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1   **EFFECT OF GENOTYPE ON RAW AND COOKED POULTRY MEAT**

2   **Fatty acid profile, oxidative status, and content of volatile organic compounds in**

3   **raw and cooked meat of different chicken strains**

4

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ABSTRACT

Chicken meat is rich in unsaturated fatty acids. Therefore, it is more susceptible to lipid oxidation and production of volatile organic compounds (**VOC**). In this study, we evaluated the fatty acids, antioxidants, and VOC profiles of raw and cooked meat samples derived from four strains of chicken differing in their growth rates, which were as follows: Slow-Growing (**SG**, Leghorn), Medium-Growing (**MG**, Hubbard and Naked Neck) and Fast-Growing (**FG**, Ross). The VOC profile of meat was measured using proton-transfer reaction mass spectrometry (**PTR–MS**). The VOC were identified using PTR–time of flight-MS (**PTR-ToF-MS**). The data were analyzed using both univariate and multivariate models. Twenty main VOC were identified, which were classified into the following chemical categories: aldehydes, alkadienes, alkenes, furans, amides, alcohols, and other compounds. Our results revealed that the chicken genotype and the method of cooking strongly influenced the VOC profile of the meat. Identifying the relationships between these traits allowed us to highlight the trade-off of the main substrates such as n-3 and n-6 Polyunsaturated Fatty Acids (**PUFA**), protective substances (antioxidants) and degradation products (VOC) of the poultry meat produced during cooking. The extent of VOC production and n-3 loss was found to be higher for the SG genotype. Reduction of n-6 was higher in MG whereas small losses in antioxidants and PUFA were observed in the FG genotype, consequently, resulting in the lowest production of VOC.

The SG and MG are genotypes more active from a kinetic point of view respect to the FG ones. For this reason, in the FG genotypes the antioxidants are less involved in the oxidative stress induced by the movement thus, they were available to protect the lipid of the meat during the cooking process. These results suggested that the use of SG and MG genotypes requires a specific dietary protocol (e.i. increasing the antioxidants content) in order to counteract the lipid oxidations in all the phases: *in vivo, post-mortem*, and during/after cooking.

Keywords: genotype, cooking, volatile organic compound, fatty acid profile, antioxidant.

INTRODUCTION

47

48

49 Humans consume diets with highly unbalanced lipid profiles and there is a consensus on the need to

50 reduce the consumption of saturated fatty acids (**SFA**) and increase that of polyunsaturated fatty

51 acids (**PUFA**), particularly of the n-3 series.

52 Fish is the main source of n-3 long-chain PUFA ($\geq$ 20 carbons) such as eicosapentaenoic (**EPA**) and

53 docosahexaenoic acid (**DHA**). However, increased fishing and aquaculture has resulted in issues of

54 sustainability as they depend on the marine trophic chain and use of wild fish as feed for farmed

55 fish, respectively (Tocher et al., 2019).

56 Thus, it is crucial to find other sustainable food sources containing long chain n-3 (Menchetti. et al.,

57 2018). Consequently, the ability of terrestrial animals to elongate and desaturate α-linolenic acid

58 (**ALA**), the precursor of EPA and DHA, needs to be carefully investigated.

59 Although standard poultry meat contains low levels of EPA and DHA, several factors such as sex,

60 feed, rearing systems, and genotypes may affect their content. Particularly, genotype is an important

61 factor affecting the fatty acid composition of poultry meat, which is as follows: usually, a higher

62 percentage of n-3 PUFA is produced by slow-growing (**SG**) chickens compared to the fast-growing

63 (**FG**) ones (Sirri et al., 2010). Castellini et al., (2002) showed that this content could increase in SG

64 birds raised in free-range and organic systems due to high pasture intake. Simultaneously, intake of

65 grass improves their antioxidant responses and prevents PUFA from oxidation (Dal Bosco et al.,

66 2016, Cartoni Mancinelli et al., 2019).

67 Nevertheless, there is a lack of knowledge on how meat processing (freezing, cooking) can affect its

68 nutritional properties and the development of unpleasant odors and aroma, which are important for

69 consumer acceptability. Volatile organic compounds (**VOC**) (Holm et al., 2013), are some of the

70 molecules responsible for such alteration in the food, the production of which, is influenced by

71 many factors (Leroy et al., 2009). For instance, degradation of nutrients on the surface of cured

72 products, particularly by lactic acid bacteria, can produce unpleasant odours (Holm et al., 2012).

73   However, hygienic conditions and use of low storage temperature, which restrict the growth of

74   spoilage microorganisms, can prevent excessive generation of VOC (Gill, 2007) in raw meat.

75   Additionally, oxidation of lipids during cooking is the main factor responsible for VOC production

76   (Angelo et al., 1987). Warmed-over flavors develop due to the thermal autoxidation of PUFA,

77   although other components such as proteins or carbohydrates can also contribute to the process. A

78   high cooking temperature induces the Maillard reaction, which also results in the development of

79   VOC (Byrne et al., 2001). Therefore, meat is a highly perishable food and its susceptibility to lipid

80   oxidation depends on the species of livestock, which particularly decreases in the order of fish >

81   poultry > pork > beef > lamb. This ranking is attributed to the different levels of PUFA and

82   presence of specific antioxidants, such as tocopherols, in the meat (Rhee et al., 1996).

83   Since the production of VOC is linked to the contents of PUFA and antioxidants, which vary with

84   the species and genotype of the livestock (Sirri et al., 2010), this study evaluates the effect of

85   cooking on lipid oxidation, PUFA, and VOC in different poultry strains. Accordingly, the amount

86   of VOC produced, fatty acid profile, and content of tocols and thiobarbituric acid-reactive

87   substances (**TBARS**) in raw and cooked meat derived from four strains of chicken with different

88   growth rates were analyzed.

89 **MATERIALS AND METHODS**

90

91 Pure linoleic acid (**LA**) and ALA, linolenic acid methyl ester, and organic solvents were obtained

92 from Sigma-Aldrich (Germany). The methyl ester of stearidonic acid (C18:4n-3) was obtained from

93 Cayman Chem (Ann Arbor, Michigan, USA).

94

95 *Experimental layout and sample preparation*

96 Poultry with 4 different genotypes and growth rates, namely SG (Leghorn), medium growing (**MG**,

97 Naked Neck and Hubbard Red JA), and FG (Ross 308) were reared at the experimental section of

98 the University of Perugia (Perugia, Italy) according to the EU Regulation 834/07 and Directive

99 2010/63/EU and transposed as per Regional Directive n.26 on animal welfare for experimental and

100 other scientific purposes.

101 The animals were reared indoors in equal densities (5 chickens/m^2) and administered the same

102 standard diets (Table S1).

103 Since the growth rate for different genetic strains differs significantly, the birds were slaughtered

104 upon attaining the same stages of maturity (about 60% of the adult weight). Leghorn, Hubbard and

105 naked neck, and Ross chickens were reared for 100, 56, and 42 days, respectively (Table S2). All

106 chickens were slaughtered in a commercial slaughtering house.

107 For each genotype, ten breast samples (five males and females, each) were collected from the

108 carcasses. From every breast sample 3 replicates (3 g of meat each) were analyzed both for raw and

109 cooked meat. From each animal, two aliquots of approximately 100 g of the breast muscle were

110 collected, vacuum-packaged, and frozen at -30 °C. After two weeks of storage, all the samples were

111 thawed at 4 °C for 6 h, of which, half were analyzed immediately, and whereas the other aliquots

112 were cooked by placing them in 250-mL glass bottles and boiling in a water bath at 80 °C for 30

113 min. With this cooking method the muscle samples reach a temperature of 60 °C ± 1. All samples

114 were stored at 5° C until completion of the analysis, which was carried out within 12 h from the

115 time of thawing.

116

117 *Proton-transfer reaction–mass spectrometry (PTR–MS) and PTR–Time-of-Flight (ToF)-MS*

118 *measurements*

119

120 Finely cut raw or cooked meat weighing 3 g was placed in a 250-mL glass bottle and equilibrated in

121 a water bath at 35 °C for 30 min. The samples analyzed on day 1 were randomized across genotypes

122 and replicates.

123 After temperature equilibration, the bottles were connected to a PTR–MS inlet, which was heated to

124 60 °C. The headspace flow rate was adjusted to 55 mL/min. A flow of 32 mL/min was directed into

125 the PTR–MS *via* a teflon tubing of diameter 0.25 mm. A constant drift voltage and pressure of 600

126 V and 2.19 ± 0.01 mbar, respectively, were maintained in the reaction chamber. A quadrupole mass

127 spectrometer was used to analyze the masses of the samples, which were detected as the ion counts

128 per second using a secondary electron multiplier. The ion intensities of the masses were converted

129 to volume mixing ratio (ppb) values, based on the method employed by Lindinger et al.,(1998).

130 Mass spectral data were collected over a range of 20–160 *m/z* with a dwell time of 200 ms.

131 Although data of 5 cycles were collected for each measurement, only the average of cycles 2 to 4

132 was used for analysis.

133 Prior to analyzing each meat sample, the PTR-MS spectrum of a blank was acquired by sampling

134 the contents from an empty bottle. This spectrum was subtracted from the sample spectra before

135 data analysis (Aprea et al., 2007).

136 To identify the different molecules in raw and cooked meat, the samples were analyzed using PTR-

137 ToF-MS (Ionicon GmbH, Innsbruck, Austria). Cubes of approximately 3 g were equilibrated at 30

138 °C for 30 min in a 250-mL screw-cap glass bottle, and the sample from the headspace was directed

139 to the inlet of the high-sensitivity (**HS**) PTR-MS system (Ionicon GmbH) with an airflow rate of 75

6

140   mL/min. The temperatures of the inlet and drift chamber were maintained at 60 °C. The HS PTR-

141   MS was operated at a standard E/N (ratio of the electric field strength across the reaction chamber,

142   E, to the number density of the buffer gas, N, within the chamber) of 138 Td (1 Td = 10-17 cm^2 V

143   molecule^{-1}) and measured in the "mass scan" mode. Thus, a complete mass spectrum in the range

144   of 20–150 atomic mass units (amu) at a mass detection rate of 0.2 s mass^{-1} was obtained in 26 s. the

145   samples were analyzed independently in triplicates and seven mass scan cycles were used to

146   measure each replicate. The background measurements were obtained by alternating seven scan

147   cycles of the sample with seven scan cycles of a blank air sample. The individual components in the

148   fraction were identified by directly comparing the mass spectra obtained from the samples with

149   those available in NIST92 and Wiley5 libraries and of pure standards.

150

151   *Chemical analysis and Fatty Acid determination*

152

153   The proximate composition of raw and cooked meat was determined using the AOAC method

154   (Anastassiades et al., 2003). The fatty acid profile of raw and cooked breast meat was determined

155   by analyzing the lipids extracted from approximately 5 g of the samples in a homogenizer using 20

156   mL of 2:1 chloroform/methanol (Folch et al., 1957), followed by filtration through a Whatman no. 1

157   filter paper. The fatty acids were identified in the form of their methyl esters using a Varian CP-

158   3800 Gas Chromatograph (Milano, Italy) and a DB wax capillary column (25 mm ø, 30 m long).

159   The percentages of fatty acids were calculated using the Chrom-Card software. Individual fatty acid

160   methyl esters (FAME) were identified by referencing against the retention time of authentic FAME

161   standards (Sigma–Aldrich, Bellefonte, PA, USA). The relative quantity of each fatty acid present in

162   the meat was calculated using heneicosanoic acid (C21:0; Sigma–Aldrich) as the internal standard

163   and expressed as mg/g of meat. The contents of the major classes of fatty acids were also calculated

164   (SFA, monounsaturated fatty acid [**MUFA**], and PUFA of n-3 and n-6 series).

165   *Tocols content*

166

167   The content of tocols (α-tocopherol [**α-T**] and its isoforms β+γ [**γ-T**] and δ [**δ-T**]; α, β+γ tocotrienol

168   [**β -T₃, γ-T₃**]) in the meat was quantified using high-performance liquid chromatography (**HPLC**)

169   (Hewavitharana et al., 2004). A single peak for γ-T was detected intermediate to those of α-T and δ-

170   T on the chromatogram, and all three were quantified.

171   Briefly, 5 mL of distilled water and 4 mL of ethanol were added to 2 g of meat and vortexed for 10

172   s. Then, 4 mL of hexane containing butylated hydroxytoluene (**BHT** 200 mg/L) was added, and the

173   mixture was carefully shaken and centrifuged. An aliquot of the supernatant (3 mL) was dried under

174   a nitrogen stream and dissolved using 300 μL of acetonitrile. From this, 50 μL was injected into the

175   HPLC system (Perkin Elmer Series 200) equipped with an autosampler system (model AS 950-10,

176   Jasco, Tokyo, Japan) and a Synergy Hydro-RP column (4 μm, 4.6 × 100 mm; Phenomenex,

177   Bologna, Italy). The peaks were identified using a Jasco FP-1525 FD detector (excitation and

178   emission wavelengths of 295 and 328 nm, respectively) and quantified through external calibration

179   curves obtained using increasing amounts of pure standards (Sigma–Aldrich, Steinheim, Germany)

180   in ethanol.

181

182   *TBARS assay*

183

184   The extent of muscle lipid oxidation was evaluated using a spectrophotometer adjusted to 532 nm

185   (Shimadzu Corp., UV-2550, Kyoto, Japan) according to the modified method of Ke et al., 1984

186   which measures the absorbance of TBARS. The oxidation products were quantified as equivalents

187   of malondialdehyde (mg **MDA**/kg muscle) using a 1,1,3,3-tetraethoxypropane calibration curve.

188

189

190

8

191   *Statistical analysis*

192

193   In addition to analyzing single qualitative traits, the differences in few major traits (n-3 and n-6

194   PUFA, tocols, VOC) between the samples of raw and cooked meat (Δ) were also evaluated. All

195   traits were expressed as dry matter (**DM**) to prevent discrepancies due to changes in the moisture

196   content of the raw and cooked samples.

197   Two different statistical approaches were employed. Firstly, univariate analysis was conducted

198   using a linear model (Muenchen, 2012; StataCorp, 2015; Wang et al., 2016; StataCorp, 2017) in

199   which, the effects of genotype, processing (raw, cooked), and their interactions were considered.

200   The effect of sex was not found to be significant and, hence, omitted from the analysis. The

201   differences were considered significant when $P \leq 0.05$. The statistical significance of values was

202   reported only for major traits for simplification purposes.

203   Secondly, multivariate analysis was performed using an SPSS Statistics version 23. A set of

204   variables was selected by inspecting the R-matrix and communalities (Righi et al., 2019, Twigg,

205   2010), and subjected to principal component analysis (**PCA**). We used the Kaiser–Meyer–Olkin and

206   Bartlett tests to verify the adequacy of sampling and associated correlations. Principal components

207   (**PC**) with eigenvalues > 1 were retained and rotated using the varimax method. Only factor

208   loadings with an absolute value greater than 0.5 were discussed (Menchetti. et al., 2018, Pituch and

209   Stevens, 2015). Regression scores were calculated and additional groups for each PC (PC1 and

210   PC2) were determined.

211

212 **RESULTS**

213

214 *Significance of effects*

215

216 The effects of genotype, processing, and their interactions on chicken meat are reported in Table S3.

217 We found that the genotype of the poultry and the meat processing affected the content of majority

218 of the analyzed compounds except butene, butanol, methyl, metacryl amide, and α-T_3. The

219 interaction of genotype × processing was always found to be significant, except in case of some

220 VOC (butene, butanol, methyl furanone, metacrylic amide, and heptanal), fatty acids (14:0,

221 22:1cis9n-9, 20:5n-3, 22:5n-3, 22:6n-3), tocols (δ-T_3, γ-T_3,), and TBARS.

222

223 *VOC development*

224

225 Twenty main VOC were identified and grouped into different chemical categories, which were as

226 follows: aldehydes, alkadiene, alkenes, furans, amides, alcohols, and other compounds (Table 1 and

227 Figure 1). Low amounts of aldehydes, alkadienes, alkenes, furans, amides, and alcohols (2.70, 0.42,

228 0.07, 0.06, 0.04, 0.02% of total VOC, respectively) were detected in the raw meat whereas other

229 compounds (acetic acid, butyramide, methyl pyrrolidine, propionic ether) constituted 96.51%.

230 Cooking had strongly impacted VOC production. The content of VOC detected in cooked samples

231 was 5.5 times that of raw meat (Figure 1). Aldehydes were the most important group of compounds

232 detected in cooked meat of all genotypes (64.85% of the total VOC), of which, ethanal was the

233 principal compound. Alkenes (2.44%, mainly 1,4-hexadiene), furans, amides, and alcohols were the

234 components of VOC detected in minor amounts.

235 We determined that the genotype of the poultry was responsible for the major differences detected

236 in the VOC profiles of their meats. Particularly, the highest increase in VOC (47 times) was

237 observed in the cooked samples of SG (Leghorn) meat compared to the raw ones. Few changes (3.5

238 and 3.8 times) were observed in the meats derived from other genotypes such as MG (Hubbard and

239 Naked Neck, respectively), while the lowest content of VOC was detected in meats from FG (3

240 times).

241

242 *Chemical composition and Fatty Acids profile*

243

244 Significant differences in the total fat content were observed in both raw and cooked samples of

245 different poultry strains. The highest values for fat content were obtained from the meat samples

246 belonging to the genotype Ross, followed by those of Hubbard and Naked Neck, while those of

247 Leghorn were the lowest (Table S4).

248 It could be elucidated that cooking had affected the chemical composition of the meat mainly due to

249 a reduction in its water content. However, the percentage of protein and ash was almost the same in

250 raw and cooked samples whereas lipid loss was approximately 13-17% in cooked meat (data

251 calculated from table S4).

252 Upon analyzing the PUFA profiles of cooked meat (Table 2), it was observed that, although values

253 of total n-6 and n-3 fatty acids were lowest in meat of SG chickens, the loss of n-3 (Δ) was the

254 highest. Contrarily, their amounts were higher in the meats of MG and FG strains, but their

255 "absolute" losses were lower.

256

257 *Antioxidant content and Oxidative stability*

258

259 The TBARS values and the antioxidant content of raw and cooked meat and its variation (Δ) due to

260 the cooking process are reported in Table 3. The Hubbard and Ross chickens showed the highest

261 value of total tocols both in raw and cooked meat, while Leghorn had the lowest ones. The Hubbard

262 and Ross birds exhibited the highest decrease in tocols (35.96 and 30.15 μg/g DM, respectively)

263 after cooking.

264 As expected, the TBARS increased in the cooked meat. The cooked Leghorn meat showed the

265 highest increase in TBARS (about 4 times) and the most decrease in tocols (about 15 times)

266 compared with the other poultry genotypes.

267

268 *Principal Component Analysis*

269

270 Multivariate analysis allowed us to determine the combined relation between the main traits of

271 cooked meat (VOC, Δ n-3, n-6, and content of tocols). PCA revealed two components, which

272 accounted for 76.06% of the total variance (Table 4). The highest loadings of VOC and Δ n-3 were

273 observed in PC1 while PC2 was mainly influenced by Δ n-6 and the content of tocols, and

274 explained 43.04% and 33.02% of the total data variance, respectively.

275 The poultry strains were differentiated based on their PC scores on the factor maps determined

276 through PC1 and PC2, according to their growth rates (Figure 2). The PC scores of SG samples

277 (Leghorn) were concentrated in the fourth quadrant with positive PC1 and negative PC2 scores. The

278 PC1 scores of FG samples (Ross) were negative and mainly located in the first and third quadrants.

279 The scores of meats from Naked Neck and Hubbard, which had similar growth rates, were positive

280 for both PC1 and PC2, since both were located in the second quadrant. Based on their position in

281 the factor map (Figure 2), VOC production and Δ n-3 were higher for the SG genotype whereas MG

282   was characterized by a higher Δ n-6. Furthermore, small antioxidant and PUFA losses and the least

283   production of VOC were observed in meats of the FG genotype.

284

285   **DISCUSSION**

286

287   *Volatile Organic Compounds of meat*

288

289   Due to its high ratio of unsaturated/saturated fatty acids and low fat content, poultry meat is

290   considered as a healthy food for humans. It is known that PUFA, due to the presence of double

291   bonds, are susceptible to lipid oxidation, which results in the generation of numerous degradation

292   products such as aldehydes, ketones, alcohols, aliphatic hydrocarbons, acids, and esters (Campo et

293   al., 2003, Menchetti et al. 2020), which are responsible for the development of flavors in meat.

294   However, their concentrations depend mainly on the method of cooking because it affects their

295   degree of degradation. For instance, compared to boiling, more compounds such as pyrazines,

296   pyridines, pyrroles, and thiozoles are generated upon roasting, grilling, and frying chicken meat (

297   Shahidi et al., 2020).

298   The present study had focused on the response of poultry genotypes with different growth rates to

299   the cooking procedures based on the generation of VOC and content of fatty acids, tocols and

300   TBARS. To our knowledge, this is one of the few studies to have explored the complex

301   mechanisms, which regulate the relationship between cooking and the dynamics of PUFA, VOC,

302   and antioxidants in meat of different poultry strains.

303   It was observed that the number of VOC in raw meat were few, which increased significantly upon

304   cooking. The main VOC detected in cooked meat belonged to the group of aldehydes (Table 1),

305   which are generally associated with the degradation of PUFA thus, confirming that the majority of

306   VOC produced during cooking originate through lipolysis (Tornberg, 2005).

307 Pentanal and hexanal derive from the β-oxidation of fatty acids, mainly α-linolenic acid (Del Pulgar

308 et al., 2011). Guerrero-Legarreta et al., (2010) have reported that some aldehydes (hexanal,

309 pentanal, heptanal, octanal, and nonanal) are responsible for off-odors. This suggests that the meat

310 from Leghorn chickens was more susceptible to the production of unpleasant odors compared to

311 that of the other genotypes.

312 The main aldehydes detected in our study (butanal, cyclohexadiene, heptanal, hexanal, nonanal,

313 norbornane, octanal, and pentanal) are characterized by the presence of saturated chains of four to

314 nine carbon atoms. These compounds are derived through the oxidation of PUFA and contain more

315 than 20 carbon atoms (C20:4, C20:5, C22:6) whereas C18:2 and MUFA do not contain sufficiently

316 long and saturated alkyl chains. However, the alkyl radicals from C20 PUFA may degrade the other

317 fatty acids (C18:2 and C18:1) as a result of a chain reaction (Tornberg, 2005). Accordingly, our

318 results demonstrated that the content of C20:4n-6 was lower in the meat of genotypes with a higher

319 alkanal content (Leghorn), suggesting the manifestation of a specific oxidation mechanism.

320 Probably, the generation of alkanals was not related to the other C20 PUFA because their content

321 was less than 0.5 mg/g DM compared to C20:4 ($\geq$1 mg/g DM).

322 Despite alcohols being the second most abundant VOC detected in the meat samples, their

323 incidence in the total VOC content was very scarce. In raw meat, alcohols are derived largely due to

324 the metabolism of hetero-fermentative lactic acid bacteria. Schuster et al., (2018) have found that

325 the alcohol levels increase in vacuum-packed lamb meat. However, it has been reported that a

326 minor percentage of alcohol production also depends on the content of PUFA (Del Pulgar et al.,

327 2011), thus explaining the presence of alcohols in cooked meat also.

328   *Fat content and Fatty Acid profiles of meat*

329

330   It is widely known that cooking largely affects the qualitative characteristics of meat. Part of these

331   changes during cooking result from the degradation of substrates (such as PUFA) and concentration

332   of nutrients due to water loss (Badiani et al., 2002).

333   Our results have confirmed that cooked meats are enriched with nutrients compared to raw ones.

334   Additionally, cooking reduced also the fat content in meat, which mainly affected the content of

335   PUFA and MUFA while that of SFA (Table 2) remained almost stable. This trend was observed in

336   meat samples of birds belonging to all genotypes with variations in the extent of impact.

337   As mentioned above, chicken meat is highly susceptible to oxidation because it contains long-chain

338   fatty acids with many double bonds. Consequently, losses, mainly in the content of n-3 PUFA, were

339   observed during cooking. Similarly, here, the effect of cooking differed among meats of different

340   genotypes, which is as follows: Δ n-6 PUFA was found to be higher in Hubbard and Naked Neck

341   (MG genotypes) compared to those of Ross (FG) and Leghorn (SG), whereas results contrary to

342   these were observed in SG. It should be noted that, while raw meat of SG was not particularly high

343   in the n-3 content (0.78 mg/g DM), its proportion was the highest (6.3 in LEG *vs*. 4.2, 3.7, and 3.5

344   % in HUB, NN and ROSS, respectively).

345   Cortinas et al., (2005) have suggested that a positive correlation exists between the content of

346   PUFA and extent of lipid oxidation in chicken meat.

347   The PUFA profile of meat could also depends on rearing system, the presence of outdoor runs allow

348   an additional intake of n-3, some authors (Dal Bosco et al., 2012, Castellini et al., 2016)

349   demonstrated a higher percentage of PUFA, in particular of the n-3 series, in SG than FG when

350   birds are reared free-range (Sirri et al., 2011) for the supplementary intake of vegetables, rich of

351   ALA.

Content of Antioxidants and Oxidative stability

Although the same diet containing 50 mg/kg tocopheryl acetate was administered to the animals, the content of antioxidants in the raw meat of different genotypes was found to differ. This trend probably depends not only on the fat content of the meat, because tocopherols and retinol are fat-soluble antioxidants, but also on the kinetic behavior of the birds of different genotypes (Mattioli et al., 2017).

The same study has revealed that the antioxidant levels in poultry meat depend on the dietary intake and genetic strains of the birds (Mattioli et al., 2017). As stated previously, SG animals are very active, which affects their oxidative metabolism, resulting in high production of reactive oxygen species (ROS) and extensive consumption of antioxidants by the body.

In extensive rearing systems, this requirement is supported by the intake of natural antioxidants (tocols, vitamin C, and polyphenols) contained in the pastures (Mugnai et al., 2014). In our study, the absence of outdoor runs prevented the intake of additional antioxidants, probably resulting in the low content of tocopherols in SG meat compared to those of the FG and MG genotypes.

Animals of the SG genotype, which demonstrate a superior kinetic behavior and oxidative metabolism, tend to consume more antioxidants enabling them to counteract the oxidative thrust induced by the generation of ROS (Pisoschi and Pop, 2015).

Animals belonging to the MG strains (Hubbard and Naked Neck), being less kinetic, were able to preserve their tissue antioxidant stock better, while the highest content of antioxidant compounds was detected in meats of the FG genotype (Ross), which are recognized as more "static" animals (Dal Bosco et al., 2012).

The process of cooking increased the level of TBARS and decreased that of α-T because the high temperature of cooking enhances oxidation. Rhee et al. (1996) compared raw and cooked meat of beef, pork, and poultry, and revealed that the TBARS content of cooked chicken drumstick was the highest due to its higher PUFA content. In our study, the lowest level of α-T was detected in the raw

378   meat of the Leghorn genotype and, consequently, the highest levels of TBARS were detected after

379   cooking it compared to that of the other genotypes. Selim et al., (2013) demonstrated the protective

380   effect of a diet supplemented with vitamin E against PUFA autoxidation, which neutralized free

381   radicals in both blood plasma and the muscles. Similarly, Castellini et al., (1998) demonstrated that

382   the supplementation of α-tocopheryl acetate could increase the contents of n-3 fatty acid in both raw

383   and cooked rabbit meat. In agreement, Nardoia et al., (2017) detected lower levels of TBARS in

384   the breast meat of chicken supplemented with vitamin E, grape skin, and grape pomace.

385

386   *Principal Component Analysis*

387

388   In addition to the trend obtained for single traits such as the content of total fat, fatty acids,

389   antioxidants, TBARS, and VOC in poultry meat, we analyzed the specific relationships among

390   these traits. Previous studies have investigated the effect of cooking on different attributes of meat

391   quality. However, only a handful have evaluated the trade-off of substrates (fatty acids), protective

392   substances (antioxidants), and oxidative and degradation products (TBARS, VOC) in the transition

393   of poultry meat from a raw to cooked state (see Fig. 2).

394   It has been previously reported that raw meat is characterized by the presence of some antioxidants

395   (mainly tocols) and PUFA (n-3 and n-6), which, during cooking, are oxidized easily, consequently

396   producing radicals. Majorly, ROS and reactive nitrogen species (NOS) are the main radicals

397   generated in this process, which, due to the presence of an unpaired electron, trigger an oxidative

398   chain reaction with other compounds (proteins) or PUFA themselves. The effect of these radicals is

399   modulated by the presence of antioxidants (which are primarily fat-soluble vitamins such as tocols)

400   on the muscle enabling it to reduce this pro-oxidant action. If the concentration of tocols is not

401   sufficient to counteract this oxidative process, the content of PUFA decreases due to VOC

402   generation. In our trial, even the presence of PUFA series affected the amount of VOC produced

403   during cooking, the results of which, are as follows: VOC generation was higher when n-3 PUFA

404   were used as substrates instead of n-6 due to a higher degree of unsaturation in the former

405   (Scislowski et al. 2005). This confirmed that n-3 PUFA, due to the presence of many double bonds,

406   were more susceptible to oxidation compared to their n-6 counterparts.

407   A multivariate approach permitted us to highlight the pattern of the aforementioned variables and

408   distinguish the effect of cooking on meat from poultry with different genotypes. Tocols (PC2) were

409   the most stable in meat of FG chickens, with the lowest variation in both n-6 and n-3, and having a

410   low content of VOC. Contrarily, SG meat, which was characterized by low levels of antioxidants

411   and the highest n-3 PUFA content, produced more VOC (PC1) compared to the others. The

412   genotypes for MG, which were characterized by the presence of the highest amount of n-6 PUFA

413   compared to n-3 PUFA, resulted in a lower production of VOC, which was consistent with this

414   assumption.

415   In figure 3, a schematic representation of the mechanisms regulating the relationship between

416   PUFA, VOC, and antioxidants in poultry meat during cooking has been reported.

417   Based on this perspective, more researches should focus on the stability of the PUFA profile during

418   cooking. Our findings have indicated that these compounds undergo significant degradation in the

419   meat after cooking, producing VOC and other substances (TBARS) with potential concerns for

420   human health. Accordingly, greater attention should be paid to protect against PUFA, particularly in

421   case of meats with high nutraceutical values.

CONCLUSIONS

422

423 VOC such as alkanes, alkenes, aldehydes, ketones, alcohols, represent the principal products of the

424 lipid oxidation and consequently are the main responsible of the reduction of the meat quality. A

425 drastic increase in the content of VOC (aldehydes and alkanals) was observed in cooked chicken

426 meat derived mainly due to the oxidation of PUFA, which was significantly correlated with the

427 genotype of the organism. Maximum changes in the VOC content due to cooking were observed in

428 the meat of breeds which could be defined as "more vulnerable", with greater susceptibility to

429 PUFA oxidation and associated with a lower content of antioxidants (Leghorn), resulting in a

430 remarkable loss of n-3. Contrarily, losses of n-3 PUFA from the cooked meat of the FG genotypes

431 were lower. Moreover, due to their lower activity, dietary antioxidants were rendered available for

432 protecting the lipids in their meats.

433 Nowadays, increasing attention towards animal welfare has promoted the use of SG and MG

434 genotypes, particularly in alternative rearing systems (organic, free-range) because these strains are

435 more adaptable with a better welfare status in outdoor conditions. This adaptation is strictly related

436 to the kinetic behavior of chickens. Hence, the relationship between activity (oxidative burst) and

437 the nutritional quality of meat (PUFA profile) needs to be better defined. Our results have suggested

438 that the use of these genotypes (MG and SG) requires a specific dietary protocol to balance the

439 oxidative status of chickens *in vivo*, post-mortem, and, possibly during/after cooking. Accordingly,

440 additional studies are needed to understand the mechanism for protecting n-3 PUFA from oxidation.

441 For example, using dietary antioxidants (natural as per organic systems or synthetic, for the

442 conventional) and/or finding more appropriate cooking methods to preserve the meat quality.

443

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564 **TABLES**

565 Table 1. Influence of genetic strain and processing (raw *vs.* cooked) on VOC composition (ppb) of

566 chicken meat

Compounds	RAW				COOKED				Pooled SE[1]
Genotypes[2]	LEG	HUB	NN	ROSS	LEG	HUB	NN	ROSS	
Aldehydes									
2-Butenal	0.9	1.9	1.0	1.0	10.5	6.5	6.9	4.2	2.1
3-Ciano-propanal	0.3	0.3	0.4	0.3	38.9	13.2	17.3	9.0	4.8
Butanal	8.1	20.4	5.5	45.6	64.1	79.2	63.9	92.5	25.8
Butene	9.7	8.3	8.2	11.7	27.3	24.7	37.8	32.6	300.2
Cyclohexadiene	0.2	0.7	0.2	0.4	3.9	1.5	1.6	1.1	0.6
Ethanal	24.2	13.1	18.4	9.0	7736.2	5359.2	5613.1	3090.8	1493.9
Heptanal	0.1	0.2	0.2	0.4	2.0	1.2	1.3	0.9	0.6
Hexanal	0.3	0.4	0.4	0.1	46.8	12.7	22.2	9.0	11.9
Nonanal	0.2	0.3	0.5	0.2	14.3	3.3	5.0	1.9	3.0
Norbornane	0.2	0.3	0.3	0.2	9.5	6.1	8.0	5.1	4.3
Octanal	0.4	0.4	0.4	0.4	2.8	1.4	1.7	1.1	0.7
Pentanal	0.7	1.4	1.7	1.5	19.9	10.5	13.2	9.5	4.3
Xylenes	0.4	0.9	0.4	0.3	9.5	10.5	8.4	9.9	5.7
Alkadiene									
1,2-Butadiene	3.9	7.8	16	2.1	557.3	122.3	222.8	73.2	81.8
Alkenes									
1,4-Hexadiene	0.7	1.3	1.7	1.1	504.7	114.7	195.5	71.8	71.5
Furanas									
Methyl furanone	0.8	0.8	0.9	1.0	12.5	12.5	10.7	10.5	4.0
2-Pentyl Furan	0.2	0.2	0.1	0.2	1.7	1.1	1.4	1.0	0.8
Ammides									
Butyramide	0.1	0.1	0.1	0.1	1.1	0.1	1.1	1.5	0.3
Methachrylic amide	0.2	0.2	0.3	0.2	3.8	4.3	3.3	3.3	1.3

Alcohols

Butanol	0.5	1.3	0.2	0.5	0.01	23.8	1.5	2.7	72.2
Σ Aldehydes	45.7	48.6	37.6	71.1	7985.7	5530.0	5800.4	3267.6	693.7
Σ Alkadiene	3.9	7.8	16	2.1	557.3	122.3	222.8	73.2	81.8
Σ Alkenes	0.7	1.3	1.7	1.1	504.7	114.7	195.5	71.8	71.5
Σ Furanas	1	1	1	1.2	14.2	13.6	12.1	11.5	3.9
Σ Ammides	0.3	0.3	0.4	0.3	4.9	4.4	4.4	4.8	1.1
Σ Alcohols	0.5	1.3	0.2	0.5	0.01	23.8	1.5	2.7	72.2
Other compounds	192.0	2417.0	2494.0	1695.0	2421.	2967.0	3462.0	1958.3	1120.2
Total VOC[3]	244.1	2477.3	2550.9	1771.3	11487.8	8775.8	9698.7	5389.9	3210.0

567

568   Abbreviations:[1] SE, standard error;

569   [2] HUB, Hubbard; NN, Naked Neck; LEG, Leghorn; ROSS, Ross 308;

570   [3] VOC, Volatile organic compounds;

571 Table 2. Influence of genetic strains and processing (raw *vs.* cooked) on the fatty acid profiles (mg/g

572 DM) of poultry meat

Compounds[1]	RAW				COOKED				Pooled SE[3]
Genotypes[2]	LEG	HUB	NN	ROSS	LEG	HUB	NN	ROSS	
14:0	0.17	0.90	0.52	0.35	0.12	0.24	0.22	0.31	0.10
16:0	2.45	5.43	4.45	6.89	2.36	6.27	5.00	6.81	1.62
18:0	1.55	2.8	2.35	3.31	1.80	2.46	1.91	2.18	1.15
Σ SFA	4.17[a]	9.13[c]	7.32[b]	10.55[c]	4.28[a]	8.97[bc]	7.13[b]	9.30[c]	0.25
16:1cis9	0.85	2.21	1.94	2.86	0.58	1.61	1.32	2.3	0.20
18:1cis9 n-9	2.62	6.42	4.94	7.73	2.36	5.36	4.17	6.83	1.35
22:1cis9	0.15	0.31	0.34	0.30	0.17	0.35	0.35	0.37	0.01
Σ MUFA	3.73[a]	9.14[bc]	7.46[b]	10.92[c]	3.38[a]	7.83[b]	6.28[b]	9.89[c]	0.20
18:2n-6 (LA)	2.5	5.58	4.68	5.72	2.17	4.18	3.36	5.20	5.58
20:4n-6 (AA)	1.1	1.72	1.32	1.08	0.91	1.20	1.07	0.89	1.72
Σ n-6 PUFA	3.60[a]	7.30[b]	6.00[b]	6.80[b]	3.08[a]	5.38[b]	4.43[ab]	6.09[b]	1.38
Δ n-6 PUFA					0.52[a]	1.92[b]	1.57[b]	0.71[a]	0.10
18:3n-3 (ALA)	0.35	0.57	0.36	0.40	0.15	0.32	0.27	0.30	0.57
20:5n-3 (EPA)	0.22	0.35	0.28	0.39	0.15	0.30	0.22	0.34	0.35
22:5n-3 (DPA)	0.11	0.11	0.09	0.16	0.08	0.08	0.07	0.13	0.11
22:6n-3 (DHA)	0.10	0.11	0.09	0.08	0.06	0.09	0.07	0.05	0.11
Σ n-3 PUFA	0.78[a]	1.14[b]	0.82[a]	1.03[b]	0.44[a]	0.79[b]	0.63[ab]	0.82[b]	0.13
Δ n-3 PUFA					0.43[b]	0.30[ab]	0.23[a]	0.20[a]	0.08

573

574 Abbrevations:[1]SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; AA, arachidonic acid; LA,

575 linolenic acid; ALA, α-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; LCP, long chain

576 PUFA;

577 [2]HUB, Hubbard; NN, Naked Neck 1; LEG: Leghorn; ROSS: Ross 308;

578 [3]SE: standard error;

579 [a, b, c] on the same row indicate the same type of processing (raw, cooked), with means of P < 0.05;

580

581 Table 3. Influence of genetic strains and processing (raw *vs*. cooked) on the content of antioxidants

582 (μg/g DM) and oxidative stability (mg/kg DM) of poultry meat

Compounds[1]	RAW				COOKED				Pooled SE[3]
Genotypes[2]	LEG	HUB	NN	ROSS	LEG	HUB	NN	ROSS	
α-T	10.73	34.46	16.01	32.98	0.98	4.22	2.93	3.35	1.73
δ-T	1.09	0.50	0.63	0.99	0.03	0.23	0.17	0.16	0.02
γ-T	0.13	0.37	0.17	0.41	0.01	0.05	0.02	0.06	0.04
α-T$_3$	5.28	6.03	8.65	6.07	0.24	1.23	0.74	0.52	0.65
δ-T$_3$	1.13	0.17	1.38	0.87	0.30	0.03	0.17	0.20	0.14
γ-T$_3$	1.00	0.35	0.85	0.89	0.27	0.03	0.12	0.18	0.09
TBARS	0.34[a]	0.70[b]	0.54[a]	1.12[c]	1.28[c]	0.95[b]	0.65[a]	1.55[c]	0.14
Σ–T	19.50[a]	41.76[c]	28.22[b]	42.19[c]	1.28[a]	5.80[b]	4.20[b]	12.04[c]	1.82
Δ tocols (raw-cooked)					18.22[a]	35.96[c]	24.02[b]	30.15[c]	1.11

583

584 Abbreviations:[1]α-T, α-tocopherol; δ-T, δ-tocopherol; γ-T, γ-tocopherol; α-T3, α-tocotrienol; δ-T3, δ-tocotrienol; γ-T3, γ-tocotrienol; TBARS,

585 Substances Reactive to Thiobarbituric acid;

586 [2]HUB, Hubbard; NN: Naked Neck 1; LEG: Leghorn; ROSS: Ross 308;

587 [3] SE, standard error;

588 [a, b, c] on the same row indicates the same type of processing (raw, cooked), with means of $P < 0.05$;

589

590   Table 4. Principal components loadings, eigenvalue and variance

	PC1	PC2
VOC	0.71	-0.11
Δ n-3	0.59	0.05
Δ n-6	0.31	0.64
Δ tocols	0.20	0.75
Eigenvalue	1.72	1.32
% variance explained	43.04	33.02
Cumulative variance explained	76.06	

591
592   Abbreviations: VOC, Volatile organic compounds;

593   Loadings ≥0.50 or ≤–0.50 are presented in bold;

594 **FIGURES**

595 Figure 1. Main VOC classes (%) in raw (a) and cooked (b) chicken meat
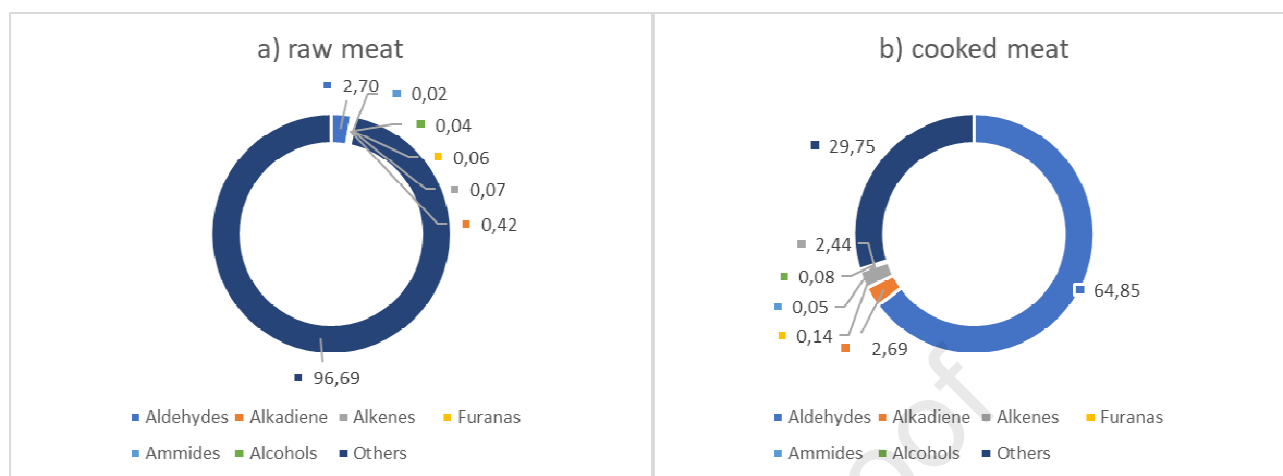
596

597 Abbreviations: VOC, Volatile Organic Compound;

598   Figure 2. Principal component analysis plot depicting the loadings and variables

599
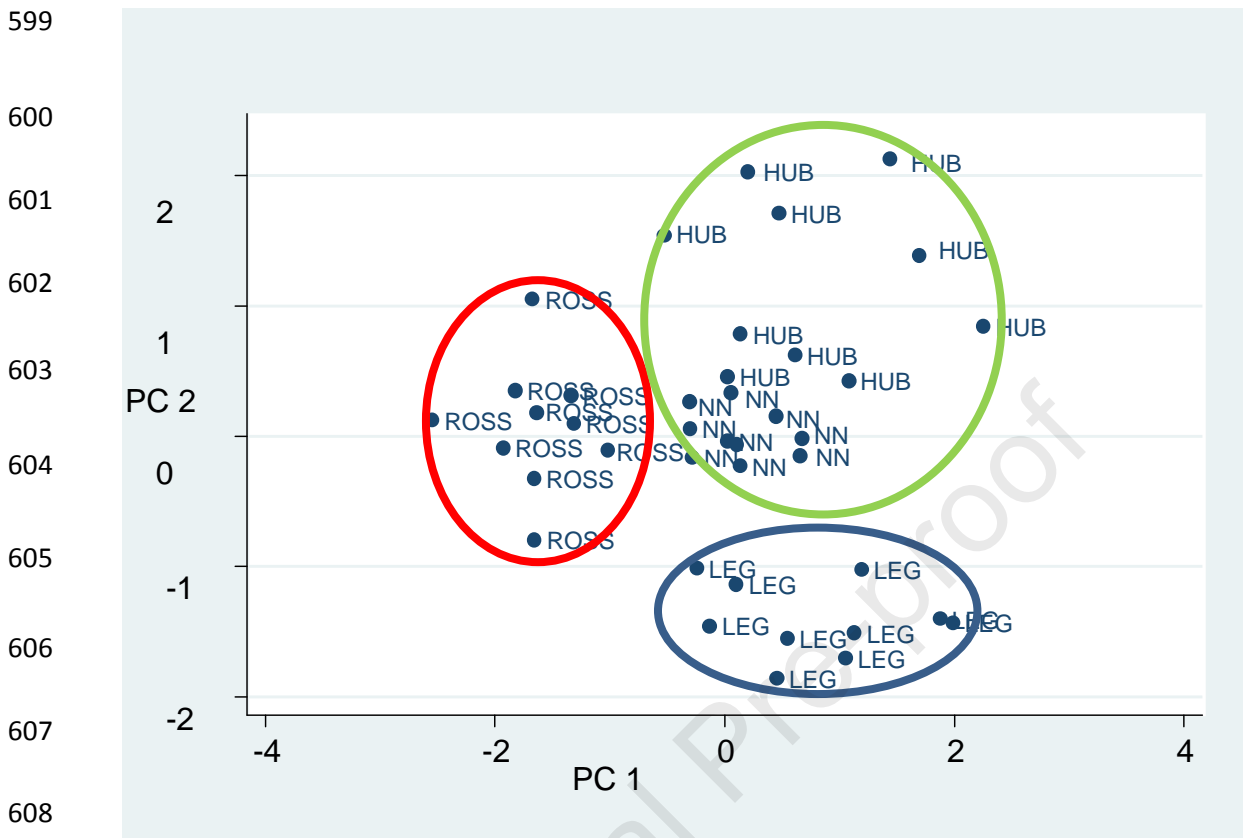
609   Abbreviations: HUB, Hubbard; NN, Naked Neck; LEG, Leghorn; ROSS, Ross 308;

610

611 Figure 3. Scheme of PUFA, VOC and antioxidant dynamics in poultry meat during cooking process

612

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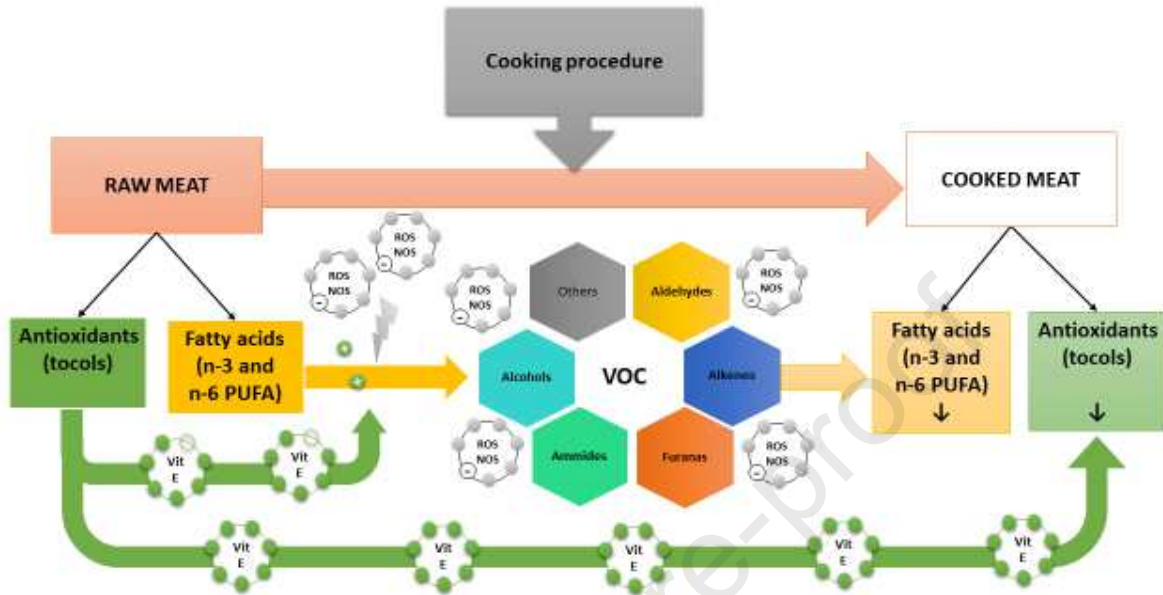
625 Abbreviations: VOC, volatile organic compound; PUFA, polyunsaturated fatty acid; NOS, reactive nitrogen species; ROS, reactive oxygen species;

626

627   **SUPPLEMENTARY MATERIAL**

628   Table S1. Formulation and chemical analysis of chicken feed

		Starter	Grower	Finisher
Ingredients				
Corn	%	53.92	55.95	53.11
soybean meal 48%	%	30.23	24.67	15.69
Extruded corn flour	%	5.08	8.90	11.45
wheat	%	5.00	5.00	15.00
Dicalcium phosphate	%	1.71	1.58	1.21
Calcium carbonate	%	1.23	1.16	1.29
Corn gluten 70	%	1.00	1.00	/
Soybean oil	%	0.62	0.54	1.15
Vitamin supplement	%	0.40	0.40	0.40
salt	%	0.20	0.18	0.23
Mineral supplement	%	0.16	0.16	0.11
Sodium bicarbonate	%	0.15	0.15	0.15
Chemical composition				
Dry Matter (DM)	%	87.80	87.89	88
Crude protein	% DM	24.01	22.16	18.41
Lipids	% DM	3.99	3.98	4.55
Crude fiber	% DM	3.48	3.58	3.60
Ashes	% DM	6.92	6.43	5.78
Metabolizable energy	kcal/kg	3245	3242	3295
Vitamin A	U.I.	11385	11377	11364
Vitamin E	Mg	36.43	36.41	36.37

647   Table S2. Productive performance of the four genotypes (slaughtered at about 60% adult weight)

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Genotypes	slaughter age (d)	slaughter weight (kg)	daily gain (g)
Leghorn	100	1.3	13
Hubbard	56	2.0	35
Naked Neck	56	2.4	42
Ross	42	3.0	71

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654   Table S3. Significance of the effects on volatile compounds (A) total lipids and fatty acids (B) and

655   oxidative parameters (C)

A) Volatile Organic Compaunds[1]	Genotype - G	Processing – P (raw *vs.* cooked)	GxP
Ethanal	***	***	***
Butadiene	***	***	***
Butene	ns	*	ns
Butenal	***	***	***
Butanal	***	***	**
Butanol	ns	ns	ns
Cyclohexadiene	***	***	***
Hexadiene	***	***	***
Cyano-propanal	***	***	***
Methyl furanone	ns	***	ns
Metacrylic amide	ns	***	ns
Pentanal	***	***	***
Butyramide	***	***	***
Norbomane	**	***	**
Hexanal	***	***	***
Xylenes	***	***	***
Heptanal	***	***	ns
Octanal	***	***	GxP
2-Pentyl furan	***	***	***
Nonanal	**	***	***
Σ Aldheydes	***	***	***
Σ Alchool	**	***	**
Σ Alkanales	***	***	***
Σ Ammine	***	***	***
Others	***	***	ns
Σ VOC	***	***	***

656   Abbreviations: [1]VOC: volatile organic compounds; *** P <0.001; ** P< 0.01; P< 0.05. ns: not significant.

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B) Fatty Acids[2]	Genotype – G	Processing – P (raw *vs.* cooked)	GxP
16:0	***	***	***
18:0	***	**	***
ΣSFA	**	**	***
16:1cis9	***	***	***
18:1cis9 n-9	***	***	***
22:1cis9	***	***	ns
ΣMUFA	***	***	***
20:4n-6 (AA)	***	***	ns
18:2n-6 (LA)	***	**	***
Σn-6	***	***	***
18:3n-3 (ALA)	***	***	***
20:5n-3 (EPA)	***	***	ns
22:5n-3 (DPA)	***	***	ns
22:6n-3 (DHA)	***	**	ns
Σn-3	***	**	***
n-3/n-6	***	***	***
ΣPUFA	***	***	***
ΣLCP	***	***	**

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658 [2]SFA, saturated fatty acids; MUFA; monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; AA, arachidonic acid; LA, linolenic acid; ALA,

659 α-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; LCP, long chain polyunsaturated fatty

660 acids;

661 *** P <0.001; ** P< 0.01; P< 0.05. ns: not significant;

662

C) Oxidative parameters [3]	Genotype - G	Processing – P (raw *vs.* cooked)	GxP
α-T	***	***	***
δ-T	***	***	**
γ-T	***	***	*
α-T$_3$	ns	***	*
δ-T$_3$	**	***	ns
γ-T$_3$	***	***	ns
TBARs	**	**	ns

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664 Abbreviations:[3]α-T, α-tocopherol; γ-T, γ-tocopherol; δ-T, δ-tocopherol; α-T3, α-tocotrienol; γ-T3, γ-tocotrienol; δ-T3, δ-tocotrienol; TBARs,

665 substances reactive to the thiobarbituric acid;

666 *** $P < 0.001$; ** $P < 0.01$; $P < 0.05$. ns: not significant.

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668   Table S4. Proximate composition of raw and cooked samples in the different poultry genotypes

Raw		LEG	HUB	NN	ROSS	Pooled SE
DM	%	23.85	24.23	23.92	24.20	1.82
Protein	g/100 g DM	93.92	92.28	92.39	92.31	8.23
Ash	"	4.61	4.62	5.02	4.17	0.32
Lipids	"	1.47^a	3.10^b	2.59^b	3.51^b	0.10
Cooked						
DM	%	33.71	34.82	35.18	34.30	3.20
Protein	g/100 g DM	93.89	92.48	92.61	92.71	9.02
Ash	"	4.89	4.88	5.12	4.37	0.40
Lipid	"	1.22^a	2.64^b	2.27^b	2.9^b	0.12

669

670   Abbreviations: HUB, Hubbard; NN, Naked Neck; LEG, Leghorn; ROSS, Ross 308;

671   a..b on the same row: $P \leq 0.05$.

672

1 **TABLES**

2 Table 1. Influence of genetic strain and processing (raw *vs.* cooked) on VOC composition (ppb) of

3 chicken meat

Compounds	RAW				COOKED				Pooled SE[1]
Genotypes[2]	LEG	HUB	NN	ROSS	LEG	HUB	NN	ROSS	
Aldehydes									
2-Butenal	0.9	1.9	1.0	1.0	10.5	6.5	6.9	4.2	2.1
3-Ciano-propanal	0.3	0.3	0.4	0.3	38.9	13.2	17.3	9.0	4.8
Butanal	8.1	20.4	5.5	45.6	64.1	79.2	63.9	92.5	25.8
Butene	9.7	8.3	8.2	11.7	27.3	24.7	37.8	32.6	300.2
Cyclohexadiene	0.2	0.7	0.2	0.4	3.9	1.5	1.6	1.1	0.6
Ethanal	24.2	13.1	18.4	9.0	7736.2	5359.2	5613.1	3090.8	1493.9
Heptanal	0.1	0.2	0.2	0.4	2.0	1.2	1.3	0.9	0.6
Hexanal	0.3	0.4	0.4	0.1	46.8	12.7	22.2	9.0	11.9
Nonanal	0.2	0.3	0.5	0.2	14.3	3.3	5.0	1.9	3.0
Norbornane	0.2	0.3	0.3	0.2	9.5	6.1	8.0	5.1	4.3
Octanal	0.4	0.4	0.4	0.4	2.8	1.4	1.7	1.1	0.7
Pentanal	0.7	1.4	1.7	1.5	19.9	10.5	13.2	9.5	4.3
Xylenes	0.4	0.9	0.4	0.3	9.5	10.5	8.4	9.9	5.7
Alkadiene									
1,2-Butadiene	3.9	7.8	16	2.1	557.3	122.3	222.8	73.2	81.8
Alkenes									
1,4-Hexadiene	0.7	1.3	1.7	1.1	504.7	114.7	195.5	71.8	71.5
Furanas									
Methyl furanone	0.8	0.8	0.9	1.0	12.5	12.5	10.7	10.5	4.0
2-Pentyl Furan	0.2	0.2	0.1	0.2	1.7	1.1	1.4	1.0	0.8
Ammides									
Butyramide	0.1	0.1	0.1	0.1	1.1	0.1	1.1	1.5	0.3
Methachrylic amide	0.2	0.2	0.3	0.2	3.8	4.3	3.3	3.3	1.3

Alcohols

Butanol	0.5	1.3	0.2	0.5	0.01	23.8	1.5	2.7	72.2
Σ Aldehydes	45.7	48.6	37.6	71.1	7985.7	5530.0	5800.4	3267.6	693.7
Σ Alkadiene	3.9	7.8	16	2.1	557.3	122.3	222.8	73.2	81.8
Σ Alkenes	0.7	1.3	1.7	1.1	504.7	114.7	195.5	71.8	71.5
Σ Furanas	1	1	1	1.2	14.2	13.6	12.1	11.5	3.9
Σ Ammides	0.3	0.3	0.4	0.3	4.9	4.4	4.4	4.8	1.1
Σ Alcohols	0.5	1.3	0.2	0.5	0.01	23.8	1.5	2.7	72.2
Other compounds	192.0	2417.0	2494.0	1695.0	2421.	2967.0	3462.0	1958.3	1120.2
Total VOC[3]	244.1	2477.3	2550.9	1771.3	11487.8	8775.8	9698.7	5389.9	3210.0

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8   Table 2. Influence of genetic strains and processing (raw *vs.* cooked) on the fatty acid profiles (mg/g

9   DM) of poultry meat

Compounds[1]	RAW				COOKED				Pooled SE[3]
Genotypes[2]	LEG	HUB	NN	ROSS	LEG	HUB	NN	ROSS	
14:0	0.17	0.90	0.52	0.35	0.12	0.24	0.22	0.31	0.10
16:0	2.45	5.43	4.45	6.89	2.36	6.27	5.00	6.81	1.62
18:0	1.55	2.8	2.35	3.31	1.80	2.46	1.91	2.18	1.15
Σ SFA	4.17[a]	9.13[c]	7.32[b]	10.55[c]	4.28[a]	8.97[bc]	7.13[b]	9.30[c]	0.25
16:1cis9	0.85	2.21	1.94	2.86	0.58	1.61	1.32	2.3	0.20
18:1cis9 n-9	2.62	6.42	4.94	7.73	2.36	5.36	4.17	6.83	1.35
22:1cis9	0.15	0.31	0.34	0.30	0.17	0.35	0.35	0.37	0.01
Σ MUFA	3.73[a]	9.14[bc]	7.46[b]	10.92[c]	3.38[a]	7.83[b]	6.28[b]	9.89[c]	0.20
18:2n-6 (LA)	2.5	5.58	4.68	5.72	2.17	4.18	3.36	5.20	5.58
20:4n-6 (AA)	1.1	1.72	1.32	1.08	0.91	1.20	1.07	0.89	1.72
Σ n-6 PUFA	3.60[a]	7.30[b]	6.00[b]	6.80[b]	3.08[a]	5.38[b]	4.43[ab]	6.09[b]	1.38
Δ n-6 PUFA					0.52[a]	1.92[b]	1.57[b]	0.71[a]	0.10
18:3n-3 (ALA)	0.35	0.57	0.36	0.40	0.15	0.32	0.27	0.30	0.57
20:5n-3 (EPA)	0.22	0.35	0.28	0.39	0.15	0.30	0.22	0.34	0.35
22:5n-3 (DPA)	0.11	0.11	0.09	0.16	0.08	0.08	0.07	0.13	0.11
22:6n-3 (DHA)	0.10	0.11	0.09	0.08	0.06	0.09	0.07	0.05	0.11
Σ n-3 PUFA	0.78[a]	1.14[b]	0.82[a]	1.03[b]	0.44[a]	0.79[b]	0.63[ab]	0.82[b]	0.13
Δ n-3 PUFA					0.43[b]	0.30[ab]	0.23[a]	0.20[a]	0.08

10

11   Abbrevations:[1]SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; AA, arachidonic acid; LA,

12   linolenic acid; ALA, α-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; LCP, long chain

13   PUFA;

14   [2]HUB, Hubbard; NN, Naked Neck 1; LEG: Leghorn; ROSS: Ross 308;

15   [3]SE: standard error;

16   [a, b, c] on the same row indicate the same type of processing (raw, cooked), with means of $P < 0.05$;

17

18 Table 3. Influence of genetic strains and processing (raw *vs*. cooked) on the content of antioxidants

19 (µg/g DM) and oxidative stability (mg/kg DM) of poultry meat

Compounds[1]	RAW				COOKED				Pooled SE[3]
Genotypes[2]	LEG	HUB	NN	ROSS	LEG	HUB	NN	ROSS	
α-T	10.73	34.46	16.01	32.98	0.98	4.22	2.93	3.35	1.73
δ-T	1.09	0.50	0.63	0.99	0.03	0.23	0.17	0.16	0.02
γ-T	0.13	0.37	0.17	0.41	0.01	0.05	0.02	0.06	0.04
α-T$_3$	5.28	6.03	8.65	6.07	0.24	1.23	0.74	0.52	0.65
δ-T$_3$	1.13	0.17	1.38	0.87	0.30	0.03	0.17	0.20	0.14
γ-T$_3$	1.00	0.35	0.85	0.89	0.27	0.03	0.12	0.18	0.09
TBARS	0.34[a]	0.70[b]	0.54[a]	1.12[c]	1.28[c]	0.95[b]	0.65[a]	1.55[c]	0.14
Σ–T	19.50[a]	41.76[c]	28.22[b]	42.19[c]	1.28[a]	5.80[b]	4.20[b]	12.04[c]	1.82
Δ tocols (raw-cooked)					18.22[a]	35.96[c]	24.02[b]	30.15[c]	1.11

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21 Abbreviations:[1]α-T, α-tocopherol; δ-T, δ-tocopherol; γ-T, γ-tocopherol; α-T3, α-tocotrienol; δ-T3, δ-tocotrienol; γ-T3, γ-tocotrienol; TBARS,

22 Substances Reactive to Thiobarbituric acid;

23 [2]HUB, Hubbard; NN: Naked Neck 1; LEG: Leghorn; ROSS: Ross 308;

24 [3] SE, standard error;

25 [a, b, c] on the same row indicates the same type of processing (raw, cooked), with means of $P < 0.05$;
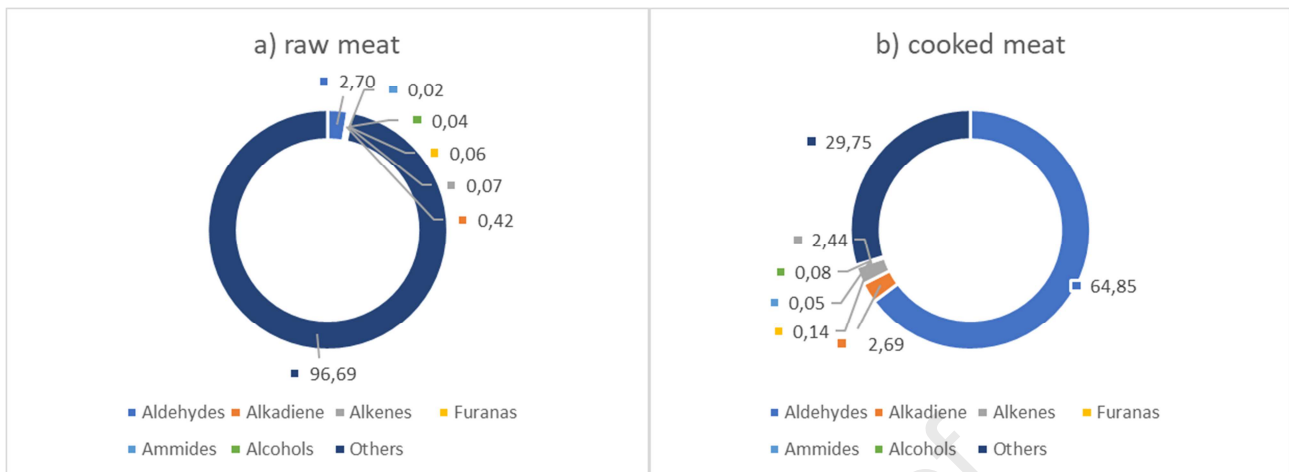
26

Table 4. Principal components loadings, eigenvalue and variance

|	PC1	PC2
VOC	0.71	-0.11
Δ n-3	0.59	0.05
Δ n-6	0.31	0.64
Δ tocols	0.20	0.75
Eigenvalue	1.72	1.32
% variance explained	43.04	33.02
Cumulative variance explained	76.06	

Abbreviations: VOC, Volatile organic compounds;

Loadings ≥0.50 or ≤−0.50 are presented in bold;

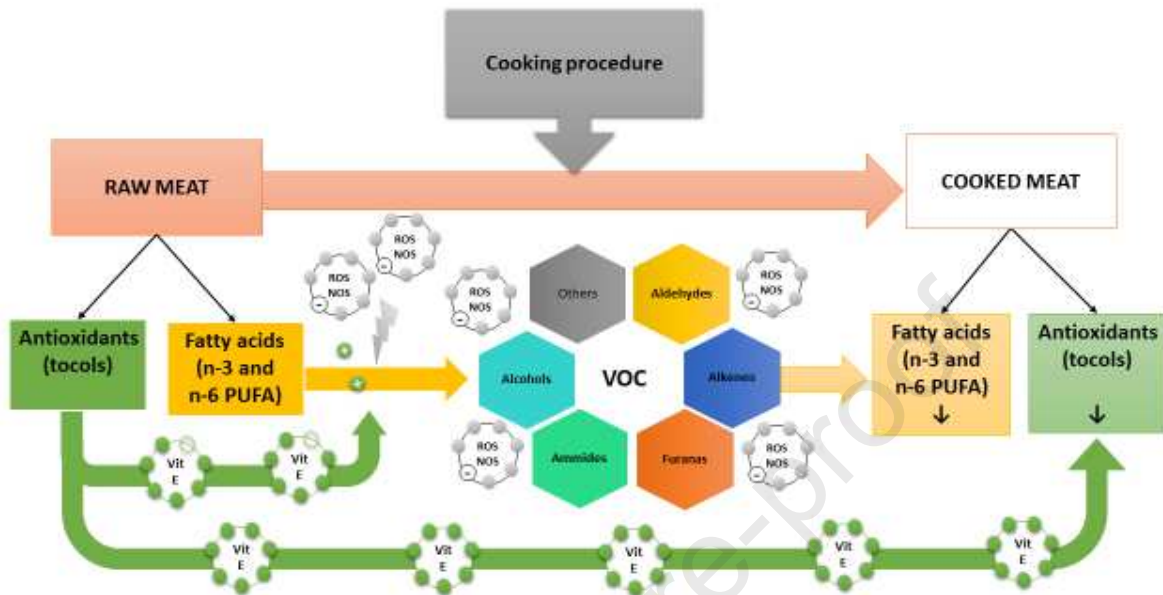Figure 1. Main VOC classes (%) in raw (a) and cooked (b) chicken meat

Abbreviations: VOC, Volatile Organic Compound;

Figure 2. Principal component analysis plot depicting the loadings and variables
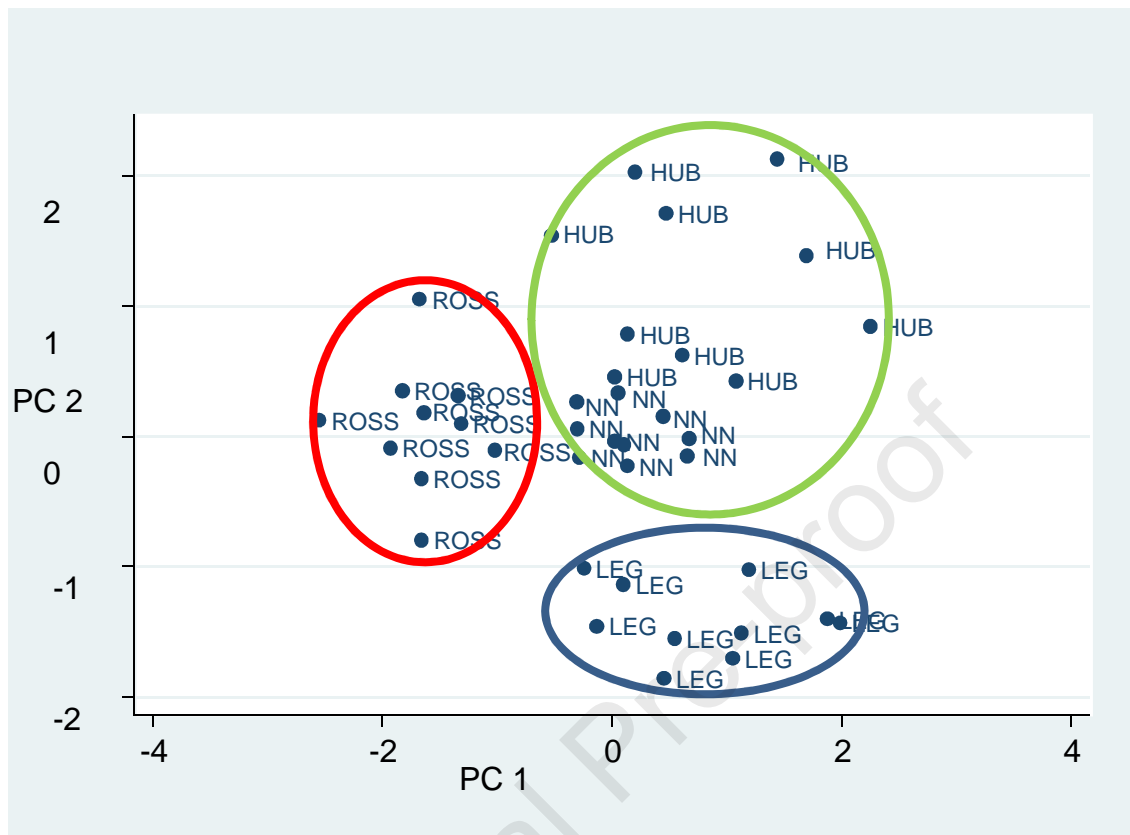
Abbreviations: HUB, Hubbard; NN, Naked Neck; LEG, Leghorn; ROSS, Ross 308;

Figure 3. Scheme of PUFA, VOC and antioxidant dynamics in poultry meat during cooking process

Abbreviations: VOC, volatile organic compound; PUFA, polyunsaturated fatty acid; NOS, reactive nitrogen species; ROS, reactive oxygen species;