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

5

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16

17

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  $\alpha$ -  
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

42

43 The decrease of the omega 6 (n-6)/omega 3 (n-3) polyunsaturated fatty acid (PUFA) ratio in human  
44 diet is recognized as one of the challenges of the modern agriculture (González-Ortiz et al., 2013;  
45 Salem and Eggersdorfer, 2015). It is well established that the modern Western diets contain excessive  
46 amounts of n-6 PUFA which can promote many diseases, including inflammatory and autoimmune  
47 disorders, cancer, cardiovascular pathology (Simopoulos, 2002). Low concentration of n-3 PUFA in  
48 blood has been correlated also with poor cognitive performance and behavior in children  
49 (Montgomery et al., 2013).

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

54 To enhance human consumption of these bioactive fatty acids several enriched food, mostly animal  
55 products, are being produced both adding directly PUFA at the end of the productive cycle or  
56 modifying animal diets. Poultry industry can be one of the most convenient sector to reach this  
57 objective, considering that consumption of poultry meat is predicted to be 50 kg per capita in 2050  
58 (Kearney, 2010). Many studies had already investigated the possibility to enrich chicken diet with  
59 different PUFA sources (Betti et al., 2009; Lopez-Ferrer et al., 2001; Gonzalez-Esquerria and Leeson,  
60 2001). It has been shown that the inclusion of fish or vegetable oils in high concentration in poultry  
61 diets can exert some negative effects such as compromise the oxidative balance in live animals and,  
62 consequently, the oxidative susceptibility of derived meat (González-Ortiz et al., 2013). In addition,  
63 oxidation can negatively affect meat healthfulness creating toxic compounds like malondialdehyde  
64 (**MDA**) and cholesterol oxidation products (Wood et al., 2004). Moreover fish and vegetable sources  
65 of PUFA can modify the organoleptic quality of meat (Betti et al., 2009) causing the recording of  
66 lower ratings when subjected to evaluation by the final consumer. The alternative could be the  
67 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,  $\alpha$ -  
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 (phytocomplexes) instead of  
93 the solvent-extract oily fraction typical of the EO. Nevertheless, AE is still rarely used if compared

94 to the EO.

95 Newest research focused on the role of plant extract in poultry nutrition, shown that oregano aqueous  
96 extract (OAE) could improve broiler performance and immune function and contribute to a balanced  
97 gut microflora. (Franciosini et al. 2016, Scocco et al. 2016). To the best of our knowledge, the effects  
98 of dietary oregano AE on the nutritional quality of poultry meat have not yet been exploited.

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

102

103

## MATERIALS AND METHODS

### 104 *Animals and Experimental Design*

105 In three consecutive trials one hundred and seventy-one day old Ross 308 chicks were randomly  
106 divided in replicates of 19 birds each and assigned to one of three experimental diets (3 replicates per  
107 treatment) and raised for 42 days according to Aviagen® (2012) recommendations. Feeds were  
108 formulated according to NRC (1994). Feed formulation and chemical composition of the basal diet  
109 (starter and finisher) can be found in Table 1. The dietary treatments were: 1) basal control diet (**C**),  
110 2) basal diet supplemented with 0.2 g/kg of oregano AE (**O**) and 3) basal diet supplemented with 150  
111 ppm of vitamin E (**E**). To better analyze the antioxidant activity of both oregano AE and vitamin E,  
112 the basal diets was enriched with a fatty acid supplement consisting in a mixture PUFA at the same  
113 dose (1.16 %) in both starter and finisher feeds. OAE composition can be found in Franciosini et al.  
114 (2016) and Scocco et al. (2016).

115 All the procedures were conducted according to Council Regulation (EC) No. 1804/1999 and Italian  
116 directives on animal welfare for experimental and other scientific purposes (DL 01/27/1992, n. 116).  
117 Experimental protocol was approved by the Council of the Department of Veterinary Medicine,  
118 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<sup>-1</sup>.

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 H<sub>2</sub>O<sub>2</sub>, 100 µL of Folin-Ciocalteu phenol reagent (Sigma-  
150 Aldrich, St. Louis, MO, USA) was added and mixed. Afterwards 20% (w/v) of Na<sub>2</sub>CO<sub>3</sub> 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 ( $y = 0.0011x + 0.023R^2 = 0.9998$ ), 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<sub>FL</sub>**) 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<sub>FL</sub> 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 peroxy radical generator, Trolox was used as a reference antioxidant standard,



171 and fluorescein was used as a fluorescent probe. A 100  $\mu\text{L}$  volume of diluted sample, blank or Trolox  
172 calibration solution (10–80  $\mu\text{mol}$ ) was mixed with 1 mL of fluorescein (80 nM); then, 200  $\mu\text{L}$  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  $\mu\text{L}$  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  $\text{ORAC}_{\text{FL}}$   
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  $\mu\text{M}$  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:

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

182 where  $C_{\text{Trolox}}$  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)

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

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<sup>®</sup> 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  $\alpha$ -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 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 ( $\varnothing$  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 testing 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<sup>2</sup>.

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 et 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  $\alpha$ -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- $\alpha$ -tocopherol or DL- $\alpha$ -tocopherol  
322 acetate for 42 days, respectively amongst control group (Cheng et al., 2016) and of turkey fed

323 different doses of D- $\alpha$ -tocopherol or DL- $\alpha$ -tocopherol acetate (Rey et al., 2015). The other colour  
324 parameters considered are generally related to myoglobin 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  $\alpha$ -white fibers, that have lower amount of iron than the  $\alpha$ -red or  $\beta$ -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- $\alpha$ -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<sub>FL</sub> measured in feed are presented in Table 4, whereas TPC, ORAC<sub>FL</sub>,  $\alpha$ -  
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,  $\alpha$ -tocopherol dietary supplementation led to an increase of  $\alpha$ -tocopherol levels in  
345 muscle. In the O group the amount of  $\alpha$ -tocopherol was not different from the control, in agreement  
346 with the results reported by Young et al. (2003). Nonetheless, the amount of muscle  $\alpha$ -tocopherol in  
347 O and E groups was not significantly different, as polyphenols exert a protection toward the oxidation  
348 of  $\alpha$ -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  $\alpha$ -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  $\alpha$ -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<sub>FL</sub> 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  $\alpha$ -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-Sąsiadek 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 attapulgite 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 ( $\Sigma$  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 (Branciarri et al., 2014;  
435 Ranucci et al., 2015; Branciarri et al., 2016). Despite dietary supplementation with  $\alpha$ -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.

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458

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### **CONFLICT OF INTEREST STATEMENT**

463 No conflict of interest reported

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## REFERENCES

- Al-Hijazeen, M., Lee, E.J., Mendonca, A., and Ahn, D.U. 2016. Effect of Oregano Essential Oil (*Origanum vulgare* subsp. *hirtum*) on the Storage Stability and Quality Parameters of Ground Chicken Breast Meat. *Antioxidants*.5: 18.
- AOAC. 1990. Official Method of Analysis. 15th ed. Association of Official Analytical Chemists Inc., Arlington, VA, USA.
- AOAC. 1996. Official Method of Analysis. 16th ed. Association of Official Analytical Chemists Inc., Arlington, VA, USA.
- AOAC. 2000. Official Method of Analysis. 17th ed. Association of Official Analytical Chemists Inc., Arlington, VA, USA.
- Bartov, I., Basker, D., and Angel, S. 1983. Effect of dietary vitamin E on the stability and sensory quality of turkey meat. *Poul. Sci.* 62: 1224-1230.
- Benjamin S., Prakasan, P., Sreedharan, S., Wright, A.D.G. and Spener, F. 2015. Pros and cons of CLA consumption: an insight from clinical evidences. *J Nutr Metab.* 12.
- Betti, M., Schneider, B.L., Wismer, W.V., Carney, V.L., Zuidhof, M. J., and Renema, R. A. 2009. Omega-3-enriched broiler meat: 2. Functional properties, oxidative stability, and consumer acceptance. *Poul. Sci.* 88: 1085-1095.
- Bhattacharya A., Banu, J., Rahman, M., Causey, J., and Fernandes, G. 2006. Biological effects of conjugated linoleic acids in health and disease. *J. Nutr. Biochem*, 17: 789-810.
- Bligh, E.G., and Dyer, W.J. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* 37: 911-917.
- Blum, J.C., Tourraillie, C., Salichon, M.R., Richard, F.H. and Frigg, M. 1992. Effect of dietary vitamin E supplies in broilers. 2. Male and female growth rate, viability, immune response, fat content and meat flavour variations during storage. *Archiv fur Gefliigelkunde* 56: 3742.

490 Bolukbasi, S.C., Erhan M.K., and Ozkan A. 2006. Effect of dietary thyme oil and vitamin E on  
491 growth, lipid oxidation, meat fatty acid composition and serum lipoproteins of broiler. South  
492 african J. Anim. Sci. 36: 189-196.

493 Botsoglou, N.A., Fletouris, D.J., Papageorgiou, G.E., Vassilopoulos, V.N., Mantis, A.J., and  
494 Trakatellis, A.G. 1994. Rapid, sensitive, and specific thiobarbituric acid method for measuring  
495 lipid peroxidation in animal tissue, food, and feedstuff samples. J. Agric. Food Chem. 42. 1931-  
496 1937.

497 Bou, R., Guardiola, F., Tres, A., Barroeta, A.C., and Codony, R. 2004. Effect of dietary fish oil,  
498  $\alpha$ -tocopheryl acetate, and zinc supplementation on the composition and consumer acceptability  
499 of chicken meat. Poult. Sci. 83: 282-292.

500 Branciarri, R., Mugnai, C., Mammoli, R., Miraglia, D., Ranucci, D., Dal Bosco, A., and Castellini,  
501 C. 2009. Effect of genotype and rearing system on chicken behavior and muscle fiber  
502 characteristics. J. Anim. Sci. 87: 4109-4117.

503 Branciarri, R., Valiani, A., Trabalza-Marinucci, M., Miraglia, D., Ranucci, D., Acuti, G., and  
504 Mughetti, L. 2012. Consumer acceptability of ovine cheese from ewes fed extruded linseed-  
505 enriched diets. Small Ruminant Res. 106: S43-S48.

506 Branciarri, R., Ranucci, D., Forte, C., Acuti, G., Miraglia, D., Olivieri, O., and Trabalza-  
507 Marinucci, M. 2014. Effects of *Saccharomyces cerevisiae* in broiler diets: 2. Meat rheological  
508 properties and consumer perception. Progr. Nutr. 16: 303-309.

509 Branciarri, R., Ranucci, D., Miraglia, D., Urbani, S., Esposito, S., and Servili, M. 2015a. Effect of  
510 dietary treatment with olive oil by-product (olive cake) on physicochemical, sensory and  
511 microbial characteristics of beef during storage. Ital. J. Food Saf. 4.

512 Branciarri, R., Ranucci, D., Trabalza-Marinucci, M., Codini, M., Orru, M., Ortenzi, R., and  
513 Valiani, A. 2015b. Evaluation of the antioxidant properties and oxidative stability of Pecorino  
514 cheese made from the raw milk of ewes fed *Rosmarinus officinalis* L. leaves. Int. J. Food Sci-  
515 Tech. 50: 558-565.

516 Branciarri, R., Balzano, M., Pacetti, D., Trabalza-Marinucci, M., Della Casa, G., Miraglia, D., and  
517 Ranucci, D. 2016. Dietary CLA supplementation of pigs confers higher oxidative stability to  
518 Ciauscolo and Fabriano salami produced from their meat with no negative impact on the  
519 physico-chemical, microbiological and sensorial characteristics. *Eur. J. Lipid Sci. Technol.*

520 Calleja, J.M., Cruz-Romero, M.C., and García-López, M.L. (2015). Antimicrobial and  
521 Antioxidant Activities of Commercially Available Essential Oils and Their Oleoresins. *J. Herb.*  
522 *Sci.* 3: 1-11.

523 Cervato, G., Carabelli, M., Gervasio, S., Cittera, A., Cazzola, R., and Cestaro, B. 2000.  
524 Antioxidant properties of oregano (*Origanum vulgare*) leaf extracts. *J. Food Biochem.* 24: 453-  
525 465.

526 Cheng, K., Niu, Y., Zheng, X.C., Zhang, H., Chen, Y.P., Zhang, M., Huang, X.X., Zhang, L.L.,  
527 Zhou, Y.M., and Wang, T. 2016. A comparison of natural (D- $\alpha$ -tocopherol) and synthetic (DL-  
528  $\alpha$ -tocopherol acetate) vitamin E supplementation on the growth performance, meat quality and  
529 oxidative status of broilers. *Asian-Australas J. Anim.* 29: 681.

530 Chilliard, Y., Ferlay, A., Rouel J., and Lamberett, G. 2003. A review of nutritional and  
531 physiological factors affecting goat milk lipid synthesis and lipolysis. *J. Dairy Sci.* 86: 1751-  
532 1770.

533 Commission International de l'Eclairage. 1976. Colourimetry: Official recommendations of the  
534 international commission on illumination. Publication CIE No. 15 (E-1.3.1). Paris, France:  
535 Bureau Central de la Commission Internationale de l'Eclairage.

536 Council Regulation (EC) No 1804/1999 of 19 July 1999 supplementing Regulation (EEC) No  
537 2092/91 on organic production of agricultural products and indications referring thereto on  
538 agricultural products and foodstuffs to include livestock production.

539 Descalzo, A., and Sancho, A. 2008. A review of natural antioxidants and their effects on oxidative  
540 status, odor and quality of fresh beef produced in Argentina. *Meat Sci.* 79: 423-436

541 DL 01/27/1992, n. 116. Attuazione della Direttiva 86/609/EEC in materia di protezione degli  
542 animali utilizzati a fini sperimentali o ad altri fini scientifici. Gazzetta Ufficiale n. 40, 18  
543 febbraio 1992 (Suppl. ord.).

544 Faustman, C., Sun, Q., Mancini, R., and Suman, S.P. 2010. Myoglobin and lipid oxidation  
545 interactions: Mechanistic bases and control. *Meat Science*. 86: 86-94.

546 Fletcher, D.L. 1999. Broiler Breast Meat Color Variation, pH, and Texture. *Poult. Sci.* 78:1323-  
547 1327.

548 Forte, C., Ranucci, D., Branciarri, R., Beghelli, D., Acuti, G., Badillo Pazmay, G.V. Cavallucci,  
549 C., and Trabalza-Marinucci, M. 2017. Dietary integration with oregano (*Origanum vulgare*)  
550 essential oil in pig reared indoor and outdoor: effects on performances, oxidative status and  
551 meat quality. *J. Anim. Physiol. Anim. Nutr.*

552 Fox, P.F., Guinee, T.P., Cogan, T.M., and McSweeney, P.L. 2000. *Fundamentals of cheese*

553 Franciosini, M.P., Casagrande-Proietti, P., Forte, C., Beghelli, D., Acuti, G., Zanichelli, D., and  
554 Trabalza-Marinucci, M. 2016. Effects of oregano (*Origanum vulgare* L.) and rosemary  
555 (*Rosmarinus officinalis* L.) aqueous extracts on broiler performance, immune function and  
556 intestinal microbial population. *J. Appl. Anim. Res.* 44: 474-479.

557 Gatellier, P., Mercier, Y., and Renerre, M. 2004. Effect of diet finishing mode (pasture or mixed  
558 diet) on antioxidant status of Charolais bovine meat. *Meat Sci.* 67: 385-394.

559 Giannenas, I.A., Florou-Paneri, P., Botsoglou, N.A., Christaki, E., and Spais, A.B. 2005. Effect  
560 of supplementing feed with oregano and/or  $\alpha$ -tocopheryl acetate on growth of broiler chickens  
561 and oxidative stability of meat. *J. Anim. Feed Sci.* 14.

562 Giannenas, I., Tzora, A., Bonos, E., Sarakatsianos, I., Karamoutsios, A., Anastasiou, I., and  
563 Skoufos, I. 2016. Effects of dietary oregano essential oil, laurel essential oil and attapulгите on  
564 chemical composition, oxidative stability, fatty acid profile and mineral content of chicken  
565 breast and thigh meat. *Eur. J. Poul. Sci.* 80.



566 Gonzalez-Esquerro, R., and Leeson, S. 2001. Alternatives for enrichment of eggs and chicken  
567 meat with omega-3 fatty acids. *Can. J. Anim. Sci.* 81: 295-305.

568 González-Ortiz, G., Sala, R., Cánovas, E., Abed, N., and Barroeta, A.C. 2013. Consumption of  
569 dietary n-3 fatty acids decreases fat deposition and adipocyte size, but increases oxidative  
570 susceptibility in broiler chickens. *Lipids.* 48: 705-717.

571 Goodman, D.L., McDonnell, J.T., Nelson, H.S., Vaughan, T.R., and Weber, R.W. 1990. Chronic  
572 urticaria exacerbated by the antioxidant food preservatives, butylated hydroxyanisole (BHA)  
573 and butylated hydroxytoluene (BHT). *J. Allergy Clin. Immunol.* 86: 570-575.

574 Habibian, M., Sadeghi, G., Ghazi, S., and Moeini, M.M. 2015. Selenium as a feed supplement for  
575 heat-stressed poultry: a review. *Biol. Trace Elem. Res.* 165: 183-193.

576 Harsini, S.G., Habibian, M., Moeini, M.M., and Abdolmohammadi, A.R. 2012. Effects of  
577 dietary selenium, vitamin E, and their combination on growth, serum metabolites, and  
578 antioxidant defense system in skeletal muscle of broilers under heat stress. *Biol. Trace Elem.  
579 Res.* 148: 322-330.

580 Hashemipour, H., Kermanshahi, H., Golian, A., & Veldkamp, T. (2013). Effect of thymol and  
581 carvacrol feed supplementation on performance, antioxidant enzyme activities, fatty acid  
582 composition, digestive enzyme activities, and immune response in broiler chickens. *Poult. Sci.*  
583 92: 2059-2069.

584 Hernandez, F., Madrid, J., Garcia, V., Orengo, J., and Megias, M.D. 2004. Influence of two plant  
585 extracts on broilers performance, digestibility, and digestive organ size. *Poult. Sci.* 83: 169-  
586 174.

587 Hewavitharana, A.K., Lanari, M.C., and Becu, C. 2004. Simultaneous determination of vitamin  
588 E homologs in chicken meat by liquid chromatography with fluorescence detection. *J.*  
589 *Chromatogr. A.* 1025: 313-317.

590 Honikel, K.O. 1998. Reference methods for the assessment of physical characteristics of meat.  
591 *Meat Scie.* 49: 447-457.

592 ISSFAL, 2004. International society for the study of fatty acids and lipids. Report of the Sub-  
593 Committee on “Recommendations for intake of polyunsaturated fatty acids in healthy adults”.  
594 Available from: <http://www.issfal.org.uk>.

595 Jang, A., Liu, X. D., Shin, M. H., Lee, B. D., Lee, S. K., Lee, J. H., Jo, C. 2008. Antioxidative  
596 potential of raw breast meat from broiler chicks fed a dietary medicinal herb extract  
597 mix. *Poultry Science*, 87(11), 2382-2389.

598 Julshamn, K., Maage, A., and Wallin, H.C. 1998. Determination of magnesium and calcium in  
599 foods by atomic absorption spectrometry after microwave digestion: NMKL collaborative  
600 study. *J. AOAC Int.* 816: 1202-1208.

601 Kalogeropoulos, N., Panagiotakos, D. B., Pitsavos, C., Chrysohoou, C., Rousinou, G., Toutouza,  
602 M., and Stefanadis, C. 2010. Unsaturated fatty acids are inversely associated and n-6/n-3 ratios  
603 are positively related to inflammation and coagulation markers in plasma of apparently healthy  
604 adults. *Clin. Chim. Acta.* 411: 584-591.

605 Kearney, J. 2010. Food consumption trends and drivers. *Philosophical transactions of the royal*  
606 *society B: biological sciences*, 365: 2793-2807.

607 Kirkpinar, F., Ünlüa, H.B., Serdaroğlu, M., and Turp, G.Y. 2014. Effects of dietary oregano and  
608 garlic essential oils on carcass characteristics, meat composition, colour, pH and sensory quality  
609 of broiler meat. *Brit. Poult. Sci.* 55: 157-166.

610 Lauridsen, C., Buckley, D. J., and Morrissey, P. A. 1997. Influence of dietary fat and vitamin E  
611 supplementation on  $\alpha$ -tocopherol levels and fatty acid profiles in chicken muscle membranal  
612 fractions and on susceptibility to lipid peroxidation. *Meat Sci.* 46: 9-22.

613 Li, W.J., Zhao G.P., Chen, J.L., Zheng M.Q and Wen. J. 2009. Influence of dietary vitamin E  
614 supplementation on meat quality traits and gene expression related to lipid metabolism in the  
615 Beijing-you chicken. *Br. Poult. Sci.* 50: 188-189.

616 López-Bote, C. J., Gray, J.I., Gomaa, E. A., Flegal, C. J. 1998. Effect of dietary administration of  
617 oil extracts from rosemary and sage on lipid oxidation in broiler meat. *Brit. Poultry Sci.* 39:  
618 235-240.

619 Lopez-Ferrer, S., Baucells, M.D., Barroeta, A.C., and Grashorn, M.A. 2001. n-3 enrichment of  
620 chicken meat. 1. Use of very long-chain fatty acids in chicken diets and their influence on meat  
621 quality: fish oil. *Poult. Sci.* 80: 741-752.

622 Lu, T., Harper, A. F., Zhao, J., and Dalloul, R.A. 2014. Effects of a dietary antioxidant blend and  
623 vitamin E on growth performance, oxidative status, and meat quality in broiler chickens fed a  
624 diet high in oxidants. *Poult. Sci.* 93: 1649-1657.

625 Meyer, B.J., Mann, N.J., L. Lewis, J.L. Milligan, G.C. Sinclair A.J., Howe, P.R.C. 2003. Dietary  
626 Intakes and Food Sources of Omega-6 and Omega-3 Polyunsaturated Fatty Acids. *Lipids.* 38:  
627 391–398.

628 Montgomery, P., Burton, J.R., Sewell, R.P., Spreckelsen, T. F., and Richardson, A.J. 2013. Low  
629 blood long chain omega-3 fatty acids in UK children are associated with poor cognitive  
630 performance and behavior: a cross-sectional analysis from the DOLAB study. *PloS one*, 8:  
631 e66697.

632 Morrissey, P. A., Quinn, P.B., and Sheehy, P.J.A. 1994. Newer aspects of micronutrients in  
633 chronic disease: vitamin E. *Proceedings of the Nutrition Society.* 53: 571-582.

634 National Research Council. 1994. Nutrient requirements of poultry, 9th rev. ed. *Natl. Acad. Press*,  
635 Washington, DC.

636 Ou, B., Hampsch-Woodill, M., and Prior, R.L. 2001. Development and validation of an improved  
637 oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *J. Agr.*  
638 *Food Chem.* 49: 4619-4626.

639 Pacetti, D., Balzano, M., Gagliardi, R., Mozzon, M., and Frega, N. G. 2014. Influence of dietary  
640 supplementation with conjugated linoleic acid, rosemary and oregano extracts on fatty acid  
641 profile of fresh pork meat. *Progr. Nutr.* 16: 284-291.

642 Poste, L.M., Butler, G., Cave, N.A., and Burrows, V.D. 1996. Sensory analysis of meat from  
643 broiler chickens fed diets containing hullless oats (*Avena nuda*). *Can. J. Anim. Sci.* 76: 313-319.

644 Prior, R. L., Hoang, H., Gu, L., Wu, X., Bacchiocca, M., Howard, L., and Jacob, R. 2003. Assays  
645 for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity  
646 (ORACFL)) of plasma and other biological and food samples. *J. Agr. Food Chem.* 51: 3273-  
647 3279.

648 Quiao, M., Fletcher, D.L., Smith, D.P., Northcutt, J.K. 2001. The Effect of Broiler Breast Meat  
649 Color on pH, Moisture, Water-Holding Capacity, and Emulsification Capacity. *Poult. Sci.* 80:  
650 676-680.

651 Rajput, N., Ali, S., Naeem, M., Khan, M.A., Wang, T. 2014. The effect of dietary  
652 supplementation with the natural carotenoids curcumin and lutein on pigmentation, oxidative  
653 stability and quality of meat from broiler chickens affected by a coccidiosis challenge. *Brit.*  
654 *Poult. Sci.* 55: 501-509.

655 Ranucci, D., Beghelli, D., Trabalza-Marinucci, M., Branciarri, R., Forte, C., Olivieri, O., and  
656 Acuti, G. 2015. Dietary effects of a mix derived from oregano (*Origanum vulgare* L.) essential  
657 oil and sweet chestnut (*Castanea sativa* Mill.) wood extract on pig performance, oxidative status  
658 and pork quality traits. *Meat Sci.* 100: 319-326.

659 Renerre, M., Poncet, K., Mercier, Y., Gatellier, P., and Métro, B. 1999. Influence of dietary fat  
660 and vitamin E on antioxidant status of muscles of turkey. *J. Agric. Food Chem.* 47: 237-244.

661 Rey, A.I., Segura, J., Olivares, A., Cerisuelo, A., Piñeiro, C., López-Bote, C.J. 2015. Effect of  
662 micellized natural (*D- $\alpha$ -tocopherol*) vs. synthetic (*DL- $\alpha$ -tocopheryl acetate*) vitamin E  
663 supplementation given to turkeys on oxidative status and breast meat quality characteristics.  
664 *Poult. Sci.* 94:1259-1269.

665 Ruiz, J. A., Guerrero, L., Arnau, J., Guardia, M. D., and Esteve-Garcia, E. (2001). Descriptive  
666 sensory analysis of meat from broilers fed diets containing vitamin E or  $\beta$ -carotene as  
667 antioxidants and different supplemental fats. *Poult. Sci.* 80: 976-982.

668 Rodriguez-Garcia, I., Silva-Espinoza, B.A., Ortega-Ramirez, L. A., Leyva, J.M., Siddiqui, M. W.,  
669 Cruz-Valenzuela, M.R., and Ayala-Zavala, J.F. 2015. Oregano essential oil as an antimicrobial  
670 and antioxidant additive in food products. *Crit. Rev. Food Sci. Nutr.* 56: 1717-1727.

671 Ross 308 broiler. 2014. Broiler Performance Objectives, Aviagen, Alabama, USA.

672 Rupasinghe, H.P., Ronalds, C.M., Rathgeber, B., and Robinson, R.A. 2010. Absorption and tissue  
673 distribution of dietary quercetin and quercetin glycosides of apple skin in broiler chickens. *J.*  
674 *Sci. Food Agr.* 90: 1172-1178.

675 Salem Jr, N., and Eggersdorfer, M. 2015. Is the world supply of omega-3 fatty acids adequate for  
676 optimal human nutrition?. *Curr. Opin. Clin. Nutr. Metab. Care.* 18: 147-154.

677 SAS. JMP Statistics and Graphics Guide, Version 4. 2001. Cary, NC, USA

678 Scocco, P., Forte, C., Franciosini, M. P., Mercati, F., Casagrande-Proietti, P., Dall'Aglio, C., and  
679 Trabalza-Marinucci, M. 2016. Gut complex carbohydrates and intestinal microflora in broiler  
680 chickens fed with oregano (*Origanum vulgare* L.) aqueous extract and vitamin E. *J Anim*  
681 *Physiol Anim Nutr.*

682 Simopoulos, A.P. 2002. The importance of the ratio of omega-6/omega-3 essential fatty acids.  
683 *Biomed. & Pharmacotherapy.* 56: 365-379.

684 Singleton, V.L., Orthofer, R., and Lamuela-Raventós, R.M. 1999. Analysis of total phenols and  
685 other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods*  
686 *Enzymol.* 299: 152-178.

687 Sirri, F., Tallarico, N., Meluzzi, A., and Franchini, A. 2003. Fatty acid composition and  
688 productive traits of broiler fed diets containing conjugated linoleic acid. *Poult. Sci.* 82: 1356-  
689 1361.

690 Suter, B., Grob, K., and Pacciarelli, B. 1997. Determination of fat content and fatty acid  
691 composition through 1-min transesterification in the food sample; principles. *Z. Lebensm.*  
692 *Unters Forsch.* 204: 252-258.

693 Tang, S.Z., Kerry, J.P., Sheehan, D., Buckley, D.J., and Morrissey, P.A. 2000. Dietary tea  
694 catechins and iron-induced lipid oxidation in chicken meat, liver and heart. *Meat Sci.* 56: 285-  
695 290.

696 Terramoccia, S., Bartocci, S., Taticchi, A., Di Giovanni, S., Pauselli, M., Mourvaki, E., Urbani,  
697 S. and Servili, M. 2013. Use of dried stoned olive pomace in the feeding of lactating buffaloes:  
698 effect on the quantity and quality of the milk produced. *Asian-Australas J Anim Sci.* 26: 971-  
699 980.

700 Ulbricht, T.L.V., and Southgate, D.A.T. 1991. Coronary heart disease: seven dietary factors.  
701 *Lancet.* 338: 985-992.

702 Van Soest, P. V., Robertson, J. B., and Lewis, B. A. 1991. Methods for dietary fiber, neutral  
703 detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74:  
704 3583-3597.

705 Windisch, W., Schedle, K., Plitzner, C., and Kroismayr, A. 2008. Use of phytogetic products as  
706 feed additives for swine and poultry. *J. Anim. Sci.* 86: E140-E148.

707 Wood, J.D., Nute, G.R., Richardson, R.I., Whittington, F.M., Southwood, O., Plastow, G.,  
708 Mansbridge, R., Da Costa, N., and Chang, K. C. 2004. Effects of breed, diet and muscle on fat  
709 deposition and eating quality in pigs. *Meat Sci.*, 67: 651-667.

710 Yanishlieva-Maslarova, N.V., and Heinonen, I.M. 2001. Sources of natural antioxidants:  
711 vegetables, fruits, herbs, spices and teas. Pages 210-2363 in *Antioxidants in food*. Eds. Pokorný  
712 J., Yanishlieva N., Gordon M., CRC Press, Boca Raton FL.

713 Young, F.J., Stagsted, J., Jensen, S.K., Karlson, A.H., and Henkel, P. 2003. Ascorbic Acid,  $\alpha$ -  
714 Tocopherol, and Oregano Supplements Reduce Stress-Induced Deterioration of Chicken Meat  
715 Quality. *Poult. Sci.* 82: 1343-1351.

716 Zeng, Z., Zhang, S., Wang, H., and Piao, X. 2015. Essential oil and aromatic plants as feed  
717 additives in non-ruminant nutrition: a review. *J. Anim. Sci. Biotechnol.* 6: 7.

718 Zdanowska-Sasiadek, Z., Michalczuk, M., Polawska, E., Damaziak, K., Niemiec, J., and Radzik-  
719 Rant, A. 2016. Dietary vitamin E supplementation on cholesterol, vitamin E content, and fatty  
720 acid profile in chicken muscles. *Can. J. Anim. Sci.* 96: 114-120.

721 Zulueta, A., Esteve, M. J., and Frígola, A. 2009. ORAC and TEAC assays comparison to measure  
722 the antioxidant capacity of food products. *Food Chem.* 114: 310-316

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**Tables**

**Table 1.** Ingredients and chemical composition of the basal diets

	<b>Starter</b>	<b>Grower - Finisher</b>
<b>Ingredients (kg/100 kg)</b>		
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 <sup>1</sup>	0.50	0.50
Lysine	0.15	0.10
Methionine	0.20	0.20
Fatty acid supplement <sup>2</sup>	1.16	1.16
<b>Composition g/kg</b>		
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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<sup>1</sup>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.

<sup>2</sup> 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	21-42	
	1	21	42			
<b>C</b>	42.93	457.95 <sup>B</sup>	1789.90 <sup>A</sup>	20.757	61.56 <sup>A</sup>	1.82
<b>E</b>	43.21	451.32 <sup>B</sup>	1832.49 <sup>AB</sup>	20.381	67.38 <sup>AB</sup>	1.75
<b>O</b>	43.28	491.41 <sup>A</sup>	1949.67 <sup>B</sup>	20.843	70.59 <sup>B</sup>	1.71
<b>SEM</b>	0.193	7.605	40.029	0.456	2.033	0.049
<b>p</b>	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 <sup>A,B</sup> 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.

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

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750 **Table 4.** Total phenolics content (TPC) and antioxidant capacity (ORAC<sub>FL</sub>) in feeds.

ORAC <sub>FL</sub>	C	E	O	SEM	p
TPC (mg GA g <sup>-1</sup> )	0.90 <sup>A</sup>	0.86 <sup>A</sup>	1.14 <sup>B</sup>	0,0418	<0.001
ORAC <sub>FL</sub> (μmol TE g <sup>-1</sup> )	28.17 <sup>A</sup>	30.27 <sup>AB</sup>	33.64 <sup>B</sup>	0.905	0.01

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

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

754 <sup>A,B</sup> Within a row, means without a common superscript differ (*p*<0.001).

755

756 **Table 5.** Total phenolics content (TPC), antioxidant capacity (**ORAC<sub>FL</sub>**),  $\alpha$ -tocopherol content and  
 757 thiobarbituric acid-reactive substances (TBARS) in breast meat.  
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	<b>C</b>	<b>E</b>	<b>O</b>	<b>SEM</b>	<b>p</b>
<b>TPC (mg GAE g<sup>-1</sup>)</b>	0.31 <sup>A</sup>	0.32 <sup>A</sup>	0.39 <sup>B</sup>	0.0116	<0.001
<b>ORAC<sub>FL</sub> (<math>\mu</math>mol TE g<sup>-1</sup>)</b>	18.02 <sup>A</sup>	18.76 <sup>B</sup>	21.43 <sup>B</sup>	0.584	<0.001
<b><math>\alpha</math>-tocopherol (<math>\mu</math>g g<sup>-1</sup> of lipid)</b>	111.23 <sup>A</sup>	189.80 <sup>B</sup>	137.77 <sup>AB</sup>	16.836	0.042
<b>TBARS (mg MDA kg<sup>-1</sup>)</b>	0.32 <sup>A</sup>	0.17 <sup>B</sup>	0.21 <sup>B</sup>	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 <sup>A,B</sup> Within a row, means without a common superscript differ ( $p<0.001$ ).  
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**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
<b>Σ SFA</b>	<b>40.34</b>	<b>39.05</b>	<b>40.20</b>	3.746	0.375
C16:1	2.56	1.75	1.95	0.563	0.111
C16:1 <i>isomer</i>	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 <i>t</i>	0.38	0.35	0.48	0.087	0.726
C20:1	0.30	0.33	0.41	0.036	0.566
<b>Σ MUFA</b>	<b>27.88</b>	<b>26.90</b>	<b>27.86</b>	<b>4.527</b>	<b>0.596</b>
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 $\Delta 9c, 11 t$ (CLA)	1.19	1.33	1.36	0.063	0.370
C18:2 $\Delta 10t, 12c$ (CLA)	0.65 <sup>A</sup>	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
<b>Σ PUFA</b>	<b>31.78</b>	<b>34.05</b>	<b>31.94</b>	<b>9.403</b>	<b>0.292</b>
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 <sup>A</sup>	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  $\Delta$ x: 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:  $(C12:0 + 4 \times C14:0 + C16:0) / (MUFA + PUFA)$ ; the thrombogenic  
777 index (TI) was calculated in accordance with Ulbricht and Southgate (1991) using the formula:  
778  $(C14:0 + C16:0 + C18:0) / (0.5 \times MUFA + 0.5 \times n-6 \text{ PUFA} + 3 \times n-3 \text{ PUFA} + n-3/ n-6 \text{ PUFA})$ .

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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 $\Delta^9c, 11t$ (CLA)	15	16	16	13.752	0.939
C18:2 $\Delta^{10t, 12c}$ (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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**Table 8.** Blind and informed liking scores for chickens meat.

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

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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; <sup>A,B</sup> 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