The effect of fresh bergamot pulp on fatty acid composition of suckling kids

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Abstracts

The objective of the study was to assess the effect of fresh bergamot pulp in lactating goat diets to determine its effects on milk and suckling kid fatty acid composition. Eighteen pregnant does were randomly assigned to two groups of two different diets: alfalfa hay (1 kg/head/day), supplemented with commercial concentrate (1 kg/head/day–Control diet) or the same concetrate (700g/head/day) supplemented with fresh bergamot pulp (2 kg/head/day–FBP diet). Kids were reared in individual pens and fed on their mother's milk. At 45 days of age, kids were slaughtered. Rumenic acid was higher in milk and in meat from animals from FBP group than Control one ($P < 0.001$). Total n-3 PUFA were higher ($P < 0.01$) in FBP kids as a result of the higher level ($P < 0.01$) of α-linolenic acid compared to Control kids. Linoleic and arachidonic acids were higher ($P < 0.001$ and $P < 0.05$, respectively) in Control kids compared to FBP ones. It was concluded that dietary supplementation with fresh bergamot pulp to goats improved nutritional quality of kid meat.

KEYWORDS: bergamot by-product; fatty acid composition; meat quality; suckling kids; phenolic compounds.

1. Introduction

Nowaday, one of the primary objectives for the scientific community is about the integration of alternative feeds in animal diets that do not compete with human foods (Salami et al., 2019). Agro-industrial by-products, among alternative feeds, are used in livestock farms for ruminants feeding as an effective strategy dispose of it and to reduce the cost of the diet. Furthermore, in the recent years many research activities have focused on the study of secondary metabolites in animal feeds and their effects on meat and milk (Valenti et al., 2018; Valenti et al., 2019; Natalello et al., 2020). Among plant secondary compounds, the effect of inclusion of those with antioxidant properties, such as polyphenols, was studied in intramuscular fat from monogastric and polygastric animals (Brenes et al., 2016; Vasta and Luciano, 2011). Furthermore in plants, some secondary metabolites as polyphenolic compounds could modulate the ruminal lipd metabolism of polyunsaturated fatty acids (PUFA), increasing contents of PUFA and desirable biohydrogenation (BH) intermediates (e.g., vaccenic and rumenic acids) on milk and meat from ruminants (Frutos et al., 2020).

Among the many agro-industrial wastes available, bergamot pulp, a by-product derived from juice and essential oil extractions from the whole fruit, has been paid less attention.

Bergamot (*Citrus bergamia Risso*) is a plant that grows wildly in Southern Italy and is used mostly for the extraction of juice and essential oil from the fruit. In Italy, the production of bergamot amounts to about 25,000 tons each year.

The chemical composition of bergamot by-products was studied by differents authors. Flavonoids and pectins from bergamot were characterized by Mandalari et al. (2006), concluding that bergamot fruit peel contains a high amount of flavonoids.

In several Mediterranean areas sheep and goat meat is traditionally produced from sucking lambs and kids, considering consumer preferences for meat from small ruminants, raised exclusively on maternal milk and slaughtered between 30 and 45 days of age (Valvo et al., 2005; Bañon et al., 2006). This is the typical case of southern Italy, where these products are very appreciated by

78 consumers. Also in other countries, such as Turkey, kids are often slaughtered at early ages, and
79 marketed as suckling goat kid (Ekiz et al., 2010).
80 Recently Gómez-Cortés et al. (2018) studied the effect of grape pomace in ewe feeding systems on
81 meat quality of suckling lambs, focusing attention on fatty acid composition. Instead, limited data
82 are available on the effects of goat diet on meat quality of sucking kids and, to date, no studies
83 investigated the effects of the solid residue resulting from the industrial processes of bergamot in
84 goat feeding systems on meat quality of suckling kids.
85 The effect of ewe diets on some meat quality parameters of sucking lambs has been widely
86 investigated, especially of grass vs concentrate feeding system (Velasco et al., 2001; Velasco et al.,
87 2004; Valvo et al., 2005; Scerra et al., 2007; Lobón et al., 2019). These authors reported a
88 correlation between ewe feeding system and meat fatty acid composition of sucking lambs.
89 In this experimental trial we studied the effect of dietary inclusion of fresh bergamot pulp in goat
90 diets on meat fatty acid composition of suckling kids.
91
92 **2. Materials and Methods**
93 The experimental trial was conducted from October 2019 to January 2020 in a goat farm oriented to
94 dairy production. All the procedures were approved (prot. n° 286946) by the Animal Welfare
95 Committee (O.P.B.A.) of the University of Catania.
96 Eighteen pregnant Aspromonte does, all in the second birth, were selected 30 days before
97 parturition, randomly assigned to two groups and fed two different diets, gradually adapted over a
98 period of 14 days to the established quantities. Each goat was housed in individual pen (3 × 3 m)
99 during the experimental period. Within 4 days after birth, kids were separated from their dams, put
100 into individual pens and fed by sucking their mother's milk twice a day, at 07:00 a.m. and 06:00
101 p.m. until slaughter.
102 The two experimental diets were based on the same alfalfa hay offered in the amount of 1
103 kg/head/day, supplemented with 1 kg/head/day of commercial concentrate (Control diet) or 700

104 g/head/day of commercial concentrate plus 2 kg/head/day of fresh bergamot pulp (FBP diet). The
105 chemical composition and the proportion of ingredients of the experimental diets are given in Table
106 1. The concentrate offered to the goats of FBP group had the same ingredients as that supplied to
107 the goats of control group but, in order to maintain a similar crude protein concentration between
108 treatments, had a higher soybean meal content and a lower percentage of barley, maize and oat.
109 The ingredients of each concentrate mixture were mechanically ground and in FBP diet fresh
110 bergamot pulp was mixed with concentrate to avoid any feed selection by animals. Fresh bergamot
111 pulp was transferred to the farm weekly. This by-product was obtained after the cold extraction of
112 bergamot juice. Kids were weighed at the beginning, after twenty days and at the end of the trial in
113 order to calculate average daily gain (ADG).
114 Samples of the feeds offered were collected from the beginning and every 15 days until the end of
115 the trial, vacuum packaged and stored at −30 °C for analyses. The experimental diets were supplied
116 at 08:00 a.m. and 07:00 p.m. The amounts of feed offered and refused were recorder every day in
117 order to calculate the daily voluntary feed intake.
118 A sample of milk from each goat was taken after kidding and every 15 days, until the end of the
119 trial, and stored at –20°C. To determine fatty acid composition, the milk was thawed 24 h 4°C and,
120 for each goat, a bulked sample was obtained by mixing 40 ml of milk from each of four samplings.
121 At 45 days of age, kids were slaughtered on the same day at a commercial abattoir. Animals were
122 stunned by a captive bolt and exanguinated. After slaughtering carcasses were stored at 4 °C for 24
123 h; then the *longissimus thoracis et lumborum muscle* (LTL) was excised from the left half-carcass
124 and immediately vacuum-packaged and stored at −30 °C for analyses of fatty acid composition and
125 proximate analysis.
126
127 *2.1. Feedstuffs and milk analysis*
128 The samples of commercial concentrate and fresh bergamot pulp were analysed for moisture, lipid,
129 ash and crude protein following the methods of Association of Official Analytical Chemists (AOAC

1995); neutral detergent fibre (NDF) was analyzed following the method of Van Soest, Robertson and Lewis (1991).

Fatty acids were extracted from 200 mg of freeze-dried sample of the experimental diets and converted to fatty acid methyl esters (FAME) with a 1-step procedure using chloroform (Sukhija & Palmquist, 1988) and 2% (v/v) sulfuric acid in methanol and nonadecanoic acid as an internal standard.

Following the procedure described by Makkar et al. (1993), total phenolic compounds were extracted from the feed samples using aqueous acetone (70% v/v), analysed by means of the Folin–Ciocalteu reagent and expressed as tannic acid equivalents.

The procedure by Sukhija and Palmquist (1988) as described by Tice et al. (1994) was used to determine milk fatty acid composition. Fatty acids were expressed as g/100 g of methyl esters.

2.2. Proximate analysis and fatty acid determination of muscle

In samples of LTL, according the AOAC methods (AOAC, 1995), ash (method no. 920.153), protein (method no. 984.13), crude fat (method no. 991.36) and moisture (method no. 950.46) were evaluated, after 24 h thawing at 4 °C. The indexes used to evaluate the risk of atherosclerosis and the potential aggregation of blood platelets, the atherogenic and the thrombogenic indexes respectively, were evaluated (Ulbricht and Southgate, 1991).

Intramuscular fat was extracted according to Folch et al. (1957). Briefly, fat from LTL was extracted from 5 g of tissue with a mixture of chloroform and methanol (2:1, v/v) and duplicates of 100 mg of lipid were methylated using 1 ml of hexane and 0.05 ml of 2 N methanolic KOH (I.U.P.A.C., 1987), using C19:0 as internal standard. Fatty acid methyl esters were separated and quantified using a Varian model CP 3900 gas chromatograph, equipped with a capillary column with a lengh of 100 m, internal diameter 0.25 mm and film thickness 0.25 μm (CP-Sil 88, Agilent J&W). One μl of sample was injected, carried by a helium flow of 0.7 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. The

156 temperature split–splitless injector (Varian model CP 3900) was set at 220 °C and the temperature
157 of FID detector at 260 °C. Retention time and area of each peak were computed using the Varian
158 Star 3.4.1. software (Varian, Inc. 2700 Mitchell Drive, Walnut Creek, USA). The individual fatty
159 acid were identified by comparing with a standard mixture of FAME (37 components from Supelco
160 Inc., Bellefont, PA). Fatty acids were expressed as g/100 g of methyl esters.
161
162 *2.3. Statistical analysis*
163 Data on LTL chemical composition, ADG, kid weight and milk and intramuscular fatty acid
164 composition were analyzed using a one-way ANOVA to test the effect of the treatments (Control vs
165 FBP).
166 Each animal was considered as experimental unit. Significance was declared at $P \leq 0.05$, whereas
167 trends toward significance were considered when $0.05 < P \leq 0.10$. Minitab software, (version 14;
168 Minitab Inc, State College, PA) was used for statistical analyses.
169
170 **3. Results**
171 *3.1. Animal performances and meat chemical composition*
172 No significant differerences between treatments were found for dry matter intake of does (data not
173 shown; P = 0.498; 1770 vs 1800 g/d, for control and FBP groups respectively). Data on kids weight
174 and ADG are shown in Table 2. The inclusion of fresh bergamot pulp in the diet of pregnant goat
175 did not affect the birth weight of kids, as the initial body weight (BW) was comparable between
176 groups (P = 0.784). Similarly, the final BW of kids was not statistically affected by FBP
177 supplementation (P = 0.415). In turn, the average daily gain (ADG) was not influenced (P = 0.965)
178 by dietary treatment.
179 The effects of the dietary treatment on the chemical composition of LTL are showed in Table 2. No
180 significant differences were observed between the treatments for the muscle concentrations of
181 moisture (P = 0.310), crude protein (P = 0.165), ether extract (P = 0.436) and ash (P = 0.169).

182 *3.2. Milk fatty acid composition*

183 The dietary treatment affected milk fatty acid composition (table 3). Levels of C15:0, C17:0 and
184 C18:0 were significantly higher ($P < 0.01$, $P < 0.001$ and $P < 0.05$, respectively) in the milk fat from
185 only concentrate-fed goats. Monoinsaturated fatty acids (MUFA), C16:1 *cis-9* and C18:l *trans-11*
186 (vaccenic acid, VA) levels were significantly higher ($P < 0.05$, $P < 0.001$, respectively) in the milk
187 fat from FBP-fed goats than in the milk from control goats, while the level of C18:1 *trans*-10 was
188 higher ($P < 0.05$) in the milk fat from control group compared with that from FBP group.

189 In comparison with animals from control group, FBP-fed goats produced milk with a significantly
190 higher ($P < 0.05$) concentration of α-linolenic acid (C18:3 n-3) and with a lower concentration of γ-
191 linolenic and arachidonic acid ($P<0.001$ and $P < 0.001$, respectively).

192 Among n-3 long-chain fatty acids, eicosapentaenoic (C20:5 n-3; EPA), docosapentaenoic (C22:5 n-
193 3; DPA) and docosahexaenoic (C22:6 n-3; DHA) acids were in higher concentration ($P < 0.001$, $P <$
194 0.001 and $P < 0.001$, respectively) in the milk fat from goats fed FBP diet than in the milk fat from
195 animals fed the control diet.

196 Furthermore, milk fat from the FBP group showed a higher proportion of the CLA (conjugated
197 linoleic acid) isomer usually most present, C18:2 *cis-9 trans-11* (rumenic acid, RA; $P < 0.001$) than
198 milk fat from the control group. The dietary treatment affected the desaturation-CLA index, with a
199 greater value ($P < 0.05$) found in milk fat from goats fed the FBP diet compared with that from
200 goats fed control diet.

201 The milk fat of goats from the FBP group tended to be poorer ($P = 0.08$) in n-6 PUFA and richer in
202 n-3 PUFA ($P < 0.001$) when compared milk fat from the control group. Therefore n-6/n-3 ratio was
203 lower ($P < 0.001$) in the milk fat from goats supplemented with 2 kg/head/day of fresh bergamot
204 pulp.

205 On the whole, the milk from the FBP goats showed higher total polyunsaturated fatty acids (PUFA)
206 content ($P < 0.01$) and lower level of saturated fatty acids (SFA; $P < 0.05$) then the milk from
207 control goats.

208 Finally, atherogenic and thrombogenic indexes were lower ($P < 0.05$) in the milk from goats fed
209 FBP diet than in the milk from goats fed control diet.
210
211 *3.3. Meat fatty acid composition*
212 Intramuscular fatty acid composition is reported in Table 4. The SFA, stearic and palmitic acids
213 had a higher concentration ($P < 0.05$) in the intramuscular fat from kids fed control diet compared
214 to FBP group. The levels of myristoleic acid (C14:1 *cis-9*), palmitoleic (C16.1 *cis-9*) and oleic
215 (C18:l *cis- 9*) acids were significantly higher ($P < 0.05$, $P < 0.001$ and $P < 0.01$, respectively) in the
216 intramuscular fat from FBP-group kids than in that from control-group kids. Among n-6 PUFA,
217 linoleic and arachidonic acids had higher levels ($P < 0.001$ and $P < 0.05$, respectively) in the
218 intramuscular fat from control group kids compared to their counterpart. Linolenic acid (C18:3 n-3)
219 content was higher ($P < 0.01$) in the intramuscular fat from FBP-group kids compared to the
220 control-group animals. Moreover, among the n-3 PUFA, eicosapentaenoic acid (C20:5 n-3) tended
221 to be higher ($P = 0.081$) in the intramuscular fat of kids from FBP group compared to the
222 intramuscular fat from animals from the other group.
223 Consequently, the intramuscular fat from the FBP-group kids was richer ($P < 0.01$) in n-3 PUFA
224 and poorer in n-6 PUFA compared to the control-group kids, affecting the value of n-6/n-3 ratio that
225 was lower ($P < 0.001$) in the intramuscular fat from kids from FBP group.
226 Kids from control group showed a higher ($P < 0.01$) concentration of SFA in the intramuscular fat,
227 whereas the level of MUFA was higher ($P < 0.001$) in the FBP-group kids.
228 The CLA *cis-9, trans-11* isomer was higher ($P < 0.001$) in the intramuscular fat from kids of the
229 FBP group. The desaturation-CLA index showed a greater value ($P < 0.05$) also in intramuscular fat
230 from kids from FBP-fed goats than in intramuscular fat from kids from only concentrate-fed goats.
231 Finally, atherogenic and thrombogenic indeces were lower ($P<0.05$ and $P < 0.01$, respectively) in
232 intramuscular fat of kids from FBP treatment.
233 **4. Discussion**

Kids fed exclusively maternal milk, from a functional point of view, act as monogastric animals considering that the rumen is not functional. Consequently, milk digestion occurs in the abomasum and the dietary unsaturated fatty acid profile is negligibly modified by the biohydrogenation normally caused by ruminal microorganisms. Therefore during the pre-weaning period the fatty acid composition of kids should be strongly linked to the milk fatty acid profile (Zygoyiannis et al., 1992).

The composition of milk fatty acids (FAs) is affected by several factors, among which the composition of diet is predominant. Feeding by-products of the crop and food processing industries to livestock could reduce the costs of expensive waste management programs and the feed to food competitiveness of grains. Furthermore, some of these by-products could have beneficial effect on the quality of milk for their high content of certain bioactive phytochemicals favourable for human health (Santos-Silva et al., 2016; Todaro et al., 2018).

In this experiment, we fed goats reducing the amount of dry matter from concentrate by replacing it with fresh bergamot pulp. In literature, no data are reported on the effects of inclusion of the solid residue resulting from the industrial processes of bergamot in goat feeding systems. The inclusion rate of bergamot pulp in the diet was chosen considering the amount of similar by-products used in ruminants which did not cause large variations in the quantity of milk produced (Todaro et al., 2017).

As it has been detailed in previous works, bergamot by-product, in addition to being a good source of unsaturated FAs (Scerra et al., 2018), molasses and pectins (Postorino et al., 2002; Mandalari et al., 2006) as other citrus fruits by-products, contain a high amount of flavonoids, some of them found in higher levels than other citrus peels (Mandalari et al., 2006; Sommella et al., 2014). Some authors (Vasta et al., 2009; Vasta and Luciano 2011; Lanza et al., 2015) obseved that phenolic compounds could reduce the ruminal biohydrogenation of PUFA. In this trial, bergamot pulp integrated in FBP diet, showed a higher amount of total phenolic compounds than concentrate, evaluated by the Folin-Ciocalteu assay (14.34 vs 1.35 g TAe/kg DM respectively). Consequently, it

would be reasonable to expect a change in the ruminal biohydrogenation pathway, and then in the bioavailability of unsaturated FAs for milk fat.

Regarding productive traits, the data showed that the dietary treatment considered did not affect the average daily gain in kids slaughtered at 45 days of life, reporting similar slaughter weight between the groups.

Meat and milk from ruminants are the main natural source of RA, the principal CLA isomer in dairy products. After lipid hydrolysis in the rumen many unsaturated fatty acids are biohydrogenated into stearic acid (Bessa et al., 2007). RA originates in the rumen due to the incomplete saturation of dietary PUFA. Moreover, this fatty acid is formed by the conversion of vaccenic acid, also originated in the rumen during biohydrogenation of dietary PUFA, flowing from the rumen to animal tissues, by the action of Δ^9-desaturase enzyme (Griinari et al., 2000).

Considering the above, the accumulation of some fatty acids in the milk of ruminants depends not only on the intake of the different fatty acids but also on the extent of the ruminal biohydrogenation of the ingested PUFA.

In this experiment RA was higher in both milk and intramuscular fat from animals from FBP group than from animals from Control group. In particular, RA was four times higher in milk from FBP-fed goats compared with milk from goats given only concentrates (1.48 vs 0.31, respectively for FBP and Control treatments).

In trials involving suckling lambs, some authors (Valvo et al., 2005; Scerra et al., 2007) found higher proportions of RA in *longissimus thoracis* muscle of lambs raised by ewes producing milk with a high level of RA compared with lambs suckled by ewes producing milk low in RA.

For milk, similar results were showed by other authors (Santos-Silva et al. 2016; Todaro et al. 2017) in lactating ewes, replacing cereals with dehydrated citrus pulp or fresh lemon pulp, while, to the best of our knowledge, no studies investigated the effects on milk fatty acid composition of the solid residue resulting from the industrial processes of bergamot in goat feeding systems. However, the data reported from these authors are not fully comparable, considering also that the results

obtained in studies on milk fatty acid composition arise from sheep and goats receiving the same diet (Tsiplakou and Zervas, 2008a; Tsiplakou and Zervas, 2008b). These authors showed that sheep milk had higher vaccenic acid and CLA content compared to goats when both animal species were fed indoors with the same diet. Tsiplakou et al. (2009) suggest that these different responses of sheep and goats, under the same dietary treatment, could be explained by the differences found in mRNA of stearoyl-CoA desaturase of their mammary adipocytes.

Looking at the results it is likely that the inclusion of a high amount of fresh bergamot pulp depressed the complete ruminal biohydrogenation pathway. High levels of PUFA may disturb rumen bacteria metabolism (cellulolytic bacteria), therefore, in the present study, the higher amount of α-linolenic acid ingested by FBP might have affected rumen biohydrogenation process. Furthermore, the inclusion of bergamot pulp in the diet increased the ingestion of phenolic compounds in goats from FBP group, compounds that, as reported above, could impair the ruminal biohydrogenation of PUFA, with a consequential increase of intermediate compounds (Vasta et al., 2009). In the milk fat from FBP-fed goats also the level of another intermediate from rumen biohydrogenation such as vaccenic acid was higher than in milk fat from control goats. In this trial, despite the bergamot-supplemented diet provided a higher amount of stearic acid than the control diet, consequently leading to a greater ingestion of it in goats from FBP treatments, a significantly higher value of this saturated fatty acid was obseved in the milk fat from control goats.

In addition to effects on ruminal biohydrogenation of PUFA, some authors observed that phenolic compounds can increase the expression of the Δ^9-desaturase enzyme (Vasta et al., 2009). So the highest level of rumenic acid in milk from FBP fed goats could be linked both to its direct formation in the rumen during biohydrogenation and also to its de-novo synthesis from vaccenic acid in goat udder through the action of the Δ^9-desaturase enzyme. In our study, the desaturation-CLA index was greater in milk from FBP goats compared with that from Control group. This finding might lead to the hypothesis that feeding the diet including bergamot pulp could have increased the rate of RA synthesis from VA in the mammary gland through the action of the

312 enzyme Δ^9-desaturase. Also the levels C16:1 *cis-9,* fatty acid exclusively synthesized by the action
313 of the Δ^9-desaturase (Palmquist et al., 2004), in milk from goats fed the FBP diet was greater than
314 in milk from goats from control group.
315 Despite the highest level of vaccenic acid in milk from goats from FBP group, no differences were
316 observed for this fatty acid in intramuscular fat from kids from both groups. Rumenic acid in kids
317 intamuscular fat was probably formed in different ways: (a) incorporated in the milk, after synthesis
318 in the rumen of goats, and subsequently in kid tissues; (b) formed in goat udder from vaccenic acid
319 by the action of Δ^9-desaturase and subsequently incorporated in kid tissues; (c) formed directly in
320 kid muscle from milk trans-vaccenic acid by the action of Δ^9-desaturase. This latter hypothesis of
321 formation of CLA in kid tissue could explain why the difference in vaccenic acid between
322 treatments was more important for milk than for meat. We suppose therefore that milk vaccenic
323 acid was partially desaturated to CLA in kids tissue by the action of Δ^9-desaturase. This hypothesis
324 is supported by the value of the desaturation-CLA index that was strongly higher in intramuscular
325 fat from kids from FBP-fed goats than in intramuscular fat from kids from control group.
326 Furthermore, a higher expression of the Δ^9-desaturase enzyme could be supported by the greater
327 level of oleic acid in intramuscular fat of kids from FBP-fed goats than in intramuscular fat of kids
328 from control goats, although its concentration was comparable between dietary treatments in milk
329 fat.
330 Regarding another important trans monounsaturated fatty acid that is formed in the rumen, the level
331 of C18:1 *trans-10* was higher in milk fat from animal fed the control diet. The level of this fatty
332 acid tended to be higher also in the intramuscular fat of kids of the Control group. In animals fed a
333 diet characterized by a high concentrate inclusion the BH pathway may be altered causing an
334 accumulation of C18:1 *trans-10* at the expense of C18:1 *trans-11* in the rumen, which is then
335 reflected in the ruminant products.
336 In this study, the proportion of n-3 PUFA in meat fat was higher in the kids of the FBP group and
337 this was mainly due to the level of α-linolenic acid (C18:3 n-3) that was higher in the kids from

338 goats of FBP group than in kids from goats of the control group. This was probably correlated with
339 the higher level of this fatty acid in the milk from FBP-fed goats compared to the milk from only
340 concentrate-fed goats. Consequently, due to higher intake of α-linolenic acid, the polyunsaturated
341 fatty acid EPA that derives from the elongation of α-linolenic acid (Raes et al., 2004), tended to be
342 higher in the intramuscular fat of kids of the FBP group.
343 Instead, the levels of the most important n-6 PUFA such as linoleic and arachidonic acids were
344 lower in intramuscular fat from kids of FBP group than in intramuscular fat from kids of control
345 group, influencing the total n-6 PUFA that showed the lowest amount in intramuscular fat from kids
346 of FBP group.
347 In accordance with higher levels of the n-3 polyunsaturated fatty acids in kids meat from FBP-fed
348 goats, the n-6/n-3 ratio was significantly lower in this group. It is strongly recommended to
349 decrease the PUFA n-6/n-3 ratio in food, which should not exceed the threshold of 4 (Department
350 of Health, 1994). In this experiment the inclusion of approximately 350 g/day of bergamot pulp DM
351 in the diet resulted in a PUFA n-6/n-3 ratio of 1.88, being this value lower compared to that
352 observed in intramuscular fat of kids from only concentrate-fed goats where was equal to 4.77.
353 However, some of these results could be explained considering that the dietary treatment affected
354 the intake of fatty acids of the goats.
355 Meat from kids reared by FBP-fed goats contained less unfavourable fatty acids for human health.
356 This is well marked by the lower content of palmitic acid ($P < 0.05$), a fatty acid characterized by a
357 high atherogenic potential. However, considering the highest level of this saturated fatty acid in
358 FBP diet, this data was not expected.
359 In this trial, consequently to the positive effects of FBP on some desirable fatty acids, the
360 atherogenic and thrombogenic indexes related to a lipid nutritional quality resulted lower in
361 intramuscular fat of FBP kids than Control ones.
362
363 **5. Conclusion**

A dietary supplementation of 2 kg/head/day of fresh bergamot pulp enhanced the nutritional quality of goat products, firstly by increasing the proportion of CLA, vaccenic and n-3 fatty acids in milk and, as a consequence, by improving fatty acid composition of meat from suckling kids. In particular, we have found that the intramuscular fat from kids raised by fresh bergamot pulp-fed goats contained two fold higher CLA proportion compared with kids raised by goats given only concentrate. Moreover, the inclusion of high amount of fresh bergamot pulp in the diet resulted in a PUFA n-6/n-3 ratio less than 4 in intramuscular fat from kids.

We can conclude that inclusion fresh bergamot pulp in diets proves to be an efficient means of improving the dietetic quality of goat products in terms of fatty acid profile, in kids raised exclusively on maternal milk.

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Table 1
Ingredients (% on DM basis) and chemical composition of experimental diets

	Control diet	FBP diet	Alfalfa hay	Bergamot pulp
Barley	300	150		
Maize	300	150		
Oat	150	80		
Soybean meal	70	90		
Faba bean	150	150		
Bergamot pulp	-	350		
Vitamin mineral premix[1]	30	30		
Chemical composition				
Dry matter (DM) g/Kg wet weight	879	611	908	175
Crude protein g/Kg DM	134	124	157	80.5
Ether extract g/Kg DM	26.3	23.9	14.5	23.8
Ash g/Kg DM	59.5	45.1	90.2	51.3
NDF g/Kg DM	279	278	522	293
Total phenolic compounds, g TAe[2]/Kg DM	1.35	5.85	4.80	14.3
fatty acids (g/100g of total fatty acid)				
C10:0	-	0.05	0.01	0.08
C12:0	0.04	0.10	0.17	0.18
C14:0	0.13	0.23	0.59	0.32
C16:0	15.8	17.7	23.9	25.1
C16:1	0.16	0.31	0.36	0.58
C18:0	1.35	2.63	3.07	4.91
C18:1 n-9	23.3	19.6	3.13	20.8
C18:2 n-6	56.3	48.8	21.3	30.4
C18:3 n-3	2.73	8.31	41.9	17.4
C20:0	0.14	0.15	1.77	0.18

[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]Tannic acid equivalent

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Table 2

Effects of goat feeding system on kid growth and meat chemical composition (g/100g wet weight).

	treatment		SEM[1]	*P* value
	Control	FBP		
No. of kids	9	9		
initial BW[2], kg	3.95	3.87	0.149	0.784
Final BW[2], kg	13.7	13.5	0.342	0.415
ADG[3](g/day)	217	215	1.290	0.965
Chemical composition				
Moisture	73.4	74.1	0.335	0.310
Crude protein	21.8	22.1	0.209	0.165
Ether extract	1.56	1.59	0.669	0.436
Ash	1.10	1.12	0.032	0.169

[1]SEM= standard error of means; [2]BW=Body weight; [3]ADG=averange daily gain.

Table 3

Effect of dietary treatment on milk fatty acid composition (g/100 g of total fatty acid methyl esters)

	Dietary Treatment			
Item	Control	FBP	SEM	*P* values
No. of animals	9	9		
fat, g/kg	65.6	64.7	6.460	0.923
C4:0	1.43	1.04	0.144	0.136
C6:0	2.14	1.72	0.128	0.099
C8:0	3.10	2.63	0.112	0.079
C10:0	11.01	9.85	0.437	0.197
C12:0	5.66	4.96	0.312	0.280
C14:0	10.49	9.43	0.321	0.098
C14:1 *cis-9*	0.37	0.45	0.025	0.127
C15:0	0.82	0.32	0.102	0.006
C15:1	0.44	0.50	0.035	0.416
C16:0	23.50	21.63	0.918	0.333
C16:1 *cis-9*	0.38	0.65	0.070	0.045
C 17:0	0.79	0.20	0.103	0.001
C17:1 *cis-9*	0.48	0.34	0.073	0.366
C18:0	11.21	9.93	0.589	0.041
C18:1 *trans-10*	0.45	0.14	0.295	0.001
C18:1 *trans-11* (VA)[1]	0.38	1.49	0.189	0.001
C18:1 *cis-9*	20.34	22.85	0.923	0.185
C18:2 *cis-9, cis-12* (LA)[1]	2.49	2.14	0.201	0.404
C18:2 *cis-9, trans-11* (RA)[1]	0.31	1.48	0.187	0.001
C18:2 *trans-10, cis-12*	0.32	0.54	0.084	0.197
C18:2 *trans-9, trans-12*	0.44	0.60	0.074	0.268
C18:2 *cis-13, trans-11*	0.30	0.34	0.035	0.626
C18:3 n-6	0.40	0.19	0.038	0.001
C18:3 n-3 (ALA)[1]	0.19	0.82	0.162	0.047
C20:5 n-3 (EPA)[1]	0.18	0.94	0.130	0.001
C20:3 n-3	0.22	0.24	0.019	0.652
C20:4 n-6	0.70	0.30	0.073	0.001
C22:2 n-6	0.26	0.15	0.053	0.321
C22:5 n-3 (DPA)[1]	0.14	0.85	0.116	0.001
C22:6 n-3 (DHA)[1]	0.12	0.53	0.074	0.001
unknown	5.06	6.06	0.288	0.566

∑ SFA[1]	70.16	61.70	1.63	0.012
∑ MUFA[1]	22.83	26.42	1.19	0.065
∑ PUFA[1]	6.08	9.14	0.626	0.006
∑ n-3	0.85	3.38	0.441	0.001
∑ n-6	4.29	3.39	0.259	0.080
n-6/n-3	5.17	1.06	0.653	0.001
∑ PUFA[1]/∑ SFA[1]	0.09	0.15	0.012	0.005
MUFA/SFA	0.33	0.43	0.027	0.032
Desaturation-CLA index[2]	43.75	50.40	3.860	0.032
Atherogenic Index[3]	2.55	1.83	0.176	0.033
Thrombogenic index[4]	2.83	1.62	0.230	0.002

[1]LA: linoleic acid; ALA: α-linolenic acid; EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid; DHA: docosahexaenoic acid; RA: rumenic acid; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

[2]Desaturation-CLA index: 100 × [(cis-9, trans-11 C18:2 CLA)/(trans-11C18:1+ cis-9, trans-11 C18:2 CLA)]

[3]Atherogenic index: (C12:0 + 4*C14:0 + C16:0)/(MUFA + PUFA n-6 + PUFA n-3)

[4]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)

Table 4

Effect of goat dietary treatment on fatty acid composition of LTL muscle of suckling kids (g/100 g of total fatty acid methyl esters)

	Dietary Treatment			
Item	Control	FBP	SEM	*P* values
No. of animals	9	9		
intramuscular fat, mg/100 g of muscle	1417	1478	8.500	0.090
C10:0	0.32	0.22	0.048	0.318
C12:0	0.60	0.44	0.065	0.236
C14:0	3.68	4.74	0.300	0.094
C14:1 *cis-9*	0.23	0.41	0.034	0.021
C15:0	0.64	0.35	0.111	0.200
C15:0 anteiso	0.35	0.40	0.036	0.143
C15:0 iso	0.77	0.52	0.137	0.397
C16:0	23.80	20.04	0.893	0.027
C16:1 *cis-9*	0.45	2.61	0.333	0.001
C17:0	1.57	1.01	0.179	0.101
C17:0 anteiso	0.55	0.58	0.056	0.848
C17:1 *cis-9*	0.63	0.74	0.042	0.187
C18:0	13.34	10.03	0.662	0.013
C18:1 *cis-11*	0.46	0.38	0.063	0.537
C18:1 *cis-9*	26.86	35.73	1.660	0.002
C18:1 *trans-9*	0.38	0.27	0.040	0.202
C18:1 *trans-10*	0.54	0.30	0.652	0.061
C18:1 *trans-11* (VA)[1]	1.71	1.68	0.065	0.868
C18:2 *cis-9, cis-12 (*LA)[1]	10.25	6.51	0.669	0.001
C18:2 *cis-9, trans-11*	0.54	1.95	0.225	0.001
C18:2 *trans-10, cis-12*	0.51	0.96	0.099	0.071
C18:3 n-3 (ALA)[1]	0.33	0.80	0.097	0.008
C18:3 n-6	0.30	0.40	0.039	0.207
C20:2 n-6	0.97	0.22	0.125	0.001
C20:3 n-3	0.35	0.57	0.078	0.173
C22:3 n-3	0.22	0.45	0.080	0.165
C20:4 n-6	6.25	3.24	0.654	0.012
C20:5 n-3 (EPA)[1]	1.00	1.41	0.120	0.081
C22:1	0.19	0.22	0.025	0.662
C22:5 n-3 (DPA)[1]	1.20	1.58	0.187	0.330
C22:6 n-3 (DHA)[1]	0.60	0.81	0.082	0.223
unknown	4.34	3.93	0.365	0.156
∑ SFA[1]	45.61	38.34	1.46	0.005
∑ MUFA[1]	31.62	42.35	1.92	0.001

∑ PUFA[1]	22.54	18.91	0.910	0.068
∑ n-3 PUFA	3.72	5.63	0.388	0.006
∑ n-6 PUFA	17.77	10.37	1.27	0.001
n-6/n-3 PUFA	4.77	1.88	0.522	0.001
∑ PUFA[1]/∑ SFA[1]	0.50	0.48	0.862	0.341
∑ MUFA[1]/∑ SFA[1]	0.68	1.08	2.270	0.303
Desaturation-CLA index[2]	23.78	53.59	4.820	0.001
Atherogenic Index[3]	0.78	0.65	0.029	0.042
Thrombogenic index[4]	1.15	0.80	0.067	0.002

[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]Desaturation-CLA index: 100 × [(cis-9, trans-11 C18:2 CLA)/(trans-11C18:1+ cis-9, trans-11 C18:2 CLA)]

[3]Atherogenic index: (C12:0 + 4*C14:0 + C16:0)/(MUFA + PUFA n-6 + PUFA n-3)

[4]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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