). The autosampler 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 1µL. For the quantitative analysis of α-tocopherol, a calibration curve was obtained by injecting standard solution at ten different concentration (0.305, 0.612, 1.25, 2.5, 5, 10, 20, 40, 80, 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 *post-mortem* using a penetrating electrode connected to a portable pH-meter (Mod SG2, Mettler Toledo AG, Schwerzenbach, CH). The colour measurements were performed 24 h after slaughter on *P. major* muscle following a 30 min bloom period at the refrigeration temperature. Colour coordinates (CIE L*a*b* colour 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 ($(a^{*2} + b^{*2})^{1/2}$), were also 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 fibre 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 hour and finally cooled under running tap water for 30 min. Samples were weighed before and after the test, and losses were calculated as 100 x (initial weight-final weight)/initial weight.

246 The Warner-Bratzler shear force measurement was also performed according to the Honikel (1998)
247 method. Three cylindrical cores (Ø 1.25 cm), which were cut parallel to the LL muscle fibres, were
248 obtained from cooking loss samples and tested for the shear force value using a Warner-Bratzler
249 (**WB**) shear device fitted to an INSTRON universal texting machine (INSTRON model 1011,
250 INSTRON Instrument, Norwood, MA, USA; 50 kg loading range, shearing velocity 100 mm/min).
251 The peak force, which was expressed in Newtons, was recorded and then converted to kg/cm^2.
252
253 ***Sensory Analysis***
254 A series of consumer tests was performed at the Department of Veterinary Medicine, University of
255 Perugia. Consumers (mainly represented by students and staff members) were asked to complete a
256 questionnaire that included information regarding their age, sex and frequency of meat consumption
257 (Branciari et al., 2012). The assessors tasted samples of the *P. major* muscle, which was placed on
258 steel trays covered with aluminium foil and oven cooked at 180 °C (10% relative humidity) for
259 approximately 25 minutes to an internal temperature of 71.1 °C, which was measured using a
260 thermometer with a handheld probe (TES-1300, TES Electrical Electronic Co., Taipei, Taiwan).
261 Breasts were cooked with salt and spices. The cooked breast was cut into 2 x 2 x 2 cm pieces and
262 kept warm until the slices were served.
263 The consumer tests were performed in three sessions under different conditions (blind, expected and
264 informed), one week apart, as reported by Branciari et al. (2016). For each session, 100 regular poultry
265 meat consumers (aged 20-60, 50 females and 50 males) were used (regular consumers were those
266 who had a consumption frequency of at least once a week). A practicing session was performed before
267 the test to allow consumers to become familiar with the use of a nine points hedonic scale (from 1,
268 "dislike extremely" to 9, "like extremely"). In the first session, 1 sample/group was monadically
269 served on white plastic plates identified by three random digit codes. Consumers received no
270 information (blind experimental condition) and were asked to rate sensory attributes using the nine-
271 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 one-way ANOVA (SAS, 2001). The model included dietary treatment (control, oregano AE and vitamin E), year of experiment (3 trials in 3 consecutive years), and the replicate (3 replicates/treatment). The Tukey test was used for comparison of the means among different dietary treatments groups 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 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 days compared to the C group and at 21 days compared to the E group. Average daily gain (**ADG**) values in the first period (1-21 days) showed no differences, even if a tendency for the O group to show higher values was observed. In the second period (21-42

297 days) the O group registered higher ADG compared to C group. Overall feed conversion ratio was
298 not different among groups.
299 Results obtained are in line to other studies conducted by the same team (Franciosini et al., 2016
300 Scocco et al., 2016) in which oregano aqueous extract resulted able to increase performances of
301 broilers. Other studies already reported the beneficial effects deriving from the administration of
302 phytogenic feed additives derived from *Origanum vulgare* (Hernandez et al., 2004; Hashemipour et
303 al. 2013). However, aqueous extracts have been poorly investigated and comparisons between results
304 are difficult to make due to the differences in chemical composition of the feed additives.
305
306 ***Meat quality measurements and proximate composition***
307 The results of the meat quality traits and proximate composition performed on *Pectoralis major*
308 muscle are reported in Table 3. Proximate composition of muscle was not affected by diet. This results
309 was in agreement with other author who found that the supplementation with antioxidant herb
310 medicinal extract did not modified the proximate composition of breast meat (Jang et al., 2008). The
311 pH decline is consistent with the L*, drip and cooking values recorded, that were not influenced by
312 the diet, as pH values are negatively correlated to L* value (Fletcher, 1999; Quiao et al., 2001) and
313 positively to water holding capacity (**WHC**) (Quiao et al., 2001). Similar results for pH and WHC
314 was reported by Young at al. (2003) on breast meat of female chickens (Ross 208) fed with 3% of
315 Turkish oregano (*Origanum onites*) or supplemented with 200 ppm of α-tocopherol and 1000 ppm of
316 ascorbic acid. Nonetheless L* values higher than oregano were recorded for the latter but not than
317 the control group. The data about antioxidant feeding integration and L* values are almost
318 contradictory as a mix of oregano and garlic oil dietary supplementation in broilers significantly
319 decreased the L* value of the meat but this data were not confirmed if only oregano was used
320 (Kirkpinar et al., 2014). No difference in the pH, L*, and drip loss values were found also in breast
321 meat of broiler chicken fed with diet supplemented with 20 IU D-α-tocopherol or DL-α-tocopherol
322 acetate for 42 days, respectively amongst control group (Cheng et al., 2016) and of turkey fed

323 different doses of D-α-tocopherol or DL-α-tocopherol acetate (Rey et al., 2015). The other colour
324 parameters considered are generally related to myoblobin oxygenation/oxydation (Faustman et al.,
325 2010) or meat pigmentation due to the feed ingredient (Rajput et al., 2014). The limited changes in
326 muscle a* values, that could be expected in meat when antioxidants are used, could be due to the
327 limited time of storage before the color determination. In meat of other species, the difference in the
328 a* value between control and animal feed with antioxidant increase only during the storage of the
329 samples (Branciari et al., 2015b; Ranucci et al., 2015). Furthermore in *P. major* muscle the main
330 muscle fibers are α-white fibers, that have lower amount of iron than the α-red or β-red type fibers
331 (Wood et al., 2004; Branciari et al., 2009) and for this reason are less susceptible to oxidation. No
332 differences were detected by other authors that fed poultry with D-α-tocopherol or oregano enriched
333 diets (Young et al., 2003; Cheng et al., 2016). Meat shear force values and proximal composition
334 were not different among the groups.
335
336 ***Antioxidant Capacity and Oxidative Stability in Feed and Meat***
337 The TPC and $ORAC_{FL}$ measured in feed are presented in Table 4, whereas TPC, $ORAC_{FL}$, α-
338 tocopherol and TBARS of meat samples are reported in Table 5.
339 The TPC value of O group was always higher ($P < 0.001$) than that of C and E groups in both feed
340 and meat. The higher value of polyphenols detected in O meat was a consequence of the higher
341 presence of polyphenols in the animal diet (Table 4). Dietary supplementation has been proved to be
342 a strategy to introduce phenolic compounds in meat (Rupasinghe et al., 2010; Ranucci et al., 2015;
343 Forte et al., 2017).
344 As expected, *α*-tocopherol dietary supplementation led to an increase of *α*-tocopherol levels in
345 muscle. In the O group the amount of *α*-tocopherol was not different from the control, in agreement
346 with the results reported by Young et al. (2003). Nonetheless, the amount of muscle *α*-tocopherol in
347 O and E groups was not significantly different, as polyphenols exert a protection toward the oxidation
348 of *α*-tocopherol (Terramoccia et al., 2013).

Animals fed with vitamin E or oregano showed lower value 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 supplements 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 et al., 1998) has 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, 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 O group. Oregano has been demonstrated to possess high antioxidant activity due to the high content of polyphenols such as protocatechinic acid and his 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 were also found in the dichloroethane extract of oregano (Cervato et al. 2000). Nonetheless α-tocopherol addition to diets do not enhance antioxidant acidity in meat as reported by other author (Gatelier et al. 2004, Descalzo and Sancho, 2008). A small effect of vitamin E was noted by Renerre (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).

As an oxidative marker, the TBARS level may be important in cheese because lipid oxidation leads to the formation of various by-products that may result in flavour defects, loss of nutritional quality, and food safety concerns (Botsoglou et al. 1994; Fox et al. 2000).

Fatty Acids Profile

The fatty acid composition (wt % of total fatty acids) of breast meat samples deriving from chicken fed with different diets were reported in Table 6.

Twenty-two FA were identified in all investigated samples. No differences were stated among the fatty acid percentage composition in the samples deriving from the three dietary treatments. The portion of the saturated FA (SFA) was the most abundant in all samples. It accounted for about 39-40 % of the total fatty acids. The level of monounsaturated FA (MUFA) was comparable to that of PUFA, which represented about one third of total fatty acids. The most abundant FA were palmitic (C16:0), oleic (C18:1) and linoleic (C18:2 n-6), showing percentages higher than 20%. As result, the dietary supplementation with vitamin E (150 mg/kg feed) or oregano AE (150 mg/kg feed) did not affect the fatty acid 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 fatty acid composition of breast meat. On the contrary, Li et al. (2009) showed as 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-Sąsiadek et al. (2016) recorded that the dietary addition of 200 mg/kg feed of vitamin E caused an increase of n-3 PUFA, especially of C22:6 n-3, and a decrease in the n-3/n-6 PUFA ratio in chicken breast meat.

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 fatty acid profile. In this contest, our results agree with previous works. Giannenas et

al. (2016) found that the supplementation of broiler diets with essential oils of oregano, laurel and attapulgite did not influence the fatty acid 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 fatty acid profile of chicken breast meat.

Taking into account all data set, it is possible to conclude that in the present experimental conditions, the inclusion of vitamin E or oregano AE 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 fatty acids in breast meat samples was not revealed. Similarly, in a previous work (Pacetti et al., 2014), the addition of rosemary and /or oregano aqueous extracts (2 g/Kg) into a CLA enriched diet was not able to affect fatty acid composition and 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), lead to chicken breast meat with an interesting nutritional quality. Taking into account the strong interest toward the role of poultry meat as functional food it is noteworthy to examine the absolute amounts (mg/100 g of meat) of bioactive fatty acids 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 CLAs to broiler meat allowed an enrichment accounted for about 21-26 mg/100g, in according to Sirri et al. (2003).

According to Meyer et al. (2003) the recommended intakes for long chain n-3 PUFA (Σ 20:5n-3, 22:5n-3 and 22:6n-3) range from 0.16-1.6 g/day, whereas for CLA as reported by Benjamin et al. (2015) some of the clinical studies suggested a positive association of the intake of 3.4 to 6.8 g/d isomeric mixture of CLA.

Sensory Analyses

The results of consumer tests performed under blind and informed conditions for chicken meat are reported in Table 8. The results of consumer expectations are reported in Figure 1. The three 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 samples with Oregano for all attributes compared than the control and higher Taste and Overall liking than the vitamin E sample. Furthermore, consumers showed a higher expectation for the Oregano, followed by vitamin E and finally for the C. 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). Despite dietary supplementation with α-tocopheryl acetate effectively controlled lipid oxidation no difference in consumer acceptability scores were found in the present study in agreement with the data reported by Bou et al. (2004) and Riuz 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 the time is 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.

CONCLUSION

The experimented dietary treatments, consisting in CLA and PUFA n-3 enriched diets, added with oregano aqueous extract (0.2 g/kg fed diet) or vitamin E (150 ppm feed), were able to obtain functional poultry meat rich of bioactive fatty acids, such as CLA and PUFA n-3.

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

Results obtained in terms of performances, meat quality and sensorial evaluations allow to state that the use of oregano aqueous extract can represent a valid solution to improve live weight of chickens, 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.

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CONFLICT OF INTEREST STATEMENT

No conflict of interest reported

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Tables

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/kg		
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

[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; choline chloride, 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.

737 **Table 2.** Growth performance of chickens.

	Live Weight			Average Daily Gain		Overall Feed Conversion Ratio
	Days					
	1	**21**	**42**	**1-21**	**21-42**	
C	42.93	457.95^{B}	1789.90^{A}	20.757	61.56^{A}	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^{B}	20.843	70.59^{B}	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

738 C, chickens fed a basal control diet; E, chickens fed a basal diet supplemented with 150 ppm of
739 vitamin E; O, chickens fed a basal diet supplemented with 0.2 g/kg of oregano aqueous extract.
740 ^{A,B} Within a row, means without a common superscript differ ($p<0.001$).
741

742 **Table 3.** Results of physical-chemical and proximate 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
Cook %	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	74.63	74.60	74.59	0.218	0.987
Protein	22.82	22.80	22.80	0.102	0.980
Lipid	1.39	1.46	1.47	0.172	0.942
Ashes	1.14	1.14	1.13	0.025	0.931

743 Results are given as mean values of 90 samples (*n*=90, 10 samples x 3 replicates x 3 dietary
744 treatments).
745 C, meat from chickens fed a basal control diet; E, meat from chickens fed a basal diet supplemented
746 with 150 ppm of vitamin E; O, meat from chickens fed a basal diet supplemented with 0.2 g/kg of
747 oregano aqueous extract.
748

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

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

Results are given as mean values of 3 samples (*n*=3).
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<0.001).

Table 5. Total phenolics 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^{A}	0.32^{A}	0.39^{B}	0.0116	<0.001
$ORAC_{FL}$ (µmol TE g^{-1})	18.02^{A}	18.76^{B}	21.43^{B}	0.584	<0.001
α -tocopherol (µg g^{-1} of lipid)	111.23^{A}	189.80^{B}	137.77^{AB}	16.836	0.042
TBARS (mg MDA kg^{-1})	0.32^{A}	0.17^{B}	0.21^{B}	0.024	0.002

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

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<0.001).

766 **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	0.040
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
C16:1*isomer*	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[A]	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[A]	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

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768 Results are given as mean values of 90 samples (*n*=90, 10 samples x 3 replicates x 3 dietary
769 treatments).
770 C, meat from chickens fed a basal control diet; E, meat from chickens fed a basal diet supplemented
771 with 150 ppm of vitamin E; O, meat from chickens fed a basal diet supplemented with 0.2 g/kg of
772 oregano aqueous extract.
773 Cm:n Δx: m=number of carbon atoms, n= number of double bonds, x=position of double bonds.
774 SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; AI,
775 atherogenic index; TI, thrombogenic index. The atherogenic index (AI) was calculated according to
776 Chilliard et al. (2003) as follows: (C12:0 + 4 × C14:0 + C16:0) / (MUFA + PUFA); the thrombogenic
777 index (TI) was calculated in accordance with Ulbricht and Southgate (1991) using the formula:
778 (C14:0 + C16:0 + C18:0)/(0.5 × MUFA + 0.5 × n-6 PUFA + 3 × n-3 PUFA + n-3/ n-6 PUFA).
779

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

	C	**E**	**O**	**SEM**	**p**
C18:2 *Δ9c, 11 t (CLA)*	15	16	16	13.752	0.939
C18:2 *Δ10t,12c (CLA)*	8	9	10	6.609	0.545
C18:3 n-3	15	15	14	16.968	0.968
C20:5 n-3	5	4	5	1.273	0.321
C22:5 n-3	10	9	9	3.91	0.569
C22:6 n-3	15	20	16	9.890	0.059

781

782 Results are given as mean values of 90 samples (*n*=90, 10 samples x 3 replicates x 3 dietary
783 treatments).
784 C, meat from chickens fed a basal control diet; E, meat from chickens fed a basal diet supplemented
785 with 150 ppm of vitamin E; O, meat from chickens fed a basal diet supplemented with 0.2 g/kg of
786 oregano aqueous extract.
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788

Table 8. Blind and informed liking scores for chickens meat.

Item	Blind			Informed			SEM	p		
	C	E	O	C	E	O		D	T	DxT
Appearance	5.64^{A}	5.36^{A}	5.84^{A}	5.62^{A}	6.09^{AB}	6.55^{B}	0.173	0.001	0.001	0.063
Texture	5.97^{A}	5.71^{A}	5.59^{A}	5.54^{A}	6.00^{AB}	6.45^{B}	0.191	0.374	0.121	0.004
Taste	5.78^{A}	5.37^{A}	5.79^{A}	5.47^{A}	5.89^{A}	6.68^{B}	0.183	<0.001	0.013	0.004
Overall liking	5.91^{A}	5.51^{A}	5.97^{A}	5.54^{A}	6.14^{B}	6.82^{C}	0.190	<0.001	0.018	0.003

Results are the mean values of 100 consumers for each test (Blind and Informed).
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; T: test; ^{A,B} Within a row, means without a common superscript differ ($p<0.001$).

Figure legend

Figure 1. Expected overall liking scores for chicken meat.
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