1 **Nutritional characteristics and quality of eggs from laying hens fed a diet**

2 **integrated with chestnut tannin extract (*Castanea sativa* Miller).**

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4 Sara Minieri[1,*], Arianna Buccioni[2], Andrea Serra[3], Ilaria Galigani[2], Antonio Pezzati[2],

5 Stefano Rapaccini[2], Mauro Antongiovanni[4].

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7 [1] Department of Veterinary Science, University of Pisa, Italy

8 [2]Department of Agrifood Production and Enviromental Sciences, Animal Science

9 Section, University of Florence, Italy.

10 [3]Department of Agricultural, Food and Agro-environmental, University of Pisa, Italy.

11 [4] Gruppo Mauro Saviola, Mantova, Italy.

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13 *Corresponding Author: Sara Minieri. Department of of Veterinary Science,

14 University of Pisa, Italy, Viale delle Piagge, 2. Italy. tel:+390502216906; e-mail

15 address: sara.minieri@unipi.it

16

17 **Abstract**

18 The trial was performed with 80 laying hens belonging to two Tuscan autochthonous

19 breeds: 40 birds of the Mugellese breed (MU) and 40 of the White Leghorn breed

20 (WL). The animals were allotted to 4 groups of 20 hens each: 2 groups were fed a

21 commercial diet and worked as the control groups (MUC and WLC); the other 2

22 groups received the same diet, integrated with 2 g of chestnut tannin extract per kg of

23 diet (MUT and WLT). 70 eggs were randomly collected and analysed for cholesterol

24 content, fatty acid profile, weight, thickness of shell and colour of yolk. Physical

25 parameters, including yolk color, and indices of egg quality were not affected by the

26 treatments. The concentration of unsaturated fatty acids increased whereas cholesterol

27 resulted significantly depressed: -17% in WLT and -9% in MUT. Dietary integration

28 with chestnut tannin extract resulted in a modification of lipid composition, toward a

29 healthful quality of eggs.

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31 **Key word**: chestnut tannin, egg yolk, fatty acids, cholesterol.

32

33 **1. Introduction**

34   The world daily consumption of eggs is high because they are inexpensive source of

35 nutrition and because they are ingredients for many food products. Hence, eggs are

36 considered the primary source of cholesterol in human diet for their chemical

37 composition. Studies on lipid metabolism have shown that most of the eggs'

38 cholesterol is synthesized in the liver and is used essentially for embryonic

39 development (Naber E.C, 1976). Cholesterol and its esters, therefore, are found only

40 in yolks where they are emulsified by high, low and very low density lipoproteins.

41 Literature reported that egg cholesterol is strongly influenced either by genetic factors

42 or by lay intensity and, hens belonging to good-laying breeds produce eggs with a

43 lower cholesterol content compared to eggs from autoctonous breeds, characterized

44 by a lower daily egg production percentage (NRC, 1994). Despite conflicting

45 evidence about the role of dietary cholesterol in cardiovascular deseases, many efforts

46 have been made to reduce its content in eggs by genetic approaches and by new

47 feeding strategies (Milinsk et al., 2003). Several authors demonstrated that the fiber

48 percentage in diet plays an important role in reducing the cholesterol in yolks,

49 especially if associated with a low supplementation of vegetable oils (Naber, 1976,

50 McNaughton, 2014). Also the dietary integration of probiotics as *Lactobacillus*

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sporogenes showed positive results in limiting cholesterol in eggs (Panda et al., 2008). Literature reported that hydrolizable polyphenols are able to reduce the cholesterol synthesis in monogastrics including humans, interfering with lipid metabolism at the liver level and that the gallic acid moiety is important in these inhibitory activities (Kim et al., 2013; Kobayashi and Ikeda, 2014; Lu and Hwang, 2008). Tannins extracted from Chestnut wood (*Castanea sativa* Miller), a common plant species in the Mediterranean area, are an example of hydrolizable polyphenols characterized by the presence of the gallic acid moiety (Campo et al., 2012). However, the use of tannins in poultry feeding is limited by their anti-nutritional effect responsible for the decrease of organic matter digestibility and consequently, of the growth performance or of a depression in egg production (Ahmed et al., 1991; Chang and Fuller, 1964; Garcia et al., 2004; Longstaff and McNab, 1991a, 1991b; Smulikowska et al., 2001; Trevino et al., 1992; Giner-Chavez et al., 1996). Literature reported controversial data probably because tannin properties are strongly linked to their origin and some of them, when used in appropriate doses, may help to prevent undesired intestinal microflora development (Scalbert, 1991; Chung et al., 1998). Several authors, investigating the influence of the polyphenol extract from chestnut wood, found that the use of these substances in poultry feeding did not affect nitrogen balance, nutrient digestibility, mineral bioavailability, body weight, feed conversion ratio and the carcass quality (Jamroz et al., 2009; Salobir et al., 2008; Schiavone et al., 2008). Moreover, chestnut tannins (CT) are also efficient against coccidiosis and necrotic enteritis in poultry (Bole-Hribovsek et al., 2012; Elizondo et al., 2010; Tosi et al., 2013). In contrast, little information is reported on the role of CT in laying hens' feeding because most of the studies deal with the use of condensed tannins (Imik, H. 2009; Jacob et al., 1996; Marzoni et al., 2005; Sell et al., 1983).

76 Hens' sensitivity to dietary tannins, varies according to their ability to denature these

77 compounds with digetive enzymes and several authors observed a marked decrease in

78 egg production and an increase frequency of egg yolk mottling also at low inclusion

79 level in the diet (Begovic et al., 1978; Chang and Fuller 1964; Fuller et al. 1967;

80 Vohra et al. 1966).

81 Consequently, the aim of the current study was to investigate the effects of a

82 commercial chestnut tannin extract (CTE) from *Castanea sativa* Miller, on cholesterol

83 content and nutritional quality of eggs from two breeds of laying hens characterized

84 by a different productive performance, White Leghorn *vs* Mugellese.

85

86 **Acronym: CT**, chestnut tannin; **CTE**, chestnut tannin extract; **MU**, Mugellese breed;

87 **WL,** White Leghorn breed; **GE**, gross energy; **MUC**, Mugellese group fed control

88 diet; **MUT**, Mugellese group fed diet enriched whith chestnut tannin extract; **WLC**,

89 White Leghorn group fed control diet; **WLT**, White Leghorn group fed diet enriched

90 with chestnut tannin extract; **DM**, dry matter; **DMI**, dry matter intake; **CP**, crude

91 protein; **EE**, ether extract; **NDF**, neutral detergent fiber; **FA**, fatty acid; **FAME**, fatty

92 acid methyl ester; **CLA**, conjugated linoleic acid; **LA**, linoleic acid; **œ-LNA**, alpha

93 linolenic acid; **VFA**, volatile fatty acid; **PO**, palmitoleic acid; **OA**, oleic acid; **PA**,

94 palmitic aid; **SA**, stearic acid; **EPA**, eicosapentaenoic acid,; **DHA**, docosahexaenoic

95 acid; **AA**, arachidonic acid ; **DI**, desaturation index; **HDEP%**, hen-day egg

96 production percentage.

97

98 **2. Methods**

99 *2.1 Animals, enviroment, experimental design and diets.*

100 All experimental procedures were approved by the Ethics Committee of the
101 University of Florence and were in compliance with the guidelines of the International
102 Animal Care and Use Committee (IACUC, 2004) for the care and use of animals in
103 research.

104 The trial was performed with 80 laying hens (aged 39 weeks), 40 belonging to the
105 dwarf breed Mugellese (MU) and 40 to the White Leghorn (WL) breed.

106 These two breeds were chosen because they are caracterized by different productive
107 performances. WL is a cosmopolitan breed because good layers of white eggs and
108 characterized by a good feed-to-egg conversion ratio. In contrast, MU is a small local
109 population very appreciated by consumers for their egg quality, characterized by a
110 lower daily egg production than WL .

111 The birds were weighed and individually allotted in 80 pens (20 pens per each
112 experimental group; one bird is considered as replicate) and maintained under semi-
113 controlled enviromental conditions with esposure to 16h photoperiod in a 2x2
114 factorial design. For each breed, a group was fed a control diet (groups MUC and
115 WLC; 603.5 ± 4.4 g and 2315.7 ± 22.5 g of life body weight, respectively); the other
116 group (groups MUT and WLT; 603.9 ± 7.0 g and 2313.7 ± 34.3 g of life body weight,
117 respectively) received the same diet, integrated with 2 g of a commercial CTE
118 (Saviotan feed ©, provided by Gruppo Mauro Saviola – Mantova - Italy) per kg of
119 diet, expressed on dry matter (DM). CTE contained 750g of tannic acid equivalent/kg
120 of DM and was titrated according to Burns (1963). The CTE chemical composition
121 has been previously investigated by Campo et al. (2012). The diets used in this trial
122 were administered as pellet and were formulated to meet the nutrient requirements of
123 laying hens consuming 100 g of feed per day according to National Research Council

124 (NRC, 1994). The trial lasted 5 weeks, after a 4 week preliminary adaptation period.

125 The ingredients and the chemical composition of the diets are shown in Table 1.

126 Diets and water were administered *ad libitum* during the study and dietary

127 consumption was measured daily for each hen considering the amount of feed offered

128 and the residuals. Individual animal body weights were measured at the beginning and

129 at the end of the experimental period.

130 *2.2 Collection of eggs.*

131 Egg production was recorded for the entire 35-day period for each hen and the percent

132 hen-day egg production (HDEP%) was calculated according to the formula published

133 by North (1984):

134 (HDEP% = Number of eggs produced on daily basis / Number of birds available in

135 the flock on that day) x 100.

136 All eggs produced were collected daily to be weighed and measured with a digital

137 compass to obtain both short and long diameters as well as thickness of shells at a ±

138 0.001mm sensitivity.

139 During the whole experimental period, 70 eggs from each group were randomly

140 collected; for each egg the yolk and albumen were separated and, immediately after

141 the collection, tested for chemical and physical assay as described below.

142 *2.3 Proximate analysis of diets and eggs.*

143 Samples of feeds (in triplicate for each treatment) were analysed for DM, crude

144 protein (CP), ash and ether extract (EE) according to the 930.15, 976.06, 942.05 and

145 920.39 procedures of AOAC (1995) respectively while neutral detergent fiber (NDF)

146 of the diets was determined according to Van Soest et al. (1991) using heat-stable

147 amylase and expressed inclusive of residual ash. The Gross Energy (GE) value was

148 calculated according to NRC (1994). Fresh individual samples of yolk and albumen

149 (70 for each treatment) were analysed for CP and ash according to the 976.06, 942.05

150 procedures of (AOAC, 1995). Fat content was determined gravimetrically according

151 to Folch et al. (1957) at the moment of lipid extraction for fatty acid (FA) profile

152 characterization as described below.

153 *2.4 Determination of fatty acid profile of diets and yolk.*

154 Diets (2g; in triplicate for each treatment) were analyzed for FA profile using a one-

155 step methylation procedure according to Suskja and Palmquist (1996). Fresh samples

156 (200 mg) of yolk (70 from each experimental group) were extracted for total lipids

157 content according to Folch et al. (1957) and FA composition was determined after a

158 double-step esterification according to Kramer et al. (2004).

159 The fatty acid methyl esters (FAME) were separated by a GC equipped with an FID

160 detector and a capillary coloumn (CP-Select CB for FAME Varian, Middelburg,

161 TheNetherlands: 100 m x 0.25 mm i.d.; film thickness 0.20 um). The injector and FID

162 detector temperatures were respectively 270°C and 300°C. The oven programmed

163 temperature was 40°C for 4 min, increased to 120°C at a rate of 10°C min^{-1},

164 maintained at 120°C for 1 min, increased to 180°C at a rate of 5°C min^{-1}, maintained

165 at 180°C for 18 min, increased to 200°C at a rate of 2°C min^{-1}, maintained at 200°C

166 for 1 min, increased to 230°C at a rate of 2°C min^{-1} and maintained at this last

167 temperature for 19 min. The split ratio was 1:100 and helium was the carrier gas with

168 a flux of 1 mL min^{-1}. Individual FAMEs were quantified using valeric acid (C5:0)

169 and nonadecanoic acid (C19:0) methyl esters (cods 14899 and N5377 respectively;

170 Sigma-Aldrich Chemical Co., St. Louis, MO) as internal standards and identified by

171 comparison to the relative retention times of FAMEs peaks from samples, with those

172 of the standard mixture 37 Component FAMEs Mix C4:0-C24:0 (cod 18919-1AMP,

173 Supelco, Bellefonte, PA), individual *trans*-9 C18:1 and *trans*-11 C18:1 (cods 46903

174 and v1381 respectively, Sigma-Aldrich Chemical Co., St. Louis, MO), individual *cis*-

175 9, *trans*-11 C18:2 (cod 1255, Matreya Inc, Pleasant GAP, PA), conjugated linoleic

176 acid (CLA) mix standard (cod 05632, Sigma-Aldrich Chemical Co., St. Louis, MO)

177 and published isomeric profile (Cruz-Hernandez et al., 2006; Kramer et al., 1997;

178 Kramer et al., 2004). The C18:1 isomers elution sequence was performed according to

179 Kramer et al. (2008). Moreover, standard mix of α-linolenic acid (α-LNA) isomers

180 (cod 47792, Supelco, Bellefonte, PA) and of linoleic acid (LA) isomers (cod 47791,

181 Supelco, Bellefonte, PA) and published isomeric profiles (Destaillats et al., 2005)

182 were used to identify the isomers of interest. Two bacterial acid methyl ester mixes

183 (cod 47080-U Supelco, Bellefonte, PA; cod GLC110, Matreya, Pleasant Gap, PA) and

184 individual standard for methyl ester of *iso* C14:0, *anteiso* C14:0, *iso* C15:0 and

185 *anteiso* C17:0 (cods 21-1211-11, 21-1210-11, 21-1312-11 and 21-1415-11

186 respectively, Larodan Malmo, SW) were used to identify branched FA profile. Inter

187 and intra-assay coefficients of variation were calculated by using a reference standard

188 butter (cod CRM 164, Community Boureau of Reference, Bruxelles, Belgium) and

189 detection threshold was 0.1g/kg of FA (Contarini et al., 2013). All results were

190 expressed as g/kg of total lipids. Intra-assay coefficients of variation ranged from 0.5

191 to 1.5% whereas inter-assay coefficients of variation ranged from 1.5 to 2.5.

192 Desaturation Index (DI) was calculated considering the concentration of C14:0 and

193 *cis*-9 C14:1 fatty acid according to the following formula (Buccioni et al., 2015):

194 $$DI = \textit{cis-9 C14:1} / (\textit{cis-9 C14:0} + C14:1)$$

195 *2.5 GC analysis of cholesterol.*

196 Fresh samples (500 mg) of yolk (70 from each experimental group) were individually

197 analyzed for total sterols (free and esterified) obtained after cold saponification

198 according to Sander et al. (1989). Gaschromatographic analysis of cholesterol was

199 carried out using a capillary column (SE 52, Macherey-Nagel GmbH & Co KG,

200 Germany; 50m x 0.25 mm ID, film thickness 0.25μm) with the temperature being

201 programmed from 220 to 310°C at a rate of 4.5 °C/min. Both injector and detector

202 temperatures were set at 350°C (Sweeley et al.,1963).

203 *2.6 Color analysis.*

204 Yolks (70 from each experimental group) were poured into a clean glass petri dish to

205 be measured for color using the portable spectrophotometer (Minolta CR 200 Chroma

206 Meter4, calibrated using a standard yellow calibration tile, model CRA471). The top

207 of the Chroma Meter measuring head was placed flat against the surface of yolk and

208 reflective color was determined from the average of three consecutive pulses from the

209 optical chamber of the spectrophotometer. Data are reported in the L*a*b* color

210 notation system with L* axis representing lightness, the a* axis representing the red-

211 green color axis (redness) and the b* axis representing the blue-yellow (yellowness)

212 color axis (Minolta, 1994).

213 *2.7 Statistical Analysis*

214 All data (e.g., animal weight, egg's physical parameters, egg's chemical composition

215 and fatty acid profile) recorded over the course of the experiment were processed as a

216 full factorial design with repeated measures using the MIXED procedure of SAS

217 (1999):

218 $$y_{ijkl} = \mu + D_i + B_j + I_{k(D)} + (D \times B)_{ij} + e_{ijkl}$$

219 where yijkl is the observation; μ is the overall mean; Di the fixed effect of diet (i = 1

220 to 3); Bj the fixed effect of Breed (j = 1 to 2); Ik is the random effect of the hen nested

221 within the diet (k = 1 to 20); (D × B)ij the interaction between diet and breed and eijkl

222 the residual error. The covariance structure was compound symmetry, which was

223 selected on the basis of Akaike's information criterion of the mixed model of SAS.

224 Statistical significance of the diet effect was tested against variance of hen nested

225 within diet according to repeated measures design theory (Littell et al. 1998).

226 Data related to percent hen-day egg production were processed with GLM using the

227 MIXED procedures of SAS (1999):

228 $$yijl = μ + Di + Bj + (D × B)ij + eijkl$$

229 where yijl is the observation; μ is the overall mean; Di the fixed effect of diet (i = 1 to

230 3); Bj the fixed effect of Breed (j = 1 to 2); (D × B)ij the interaction between diet and

231 breed and eijkl the residual error.

232

3. Results

234 *3.1 Feed intake, egg production and egg quality.*

235 During the experimental period no animal loss was registered. Moreover, no

236 differences in average dry matter intake (DMI) were found in treated groups respect

237 to their related control groups (MUC= 93.02g /head and day *vs* MUT= 97.93g / head

238 and day, s.e.m 2.65, P=0.732; WLC= 120.34g /head and day, WLT= 118,32g /head

239 and day, s.e.m. 1.99, P=0.902). At the end of the trial no variation in body weight nor

240 in egg production was registered as consequence of a D effect (Table 2). Instead, a B

241 effect for animal body weight, percent hen-day egg production and their physical

242 characteristics was observed according to the higher live-weight and better

243 performances of WL respect to that of MU (Table 2). The yolk color and shell

244 thickness were not affected by D, B or by their interaction D x B (Table 3). Only a

245 significant B effect was found for yolk and albumen protein, fat and ash contents that

246 were higher in eggs from MU than WL (Table 3).

3.2 Fatty acid profile and cholesterol content of eggs yolk.

248 Yolk FA composition was affected by CTE for both breeds of laying hens (Table 4).

249 The main effects were shown for unsaturated fatty acid (UFA). In fact, the dietary

250 inclusion of CTE increased *cis*-9 C16:1 (palmitoleic acid, PO) and decreased *cis*-9,

251 *cis*-12 C18:2 (linoleic acid, LA) content with a significant effect due to the D and B

252 factors and their interaction D X B (P< 0,05). In contrast, *cis*-9 C18:1 (oleic acid, OA)

253 was enhanced in MUT and WLT with a significant effect of the D factor and

254 interaction D X B but not of B. The interation D X B was significant for C16:0

255 (palmitic acid, PA) which decreased in MUT and increased in WLT compared to the

256 related control groups. *Cis*-9 C14:1 concentration is decreased by CTE inclusion in

257 the diet only in yolk from MUT respect to MUC. Only WL hens, fed with diet WLT,

258 showed a significant decrease of *iso* C17:0, C18:0 (stearic acid, SA), and *cis*-11 *cis*-14

259 C20:2. Instead, the concentration of LA decreased in both WLT and MUT. The

260 content of *cis*-5 *cis*-8 *cis*-11 *cis*-14 *cis*-17 C20:5 (eicosapentaenoic acid, EPA) and

261 *cis*-4 *cis*-7 *cis*-10 *cis*-13 *cis*-16 *cis*-19 C22:6 (docosahexaenoic acid, DHA) remained

262 constant regardless of breed or CTE dietary integration. Also *cis*-5 *cis*-8 *cis*-11 *cis*-14

263 C20:4 (arachidonic acid, AA) content remained constant in the two groups of eggs for

264 both breeds of hens.

265   Considering DI, this parameter was affected by D and showed an opposite trend

266   (significant effect of D X B) in treated groups because it decreased significantly in

267   MUT while in WLT increased.

268   The main effect on lipid fraction resulted in cholesterol content that tended to

269   decrease in the group treated with CTE, regardless breeds with a D and DxB

270   significant effect (Table 2).

271

272   **4. Discussion.**

273   No differences were observed in Dry Matter Intake (DMI) between hens fed control

274   or CTE diets. As consequence, the inclusion of CTE in the diets, at the percentage

275   adopted in this trial, did not affect the dietary palatability. Moreover, the differences

276   found in egg production and egg weight were due only to the breed effect according

277   to the better performance of WL respect to the MU and not to the dietary

278   supplementation of CTE. WL, in fact, is largely diffused in the world for its high

279   productivity and for its good feed-to-egg conversion ratio, also when reared in free-

280   range farming. In contrast, MU is a small population, particularly adapted for a free-

281   range management but with medium productivity, even if appreciated by consumers

282   for the egg quality. Literature reported that tannins can interfere with Calcium

283   absorption in hens, affecting the shell thickness (Salobir et al., 2008). In our study this

284   parameter is not changed among groups, suggesting that presumably the

285   bioavailability of Calcium is ensured also in animals fed the diet integrated with CTE.

286   Moreover, in this trial CTE integration did not cause discoloration and mottling in

287   yolks, suggesting that CTE did not interfere with pigment metabolism, in contrast to

288   what has been found in other studies which reported the passage of undesirable

289   pigments from gut to yolk when condensed polyphenols were added to feed at a

290   percentage comparable to that used in the present experiment (Hughes, 1972; Potter,

291   1967; Weber, 1970). No differences were observed in CP content of eggs among

292   groups, regardless of diet or breed. Literature reported controversial data on the effect

293   of tannins in poultry feeding and it could be related to the dose and the kind of tannin

294   used in the trials as a consequence of their chemical structure and their solubility

295   strongly linked to their chemical structure. Observing the FA profile of yolks, the

296   absorption of MUFA seems to be encouraged by the inclusion of tannins in the diet,

297   especially in the case of OA that increased with CTE diet (+7% in MUT and +9% in

298   WLT respectively) regardless of breeds. α-LNA content was affected by both diet and

299   breed effect. FA profile in egg yolk varies considerably with the dietary ingredients

300   that affect the efficiency of FA transfer with particular attention to the UFA fraction

301   (Gonzalez-Esquerra and Leeson, 2001). Even if the role of tannin in lipid metabolism

302   in monogastrics is not yet completely elucidated, several authors demonstrated that

303   hydrolysable polyphenols can limit the lipid solubility and consequently the intestinal

304   absorption of fat (Zhao et al., 2014). For this reason, it is hypothetical that CTE could

305   interfere with selective FA absorption at the gut level causing a different uptake

306   according to the FA molecular structure. The DI values, calculated as *cis*-9 C14:1 /

307   total C14 fatty acids ratio to evaluate the index of the Stearoyl CoA gene expression

308   in tissues, suggested that CTE could decrease the Δ^9 desaturase activity in WL breed

309   but not in MU. In fact, several authors demonstrared that tannins are able to interfere

310   with gene expression in cells and that their solubility plays an important role in both

311   inhibiting the enzymatic activity as well as being metabolized by cells to bioactive

312   monomers (Landete, 2011, Buccioni et al., 2015). Despite conflicting studies about

313   the role of cholesterol in cardiovascular diseases (Vos, 2010), some authors have

314   proposed reducing dietary cholesterol (Houston et al., 2011; Plourde and Cunnane,

315   2007; Spence et al., 2010) and encouraged the production of novel foods with low

316   content of this lipid and high levels in Omega-3 FA, vitamin E and vitamin D

317   (Cherian, 2009; Elkin, 2007; Kassis et al., 2010; Lawlor et al., 2010; Naber, 1993).

318   In the current study, the soluble tannin extract was able to reduce cholesterol content

319   in yolks regardless of breed. This finding could be related to cholesterol biosynthesis

320   inhibitory activities, as a consequence of the presence of polyphenolic compounds in

321   the diet (Lu and Hwang, 2008). Unfortunately, few studies have been done on the

322   effect of hydrolisable tannins on hens cholesterol metabolism. In literature several

323   trials have demonstrated that a constant consumption of hydrolysable polyphenols

324   contributes to reduced serum cholesterol concentration in monogastrics including

325   humans and that the gallic acid moiety, present also in CTE, may play an inhibitory

326   role in the cholesterol biosynthesis or uptake (Kim et al., 2013; Kobayashi and Ikeda,

327   2014; Lu and Hwang, 2008; Romani et al., 2013). WL breed seemed to be more

328   sensitive than MU breed to the CTE effect in reducing cholesterol content in eggs.

329   Usually, cholesterol content in eggs from autochthonous hens is higher when

330   compared to that in eggs from commercial laying hens based on the fact that the

331   cholesterol content is strongly related to genetic factors, lay intensity, dietary

332   composition and layer age (Mikec and Dinarina-Sablic, 2007; Millet et al., 2006;

333   Vorlovà et al., 2001).

334

335   **Conclusions**

336   The dietary integration of chestnut tannin extract in a practical dose can contribute to

337   decrease the cholesterol and to increase OA concentration in egg yolks. For human

14

338 consumption, eggs with a lower cholesterol content and a higher functional fatty acid

339 percentage could be recommended as a support to controlling heart disease. However,

340 the MU breed seemed to be less sensitive to polyphenol dietary inclusion. In terms of

341 egg nutritional value an improvement of their quality enhancing the healthy

342 components present in lipid fraction and, at the same time, lowering the harmful ones,

343 is however desirable.

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584   Table 1. Ingredients and chemical composition diets administered to birds.

		Diet C[1]	Diet T[1]
Ingredients			
Maize meal	g /kg	337.8	337.8
Rice Bran	"	185.0	185.0
Soybean meal	"	308.3	308.3
Soybean oil	"	95.2	95.2
Limestone	"	14.5	14.5
Calcium Phosphate	"	40.1	40.1
Vitamin and mineral premix[2]	"	1.50	1.50
Lysine-HCl	"	2.1	2.1
Chestnut extract tannin[3]	"	-	2.0
Bentonite	"	2.0	-
Chemical composition			
DM [4]	g/kg	945.0	945.0
CP [5]	g /kg DM	188.1	188.1
NDF [6]	"	109.2	109.2
CF [7]	"	47.3	47.3
Ash [8]	"	55.5	55.5
GE [9]	MJ/kg DM	15.6	15.6
Tannic acid equivalent	g/ kg DM	-	1.5

Fatty acid profile (g /100g of total fatty acids)

C16:0	9.60	9.58
C18:0	3.41	3.45
C18:1 *cis*-9	22.60	22.64
C18:2 *cis*-9 *cis*-12	54.32	54.29
C18:3 *cis*-9 *cis*-12 *cis*-15	6.90	6.88
Others	3.17	3.16

585 [1] diet C, control diet; diet T, diet supplemented with chestnut tannin extract.

586 [2] The vitamin and mineral mixture provided per kilogram of diet: cholecalciferol,

587 180000 mg; vitamin E (DL-α-tocopheryl acetate), 4000 mg; retinol (retinyl acetate),

588 1620000 mg; menadione, 300 mg; thiamin 120 mg; riboflavin, 180 mg; pyridoxine,

589 120 mg; folic acid, 100 mg; biotin, 200 μg; cyanocobalamin, 20 μg; Calcium-D

590 pantothenate, 1.08 g; $FeCO_3$, 4000 mg; ZnO, 5000 mg; MnO, 6000 mg; $CuSO_4.5H_2O$,

591 300 mg; KI, 1000 μg; Na_2SeO_3, 200 μg and $CoCO_3$, 200 μg.

592 [3] SaviotaN®, provided by Gruppo Mauro Saviola – Radicofani – Si- Italy.

593 DM [4], dry Matter [5]; CP [6], Crude Protein; NDF [7], neutral detergent fiber assayed with a

594 heat stable amylase and expressed inclusive of residual ash; CF [8], Crude Fat; GE [9],

595 GrossEnergy.

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602   Table 2. Influence of diet on egg production and egg quality from hens consuming

603   chestnut tannin extract (Data shown are the means of 70 replicates).

	Mugellese		White legorn				P	P[2]
	[3]MUC	[3]MUT	[3]WLC	[3]WLT	SEM[1]	D	B	DXB
Hen weight (g)	604.7	604.0	2319.0	2320.8	6.26	0.867	<0.0001	0.781
Percent hen-day egg production (%)	56.6	55.1	72.2	71.6	0.78	0.173	<0.0001	0.649
Egg weight (g)	31.9	33.0	49.3	51.1	1.44	0.323	<0.0001	0.787
Egg shell thickness(mm)	0.4	0.4	0.4	0.4	0.02	0.402	0.086	0.310
g/ 100g on DM								
Yolk crude protein	28.31	28.52	29.38	29.73	4.12	0.508	0.016	0.508
Albumen crude protein	82.03	82.15	83.61	83.92	2.73	0.600	0.021	0.927
Yolk crude fat	58.07	58.65	55.82	55.15	7.34	0.948	0.002	0.412
Albumen crude fat	0.13	0.13	0.11	0.12	0.91	0.847	0.011	0.723
Yolk ash	3.34	3.02	3.95	3.57	2.14	0.130	0.019	0.886
Albumen ash	5.31	5.62	5.44	5.58	1.93	0.567	0.042	0,756
Colesterol	2.317a	2.117b	2.478a	2.066b	1.13	0.041	0.615	0.025

604   [1] Standard error mean.

605   [2] Probability of significant effect due to experimental diet (D), breeds (B) and their

606   interaction D x B.

607   [3] MUC, Mugellese hens fed control diet; MUCT, Mugellese hens fed diet

608   supplemented with chestnut tannin extract; WL, White Leghorn hens fed control diet;

609   WLT, WL, White Leghorn hens fed diet supplemented with chestnut tannin extract.

610   [a-c] Means within row with different letters differs (P<0.05).

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613   Table 3. Influence of diet on yolk color (Data shown are the means of 70 replicates).

	Mugellese		White Legorn			P[2]		
Color	[3]MUC	[3]MUT	[3]WLC	[3]WLT	SEM[1]	D	B	DXB
L*	58.2	56.7	57.9	57.9	1.19	0.554	0.720	0.521
a*	4.3	4.7	5.1	4.9	0.41	0.840	0.190	0.633
b*	39.8	38.6	39.9	40.4	2.17	0.863	0.669	0.706

614   [1] Standard error mean.

615   [2] Probability of significant effect due to experimental diet (D), breeds (B) and their

616   interaction D x B.

617   [3] MUC, Mugellese hens fed control diet; MUCT, Mugellese hens fed diet

618   supplemented with chestnut tannin extract; WL, White Leghorn hens fed control diet;

619   WLT, WL, White Leghorn hens fed diet supplemented with chestnut tannin extract.

620   [a-c] Means within row with different letters differs (P<0.05).

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632 Table 4. Fatty acid profile of yolk (g /100g of total lipids; Data shown are the means

633 of 70 replicates).

Fatty acid	Mugellese		White leghorn		
	[3]MU	[3]MUT	[3]WL	[3]WLT	SEM[1]
C14:0	0.201	0.251	0.268	0.239	0.33
C14:1 *cis*-9	0.082a	0.033b	0.040b	0.038b	0.10
C15:0	0.049	0.056	0.070	0.057	0.11
C15:1 *trans*-9	0.107	0.082	0.082	0.111	0.15
C16:0	24.264b	24.041c	23.000d	24.849a	0.80
C16:1 *cis*-9	1.673c	1.739b	1.473d	2.009a	0.21
C17*iso*	0.039b	0.032b	0.056a	0.016c	0.08
C17*ante*	0.064	0.037	0.071	0.037	0.23
C17:0	0.215	0.218	0.221	0.163	0.60
C17:1 *cis*-9	0.056b	0.071a	0.051b	0.044b	0.09
C18:0	11.813b	11.761b	12.409a	10.779c	0.31
C18:1 *trans*-9	0.094	0.108	0.335	0.101	0.95
C18:1 *cis*-9	3.0651b	3.2677a	2.9343b	3.2078c	0.81
C18:1 *cis*- 11	1.277	1.413	1.413	1.291	0.93
C18:2 *trans*-9, *trans*-12	0.041b	0.040b	0.061a	0.029c	0.07
C18:2 *cis*-9, *cis*-12	14.986b	14.275c	15.908a	14.602b	0.90
C18:3 *cis*-9, *cis*-12, *cis*-15	0.183a	0.114b	0.058c	0.115b	0.11
C20:0	0.051	0.038	0.048	0.028	0.06
C20:1 *cis-11*	0.118	0.154	0.124	0.133	0.01
C20:2 *cis-11*, *cis-14*	0.133b	0.137b	0.174a	0.116b	0.14

C20:3 *cis-*11*, cis-*14*, cis-*17	0.211a	0.210a	0.154c	0.181b	0.04
C20:4 *cis-5, cis-*8*, cis-*11*, cis-14*	3.516	4.210	4.201	3.381	4.02
C22:1 *cis-*15	0.064	0.055	0.064	0.045	0.08
C20:5 *cis-5, cis-*8*, cis-11, cis-14, cis-*17	0.071	0.037	0.054	0.042	0.14
C24:0	0.045	0.050	0.042	0.057	0.12
C24:1 *cis-*15	0.095	0.067	0.041	0.061	0.17
C22:6n3 *cis-4, cis-7, cis-10, cis-13, cis-16*	1.615	1.769	2.102	1.554	0.27
SFA	36.638a	36.415a	36.058b	36.172b	0.81
MUFA	34.217a	36.399b	32.966d	35.911c	0.43
PUFA	20.644ab	20.715ab	22.597a	19.949b	0.99
DI[4]	0.289a	0.116 b	0.129b	0.137b	0.34

634   [1] Standard error mean.

635   [2] Probability of significant effect due to experimental diet (D), breeds (B) and their

636   interaction D x B, a,b,c,d for P< 0,05.

637   [3] MUC, Mugellese hens fed control diet; MUCT, Mugellese hens fed diet

638   supplemented with chestnut tannin extract; WL, White Leghorn hens fed control diet;

639   WLT, WL, White Leghorn hens fed diet supplemented with chestnut tannin extract;

640   [4] Desaturation Index, calculated as C14:1 *cis-*9 / (C14:1*cis-*9 + C14:0).

641   [a-c] Means within row with different letters differs (P<0.05).

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