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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.

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18 **ABSTRACT**

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

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

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

39

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

41

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-Esquerria 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 as 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 levels 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, turned 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. Phytogetic 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 (phytochemicals) 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 nutritional 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.

119 For each experiment, at day 1, 21 and at the end of the trial (day 42) all the birds were weighed and
120 feed intake was evaluated for the calculation of average daily gain and overall feed conversion ratio.
121 At day 42 of each trial, 10 broilers from each replicate were slaughtered in a local slaughterhouse and
122 meat samples were collected for further analyses.

123

124 ***Physicochemical Analysis of Feed and Meat***

125 Samples of the feeds were collected weekly during the trial and the chemical composition of the
126 samples was analysed. The composition of the basal diets (starter and finisher) is indicated in Table
127 1. The dry matter was evaluated using AOAC method 934.01 (AOAC, 2000). The crude protein,
128 crude fat and ash were determined according to AOAC procedures 976.06, 920.39 and 942.05,
129 respectively (AOAC, 1990). The methods of Van Soest et al. (1991) were used for the analyses of
130 the neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin (sa). Sodium sulphite, but
131 not amylase, was used in the NDF procedure. Both the NDF and ADF are expressed inclusive of ash.
132 The calcium and phosphorous concentrations were determined following AOAC method 985.35
133 (Julshamn et al., 1998) and AOAC method 964.06 (AOAC, 1996), respectively.

134 Samples of *P. major* muscle of 10 animals for each replicate in all the 3 trials were analysed for
135 chemical composition according to the Association of Analytical Chemists methods (AOAC, 2000).
136 The moisture content was obtained by oven drying meat samples (125 °C for 2 h) (method 950.46).
137 The fat content was gravimetrically determined using ether solvent extraction (method 960.30). The
138 nitrogen content was determined using the Kjeldahl method (method 992.15). The protein content
139 was obtained multiplying the total Kjeldahl nitrogen with a coefficient factor of 6.25. The ash content
140 was obtained using a muffle furnace at 600 °C (method 923.03). The TBARS (2-ThioBarbituric Acid
141 Reactive Substances) value was determined according to Ranucci et al. (2015) and the results were
142 expressed as mg malonaldehyde (MDA)·kg⁻¹.

143

144 ***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 supernatant were
149 transferred into tube containing 1.58 mL of H₂O₂, 100 µL of Folin-Ciocalteu phenol reagent (Sigma-
150 Aldrich, St. Louis, MO, USA) was added and mixed. Afterwards 20% (w/v) of Na₂CO₃ 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, 2,2'-azobis (2-methylpropionamide) dihydrochloride (AAPH; Sigma-
170 Aldrich) was used as a peroxy radical generator, Trolox was used as a reference antioxidant standard,

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

$$181 \quad \text{Orac}(\mu\text{molTE}) = \frac{C_{\text{Trolox}}(AUC_{\text{Sample}} - AUC_{\text{Blank}})k}{(AUC_{\text{Trolox}} - AUC_{\text{Blank}})}$$

182 where C_{Trolox} is the concentration of Trolox, k is the sample dilution factor, and AUC is the area
 183 below the fluorescence decay curve of the sample, the blank and Trolox, respectively, calculated by
 184 applying the following formula (Ou et al., 2001) in a Microsoft Excel spreadsheet (Microsoft,
 185 Washington, DC, USA)

$$186 \quad AUC = 0.5 + f_1/f_0 + \dots f_i/f_0$$

187 where f_1 is the initial fluorescence reading at $t = 0$ min and f_i is the fluorescence reading at time i .
 188 The net AUC for each sample was obtained by subtracting the AUC of the corresponding blank from
 189 that of the sample.

190 ***Fatty Acids Analysis of meat***

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

194 Fatty acid methyl esters (**FAMES**) were obtained from total lipids through alkaline transmethylation
 195 (Suter et al. 1997). The qualitative analysis of FAMES was carried out using a Focus gas

196 chromatograph (Thermo Electron Corporation, West Palm Beach, FL, USA) equipped with a CP-
197 Sil88 fused silica capillary column (100 m × 0.25 mm i.d., film thickness 0.2 µm, Chrompack,
198 Middelburg, The Netherlands) and a quadrupole mass detector (FocusDSQ). The carrier gas was
199 helium at a flow rate of 1.6 mL/min; the oven temperature program started from 160 °C, raised to
200 240 °C at a rate of 4 °C/min and remained at 240 °C for 10 minutes. The injector temperature was
201 260 °C. The sample was injected into a split/splitless system. The ion source temperature of the mass
202 detector was set at 260 °C. The mass spectrum was acquired using Xcalibur Data System ver. 1.4.
203 Peaks were identified by comparison with known standards and using the NIST mass spectral
204 database. The quantitative analysis of FAMES was performed by means of gas chromatography using
205 a CP-9002 apparatus (Chrompack, Middelburg, The Netherlands) equipped with a flame ionization
206 detector (**FID**) and the same column and operative conditions reported above. The temperature of the
207 detector was set at 260 °C. A Supelco (Bellefonte, PA, USA) standard solution containing a mixture
208 of 37 FAMES was used for identification of peaks and for the calculation of correction factor of the
209 individual fatty acid peak areas. Fatty acid compositions (wt %) were calculated by the corrected
210 peak area normalization method. The concentrations of fatty acids in mg/100g of meat were measured
211 against nonadecanoic acid methyl ester (C19:0) as an internal standard.

212

213 ***α-Tocopherol Analysis***

214 The lipid extraction for the determination of the tocopherol fraction was performed according to
215 (Hewavitharana et al., 2004) with slight modification. A representative portion of raw chicken breast
216 (1g) was placed in 50 mL of absolute ethanol, the mixture was homogenized for 30 s. Subsequently,
217 5 mL of distilled water were added and the content was homogenized for 15 s. Then 4 mL of hexane
218 were added and the sample was homogenized for further 15 s. The tube was capped and centrifuged
219 at 2500 rpm (1750 g; t = 10 min; T = 20 °C). After separation of two phases, the upper phase (hexane)
220 containing the lipids was vacuum dried in a rotary evaporator and used for UPLC analysis.

221 UPLC analysis was carried out using ACQUITY UPLC H-Class (Milford, MA, USA), an isocratic
222 flow consisting of a mixture of hexane/2-propanol/glacial acetic acid (99.5 : 0.5 : 0.1; v/v). The
223 column was a Ascentis® Express HILIC (2.7 nm 150 mm x 2.1 mm SUPELCO, Bellefonte, PA,
224 USA). The autosampler and the column were maintained at 30 °C.
225 The detector was a fluorimeter (FLR ACQUITY UPLC) at an excitation wavelength of 290 nm and
226 an emission wavelength of 330 nm. The flow rate was 0.3 mL/min and the volume of injection was
227 1µL. For the quantitative analysis of α -tocopherol, a calibration curve was obtained by injecting
228 standard solution at ten different concentration (0.305, 0.612, 1.25, 2.5, 5, 10, 20, 40, 80, 160 mg/L).
229 The coefficient of the determination of the calibration curve was higher than 0.9774.

230

231 ***Meat Quality Measurements***

232 The pH was measured on *P. major* muscle after 45 min and 24 h *post-mortem* using a penetrating
233 electrode connected to a portable pH-meter (Mod SG2, Mettler Toledo AG, Schwerzenbach, CH).
234 The colour measurements were performed 24 h after slaughter on *P. major* muscle following a 30
235 min bloom period at the refrigeration temperature. Colour coordinates (CIE L*a*b* colour system,
236 1976) were determined using a Minolta Chromameter CR400 (Minolta, Osaka, Japan - light source
237 of D65 calibrated against a standard white tile). The results were expressed as lightness (L*), redness
238 (a*), and yellowness (b*). The hue value ($\tan^{-1} b^*/a^*$) and saturation index, or chroma ($((a^{*2} + b^{*2})^{1/2})$,
239 were also calculated.

240 Drip loss and cooking loss were performed on *P. major* muscle as described by Honikel (1998). For
241 drip loss determination, meat samples were held in a plastic box on a grid parallel to the fibre direction
242 and then stored at 4 °C for 24 hours. For cooking loss determination, meat samples were held in
243 plastic bags, then cooked in a water-bath at 80 °C for 1 hour and finally cooled under running tap
244 water for 30 min. Samples were weighed before and after the test, and losses were calculated as 100
245 $\times (\text{initial weight} - \text{final weight}) / \text{initial weight}$.

246 The Warner-Bratzler shear force measurement was also performed according to the Honikel (1998)
247 method. Three cylindrical cores (\varnothing 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/cm².

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

272 participants were asked to assess on the same hedonic scale their liking expectation from chicken
273 meat (expectation test) when given the following information regarding the animal diet: 1) meat from
274 a chicken fed a standard diet; 2) meat from a chicken fed a standard diet enriched with oregano
275 (aqueous extract), a natural active compound with many potential health benefits and 3) meat from a
276 chicken fed a standard diet enriched with vitamin E, an antioxidant already used in feed industries.
277 In the third session the participants rated the samples in the informed condition, similar to the
278 procedures followed in the blind test, except these samples were accompanied by label information
279 regarding the animal feeding system used in the expectation test.

280

281 *Statistical Analysis*

282 Data were reported as least square means and SEM. Homogeneity of variance was confirmed, and
283 the comparison between means was done by one-way ANOVA (SAS, 2001). The model included
284 dietary treatment (control, oregano AE and vitamin E), year of experiment (3 trials in 3 consecutive
285 years), and the replicate (3 replicates/treatment). The Tukey test was used for comparison of the
286 means among different dietary treatments groups and significance was accepted at a probability of
287 0.05 ($P < 0.05$), according to the MSD (minimum significant differences) test. Tendencies were
288 discussed for $0.5 < P < 1.0$.

289

290 **RESULTS AND DISCUSSION**

291 *Growth Performance*

292 Growth performance of chickens are reported in Table 2. Results obtained in the trials showed the
293 positive effects of the oregano supplemented diet on animal performance. Live weight values were
294 higher in the O group at both 21 and 42 days compared to the C group and at 21 days compared to
295 the E group. Average daily gain (**ADG**) values in the first period (1-21 days) showed no differences,
296 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 phytogetic 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

different doses of D- α -tocopherol or DL- α -tocopherol acetate (Rey et al., 2015). The other colour parameters considered are generally related to myoglobin oxygenation/oxydation (Faustman et al., 2010) or meat pigmentation due to the feed ingredient (Rajput et al., 2014). The limited changes in muscle a^* values, that could 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 animal feed with antioxidant increase only during the storage of the samples (Branciari et al., 2015b; Ranucci et al., 2015). Furthermore in *P. major* muscle the main muscle fibers are α -white fibers, that have lower amount of iron than 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 that fed 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.

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).

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

362 The results obtained for the ORAC_{FL} determinations in feed and meat samples are reported in Table
363 4 and Table 5. As for the feed, differences were recorded only between C and O diets. The same trend
364 was confirmed for the meat samples, where an increase in the antioxidant activity, compared to
365 control, was found in O group. Oregano has been demonstrated to possess high antioxidant activity
366 due to the high content of polyphenols such as protocatechinic acid and his phenyl glucoside, caffeic
367 acid, rosmarinic acid and a phenolic derived of rosmarinic acid (Cervato et al., 2000). Rosmarinic
368 acid exhibits the highest antioxidant activity among all the compounds detected in the aqueous extract
369 (Branciari et al., 2015b). Tocopherol homologues were also found in the dichloroethane extract of
370 oregano (Cervato et al. 2000). Nonetheless α -tocopherol addition to diets do not enhance antioxidant
371 acidity in meat as reported by other author (Gatelier et al. 2004, Descalzo and Sancho, 2008). A small
372 effect of vitamin E was noted by Renerre (1999) only in thigh muscle. As vitamin E is a free radical
373 chain breaking antioxidant, it is likely that would protect different antioxidant defense systems
374 present which are not of an enzymatic nature (Renerre et al. 1999).

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

378

379 ***Fatty Acids Profile***

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

382 Twenty-two FA were identified in all investigated samples. No differences were stated among the
383 fatty acid percentage composition in the samples deriving from the three dietary treatments. The
384 portion of the saturated FA (SFA) was the most abundant in all samples. It accounted for about 39-
385 40 % of the total fatty acids. The level of monounsaturated FA (MUFA) was comparable to that of
386 PUFA, which represented about one third of total fatty acids. The most abundant FA were palmitic
387 (C16:0), oleic (C18:1) and linoleic (C18:2 n-6), showing percentages higher than 20%. As result, the
388 dietary supplementation with vitamin E (150 mg/kg feed) or oregano AE (150 mg/kg feed) did not
389 affect the fatty acid composition of breast meat.

390 Concerning the vitamin E supplementation, its effect on the meat fatty acid composition remains
391 controversial. Our results partially agree with previous studies. Bolukbasi et al. (2006) found that the
392 addition of vitamin E (100 and 200 mg/Kg) to broiler diets did not affect the fatty acid composition
393 of breast meat. On the contrary, Li et al. (2009) showed as dietary supplementation with vitamin E
394 (200 mg/kg feed) led to lower SFA and greater PUFA proportions in chicken breast meat compared
395 to control and 10 mg/kg vitamin E treatments. Furthermore, Zdanowska-Sasiadek et al. (2016)
396 recorded that the dietary addition of 200 mg/kg feed of vitamin E caused an increase of n-3 PUFA,
397 especially of C22:6 n-3, and a decrease in the n-3/n-6 PUFA ratio in chicken breast meat.

398 Regarding the oregano dietary supplementation, despite the intensive investigation about
399 performance and meat oxidative stability in chickens, little information is available concerning its
400 effects on meat fatty acid profile. In this contest, our results agree with previous works. Giannenas et

401 al. (2016) found that the supplementation of broiler diets with essential oils of oregano, laurel and
402 attapulgate did not influence the fatty acid profile of breast meat. Similarly, Hashemipour et al. (2013)
403 reported that a dietary supplementation with carvacrol and thymol, the main components of oregano
404 essential oil, did not alter the fatty acid profile of chicken breast meat.

405 Taking into account all data set, it is possible to conclude that in the present experimental conditions,
406 the inclusion of vitamin E or oregano AE in CLA and PUFA n-3 enriched diets was not able to
407 improve the stability of PUFA and, consequently, a selective increase of these bioactive fatty acids
408 in breast meat samples was not revealed. Similarly, in a previous work (Pacetti et al., 2014), the
409 addition of rosemary and /or oregano aqueous extracts (2 g/Kg) into a CLA enriched diet was not
410 able to affect fatty acid composition and CLA accumulation in pork meat from the *Longissimus*
411 *lomborum* muscle.

412 However, the use of feed enriched with CLA (C18:2 9c,11t; C18:2 10t, 12c) and PUFA n-3 (C20:5
413 n-3 and C22:6 n-3) (Table 1), lead to chicken breast meat with an interesting nutritional quality.
414 Taking into account the strong interest toward the role of poultry meat as functional food it is
415 noteworthy to examine the absolute amounts (mg/100 g of meat) of bioactive fatty acids in samples
416 obtained by our feeding strategies (Table 7). The total PUFA n-3 amounts revealed in broiler meat
417 accounted for about 40 mg/100 g breast meat. The CLA fraction of all samples was composed by
418 C18:2 9c,11t; C18:2 10t, 12c isomers. The transfer of dietary CLAs to broiler meat allowed an
419 enrichment accounted for about 21-26 mg/100g, in according to Sirri et al. (2003).

420 According to Meyer et al. (2003) the recommended intakes for long chain n-3 PUFA (Σ 20:5n-3,
421 22:5n-3 and 22:6n-3) range from 0.16-1.6 g/day, whereas for CLA as reported by Benjamin et al.
422 (2015) some of the clinical studies suggested a positive association of the intake of 3.4 to 6.8 g/d
423 isomeric mixture of CLA.

424

425 ***Sensory Analyses***

426 The results of consumer tests performed under blind and informed conditions for chicken meat are
427 reported in Table 8. The results of consumer expectations are reported in Figure 1. The three groups
428 received the same scores in the blind test. This result was not confirmed when the sample was
429 accompanied by a label (informed condition). Consumers were positively affected by the information,
430 giving a higher score to samples with Oregano for all attributes compared than the control and higher
431 Taste and Overall liking than the vitamin E sample. Furthermore, consumers showed a higher
432 expectation for the Oregano, followed by vitamin E and finally for the C. Consumer choice can be
433 influenced by product information and different studies on several meat species have shown how the
434 effect of the label modifies sensory perception and hedonic expectations (Branciari et al., 2014;
435 Ranucci et al., 2015; Branciari et al., 2016). Despite dietary supplementation with α -tocopheryl
436 acetate effectively controlled lipid oxidation no difference in consumer acceptability scores were
437 found in the present study in agreement with the data reported by Bou et al. (2004) and Riuz et al.
438 (2001). Similar results were found by Blum et al. (1992) in chicken and by Bartov et al. (1983) in
439 turkey who found that vitamin E addition in animal diets did not improve meat sensory quality.
440 Probably the time is too short for oxidative processes to decrease meat sensorial quality, indeed, Poste
441 et al. (1996) found that 4 d of storage were necessary for cooked poultry meat to show high rancidity
442 scores.

443

444

CONCLUSION

445 The experimented dietary treatments, consisting in CLA and PUFA n-3 enriched diets, added with
446 oregano aqueous extract (0.2 g/kg fed diet) or vitamin E (150 ppm feed), were able to obtain
447 functional poultry meat rich of bioactive fatty acids, such as CLA and PUFA n-3.
448 However, despite the well-know and testified antioxidant activity of oregano and Vitamin E, in the
449 present experimental condition, the dietary supplementation with oregano water extracts or vitamin
450 E was not able to provide a selective increase of CLA and PUFA in chicken breast samples.

451 Results obtained in terms of performances, meat quality and sensorial evaluations allow to state that
452 the use of oregano aqueous extract can represent a valid solution to improve live weight of chickens,
453 resistance to oxidation of meat and increase consumer acceptance and perception. Moreover the use
454 of this phytogetic feed additive could help producers in meeting the increased consumers' demand
455 for a more environmentally-friendly product, a reduction of the use of synthetic antioxidants and a
456 functional food.

457

458

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461

CONFLICT OF INTEREST STATEMENT

463 No conflict of interest reported

464

465

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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

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¹Supplied 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).
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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/cm²)	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

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

759 Results are given as mean values of 90 samples ($n=90$, 10 samples x 3 replicates x 3 dietary
 760 treatments).
 761 C, meat from chickens fed a basal control diet; E, meat from chickens fed a basal diet supplemented
 762 with 150 ppm of vitamin E; O, meat from chickens fed a basal diet supplemented with 0.2 g/kg of
 763 oregano aqueous extract.
 764 ^{A,B} Within a row, means without a common superscript differ ($p<0.001$).
 765

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
C18:1 _t	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 <i>Δ</i> 9 _c , 11 <i>t</i> (CLA)	1.19	1.33	1.36	0.063	0.370
C18:2 <i>Δ</i> 10 _t , 12 _c (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 <i>Δ</i> 9c, 11 t (CLA)	15	16	16	13.752	0.939
C18:2 <i>Δ</i> 10t, 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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789 **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

790
791 Results are the mean values of 100 consumers for each test (Blind and Informed).
792 C, meat from chickens fed a basal control diet; E, meat from chickens fed a basal diet supplemented
793 with 150 ppm of vitamin E; O, meat from chickens fed a basal diet supplemented with 0.2 g/kg of
794 oregano aqueous extract.
795 D: diet; T: test; ^{A,B} Within a row, means without a common superscript differ (p<0.001).
796

797 **Figure legend**

798

799 **Figure 1.** Expected overall liking scores for chicken meat.

800 C: meat from chickens fed a basal control diet,

801 E: meat from chickens fed a basal diet supplemented with 150 ppm of vitamin E

802 O: meat from chickens fed a basal diet supplemented with 0.2 g/kg of oregano aqueous extract