Effect of feeding pigs with bergamot by-product on fatty acid composition and oxidative stability of meat and salami

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Abstracts

This work investigated the effects of feeding ensiled bergamot pulp to pigs on meat and salami quality. Eighteen pigs were assigned to two experimental treatments and fed a cereal-based concentrate diet (control) or the same diet in which ensiled bergamot pulp replaced 15% dry matter of the diet fed (BP). The BP treatment increased α-linolenic acid (+250%; $P<0.05$), docosapentaenoic acid (+62%; $P<0.05$), docosahexaenoic acid (+43%; $P<0.05$) and consequently n-3 PUFA (+15%; $P<0.01$) in meat. In salami, the content of α-linolenic acid, total PUFA and n-3 PUFA increased (+320%, +25% and +258%, respectively) by feeding the BP diet ($P<0.001$). The inclusion of bergamot pulp in the diet did not alter the oxidative stability in raw and cooked meat and color descriptors. In salami, TBARS values were lower after 5 days of storage ($P<0.001$) in BP group (1.54 vs 2.96). Finally, dietary supplementation with ensiled bergamot pulp to pigs improved the nutritional value of meat and meat products.

Keywords: Pork, Meat quality, Bioactive compounds, Industrial by-products, n-3 PUFA.

1. Introduction

Bergamot (*Citrus bergamia Risso*) is a typical fruit grown wildly in Calabria (Italy), less commercialized than other Citrus fruits and mostly used for the extraction of juice or its essential oil. In Italy, the annual production of bergamot amounts to 25.000 tons.

After the industrial processing of bergamot for juice and essential oil extractions, a residue remains which if not further processed or disposed, it can give rise to serious environmental pollution.

Some studies have reported data on the chemical composition of bergamot industrial by-products. Mandalari et al. (2006) characterized flavonoids and pectins from bergamot and have proved that peel of bergamot fruit contains a remarkable amount of flavonoids, some of which are present at higher levels than in by-products from other citrus fruits, compounds that have been found to possess health-promoting properties, especially related to their antioxidant activity (Shahidi & Ambigaipalan, 2015). Moreover, bergamot fruits are rich in limonoid compounds (Russo, Arigo, Calabrò, Farnetti, Mondello & Dugo, 2016), which are human health-promoters as they have many pharmacological properties: anticancer, antioxidant, antibacterial andantifungal (Kaur & Kaur, 2015).

During the last years, the use of industrial by-products in animal production has been evaluated as a strategy to increase sustainability of food production systems, allowing food processing industries to profitably mitigate the costs generated by waste disposal (Kasapidou, Sossidou, & Mitlianga, 2015). On the other hand, feeding animals with agro-industrial by-products could be a valid strategy to reduce feeding costs and, in some cases, to enrich diets with bioactive compounds (Biondi et al., 2020; Natalello et al., 2020).

Citrus pulps in general are by-products of the citrus juicing industry that are often included as an energy supplement in ruminant diets (Inserra et al., 2014; Lanza et al., 2015), especially in citrus-producing regions. While in the past these by-products as other fibrous feedstuffs were considered of marginal quality for monogastrics (Cunha, Pearson, Glasscock, Buschman & Folks, 1950), now they are being reevaluated for use in swine diets (Crosswhite, Myers, Adesogan, Brendemuhl,

78 Johnson & Carr, 2013). As outlined by Watanabe et al. (2010) the inclusion of a source of fiber for
79 feeding swine could be a strategy to control fat deposition, e.g. in local breeds that are generally
80 reported to have a higher propensity for fat deposition (Pugliese & Sirtori, 2012).
81 Recently there has been a growing interest in local pig breeds, such as Apulo-Calabrese, due to high
82 added value and eating quality of their meat, with quality features suitable for the production of
83 Protected Designation of Origin (PDO) salami (Micari, Racinaro, Sarullo, Carpino, & Marzullo,
84 2009). The Apulo-Calabrese pig is an Italian autochthonous breed from Calabria region well
85 adaptable to different production systems (Micari et al., 2009; Pugliese & Sirtori, 2012).
86 In the literature there are some studies on the use of citrus pulp in ruminant nutrition (Bueno,
87 Ferrari, Bianchini, Leinz & Rodrigues, 2002; Inserra et al. 2015; Lanza et al. 2015). However, few
88 papers have been reported on the utilization of bergamot pulp in diet for growing ruminants (Scerra
89 et al., 2018), and to the best our knowledge, no studies investigated the effects of feeding pigs with
90 bergamot by-products on the quality of meat and typical products deriving from it.
91 Therefore, here we have investigated, for the first time, the effect of feeding bergamot by-product to
92 finishing pigs on the quality of meat and salami.
93
94 **2. Materials and methods**
95 The present study was conducted from October 2019 to March 2020 in a farm oriented on heavy pig
96 production, where all the procedures were approved (prot. No. 286946) by the Animal Welfare
97 Committee (O.P.B.A) of the University of Catania.
98 In the native farm, 18 Apulo-Calabrese barrows were selected from the same group of animals, all
99 fed equal amounts of the same commercial concentrate. Animals were weighed (115.4±8.70 kg
100 initial body weight and 17±0.5 months of age), individually identified and allocated in individual
101 pens.
102 Pigs were fed *ad libitum* during the 120-day finishing period with two dietary treatments formulated
103 to contain: only concentrate (control group; 9 pigs), concentrate and ensiled bergamot pulp at the

104 level of 15 % dry matter (DM) on the diet fed (BP group, 9 pigs). Diet with bergamot pulp was a
105 mixture of concentrate with the respective amount of ensiled bergamot pulp.
106 The concentrate offered to the pigs of BP group had the same ingredients of the concentrate
107 supplied to the pigs of control group but, in order to maintain a similar crude protein concentration
108 between treatments, had a higher soybean meal content and a lower percentage of barley and maize
109 (table 1).
110 Fresh Bergamot pulp was obtained from a juice citrus industry and ensiled for 90 days.
111 All the pigs were fed the experimental diets twice daily (0700 and 1600 h). The amounts of feed
112 offered and refused were recorded every day in order to measure the daily feed intake. Fresh water
113 was continuously available. Pigs were weighed every 15 days from the beginning to the end of the
114 experimental trial to determine average daily gain (ADG).
115 All the animals were slaughtered in a commercial abattoir 120 days after the beginning of the trial.
116 Animals were electrically stunned and exsanguinated. The carcass weight was recorded and the
117 *longissimus thoracis et lumborum* (LTL) muscle was removed from each carcass and immediately
118 transported and refrigerated to the laboratory.
119 The back fat and meat from each pig were used to prepare an individual salami. Thus, a total of 18
120 salamis were obtained (9 per treatment). Salami samples were manufactured and provided by a
121 local Calabrian company. All the salami were manufactured on the same day, using the same
122 technology, ingredients and formulation, which were: raw material (% w/w), pork meat (90),
123 backfat (10), combined with 25 g/kg of NaCl. The ingredients were mixed and the paste obtained
124 was stuffed into the casings, subsequently manually tied, hung from steel racks and placed in a
125 fermentation chamber. The ripening was performed as follows: for the first 24 hours a temperature
126 of 20 ± 1 °C and 75% relative humidity (RH); within five days, the temperature was gradually
127 reduced to 12 °C, while the RH was gradually increased to 80% ± 5%. Ripening was carried out for
128 30 days. Matured salamis were packed under vacuum and stored frozen at - 20 C.
129

130 *2.1. Feedstuff analyses and meat proximate analyses*

131 Feed samples were analysed using the methods proposed by AOAC (1995), method 984.13 for
132 crude protein evaluation, method 920.39 for crude fat and method 942.05 for ash evaluation, while
133 neutral detergent fibre (NDF) was analysed according to the method used by Van Soest, Robertson
134 and Lewis (1991). Following the procedure described by Makkar, Blümmel, Borowy and Becker
135 (1993), total phenolic compounds were extracted from the feed samples using aqueous acetone
136 (70% v/v), analysed by means of the Folin–Ciocalteu reagent and expressed as tannic acid
137 equivalents. The method of Gray, Rumsby and Hawke (1967) was used to analyse the fatty acid
138 composition of feed.

139 Liposoluble vitamins were extracted in the feedstuffs following the methodologies described by
140 Rufino-Moya, Joy, Lobón, Bertolín and Blanco (2020). Briefly, 50 mg of bergamot pulp and 200
141 mg of concentrates were extracted three times with 3 mL of methanol:acetone:petroleum ether
142 (1:1:1, v:v:v, 0.01% (w/v) of 2,6-di-tert-butyl-4-methylphenol (BHT)) solution. Then, the
143 supernatant (1 mL for the bergamot pulp samples and all the supernatant for the concentrate
144 samples) was evaporated. The dry residues obtained in the extractions were dissolved in 1 mL of
145 methanol HPLC grade, filtered through a 0.22 µm polytetrafluoroethylene (PTFE) filter, and
146 transferred into a 2 mL glass screw-top vial for automatic sampling using 5 µL for ultra-high
147 performance liquid chromatography (UHPLC). Chromatographic conditions were as described later
148 for the meat.

149 In samples of LTL and salami, according to AOAC (1995) procedures, method no. 950.46 was used
150 for moisture evaluation, method no. 991.36 for crude fat, method no. 920.153 for ash and method
151 no. 984.13 for protein evaluation, after 24 h thawing at 4 °C.

152

153 *2.2. Meat and salami antioxidant vitamins determination*

154 In muscle and salami samples, antioxidant vitamins were extracted as described by Bertolín, Joy,
155 Rufino-Moya, Lobón and Blanco, (2018). Briefly, 500 mg of lyophilised sample was placed in a 15

mL polypropylene tube. Subsequently were added 0.2 g of L-ascorbic acid and 7.5 mL of saponification solution (10% w/v KOH in 50:50 v:v ethanol:distilled water mixture). The mixture was vortexed for 30 seconds under nitrogen atmosphere. The saponification procedure was performed overnight at room temperature in an orbital shaker (250 rpm).

Then, 5 mL of n-hexane:ethyl acetate 9:1 v:v 25 (with 25 μg/mL of BHT) mixture were added. The tubes were vortexed for 30 s, and centrifuged for 5 minutes at 2000×g at 10 °C. The supernatant was recovered in a pyrex tube. This procedure was repeated three time. The organic solution was evaporated under nitrogen flow at 40°C. Then, the residue was dissolved in 1 mL of methanol, the tube leaves at 40 °C for a few minutes, vortexed for 30 seconds and filtered through a 0.2 μm–13mm PTFE filter into a 2 mL amber vial for UHPLC.

The chromatographic system was a Nexera UHPLC (Shimadzu Corporation, Milan, Italy) equipped with a Zorbax ODS column (250 mm × 4.6 mm, 5 μm; Supelco, Bellefonte, PA), a photodiode array detector (PDA; SPD-M40, Shimadzu) and a spectrofluorometric detector (RF-20AXS, Shimadzu). The UHPLC system was controlled by the LabSolutions software. The mobile phase was methanol with a flow rate of 1.3 mL min^{-1}. The temperature of the samples and the column were adjusted to 25 °C and 40 °C, respectively. The injection volume was 10 μL.

Tocopherols were detected by fluorescence emission at 295 excitation wavelength and 330 nm emission wavelength, retinol by absorbance at 325 nm. The analytes in the different matrices were identified by comparison of the retention times and spectral analysis with those of the pure standards.

2.3. Meat, backfat and salami fatty acid determination

Fatty acid composition was analysed on total lipids extracted according to Folch, Lees and Stanley (1957). Briefly, fat from 5 g of homogenized samples with solvent chloroform/methanol (2:1, v/v) was extracted and 100 mg of lipid were methylated adding 1 mL of hexane and 0.05 mL of 2 N methanolic KOH (I.U.P.A.C., 1987), containing C9:0 as an internal standard. Fatty acid methyl

esters, obtained from meat, back-fat and salami, were separated and quantified using a Varian CP 3900 Gas chromatograph, equipped with a capillary column 100 m long, internal diameter 0.25 mm and film thickness 0.25 μm (CP-Sil 88). One μl of sample was injected, carried by a helium flow of 0.7 mL/min. The temperature of FID detector was set at 260 °C and at 220 °C the temperature of split–splitless injector with an injection rate of 120 mL/min. The temperature program of the column was: 4 min at 140 °C and a subsequent increase to 220 °C at 4 °C/min. Retention time and area of each peak were computed using the Varian Star 3.4.1. software. The individual fatty acid was identified by comparison with retention times of known mixtures of standard fatty acids (FAME mix 37 components from Supelco Inc., Bellefont, PA). Fatty acids were expressed as g/100 g of total fatty acids.

The thrombogenic index was estimated as reported by Ulbricht and Southgate (1991).

2.4. Lipid oxidation and colour measurements

Three slices 2 cm thick of raw meat were used for monitoring the oxidative stability under aerobic refrigerated storage. The slices were placed in polystyrene trays, covered with PVC film and stored at 4 °C in the dark for 2 hours (day 0), 3 and 7 days for measurements of colour stability and lipid oxidation extent. To assess the extent of lipid oxidation in cooked meat, three more slices were used. The slices were vacuum-packaged and cooked for 30 min by immersion of the bags into a water bath set at 75 °C. One of these was immediately used for measuring the extent of lipid oxidation (day 0), whereas the remaining slices were placed into polystyrene trays, overwrapped with PVC film and stored at 4 °C in the dark. Lipid oxidation was measured after 2 and 5 days (Luciano et al., 2013).

Three slices from each salami were used for assessing oxidative stability under aerobic refrigerated storage as described above for meat samples, where one slice of salami was immediately used for measuring the extent of lipid oxidation (day 0), whereas the remaining slices were placed into

polystyrene trays, overwrapped with PVC film and stored at 4 °C in the dark. The extent of lipid oxidation was measured after 2 and 5 days of storage.

Lipid oxidation was monitored in salami, raw and cooked meat by measuring thiobarbituric acid reactive substances (TBARS) at each day of storage (Siu & Draper, 1978). In brief, meat and salami samples (2.5 g) were homogenized with distilled water (12.5 mL) using an Ultra-Turrax T25 (IKA-Werke GmbH & Co.KG, Staufen, Germany), maintaining in a cold-water bath during homogenization. After homogenization, 12.5 mL of 10% (w/v) trichloroacetic acid were added and samples were vigorously vortexed. Homogenates were filtered through Whatman No.1 filter paper. In pyrex glass tubes, 4 mL of clear filtrate was mixed with 1 mL of 0.06 M aqueous thiobarbituric acid and incubated in a water bath at 80 °C for 90 min. The absorbance of the samples at 532 nm was measured using a UV-1800 Shimadzu spectrophotometer (Shimadzu Corporation, Milan, Italy). The assay was calibrated using solutions of known concentrations of 1,1,3,3,-tetra-ethoxypropane in distilled water ranging from 5 to 65 nmoles/4 mL. Results were expressed as mg of malonaldehyde (MDA)/kg of meat.

A Minolta CR300 colour-meter (Minolta Co. Ltd. Osaka, Japan) was used, at the end of the respective storage time (0, 3 and 7 days), for measuring colour stability on each slice of raw meat. Hue angle (H*) was calculated as: $H^* = \tan{-1}\ (b^*/a^*) \times (180/\pi)$. Measurements were performed using illuminant A and 10° standard observer. For each muscle slice, average values were calculated from triplicate readings made on nonoverlapping areas of the sample.

2.5. Statistical analysis

Data on intramuscular FA composition and animal performance were analysed using a one-way ANOVA to evaluate the effect of the dietary treatment. Data of meat colour stability descriptors (L*, a*, b*, C*, H*) and lipid oxidation (TBARS values) in raw, cooked meat and salami were analyzed using a mixed model to study the effect of dietary treatment and of the time of storage, as

well as of their interaction as the fixed factors, while individual animal was included as a random factor.

Differences between means were assessed using Tukey's multiple comparison test. Significance was declared at $P \leq 0.05$, whereas trends toward significance were considered when $0.05 < P \leq 0.10$. Minitab software (version 14, Minitab Inc, State College, PA) was used for statistical analyses.

3. Results

3.1. Animals Performance

As shown in Table 2, no effect of the dietary treatment was found on the final body weight, dry matter intake (DMI), feed conversion ratio (FCR), average daily gain (ADG) and carcass weight.

As for meat proximate analyses, there were no significant differences between treatments for crude protein, moisture, ether extract and ash.

The intake of total fatty acids (FA), expressed on a dry matter basis, was higher ($P < 0.01$) for the pigs from BP treatment compared to control.

Regarding individual fatty acids, α-linolenic acid intake was higher ($P = 0.005$) in BP group then in control group and stearic acid intake tended to increase ($P = 0.057$) in animal from BP group.

3.2. Fatty Acid Composition of Intramuscular fat and antioxidant vitamins

Table 3 reports the concentration of vitamins E and A in meat. Vitamin E (VE) was mainly represented by α-tocopherol and its concentration was not affected by supplementing bergamot pulp in the finishing diet of pigs. Also, the concentration of retinol (vitamin A) was not affected by the dietary treatment.

The effects of dietary treatment on the individual FA in intramuscular fat (IMF) are reported in table 3. In the present study, the dietary administration of 15% bergamot pulp tended to reduce the accumulation of IMF ($P = 0.082$) in meat.

The total of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) was not different between groups. Within the SFA class, the BP diet specifically tended to reduce the concentration of C12:0 ($P = 0.085$).

The level of polyunsaturated fatty acids (PUFA) tended to increase by feeding the BP diet ($P = 0.081$). Consequently, the PUFA to SFA ratio increased ($P < 0.05$) by BP treatment compared to the control treatment. Among the individual PUFA, the BP diet increased the concentration in muscle of α-linolenic acid (C18:3 cis-9, cis-12, cis-15; $P < 0.05$), docosapentaenoic acid (DPA, C22:5 *n-3*; $P < 0.05$) and docosahexaenoic acid (DHA, C22:6 *n-3*; $P < 0.05$).

The dietary treatment affected the sum of *n-3* PUFA, with a greatest concentration found in meat from BP group ($P < 0.01$). Consequently, the BP treatment reduced the *n-6* to *n-3* ratio ($P < 0.01$) compared to the control treatment. The same statistical results were observed when the individual FA were expressed as mg/100 g of meat (Supplementary Table S1), although the slight difference in IMF concentration.

The concentration in meat of the highly peroxidisable (HP) PUFA, with unsaturation degree ≥ 3, increases ($P < 0.05$) by feeding pigs with the diet containing bergamot pulp. Considering the above-mentioned effect of the dietary treatment on the concentration of vitamin E, HP-PUFA ÷ VE ratio tended to increase ($P = 0.059$) in meat from the pigs fed with diet containing bergamot pulp compared to the control treatment.

Finally, the thrombogenic index was lower in BP meat ($P < 0.01$) compared control meat.

3.3. Fatty Acid Composition of backfat

Table 4 reports the effect of the dietary treatment on the fatty acid composition of backfat. The inclusion of bergamot pulp in the diet did not affect the total of SFA, MUFA and PUFA.

The only fatty acid that showed a difference ($P < 0.05$) was α-linolenic acid, with the highest value found in backfat from pigs fed BP diet. Consequently, the proportion of *n-3* PUFA tended to increase ($P = 0.074$) in backfat of pigs from BP group compared to the control group.

283 *3.4. Fatty Acid Composition of salami and antioxidant vitamins*

284 The fatty acid composition of salami is shown in Table 5. The dietary administration of 15%
285 bergamot pulp reduced the content of saturated fatty acids in salami ($P < 0.01$).

286 Instead, the content of PUFA increased by feeding the BP diet ($P < 0.001$). Among the individual
287 PUFA, the BP diet in salami increased the concentration of α-linolenic acid ($P < 0.001$).

288 The dietary treatment affected the sum of *n-3* PUFA, with a greater concentration found in salami
289 from BP group ($P < 0.001$). Consequently, the BP treatment reduced the *n-6* to *n-3* ratio ($P < 0.001$)
290 and increased the PUFA to SFA ratio ($P < 0.001$) compared to the control treatment. Finally, the
291 thrombogenic index was lower in BP salami ($P < 0.001$) compared control salami.

292 As for the concentration of vitamins E and A (table 5), also in salami the levels α-tocopherol and γ-
293 tocopherol were not affected by supplementing bergamot pulp in the finishing diet of pigs. Also, the
294 concentration of retinol (vitamin A) was not affected by the dietary treatment. As in meat, the
295 concentration of the HP-PUFA increases ($P<0.001$) by feeding pigs with the diet containing
296 bergamot pulp in salami, influencing the HP-PUFA ÷ VE ratio that was higher ($P<0.007$) in salami
297 from BP group compared to the control treatment.

298

299 *3.5. Meat colour and oxidative stability*

300 In fresh meat, lipid oxidation (TBARS values) was not affected by the dietary treatments (table 6).
301 Regarding colour descriptors, the redness (a*) and lightness (L*) descriptors were not affected by
302 the time of storage or by the dietary treatment. Instead, meat saturation (C*), yellowness (b*) and
303 hue angle (H*) values were affected by the time of storage (respectively $P < 0.001$ for b* and H*
304 values and $P < 0.05$ for C* value), with values overall increasing from 0 to 3 days and stabilizing
305 thereafter.

306 Also, in cooked meat, lipid oxidation was not affected by the dietary treatments (table 6), while
307 increased for all treatments over 2 days of storage ($P < 0.001$).

308

309 *3.6. Salami oxidative stability*

310 The oxidative stability parameters measured in salami are reported in Fig. 1. The TBARS values
311 increased over storage duration ($P < 0.001$) and the BP diet reduced the extent of lipid oxidation
312 overall measured in salami over time ($P < 0.001$). A significant diet × time interaction was found (P
313 < 0.001). Specifically, compared to day 0, while the TBARS values increased already after 2 days
314 in salami from only concentrate-fed animals, in salami from animals fed BP diet lipid oxidation
315 increased after 5 days ($P < 0.01$). Salami from BP treatment had lower TBARS values compared to
316 salami from control treatment after 5 days of storage ($P < 0.001$; 1.54 vs 2.96 respectively).

317

318 ***4. Discussion***

319 *4.1. Fatty acid composition*

320 The dietary treatment did not result in differences in the main performance parameters. As shown in
321 Table 2, the final weight of the animals, as well as carcass weight were comparable between
322 treatments.

323 In literature, no information has been provided on the effects of supplementing bergamot pulp in the
324 finishing diet of pigs on animal performance and meat quality. However, some data have been
325 provided on the effects of supplementing citrus pulp (from other species than bergamot) in the
326 finishing diet of pigs on animal performance. Crosswhite et al. (2013) observed no differences in
327 ADG values in finishing pigs fed a diet with 15% DM of ensiled citrus pulp. Other author also
328 showed similar results (Strong, Brendemuhl, Johnson & Car, 2015). Cerisuelo et al. (2010)
329 observed that the inclusion of up to 10% of ensiled citrus pulp tended to reduce animal growth
330 performance. However, the authors suggested that the lower performance was due to a lower DM
331 feed intake during the first 4 weeks on trial, thereafter differences disappeared.

332 Apulo-Calabrese is a breed characterized by reduced growth and carcass performance (Aboagye,
333 Zappaterra, Pasini, Dall'Olio, Davoli & Costa, 2020), and this justifies the lower ADG values

334 registered in all treatments of our trial compared to the data reported from the authors mentioned
335 above.
336 In animal nutrition, in both ruminants and monogastrics, a main objective is to find strategies to
337 increase polyunsaturated fatty acids, especially of *n-3* series, and reduce saturated fatty acids in
338 zootechnical products (Scollan, Hocquette, Nuernberg, Dannenberger, Richardson & Moloney,
339 2006; Wood et al., 2008).
340 In ruminants, after lipid hydrolysis in the rumen, many unsaturated fatty acids are hydrogenated by
341 ruminal micro-organisms, changing dietary fatty acids. Differently from ruminants, in monogastric
342 animals, dietary fatty acids do not undergo substantial changes along the digestive tract and, after
343 absorption, accumulate in animal tissues (Wood et al., 2008). Moreover, Aboagye et al. (2020)
344 indicated that when Apulo-Calabrese pigs are reared in the indoor system and fed the same
345 commercial diet as crossbreeds, their *longissimus thoracis* muscle fatty acid composition is similar
346 to those observed in commercial crossbreed pigs, excluding effects of the genetic type over muscle
347 FA synthesis and storage. For these reasons, the results found in the present study for the fatty acid
348 composition of meat can be mainly explained by the fatty acid profile of the two experimental diets.
349 To date, no studies investigated the effects of feeding pigs with the solid residue resulting from the
350 industrial processes of bergamot on meat fatty acids composition and also on the use of citrus pulp,
351 which is well documented in ruminants, little is known about its effects on meat quality for pigs.
352 Cerisuelo et al. (2010), in a study where pigs were fed 100 g of ensiled citrus pulp per kg of diet
353 (DM basis), observed no significant differences in total SFA and in total PUFA. However, in our
354 trial, taking into account the fatty acid composition of the C and BP diets, a greater concentration of
355 α-linolenic acid in BP pork meat was expected. This fatty acid was found at a greater amount in
356 meat from BP group than in meat from control group. Also, the level of total PUFA tended to be
357 higher in the meat of animals fed bergamot processing by-products. These results are in agreement
358 with the data of our previous research, where bergamot processing by-products supplementing in

359 the diet increased α-linolenic acid and tended to increase total PUFA in lamb meat (Scerra et al.,
360 2018).
361 Consistent with the higher level of α-linolenic acid in meat from the BP group, some
362 polyunsaturated fatty acid derived from it through the action of Δ^5 and Δ^6 desaturase enzymes and
363 elongase (Gurr & Harwood, 1996), were higher in meat from animals of this group. In fact, the
364 levels of docosapentaenoic (DPA, C22:5 *n-3*) acid and docosahexaenoic acid (DHA, C22:6 *n-3*)
365 were greater in meat from BP group than in meat from C group. Durand-Montgé, Realini, Barroeta,
366 Lizardo and Esteve-Garcia (2010) reported that diets rich in linolenic acid, for example diet with
367 linseed oil, may result in an increased level of docosapentaenoic (DPA) in pork.
368 These findings are of relevance as it is well recognized that long chain *n-3* PUFA have a wide
369 range of biological effects, which are believed to be beneficial for human health (Kromhout, 1989;
370 Barlow, Young, & Duthie, 1990). However, considering that the adequate intake estimated for
371 human is 2.22 g/d for ALA and 0.65 g/d for DHA + EPA (Simopoulos, 2000), the contribution
372 provided by the integration of bergamot pulp on increased levels of ALA and long chain n-3 PUFA
373 in the meat was small.
374 These data obviously influenced the total *n-3* PUFA content that was higher in meat from BP group
375 than in meat from the other group. Instead, the levels of the most important *n-6* PUFA such as
376 linoleic and arachidonic acids were comparable between treatments. Consequently, the level of *n-6*
377 to *n-3* PUFA ratio was significantly lower in meat from pigs fed with BP diets than in meat from
378 pigs fed only concentrate.
379 In this study, consequently to the positive effects of bergamot pulp on some desirable fatty acids,
380 the thrombogenic index, a lipid nutritional quality index, tended to be lower in meat from pigs fed
381 BP diet than in meat from pigs fed the control diet.
382 Similarly to meat, the concentration of total *n-3* PUFA tended to increase in backfat from BP fed
383 pigs. In fact, feeding diets with a high concentration of α-linolenic acid to pigs generally results in
384 the accumulation of this fatty acid and its long-chain derivatives in the backfat. Consistent with this,

385 we found a greater concentration of α-linolenic acid in the backfat from the BP fed animals.
386 However, its long-chain derivatives were found at comparable amounts between the two groups.
387 As in meat and backfat, the use of bergamot pulp influenced the salami fatty acid composition
388 (table 5). In all the salami, the major FA was C18:1 *cis-9*, which occurred at comparable levels
389 between treatments. Among the SFA, significant differences were found for C14:0, C16:0 and
390 C18:0, with higher values observed in salami from animals fed C diet than in salami from animals
391 fed BP diet.
392 Salami produced from pigs fed with bergamot pulp had a four-fold greater content of C18:3 *n-3*
393 compared to the Control group. Considering the above described effect of feeding bergamot by-
394 products on the content of α-linolenic acid in meat and in backfat, this result was expected. This
395 result strongly influenced the total *n-3* PUFA content which was higher in meat from BP group than
396 in meat from the control group.
397 Similar to meat, also in salami the levels of the most important *n-6* PUFA such as linoleic and
398 arachidonic acids were comparable between treatments. Consequently, the level of *n-6* to *n-3* PUFA
399 ratio was significantly lower in salami from pigs fed with BP diet than in salami from pigs fed
400 control diet. Furthermore, the data reported above influenced the level of total PUFA, which was
401 higher in the meat of animals from BP group. In this trial, the PUFA content was comparable with
402 those reported by Zanardi, Dorigoni, Badiani and Chizzolini (2002) and Warnants, Van Oeckel and
403 Boucque (1998) for salami, who described values of approximately 13.5 and 16.3 %, respectively.
404 Regarding the thrombogenic index, also in salami the values was lower in BP group than in control
405 group.
406
407 *4.2. Oxidative stability*
408 The deterioration of colour and the development of rancid off-flavours in meat over time of storage
409 is primarily affected by the oxidation of lipids and myoglobin (Faustman, Sun, Mancini, & Suman,

2010). The inherent susceptibility of meat to oxidation is the result of a complex balance in muscle between anti-oxidant and pro-oxidant factors.

The trend of lipid oxidation depends strongly on the dietary background of the animals. Different authors reported that a higher deposition of PUFA, especially n-3 fatty acids, is associated with an increased susceptibility of the meat to lipid oxidation (Dunne, Rogalski, Childs, Monahan, Kenny & Moloney, 2011; Moloney, Kennedy, Noci, Monahan & Kerry, 2012), while the increase of antioxidants compounds, derived directly and indirectly from the diet such as vitamin E, is commonly associated with the improvement of the antioxidant capacity (Luciano et al., 2017).

The results of the present study showed that, although the concentration of vitamin E (α-Tocopherol and γ-Tocopherol) was higher in the bergamot pulp than in the concentrate and consequently in BP treatment than in control treatment, not difference in vitamin E content was observed in meat from both experimental groups. This latter result was not expected. However, despite the data reported on vitamin E in meat and the higher levels of total PUFA and especially HP- PUFA in meat from BP animals, the inclusion of bergamot pulp in the diet did not alter the shelf-life in raw meat. Similarly, colour coordinates mostly related to meat browning (a*, C* and H* values) did not differ between treatments. Probably, the high level of vitamin E in the meat of both experimental groups was enough to protect it from oxidation during the 7 days of storage.

Different authors showed similar trend in fresh pork stored for up to 5 or more days (Inserra et al., 2015; Biondi et al., 2020). Aerobic storage of fresh meat in darkness may represent a low oxidative challenge for meat to fully express its resistance to oxidative deterioration. Indeed, it has been reported that differences in oxidative stability between meat samples may be masked under refrigerated storage of fresh meat, but become evident when meat is subjected to stronger oxidative challenges (Luciano et al., 2019). Therefore, in the present study, we also assessed oxidative stability in cooked meat to assess possible diet-related differences of meat oxidative stability under more pro-oxidant conditions. Also, under these conditions, no effect of dietary treatment was observed, although TBARS values increased during the 5 days of refrigerated storage, reaching

much higher values than those recorded in fresh meat. Recently some authors (Scerra et al. 2018) observed a stronger effect of bergamot pulp supplementation in ruminant diet, showing a lower TBARS value in meat from lambs fed concentrate and fresh bergamot pulp at the level of 20% DM on the diet fed than in meat from lambs fed only concentrate.

In contrast to the results observed on the meat, significant differences were observed between treatments in salami oxidative stability (figure 1). Already on the first day of oxidative stability test, TBARS values in salami from animals of control group were significantly higher than in salami from animals of BP treatment. Furthermore, while TBARS values strongly increased during the 5 days of refrigerated storage in salami from control group, already exceeding after 2 days of storage the suggested value of TBARS (2 mg MDA/Kg of meat) considered a maximum level for positive sensory perception (Campo, Nute, Hughes, Enser, Wood & Richardson, 2006), in salami from BP group TBARS values increased slightly during the 5 days of refrigerated storage, remaining below the threshold value of 2 mg MDA/Kg for the entire monitoring period.

As in raw meat, not difference in vitamin E content was observed in salami between the experimental groups. However, the level of vitamin E in salami was 4 times lower than in fresh meat, which may be related to the consumption of vitamin E due to pro-oxidation challenges linked to salami production process and ageing. Therefore, it may be supposed that this level was probably not enough to protect salami from oxidation during storage. Nonetheless, TBARS values in salami from animals of BP group were significantly lower, despite the highest level of HP-PUFA, than in salami from animals of control treatment after 5 days of refrigerated storage. As mentioned above, aerobic storage of fresh meat in darkness may represent a low oxidative challenge for meat to fully express its resistance but can be more evident when the meat is subjected to strong oxidative stress. Some authors (Warnants et al., 1998) observed that oxidative stability of the salami decreased with increasing ripening time and PUFA content. The long storage time has probably allowed to show the resistance to oxidative deterioration of the meat from animals of BP group.

We could speculate that bergamot pulp may offer additional antioxidant effects, which could be due to the occurrence of antioxidant compounds other than tocopherols. Bergamot fruit contain a very high amount of flavonoids, especially naringin and neosperidin, and the highest concentrations of these compounds occur in the peel (Tsiokanos et al., 2021). Citrus flavonoids are polyphenolic compounds, secondary metabolites of plants that have been found to have different properties, especially based on their antioxidant activity (Kawaii, Tomono, Katase, Ogawa & Yano, 1999). However, some authors (Bieger et al., 2008; López-Andrés et al., 2013) confirmed the poor bioavailability of these compounds in animal tissue and that it is not yet possible to reach conclusions on the effects of polyphenols. Nevertheless, several authors suggest an indirect antioxidant effects of phenolic compounds, such as an antioxidant activity on the gastrointestinal tract, interrupting lipid oxidation propagation and formation of toxic molecules (Kerem, Chetrit, Shoseyov & Regev-Shoshani, 2006) or a possible protective effect in the gastrointestinal tract of phenols towards other more bioavailable antioxidant compounds (Halliwell, Rafter & Jenner, 2005). In our trial, bergamot pulp integrated in BP diet, showed a higher amount of total phenolic compounds than concentrate, evaluated by the Folin-Ciocalteu assay (14.15 vs 1.55 g TAe/kg DM respectively) and these data indicate that animals from BP group ingested a higher quantity of reducing compounds than animals from control group. Furthermore, it should be pointed out that bergamot peel generally contains essential oils, a complex mixtures of plant metabolites able to exert a wide spectrum of biological activities such as antioxidant (Mandalari et al., 2006; Cui, Che & Wang, 2020), in a phytochemical fraction other than phenolic compounds and that these substances could partly contribute to the overall antioxidant capacity.

Therefore, it may be supposed that all these compounds exerted antioxidant protection which was evident in condition of low levels of vitamin E, as observed in the case of salami.

5. Conclusion

To date, this is the first study that investigated the effect of feeding pigs with the solid residue resulting from the industrial processes of bergamot on meat and meat products on fatty acids composition and oxidative stability. The results showed that the replacement of part of the concentrate with bergamot pulp in the diet increased the content of some of the most desirable fatty acids from health point of view in meat and salami, primarily linolenic acid.

The inclusion of bergamot pulp in the diet did not alter the oxidative stability in raw and cooked meat. Similarly, colour descriptors mostly related to meat browning did not differ between treatments in fresh meat. Instead, in salami TBARS values were reduced over aerobic storage in BP group. Probably, the strong oxidative stresses condition, such as the long storage time, has allowed to show the resistance to oxidative deterioration of the meat from animals of BP group.

In conclusion, the integration of fresh bergamot pulp at up to 15% in diets for fattening pigs could represent a strategy, in the Mediterranean areas, to naturally improve nutritional value of meat and meat products and to promote the exploitation of this local feed resource.

In the light of this, it would be interesting that future studies assess the effect of bergamot pulp on pig performances and meat quality by including it also in other form, such as dried, among the ingredients of a concentrate-based diet.

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Table 1

Ingredients (% on DM basis) and chemical composition of the experimental diets

	control diet	BP diet	Ensiled bergamot pulp
barley	30	23	
Maize	30	23	
Oat	16	12	
Soybean mail	7	10	
Faba bean	14	14	
Ensiled bergamot pulp	-	15	
Vitamin mineral premix[1]	3	3	
Chemical composition			
Dry matter (DM) g/Kg wet weight	885	606	185
Crude protein g/Kg DM	136	132	50.8
Ether extract g/Kg DM	31.9	23.7	13.7
Ash g/Kg DM	39.7	44.8	53.8
NDF g/Kg DM	436	360	246
Total phenolic compounds (g TAe[2]/Kg DM)	1.55	6.58	14.2
Tocopherols, μg/g dry matter			
α-Tocopherol	2.52	68.8	169
γ-Tocopherol	5.32	7.67	11.3
fatty acids (g/100g of total fatty acids)			
C10:0	-	0.04	0.09
C12:0	0.04	0.09	0.16
C14:0	0.13	0.24	0.30
C16:0	14.5	16.3	19.4
C16:1	0.16	0.34	0.58
C18:0	2.52	2.79	2.97
C18:1 *n*-9	31.3	29.9	25.1
C18:2 *n*-6	44.4	39.5	34.1
C18:3 *n*-3	2.13	4.91	8.92
C20:0	0.12	0.15	0.19

[1]The mineral vitamin premix consisted of vitamin 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]tannic acid equivalent

691

692

Table 2
Pig performances in vivo and chemical composition of *longissimus dorsi* muscle (g/100g wet weight).

	Dietary treatment[1]		SEM[6]	*P* value
	Control	BP		
Final BW[2], kg	157	154	2.276	0.415
Carcass weight, kg	133	131	5.780	0.965
Total DMI[3], g/d	3.53	3.35	0.167	0.478
ADG[4], g/d	454	431	8.231	0.692
FCR[5], g DMI[3]/g ADG[4]	7.76	8.18	0.392	0.285
Total FA[7] intake, g/d	41.2	51.5	2.321	0.002
SA[8] intake, g/d	1.0	1.4	0.123	0.057
LA[9] intake, g/d	18.3	20.7	0.362	0.129
ALA[10] intake, g/d	0.9	2.2	0.123	0.005
Chemical composition				
Moisture	71.5	72.1	0.325	0.300
Crude protein	21.4	22.2	0.202	0.065
ether extract	2.79	2.29	0.869	0.286
ash	1.23	1.13	0.031	0.135

[1]Treatments were: only concentrate (control) or concentrate and bergamot pulp at the level of 15% dry matter on the diet fed (BP)

[2]BW=Body weight; [3]DMI=dry matter intake; [4]ADG=averange 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.

Table 3

Effect of the dietary treatment on the antioxidant vitamins (µg/g muscle) and fatty acid composition of *LTL* (g/100 g of total fatty acids)

	Dietary Treatment			
Item	Control	BP	SEM	*P* value
Tocopherols and retinol, µg/g muscle				
α-Tocopherol	2.37	2.38	0.060	0.880
γ-Tocopherol	0.09	0.11	0.009	0.180
Retinol	7.26	6.52	0.392	0.367
intramuscular fat, mg/100 g of muscle	2840	2160	195	0.082
C10:0	0.08	0.12	0.011	0.113
C12:0	0.10	0.07	0.010	0.085
C14:0	1.24	1.19	0.027	0.400
C14:1 *cis-9*	0.03	0.03	0.002	0.582
C15:0	0.04	0.05	0.005	0.864
C16:0	20.4	20.5	0.144	0.927
C 17:0	0.41	0.36	0.014	0.215
C16:1 *cis-9*	3.38	3.58	0.089	0.262
C17:1 *cis-9*	0.22	0.20	0.005	0.200
C18:0	9.49	9.55	0.151	0.841
C18:1 *cis-9*	40.1	37.7	0.912	0.201
C18:1 *trans-11* VA[1]	4.68	4.91	0.091	0.208
C18:2 *cis-9, cis-12* LA[1]	11.9	12.3	0.442	0.650
C18:3 n-3 ALA[1]	0.42	1.47	0.235	0.018
C 20:0	0.28	0.25	0.014	0.377
C 20:1 *cis-9*	0.90	0.88	0.020	0.627
C20:2 n-6	0.50	0.49	0.013	0.800
C20:3 n-3	0.22	0.33	0.053	0.344
C20:4 n-6	2.08	2.51	0.353	0.203
C20:5 n-3 EPA[1]	0.11	0.15	0.025	0.409
C22:5 n-3 DPA[1]	0.24	0.39	0.037	0.036
C22:6 n-3 DHA[1]	0.21	0.30	0.021	0.031
∑ SFA[1]	32.0	31.9	0.270	0.951
∑ MUFA[1]	49.3	47.3	0.798	0.346
∑ PUFA[1]	15.7	18.0	0.644	0.081
∑ n-3	1.20	2.64	0.278	0.004
∑ n-6	14.5	15.3	0.710	0.361
n-6/n-3	12.1	6.87	0.928	0.002
∑ PUFA[1]/∑ SFA1	0.49	0.56	0.022	0.039
Thrombogenic index[2]	0.84	0.76	0.016	0.005
HP-PUFA[3](mg/g muscle)	0.65	0.84	0.043	0.026

HP-PUFA ÷ VE [4]	2.42	2.52	0.026	0.059

[1]VA: vaccenic acid; 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.

[2]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)

[3]Highly peroxidizable (HP) PUFA: calculated as the sum of PUFA with ≥3

[4]Calculated as the ratio between HP-PUFA and total vitamin E, both expressed as mg/g muscle. Original values obtained were not normally distributed according to the Anderson-Darling test. Therefore, logarithmic transformation was adopted and values in table are presented as LOG10.

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Table 4

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

	Dietary Treatment			
Item	Control	BP	SEM	*P* value
C10:0	0.07	0.07	0.013	0.726
C12:0	0.13	0.11	0.010	0.639
C14:0	1.46	1.46	0.438	0.963
C14:1 *cis-9*	0.03	0.03	0.002	0.183
C15:0	0.07	0.07	0.005	0.620
C16:0	21.0	21.2	0.259	0.672
C 17:0	0.47	0.41	0.020	0.198
C16:1 *cis-9*	2.03	2.01	0.029	0.715
C17:1 *cis-9*	0.26	0.27	0.020	0.791
C18:0	8.89	10.5	0.711	0.285
C18:1 *cis-9*	42.6	40.1	0.777	0.115
C18:1 *trans-11* VA[1]	2.95	2.83	0.045	0.174
C18:2 *cis-9. cis-12* LA[1]	14.2	14.3	0.361	0.877
C18:3 n-3 ALA[1]	0.55	1.29	0.183	0.036
C 20:0	0.26	0.26	0.006	0.750
C 20:1 *cis-9*	1.15	1.19	0.043	0.633
C20:2 n-6	0.65	0.71	0.080	0.688
C20:3 n-3	0.13	0.09	0.015	0.150
C20:4 n-6	0.20	0.20	0.012	0.955
C20:5 n-3 EPA[1]	0.19	0.18	0.005	0.635
C22:5 n-3 DPA[1]	0.20	0.21	0.019	0.753
C22:6 n-3 DHA[1]	0.19	0.22	0.031	0.729
∑ SFA[1]	32.2	34.0	0.827	0.306
∑ MUFA[1]	49.0	46.4	0.825	0.126
∑ PUFA[1]	16.3	17.2	0.386	0.246
∑ n-3	1.26	1.99	0.207	0.074
∑ n-6	15.1	15.3	0.392	0.817
n-6/n-3	13.1	9.02	1.280	0.110
∑ PUFA[1]/∑ SFA[1]	0.51	0.51	0.020	0.919

[1]VA: Vaccenic acid; 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.

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Table 5

Effect of the dietary treatment on the antioxidant vitamins (µg/g salami) and fatty acid composition of salami (g/100 g of total fatty acids)

	Dietary Treatment			
Item	Control	BP	SEM	*P* value
Tocopherols and retinol, µg/g salami				
α-Tocopherol	0.54	0.54	0.083	0.977
γ-Tocopherol	0.05	0.05	0.005	0.472
Retinol	3.38	2.81	0.554	0.628
Total fat, g/100 g of salami	19.7	17.0	0.479	0.333
C12:0	0.12	0.12	0.004	0.187
C14:0	1.58	1.32	0.066	0.024
C16:0	22.1	20.2	0.420	0.018
C16:1 *cis-9*	2.18	2.19	0.071	0.776
C18:0	10.9	9.55	0.341	0.040
C18:1 *cis-9*	44.9	45.1	0.336	0.803
C18:2 *cis-9. cis-12* LA[1]	11.3	12.3	0.270	0.098
C18:3 n-3 ALA[1]	0.50	2.10	0.240	0.001
C 20:1 *cis-9*	1.04	1.10	0.028	0.342
C20:2 n-6	0.51	0.68	0.048	0.088
C20:4 n-6	0.28	0.19	0.052	0.367
C20:5 n-3 EPA[1]	0.20	0.35	0.061	0.216
C22:5 n-3 DPA[1]	0.15	0.25	0.028	0.327
C22:6 n-3 DHA[1]	0.14	0.24	0.048	0.288
∑ SFA[1]	34.7	31.2	0.733	0.009
∑ MUFA[1]	48.1	48.4	0.342	0.790
∑ PUFA[1]	12.9	16.1	0.537	0.001
∑ n-3	0.82	2.94	0.288	0.001
∑ n-6	12.1	13.2	0.288	0.094
n-6/n-3	14.7	4.48	1.410	0.001
∑ PUFA[1]/∑ SFA[1]	0.37	0.52	0.023	0.001
Thrombogenic index[2]	1.06	0.78	0.043	0.001
HP-PUFA[3](mg/g salami)	1.23	3.81	0.456	0.001
HP-PUFA ÷ VE [4]	3.25	3.87	0.124	0.007

[1]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.

[2]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)

[3]Highly peroxidizable (HP) PUFA: calculated as the sum of PUFA with ≥3

[4]Calculated as the ratio between HP-PUFA and total vitamin E. both expressed as mg/g salami. Original values obtained were not normally distributed according to the Anderson-Darling test. Therefore. logarithmic transformation was adopted and values in table are presented as LOG10.

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Table 6
Effect of the dietary treatment and time of refrigerated storage on meat colour stability and lipid oxidation

	Dietary treatment[1]		Time [2]			SEM	*P* values		
	Control	BP	0	1	2		Diet	Time	Diet x Time
L* values[3]	41.7	43.8	42.0	43.3	43.0	0.583	0.084	0.650	0.984
a* values[3]	6.0	6.0	6.6	6.2	5.1	0.306	0.986	0.106	0.320
b* values[3]	8.2	8.2	6.5^{x}	9.6^{y}	8.4^{y}	0.316	0.953	0.001	0.069
C* values[3]	10.3	10.3	9.3^{x}	11.5^{y}	10.1^{xy}	0.337	0.985	0.023	0.065
H* values[3]	53.6	54.2	45.4^{x}	57.5^{y}	58.8^{y}	1.64	0.835	0.001	0.965
TBARS raw meat mg MDA/kg	0.50	0.52	0.46	0.56	0.50	0.019	0.677	0.101	0.635
TBARS cooked meat mg MDA/kg	3.08	3.04	2.35^{x}	2.83^{x}	4.01^{y}	0.132	0.827	0.001	0.833

[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 (C); concentrate and bergamot pulp at the level of 15% dry matter on the diet fed (BP).
[2]Times 0. 1. 2 = days 0. 3. 7 for raw meat and 0. 2. 5 for cooked meat at 4 °C under aerobic conditions (meat slices).

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