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Effects of feeding fresh bergamot (*Citrus Bergamia* Risso) pulp at up to 35% of dietary dry matter on growth performance and meat quality from lambs

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Abstract

The effect of fresh bergamot pulp (FBP) was tested on lamb performances and meat quality. Twenty-seven Italian Merino ram-lambs were allotted into three experimental groups and for 90 days were fed a cereal-based concentrate diet (BERG-0) or two diets in which cereals were replaced with 20% (BERG-20) or 35% (BERG-35) FBP on DM basis. No significant differences between treatments were found for final weight, average daily gain, dry matter intake, feed conversion ratio and carcass weight. The BERG-35 treatment tended to increase total polyunsaturated fatty acids (PUFA; P=0.081) and vaccenic acid (P=0.068) and increased PUFA n-3 (P<0.001), rumenic acid (P<0.001), α-linolenic acid (P<0.001) and docosapentaenoic acid (P<0.01), leading also to a value of PUFA n-6/n-3 ratio that not exceed a threshold value of 4. After 6 days of refrigerated storage BERG-20 and BERG-35 treatments reduced meat lipid oxidation (P<0.01), while about colour parameters no effect of the treatment was observed. Therefore, present results suggest that inclusion fresh bergamot pulp at up to 35% in diets for fattening lamb might be an excellent strategy to reduce the amount of cereal concentrates without compromising animal growth performances and to improve meat quality.

Keywords: Fresh bergamot pulp; Lamb's growth; Meat quality; oxidative stability; fatty acid profile.

53

1. Introduction

Bergamot (*Citrus Bergamia* Risso) is a natural hybrid fruit derived from bitter orange and lemon that is used mostly for juice and the extraction of its essential oil. The annual Italian production of bergamot amounts to 25500 tons and it is produced almost exclusively in province of Reggio Calabria (South Italy), where the cultivated area is about 1500 ha, contributing for more than 90% of world supply (ISMEA, 2012).

During the industrial processing of bergamot or in general of citrus fruits for juice and essential oil extractions, remains a solid residue which represents about 55-60% of the fruit.

Some studies have elucidated the chemical composition of bergamot derivatives and its industrial by-products and have been proved to exert, in addition to being a good source of molasses and pectin as other citrus fruits by-products, important biological activities as protective agents on human cells exposed to tumour necrosis and antioxidants (Leopoldini et al., 2010; Trombetta et al., 2010). In fact the peel of bergamot fruit contain a significant amount of flavonoids, found in lower levels in other *Citrus* peels (Mandalari et al., 2006; Sommella et al., 2014), compounds that have been found to have health-related properties, especially based on their antioxidant activity (Kawaii et al., 1999). Moreover, for their content of polyphenols and others bioactive phytochemicals such as unsaturated fatty acids, sterols, tocopherols, carotenes, terpenes, several agro-industrial by-products can be considered as functional feedstuffs (Kalogeropoulos et al., 2012).

Obviously, if the solid residues resulting from the industrial processes of bergamot are not further processed or disposed, it can give rise to serious environmental pollution. In Calabria region these solid residues are not further processed due to the quantities produced. An interesting alternative to dispose this surplus, during the harvesting period of bergamot and subsequently of industrial processing, could be to use them for animal feeding.

In the literature there are some research on the use of dried citrus pulp in ruminant nutrition (Bueno et al., 2002; Caparra et al., 2007) and recently Inserra et al. (2014) and Lanza et al. (2015) studied the

79 oxidative stability and metabolism of fatty acid in lambs fed dried citrus pulp. However, few paper
80 have been reporter on the utilization of fresh citrus pulp in diet for growing lambs, and to the best our
81 knowledge, no studies investigated the effects of feeding ruminants with the solid residue resulting
82 from the industrial processes of bergamot on meat quality.
83 Therefore, the aims of this study were to assess *i*) if fresh bergamot pulps does not compromise growth
84 performances and *ii)* if fresh bergamot pulp affects meat quality traits, intramuscular fatty acid
85 composition and oxidative stability.
86
87 **2. Materials and Methods**
88 **2.1 Animals and diets**
89 The experimental protocol was approved by the University of Reggio Calabria, and animals were
90 handled by specialized personnel following European Union (2010) guidelines (EU Directive
91 2010/63). A total of twenty-seven Italian Merino male lambs, born within two weeks in September,
92 were weaned at 60 d of age [average initial body weight 15.79 ± 0.50 (SD) kg] and individually kept
93 in straw-bedded pens. Lambs were randomly assigned to three dietary treatments and adapted to the
94 respective experimental diets for 10 days. The experimental period lasted 90 days. During this period
95 lambs were fed on the following dietary treatments: concentrate (BERG-0; n=9 lambs), concentrate
96 and fresh bergamot pulp at the level of 20% dry matter on the diet fed (BERG-20; n=9 lambs) and
97 concentrate and fresh bergamot pulp at the level of 35% DM on the diet fed (BERG-35; n=9 lambs).
98 The proportion of ingredients and the chemical composition of the experimental diets are given in
99 Table 1.
100 The ingredients of each concentrate mixture were ground with mechanical mixer to make it decrease
101 selective feeding and in BERG-20 and BERG-35 groups bergamot pulp was mixed with concentrate.
102 The fresh bergamot pulp used in the study, consisting of peel, pulp, and seeds, was obtained after the
103 cold extraction of bergamot juice and transferred to the farm once week from the start of the
104 experiment. Samples of the feeds offered were collected 4 times during the trial, vacuum packaged

105 and stored at -30 °C for analyses. Every second day after feed administration, individual feed refusals
106 were weighed before fresh feed was given at 0830 h, in order to measure dry matter intake (DMI) on
107 an individual basis.
108 The amounts of feed offered and refused were recorded every day in order to measure the daily
109 voluntary feed intake.
110 The lambs were weighed at weekly intervals prior to feeding in order to calculate average daily gain.
111 At the end of the experimental period, following overnight fasting, the lambs were slaughtered at a
112 commercial abattoir.
113

114 **2.2. Muscle sampling**

115 After slaughtering carcasses were weighed to obtain hot carcass weight and stored at 4°C for 24h;
116 then the *longissimus thoracis et lumborum* muscle (LTL) was excised from the left half.
117 Subsequently, muscle was divided into five portions approximately 50g each. One portion of LTL
118 was vacuum-packaged, delivered to the laboratory and stored at 4°C for 24 h for the determination of
119 lipid oxidation and colour stability in raw muscle slices. The remaining portions of LTL were
120 immediately vacuum-packaged and stored at -30°C for analyses of lipid oxidation in cooked meat,
121 fatty acid composition and proximate analysis.
122

123 **2.3. Feedstuffs and meat proximate analysis**

124 Feed samples were analyzed for neutral detergent fibre (NDF) (Van Soest et al., 1991), crude protein
125 (AOAC, 1995a; method 984.13; Kjeldahl method), crude fat (AOAC, 1995b; method 920.39; The
126 solvent used for fat extraction was the petroleum ether) and ash (AOAC, 1995c; method 942.05).
127 Following the procedure described by Makkar et al. (1993), total phenolic compounds were extracted
128 from the feed samples using aqueous acetone (70% v/v), analysed by means of the Folin–Ciocalteu
129 reagent and expressed as tannic acid equivalents.

130 In LTL samples, moisture (method no. 950.46), crude fat (method no. 991.36), ash (method no.
131 920.153) and protein (method no. 984.13) were assessed according to AOAC procedures (AOAC,
132 1995), after 24 h thawing at 4 °C.
133

134 **2.4. Intramuscular fatty acid determination**

135 Following the procedure described by Folch et al. (1957) fat was extracted from feed samples and
136 fatty acids were determinate according to Gray et al. (1967).
137 Briefly, a 5 g of homogenized LTL samples were blended with extraction solvent
138 chloroform/methanol (2:1, v/v) twice, filtered, placed in separator funnels and mixed with saline
139 solution (0.88% KCl). After separation in two phases, the aqueous methanol fraction was discarded,
140 whereas the chloroform lipid fraction was washed with distilled water/methanol (1:1, v/v). After a
141 further filtration and evaporation by means of a rotary evaporator, lipid extracts were transferred to
142 test tubes for subsequent gas chromatographic analysis. Duplicates of 100 mg of lipid, were
143 methylated adding 1 ml of hexane and 0.05 ml of 2 N methanolic KOH (IUPAC 1987). Gas
144 chromatograph analysis was performed on a Varian model CP 3900 instrument equipped with a CP-
145 Sil 88 capillary column (length 100 m, internal diameter 0.25 mm, film thickness 0.25 μm). Operating
146 conditions were: a helium flow rate of 0.7 ml/min, an FID detector at 260 °C, a split–splitless injector
147 at 220 °C with an injection rate of 120 ml/min, an injection volume of 1 μl. The temperature program
148 of the column was: 4 min at 140 °C and a subsequent increase to 220 °C at 4 °C/min. Retention time
149 and area of each peak were computed using the Varian Star 3.4.1. software. The individual fatty acid
150 peaks were identified by comparison of retention times with those of known mixtures of standard
151 fatty acids (FAME mix 37 components from Supelco Inc., Bellefont, PA) run under the same
152 operating conditions. Fatty acids were expressed as percent of total methylated fatty acids.
153

154 **2.5. Preparation of muscle samples for oxidative stability measurements**

155 For monitoring the oxidative stability of raw meat, 4 slices of LTL (2 cm thick) were used under
156 aerobic refrigerated storage.
157 The slices were placed in polystyrene trays, covered with PVC film and stored in the dark at 4 °C.
158 Colour and lipid oxidation measurements were performed after 2 h of blooming (day 0) and after 3,
159 6 and 8 days of storage, using one slice for each day of storage. Three more slices were used for
160 assessing the extent of lipid oxidation in cooked meat under aerobic refrigerated storage. The slices
161 were vacuum-packaged and cooked by immersion of the bags into a water bath set at 75 °C. After 30
162 min of cooking, the samples were cooled by immersion of the bags into a water/ice bath. The bags
163 were opened and 1 slice was used immediately for measuring the extent of lipid oxidation (day 0). A
164 remaining slice were placed into polystyrene trays, overwrapped with PVC film and stored in the
165 dark at 4 °C. Lipid oxidation of stored cooked meat was measured after 2 days of storage.
166

167 **2.5.1. Lipid oxidation measurements and colour stability**

168 Lipid oxidation was monitored in raw and cooked meat by measuring thiobarbituric acid reactive
169 substances (TBARS) at each day of storage (Siu and Draper, 1978).
170 Briefly, 2.5 g of LTL were homogenized with 12.5 ml of distilled water using a Ultra-Turrax T25
171 (IKA-Werke GmbH & Co.KG, Staufen, Germany). Samples were maintained in a water/ice bath
172 during homogenization. Then, 12.5 ml of 10% (w/v) trichloroacetic acid were added to precipitate
173 proteins and samples were vigorously vortexed. Homogenates were filtered through Whatman No.1
174 filter paper. In 15-ml screw-cap glass tubes, 4 ml of clear filtrate was mixed with 1 ml of 0.06 1M
175 aqueous thiobarbituric acid and samples were incubated in a water bath at 80 °C for 90 min. The
176 absorbance of the samples at 532 nm was measured using a Shimadzu double beam
177 spectrophotometer (model UV-1800; Shimadzu Corporation, Milan, Italy). The assay was calibrated
178 using solutions of known concentrations of TEP (1,1,3,3,-tetra-ethoxypropane) in 5% (w/v)
179 trichloroacetic acid ranging from 5 to 65 nmoles/4 ml. Results were expressed as TBARS values (mg
180 of malonaldehyde (MDA)/kg of meat).

181 The colour was measured in the fresh LTL slices using a Minolta CR300 colour-meter (Minolta Co.
182 Ltd. Osaka, Japan). Hue angle (H*) was calculated as: $H^* = \tan^{-1}(b^*/a^*) \times (180/\pi)$. Measurements
183 were performed using illuminant A and 10° standard observer. For each muscle slice, average values
184 were calculated from triplicate readings made on nonoverlapping areas of the sample.
185

186 **2.6. Statistical analysis**

187 Data on animal performance and intramuscular FA composition were analyzed using a one-way
188 ANOVA to test the effect of the dietary treatment (BERG-0, BERG-20 and BERG-35). Data of meat
189 colour stability descriptors (L*, a*, b*, C*, H*) and lipid oxidation (TBARS values) in raw and
190 cooked meat were analyzed using a GLM mixed model to test the effect of dietary treatment and of
191 the time of storage, as well as of their interaction as the fixed factors, while individual animal was
192 included as a random factor.
193 Differences between means were assessed using Tukey's multiple-comparison test. Significance was
194 declared at $P \leq 0.05$, whereas trends toward significance were considered when $0.05 < P \leq 0.10$.
195 Statistical analyses were performed by the statistical software Minitab, version 14 (Minitab Inc, State
196 College, PA).
197

198 **3. Results**

199 **3.1 Animal performance**

200 Data on animal performances are reported in Table 2. No significant differences between dietary
201 treatments were found for final weight (P=0.415), average daily gain (ADG; P=0.571), DMI
202 (P=0.426), feed conversion ratio (FCR; P=0.367) and carcass weight (P=0.378). Obviously,
203 considering the replacement of part of the concentrate with FBP, lambs from BERG-20 and BERG-
204 35 groups consumed less (P<0.05) concentrate (710 and 568 g/d respectively for BERG-20 and
205 BERG-35 groups) as compared to animals from the BERG-0 group (823 g/d).

206 The intake of total fatty acids (FA), expressed on a dry matter basis, was higher (P=0.002) for the
207 lambs from BERG-20 and BERG-35 treatments (14.06 and 15.50 g/d respectively) compared to
208 BERG-0 (10.21 g/d).
209 Regarding individual fatty acids, the BERG-35 group had higher (P<0.01) intakes of stearic acid
210 (0.16 vs 0.45 g/d, respectively for BERG-0 and BERG-35 treatments) and α-linolenic acid (0.62 vs
211 1.49 g/d, respectively for BERG-0 and BERG-35 treatments) compared to the BERG-0 group.
212 The proximate analyses of the meat samples are presented in Table 2. No statistical differences
213 between groups were found for crude protein (P=0.349), moisture (P=0.297), ether extract (P=0.346)
214 and ash (P=0.283).
215

216 **3.2 Intramuscular fatty acid composition**

217 The intramuscular fatty acid composition is reported in Table 3. The intramuscular fat (IMF) was not
218 affected by dietary treatment. BERG-35 treatment tended to increase the proportion of total PUFA
219 (P=0.081) in meat compared to BERG-0 and BERG-20 treatments, while no effect was found for the
220 proportion of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA).
221 Meat from lambs fed with BERG-35 diet had a greater proportion of α-linolenic acid (P<0.001; 1.91
222 g/100g of FA) and docosapentaenoic acid (P<0.001; DPA; 0.86 g/100g of FA) and tended to have a
223 greater proportion of vaccenic acid (P=0.068; 1.42 g/100g of FA) compared to meat from lambs fed
224 with BERG-0 and BERG-20 diets, while no effect was found for the proportion of other important
225 long-chain n-3 PUFA, eicosapentaenoic (EPA) and docosahexaenoic (DHA), and for linoleic acid
226 and arachidonic acid.
227 Overall, the proportion of n-3 PUFA in meat was affected by the dietary treatment (P < 0.001), with
228 the highest value found in the LTL from lambs fed with BERG-35 diet (3.76 g/100g of FA). The n-
229 6/n-3 ratio was the lowest for BERG-35 diet (3.27) and the highest for BERG-0 one (P < 0.001; 8.91);
230 the BERG-20 diet had an intermediate value (5.41).

231 The BERG-35 group had the highest value (P = 0.003) of conjugated linoleic acid (rumenic acid,
232 C18:2 cis-9 trans-11; 1.43 g/100g of FA).
233 The thrombogenic index tended to reduce (P < 0.069) by feeding lambs the BERG-35 diet compared
234 with the BEG-20 and BERG-0 treatments, with the highest value found in the latter group.
235

236 **3.3 Meat oxidative stability**

237 The oxidative stability parameters measured in raw and cooked meat are reported in Figure 1. The
238 lipid oxidation (TBARS values) of raw meat (Fig. 1a) was strongly affected (P < 0.001) by the diet,
239 the time of storage (P < 0.001) and their interaction (P < 0.05). From the 6th day, TBARS value of
240 meat from BERG-0 diet was twice higher (2.98 mg MDA/kg of meat) than meat from lambs of both
241 groups fed bergamot (1.65 and 1.43 mg MDA/kg of meat, respectively for BERG-20 and BERG-35
242 treatments).
243 Also, the lipid oxidation of cooked meat (Fig. 1b) increased for all treatments over 2 days of storage
244 (P < 0.001) with higher value (P < 0.05) for BERG-0 than BERG-20 and BERG-35 dietary
245 treatments.
246 As regard colour stability, meat lightness (L*) was not affected by the dietary treatment and by storage
247 time. Redness index (a*) was comparable between dietary treatments and decreased across the storage
248 time (P<0.001). Conversely, yellowness index (b*) increased over the storage time (P<0.001); also
249 this index was not affected by dietary treatment. The hue angle (H*) values overall increased after 3
250 days of storage (P<0.001) and were not affected by the dietary treatment.
251

252 **4. Discussion**

253 Although these dietary treatments obviously decreased the DMI from concentrate no significant
254 differences were found between treatments for the main performance parameters. These data were
255 probably influenced by the composition of the three concentrates. In fact, considering the low protein
256 level and the high non-fibre carbohydrates (NFC) level of FBP, the concentrate offered to the lambs

257 of BERG-20 and BERG-35 groups had a higher soybean meal and faba bean contents and
258 consequently a lower percentage of cereal mix than the concentrate offered to the lambs of BERG-0
259 group, maintaining a similar protein concentration among the diets.
260 In this experiment, one of the most important finding was the increase of concentration of desirable
261 PUFA in meat consequently to the integration of fresh bergamot pulp in the diet (table 3).
262 In ruminants, after lipid hydrolysis in the rumen many unsaturated fatty acids are biohydrogenated,
263 reducing their amount in meat (Bessa et al., 2007). Consequently, the deposition of some fatty acids
264 in the muscle of ruminants depends not only on the intake of the different fatty acids but also on the
265 extent of the ruminal biohydrogenation of the ingested PUFA.
266 In this trial, among the n-3 PUFA, α-linolenic and DPA acids levels were higher in meat from BERG-
267 35-fed lambs than in meat from lambs fed with BERG-20 and BERG-0 diets. In ruminants,
268 differences in the proportions of the individual PUFA in muscle depend on differences in the intake
269 of PUFA, whereby a high intake of PUFA with the diet results in a greater proportion of PUFA that
270 escape saturation during the ruminal biohydrogenation (Raes et al., 2004). For example, α-linolenic
271 acid are essential fatty acid derived from the diet. In the present study it proportion was greater in the
272 meat from animals fed FBP than in meat from animals fed BERG-0 diet, which is in agreement with
273 the greater intake of this fatty acid from animals fed the bergamot-supplemented diets. Furthermore
274 differences in the intake of essential PUFA between treatments could partially explain the differences
275 observed in the deposition of long-chain n-3 PUFA in the intramuscular fat. The greater proportion
276 of DPA in the muscle of the lambs fed BERG-35 diet, could be partially explained by the greater
277 intake of α-linolenic acid of these animals (Raes et al., 2004).
278 These data obviously influenced the total n-3 PUFA that was higher in meat from BERG-35 group
279 than in meat from the other two groups. Instead, the levels of the most important n-6 PUFA such as
280 linoleic and arachidonic acids were comparable between treatments. Consequently, the level of PUFA
281 n-6/n-3 ratio was significantly lower in meat from lambs fed with FBP-diets than in meat from lambs
282 fed with only concentrate, especially in the group where fresh bergamot pulp was replaced at up to

283 35% of dietary DM. Nutritional guidelines recommend decreasing the PUFA n-6/n-3 ratio in food,
284 which should not exceed a threshold value of 4 (Department of Health, 1994). In our experiment the
285 inclusion of 35% of FBP in the diet resulted in a PUFA n-6/n-3 ratio of 3.27, value lower compared
286 to that observed in meat from animals fed with BERG-20 diet (inclusion of 20% of bergamot pulp in
287 the diet) where the value was 5.41 and in meat from animals fed with BERG-0 diet (only concentrate)
288 where the ratio was almost 9.
289 In this trial, consequently to the positive effects of FBP on some desirable fatty acids, the
290 thrombogenic index, a lipid nutritional quality index, tended to be lower in meat from lambs fed
291 BERG-35 diet than in meat from lambs fed the other two diets.
292 Meat and milk from ruminants are the main natural source of conjugated linoleic acid (CLA) but
293 supply only a small contribution towards nutritionally significant levels of CLA in the human diet
294 (700-800 mg/day; Parodi, 2003) and thus the development of feeding strategies for increasing CLA
295 content in meat, like PUFA supplementation, are desirable. According to Griinari et al. (2000) and
296 Sackmann et al. (2003), the most effective way to enhance the concentration of the most present
297 conjugated isomer of linoleic acid, rumenic acid, in ruminant products is to favor the ruminal
298 production of vaccenic acid. In the muscle (Santora et al., 2000), vaccenic acid is partially converted
299 to rumenic acid by the action of Δ^9-desaturase enzyme. In this experiment rumenic acid showed the
300 highest level in meat from BERG-35 treatment, values four times higher compared to those ones
301 observed in meat from lambs of BERG-0 group. It has been reported that feeding ruminants with a
302 concentrate-based diets often causes a shift in the biohydrogenation pathways, which in turn favours
303 the accumulation of *trans*-10 C18:1 instead of vaccenic acid as the major *trans* C18:1 isomer (Griinari
304 et al., 1998). All experimental groups showed a value for the *trans*-10 C18:1 comparable, probably
305 because in any case the concentrate was present in all three treatments, while the proportion of
306 vaccenic acid tended to be affected by the dietary treatment, with the lowest numerical value found
307 in the intramuscular fat from lambs fed the BERG-0 diet. Considering these values, it is likely that
308 the inclusion of high amount of fresh bergamot pulp depressed the complete ruminal

309 biohydrogenation pathway. Furthermore, it is interesting to note how, although the bergamot-
310 supplemented diets provided a higher amount of stearic acid than the BERG-0 diet, consequently
311 leading to a greater ingestion of it in lambs from FBP treatments and especially from BERG-35
312 treatment, no significant differences were found between treatments.
313 High levels of PUFA may disturb rumen bacteria metabolism, therefore, in the present study, the
314 higher amount of PUFA ingested by FBP might have affected rumen biohydrogenation. Furthermore,
315 Vasta et al. (2009) observed, in a study in vitro, that some secondary metabolites of plants such as
316 tannins, polyphenolic compounds, could impair the ruminal biohydrogenation of PUFA reducing the
317 rate of PUFA biohydrogenation via modification of the bacterial population in the rumen and the
318 inhibition of biohydrogenation steps, such as the conversion of vaccenic acid to stearic acid. In
319 subsequent studies, Vasta and Luciano (2011) further demonstrated that phenolic compounds could
320 impair the ruminal biohydrogenation of PUFA, with a consequential increase of PUFA in muscle.
321 Furthermore Vasta et al. (2009) observed, in addition to effects on ruminal biohydrogenation of
322 PUFA, that some phenolic compounds can increase the expression of the Δ^9-desaturase enzyme. The
323 occurrence of phenolic substances in bergamot constituents (including pulp and peels) has been
324 extensively documented, with most of these compounds being identified as flavonoids (Postorino et
325 al., 2002), and the highest concentrations of these compounds occur in the peel (Kanes et al., 1993).
326 In the present study, unfortunately, it was not possible to measure concentration of flavonoids and of
327 important antioxidant compounds such as vitamin E. However we studied the level of total extractable
328 phenolic compound that was almost eight times greater in FBP than in concentrates used.
329 On the other hand, possible effects of the diet on the ruminal biohydrogenation of fatty acids could
330 contribute to the deposition of PUFA in meat. Some of our results could lead to the conclusion that
331 feeding bergamot containing diets exerted an effect of the ruminal biohydrogenation of PUFA and
332 could lead to the speculation that secondary compounds in citrus pulp, such as phenolic, might have
333 exerted an effect on fatty acid metabolism in lambs. However, comparisons between studies on the
334 biological effects of dietary plant secondary compounds should be made with caution, considering

335 also that most of the studies considered have focused on tannins and that the proportion of the
336 different classes of these compounds can greatly vary between different sources.
337 Recently Lanza et al. (2015) studied fatty acid metabolism in lambs fed dried citrus pulp. Also these
338 authors reported a similar effect resulting from the inclusion of citrus pulp in diets for growing lambs.
339 In particular the reported data showed a greater concentration of rumenic acid in meat from animals
340 fed citrus pulp diets, while no difference was observed on the level of α-linolenic acid between
341 treatments. This result could be explained considering that in the trial of Lanza et al. the levels of α-
342 linolenic were comparable between treatments while in our experiment the amount of this fatty acid
343 was higher in the diets where part of the concentrate was replaced by fresh bergamot pulp. Moreover,
344 it is interesting to note that the level of rumenic acid reported by Lanza et al. in meat from lambs fed
345 with a diet with a high level of dried citrus pulp was lower compared to the value observed in our
346 trial in meat from lambs fed with high amounts of FBP.
347 The effects of bergamot pulps supplementation in diets on oxidative stability of raw meat are reported
348 in fig. 1a. The data indicate that the time of storage and the dietary treatment affect TBARS values
349 measured in raw LTL slices during 8 days of refrigerated storage. Furthermore, also the Diet x Time
350 interaction affected this parameter. These data indicated a different trend of development of fatty acid
351 oxidation, depending strongly on the dietary background of the animals. Indeed, after 3 days of
352 storage TBARS values were different but not significantly between the experimental groups.
353 However, after 6 days of storage, TBARS values in meat from lambs of BERG-0 group significantly
354 increase than in meat from lambs of BERG-35 treatment, while the BERG-20 treatment resulted in
355 intermediate value. Also after 8 days of storage the use of fresh bergamot pulp has positively
356 influenced fatty acid oxidation, leading to lower TBARS values in meat from lambs of both BERG-
357 20 and BERG-35 groups compared to BERG-0 group. Clearly, lipid oxidation is a major cause of
358 deterioration in meat (Gray and Pearson, 1987; Gray, Gomaa and Buckley, 1996) and the products of
359 fatty acid oxidation produce off-flavours and odours usually described as rancid (Gray and Pearson,
360 1994). Campo et al. (2006) suggested as the TBARS value of 2 mg MDA/Kg of meat could be

361 considered a maximum level for positive sensory perception of meat and from that point can expect
362 a sensory perception of lipid oxidation. After 6 days of storage TBARS value of BERG-0 group
363 exceed this threshold value, while the values measured in meat from all the lambs fed with FBP diets
364 were 1.65 and 1.43 mg MDA/Kg of meat and 1.79 and 1.75 mg MDA/Kg of meat after 8 days of
365 refrigerated storage, respectively for BERG-20 and BERG-35 treatments. Different authors showed
366 as a higher deposition of PUFA, especially n-3 fatty acids, is associated with an increased
367 susceptibility of the meat to lipid oxidation (Dunne et al., 2011; Moloney et al., 2012). However,
368 Descalzo and Sancho (2008) showed that generally, if the levels of muscle antioxidant compounds
369 increases the oxidative stability improves. Citrus flavonoids are polyphenolic compounds, secondary
370 metabolites of plants that have been found to have different properties, especially based on their
371 antioxidant activity (Kawaii et al., 1999*)*. Bergamot fruit contain a very high amount of flavonoids,
372 especially naringin and neosperidin (Postorino et al., 2002), and the highest concentrations of these
373 compounds occur in the peel (Kanes et al., 1993). Moreover, a small amount of flavone O- and C-
374 glycosides, not previously found in orange and lemon peels, have been identified in bergamot peel
375 (Mandalari et al. 2006). Unfortunately, as mentioned above, was not possible to measure
376 concentration of flavonoids and of important antioxidant compounds such as vitamin E. However we
377 studied the level of total extractable phenolic compound that was almost eight times greater in FBP
378 than in concentrates used. Therefore, the protective effects of the diets of BERG-20 and BERG-35
379 groups against lipid oxidation observed in our experiment were probably due to the presence of high
380 levels of antioxidant compounds such as flavonoids in fresh bergamot pulp. Recently Inserra et al.
381 (2014) also observed a similar trend after 3 days of refrigerated storage in meat from animals fed a
382 diet with a similar inclusion of dried citrus pulp in the diet than in meat from animals fed only
383 concentrate.
384 With regard to lipid oxidation in cooked muscle over 2 days of refrigerated storage (Fig. 1b), TBARS
385 values increased over time. An effect of the dietary treatment and of Diet × Time interaction were
386 found. After 2 days of refrigerated storage TBARS values in muscle from lambs fed with BERG-20

387 and BERG-35 diets were lower compared to the values observed in meat from lambs from BERG-0-
388 fed lambs. Discussion points similar to those raised for raw LTL slices can apply to the results
389 observed by measuring lipid oxidation in cooked LTL during refrigerated storage even if the effects
390 of the experimental diets were lighter.
391 Moreover, cooked meat after 2 days of refrigerated storage reached TBARS values similar for BERG-
392 0 treatments and higher for BERG-20 and BERG-35 treatments to those measured in raw LTL slices
393 after 8 days of refrigerated storage. These results could be explained considering that
394 freezing/thawing and cooking represent a significant stress by disrupting cell compartmentalization
395 and by inactivating most of the inherent antioxidant defence systems (Gray et al., 1996).
396 Colour stability is another important aspect to consider when meat shelf life is studied. The
397 deterioration of meat colour is mainly linked to the oxidation of myoglobin and this process causes
398 meat browning over storage time (Mancini and Hunt, 2005).
399 Generally, the loss of meat redness (a*) and the increase in hue angle (H*) over time of storage have
400 been used to describe meat browning (Mancini and Hunt, 2005, Insausti et al., 2008). In the present
401 study, a general decrease in meat redness over storage was observed. Also the other descriptor of
402 meat discolouration H* showed trend that is usually observed during refrigerated storage, increasing,
403 giving a reliable perspective of meat browning over time (table 4).
404 Some research report as the oxidation of myoglobin is promoted by fatty acids oxidation (Alderton
405 et al., 2003; Faustman et al., 2010) and consequently, strategies to reduce lipid oxidation should bring
406 positive effects in terms of colour stability. In this experimental trial, while the dietary treatment
407 affected lipid oxidation in fresh muscle, no effect was observed on colour stability. Moreover, the
408 Diet X Time interaction was not significant for any of the colour stability parameters, as also observed
409 in the report of Inserra et al. (2014), indicating that dietary treatment not influenced the trend of
410 variation of the color descriptors.
411
412 **5. Conclusions**

413 The results showed that the replacement of part of the concentrate with fresh bergamot pulp in the
414 diets increases the contents in meat of rumenic acid, α-linolenic acid and docosapentaenoic acid, fatty
415 acids that are all considered beneficial for human health. Moreover, in our experiment the inclusion
416 of high amount of fresh bergamot pulp in the diet resulted in a PUFA n-6/n-3 ratio that not exceed
417 the threshold value of 4. Furthermore, dietary FBP treatments increased meat oxidative stability.
418 Therefore, present results suggest that inclusion fresh bergamot pulp at up to 35% in diets for fattening
419 lamb might be an excellent strategy to reduce the amount of cereal concentrates without
420 compromising animal growth performances and to improve meat quality.
421 In the light of this, it would be interesting that future studies assess the effect of bergamot pulp by
422 including it, dried, among the ingredients of a pelleted concentrate-based diet on lamb performances
423 and meat quality.
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Table 1
Ingredients of concentrate (g/100g as fed) and chemical composition of diet constituents

	Concetrate BERG-0	Concentrate BERG-20	Concentrate BERG-35	Fresh Bergamot pulp
Barley	20	17	15	
Maize	20	17	15	
Wheat	20	17	15	
Soybean meal	10	15	20	
Faba bean	10	14	15	
Wheat bran	17	17	17	
Vitamin mineral premix[1]	3	3	3	
Chemical composition				
Dry matter (DM), g/kg wet weight	892.5	892.7	891.1	146.8
Crude protein, g/kg DM	164.2	200.6	206.9	65.6
Ether extract, g/kg DM	25.3	24.6	24.4	41.4
Ash, g/kg DM	52.5	60.8	53.7	53.7
NDF[2], g/kg DM	317.4	296.3	304.8	347.8
NFC[3]	440.6	417.7	410.2	491.5
Total phenolic compounds, g TAN[4]/kg DM	2.42	2.46	2.43	18.9
Total fatty acids, g/100 DM	1.17	1.18	1.16	3.04
fatty acids (g/100g of total fatty acid)				
C14:0	0.25	0.38	0.52	1.8
C16:0	20.01	19.42	18.89	20.1
C16:1	0.88	0.84	0.60	5.6
C18:0	1.59	2.07	2.49	3.2
C18:1 *n*-9	22.88	24.08	24.46	24.0
C18:2 *n*-6	48.36	46.89	45.26	32.7
C18:3 *n*-3	6.04	6.32	6.78	12.5

[1]The mineral vitamin premix consisted of vitamina A=6750 UI; vitamin D3=1000UI; vitamin E 2 mg; vitamin B12 0,01 mg; vitamin B1 1mg; folic acid 0,2 mg; D-pantotenic acid 5 mg; Co 0,05 mg; Mn 12,5 mg; Zn 15 mg; Mo 0,5mg;

[2]Neutral detergent fiber

[3]Non-fibre carbohydrates = 1000–(Crude Protein + Ether Extract+ ash +NDF).

[4]tannic acid equivalent

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Table 2
Effects of dietary treatment on lambs performances *in vivo* and LTL chemical composition (g/100g wet weight).

	Dietary treatments[1]			SEM[6]	*P* value
	BERG-0	BERG-20	BERG-35		
No. Of lambs	9	9	9		
BW[2] at 70 days, kg	15.29	16.19	15.9	0.754	0.715
BW[2] at 160 days, kg	31.83	32.5	32.6	0.643	0.415
Total DMI[3], g/d	823	897	861	22.04	0.426
Concentrate DMI[3], g/d	823[a]	710[ab]	568[b]	24.30	0.012
ADG[4], g/d	183	181	185	4.279	0.571
FCR[5], g DMI[3]/g ADG[4]	4.49	4.95	4.65	0.198	0.367
Carcass weight, kg	14.41	14.63	14.71	0.312	0.378
Total FA[7] intake, g/d	10.21[a]	14.06[b]	15.50[c]	1.546	0.002
SA[8] intake, g/d	0.16[a]	0.36[ab]	0.45[b]	0.064	0.010
LA[9] intake, g/d	4.94	5.79	5.89	0.169	0.126
ALA[10] intake, g/d	0.62[a]	1.24[ab]	1.49[b]	0.096	0.006
Chemical composition					
Moisture	73.2	73.1	73.3	0.223	0.297
Crude protein	22.4	22.7	22.5	0.156	0.349
Ether extract	2.63	2.59	2.65	0.134	0.346
Ash	1.21	1.16	1.18	0.0205	0.283

[1]Treatments were: only concentrate (BERG-0); concentrate and fresh bergamot pulp at the level of 20% dry matter on the diet fed (BERG-20) and concentrate and fresh bergamot pulp at the level of 35% DM on the diet fed (BERG-35).

[a,b,c] Within a row different superscript letters indicate significant differences ($P<0.05$) tested using Tukey's adjustment for multiple comparisons.

[2]BW=Body weight; [3]DMI=dry matter intake;[4]ADG=average daily gain;[5]FCR=feed conversion ratio; [6]SEM= standard error of means; [7]FA=Fatty acid; [8]SA=Stearic acid; [9]LA=Linoleic acid; [10]ALA=α-linolenic acid.

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Table 3
Effect of the dietary treatment on LTL fatty acid composition (g/100 g of total fatty acids)

	Dietary treatment[1]				
Item	BERG-0	BERG-20	BERG-35	SEM	*P* values
intramuscular fat, mg/100g of muscle	2462	2279	2361	301	0.534
total fatty acids, mg/100g of muscle	2018	1891	1912	201	0.193
C12:0	0.08	0.10	0.09	0.009	0.769
C14:0	1.71	1.85	1.66	0.115	0.808
C14:1 *cis-9*	0.06	0.06	0.06	0.004	0.874
C15:0	0.18	0.20	0.18	0.006	0.437
C15:0 *anteiso*	0.11	0.10	0.10	0.007	0.276
C15:0 *iso*	0.04	0.03	0.03	0.002	0.247
C16:0	20.80	21.45	20.85	0.574	0.894
C16:1 *cis-9*	1.36	1.33	1.29	0.047	0.853
C17:0	0.31	0.32	0.26	0.009	0.231
C17:0 *anteiso*	0.07	0.06	0.05	0.006	0.367
C17:0 *iso*	0.16	0.10	0.09	0.008	0.123
C17:1 *cis-9*	0.49	0.55	0.56	0.027	0.559
C18:2 *cis-9, trans-11* RA[2]	0.33[b]	0.60[b]	1.43[a]	0.098	<0.001
C18:0	16.33	15.51	15.10	0.319	0.786
C18:1 *cis-9*	36.18	36.83	34.79	0.691	0.466
C18:1 *trans-9*	0.32	0.43	0.40	0.027	0.220
C18:1 *trans-10*	0.97	0.78	0.71	0.101	0.124
C18:1 *trans-11* VA[2]	0.98	1.11	1.42	0.103	0.068
C18:2 *cis-9, cis-12* LA[2]	7.96	7.04	7.74	0.457	0.767
C18:2 *trans-9, trans-12*	0.32	0.28	0.35	0.040	0.241
C18:3 n-3 ALA[2]	0.41[b]	0.62[b]	1.91[a]	0.162	<0.001
C18:3 n-6	0.10	0.11	0.11	0.012	0.511
C20:2 n-6	0.65	0.68	0.64	0.069	0.194
C20:3 n-3	0.19	0.31	0.40	0.040	0.321
C20:3 n-6	0.07	0.09	0.07	0.010	0.694
C20:4 n-6	4.23	3.65	3.55	0.276	0.213
C20:5 n-3 EPA[2]	0.29	0.32	0.32	0.023	0.385
C21:0	0.11	0.12	0,10	0.008	0.548
C22:5 n-3 DPA[2]	0.41[b]	0.66[b]	0.86[a]	0.053	0.003
C22:6 n-3 DHA[2]	0.20	0.29	0.27	0.020	0.788
unknown	4.47	4.36	4.65	0.423	0.962
∑ SFA[2]	39.64	39.55	38.23	0.757	0.800
∑ MUFA[2]	40.66	41.09	39.22	0.657	0.534
∑ PUFA[2]	14.97	14.04	16.42	0.676	0.081

∑ OBCFA[2]	0.87	0.81	0.71	0.113	0.127
∑ n-6	13.28	11.85	12.66	0.848	0.235
∑ n-3	1.49[b]	2.19[b]	3.76[a]	0.229	<0.001
n-6/n-3	8.91[c]	5.41[b]	3.27[a]	0.393	<0.001
Thrombogenic index[3]	1.19	1.15	1.03	0.043	0.069

[a,b,c] Within a row different superscript letters indicate significant differences ($P < 0.05$) tested using Tukey's multiple comparison test

[1]Treatments were: only concentrate (BERG-0); concentrate and fresh bergamot pulp at the level of 20% dry matter on the diet fed (BERG-20) and concentrate and fresh bergamot pulp at the level of 35% DM on the diet fed (BERG 35).

[2]CLA: Rumenic acid (*cis-9 trans-11* 18:2 coeluted with *trans-7 cis-9* 18:2); LA: linoleic acid; ALA: α-linolenic acid; EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid; DHA: docosahexaenoic acid; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; OBCFA: total odd and branched chain fatty acids.

[3]Thrombogenic index: (C14:0 + C16:0 + C18:0)/(0.5 MUFA + 0.5 PUFA n-6 + 3 PUFA n-3 + PUFA n-3/PUFA n-6)

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Table 4

Effect of the dietary treatment and time of refrigerated storage on meat colour stability. Values presented are the least squares means with their pooled standard error.

	Dietary treatment[1]			Days of storage				SEM	*P* values		
	Control	Berg 20%	Berg 35%	0	3	6	8		Diet	Time	Diet x Time
L* values[2]	45.29	45.28	45.73	46.02	46.01	45.66	45.04	0.256	0.692	0.125	0.494
a* values[2]	9.02	9.2	8.96	10.31^{x}	8.90^{y}	9.10^{y}	7.92^{z}	0.157	0.745	<0.001	0.836
b* values[2]	9.69	9.88	9.53	6.61^{x}	10.39^{y}	10.97^{y}	10.82^{y}	0.226	0.289	<0.001	0.083
C* values[2]	13.40	13.53	13.07	12.70	12.42	12.26	11.97	0.171	0.457	0.175	0.326
H* values[2]	46.50	46.41	45.89	32.65^{x}	49.55^{y}	50.43^{y}	52.44^{y}	0.969	0.650	<0.001	0.573

^{x,y,z}Within row, different superscripts indicate differences between days of storage (P<0.05) tested using the Tukey's adjustment for multiple comparisons

[1]Treatments were: only concentrate (BERG-0); concentrate andfresh bergamot pulp at the level of 20% dry matter on the diet fed (BERG-20) and concentrate and fresh bergamot pulp at the level of 35% DM on the diet fed (BERG-35).

[2]L*=lightness; a*=redness; b*=yellowness; C*=Chrome; h*=hue angle, measured in degrees.

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