1	This is the peer reviewed version of the following article
2	Scerra M, Foti F, Caparra P, Lanza M, Natalello A, Cilione C, Rao R, D'Agui'G, Chies L,
3	2021. The effect of fresh bergamot pulp on fatty acid composition
4	of suckling kids
5	Small Ruminant Research, volume 203, article 106483. ISSN: 0921-4488
6	which has been published in final doi: https://doi.org/10.1016/j.smallrumres.2021.106483
7	
8	The terms and conditions for the reuse of this version of the manuscript are specified in the
9	publishing policy. For all terms of use and more information see the publisher's website
10	
11	
12	
13	
14	
15	
16	
17	
18	
19	
20	
21	
22	
23	
24	
25	
26	

27	The effect of fresh bergamot pulp on fatty acid composition
28	of suckling kids
29	Manuel Scerra <sup>*a</sup> , Francesco Foti <sup>a</sup> , Pasquale Caparra <sup>a</sup> , Massimiliano Lanza <sup>b</sup> , Antonio
30	Natalello <sup>b</sup> , Caterina Cilione <sup>a</sup> , Rosa Rao <sup>a</sup> , Giovanni D'Agui' <sup>a</sup> , Luigi Chies <sup>a</sup>
31	<sup>a</sup> University of Reggio Calabria, Dipartimento di Agraria, Produzioni Animali, Via dell'Università,
32	25, 89124, Reggio Calabria , Italy
33	<sup>b</sup> University of Catania, Dipartimento di Agricoltura, Alimentazione e Ambiente (Di3A), Via
34	Valdisavoia 5, 95123, Catania, Italy
35	
36	*Corresponding author. E-mail address: manuel.scerra@unirc.it
37	
38	Abstracts
39	The objective of the study was to assess the effect of fresh bergamot pulp in lactating goat diets to
40	determine its effects on milk and suckling kid fatty acid composition. Eighteen pregnant does were
41	randomly assigned to two groups of two different diets: alfalfa hay (1 kg/head/day), supplemented
42	with commercial concentrate (1 kg/head/day-Control diet) or the same concetrate (700g/head/day)
43	supplemented with fresh bergamot pulp (2 kg/head/day-FBP diet). Kids were reared in individual
44	pens and fed on their mother's milk. At 45 days of age, kids were slaughtered. Rumenic acid was
45	higher in milk and in meat from animals from FBP group than Control one (P < $0.001$ ). Total n-3
46	PUFA were higher (P < 0.01) in FBP kids as a result of the higher level (P < 0.01) of $\alpha$ -linolenic
47	acid compared to Control kids. Linoleic and arachidonic acids were higher ( $P < 0.001$ and $P < 0.05$ ,
48	respectively) in Control kids compared to FBP ones. It was concluded that dietary supplementation
49	with fresh bergamot pulp to goats improved nutritional quality of kid meat.
50	KEYWORDS: bergamot by-product; fatty acid composition; meat quality; suckling kids; phenolic
51	compounds.

### 53 **1. Introduction**

54 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-55 industrial by-products, among alternative feeds, are used in livestock farms for ruminants feeding as 56 an effective strategy dispose of it and to reduce the cost of the diet. Furthermore, in the recent years 57 many research activities have focused on the study of secondary metabolites in animal feeds and 58 their effects on meat and milk (Valenti et al., 2018; Valenti et al., 2019; Natalello et al., 2020). 59 Among plant secondary compounds, the effect of inclusion of those with antioxidant properties, 60 such as polyphenols, was studied in intramuscular fat from monogastric and polygastric animals 61 62 (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 63 acids (PUFA), increasing contents of PUFA and desirable biohydrogenation (BH) intermediates 64 65 (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 juiceand 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 consumers. Also in other countries, such as Turkey, kids are often slaughtered at early ages, and
marketed as suckling goat kid (Ekiz et al., 2010).

Recently Gómez-Cortés et al. (2018) studied the effect of grape pomace in ewe feeding systems on meat quality of suckling lambs, focusing attention on fatty acid composition. Instead, limited data are available on the effects of goat diet on meat quality of sucking kids and, to date, no studies investigated the effects of the solid residue resulting from the industrial processes of bergamot in goat feeding systems on meat quality of suckling kids.

The effect of ewe diets on some meat quality parameters of sucking lambs has been widely investigated, especially of grass vs concentrate feeding system (Velasco et al., 2001; Velasco et al., 2004; Valvo et al., 2005; Scerra et al., 2007; Lobón et al., 2019). These authors reported a correlation between ewe feeding system and meat fatty acid composition of sucking lambs.

In this experimental trial we studied the effect of dietary inclusion of fresh bergamot pulp in goatdiets on meat fatty acid composition of suckling kids.

91

### 92 2. Materials and Methods

The experimental trial was conducted from October 2019 to January 2020 in a goat farm oriented to
dairy production. All the procedures were approved (prot. n° 286946) by the Animal Welfare
Committee (O.P.B.A.) of the University of Catania.

Eighteen pregnant Aspromonte does, all in the second birth, were selected 30 days before parturition, randomly assigned to two groups and fed two different diets, gradually adapted over a period of 14 days to the established quantities. Each goat was housed in individual pen  $(3 \times 3 \text{ m})$ during the experimental period. Within 4 days after birth, kids were separated from their dams, put into individual pens and fed by sucking their mother's milk twice a day, at 07:00 a.m. and 06:00 p.m. until slaughter.

The two experimental diets were based on the same alfalfa hay offered in the amount of 1
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.

The ingredients of each concentrate mixture were mechanically ground and in FBP diet fresh bergamot pulp was mixed with concentrate to avoid any feed selection by animals. Fresh bergamot pulp was transferred to the farm weekly. This by-product was obtained after the cold extraction of bergamot juice. Kids were weighed at the beginning, after twenty days and at the end of the trial in order to calculate average daily gain (ADG).

Samples of the feeds offered were collected from the beginning and every 15 days until the end of the trial, vacuum packaged and stored at -30 °C for analyses. The experimental diets were supplied at 08:00 a.m. and 07:00 p.m. The amounts of feed offered and refused were recorder every day in order to calculate the daily voluntary feed intake.

A sample of milk from each goat was taken after kidding and every 15 days, until the end of the trial, and stored at –20°C. To determine fatty acid composition, the milk was thawed 24 h 4°C and, 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

130 1995); neutral detergent fibre (NDF) was analyzed following the method of Van Soest, Robertson131 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.

141

# 142 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 148 extracted from 5 g of tissue with a mixture of chloroform and methanol (2:1, v/v) and duplicates of 149 100 mg of lipid were methylated using 1 ml of hexane and 0.05 ml of 2 N methanolic KOH 150 (I.U.P.A.C., 1987), using C19:0 as internal standard. Fatty acid methyl esters were separated and 151 quantified using a Varian model CP 3900 gas chromatograph, equipped with a capillary column 152 with a lengh of 100 m, internal diameter 0.25 mm and film thickness 0.25 µm (CP-Sil 88, Agilent 153 J&W). One µl of sample was injected, carried by a helium flow of 0.7 ml/min. The temperature 154 program of the column was 4 min at 140 °C and a subsequent increase to 220 °C at 4 °C/min. The 155

temperature split–splitless injector (Varian model CP 3900) was set at 220 °C and the temperature
of FID detector at 260 °C. Retention time and area of each peak were computed using the Varian
Star 3.4.1. software (Varian, Inc. 2700 Mitchell Drive, Walnut Creek, USA). The individual fatty
acid were identified by comparing with a standard mixture of FAME (37 components from Supelco
Inc., Bellefont, PA). Fatty acids were expressed as g/100 g of methyl esters.

161

## 162 2.3. Statistical analysis

Data on LTL chemical composition, ADG, kid weight and milk and intramuscular fatty acid
composition were analyzed using a one-way ANOVA to test the effect of the treatments (Control vs
FBP).

Each animal was considered as experimental unit. 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.

169

## 170 **3. Results**

#### 171 *3.1. Animal performances and meat chemical composition*

No significant differences between treatments were found for dry matter intake of does (data not shown; P = 0.498; 1770 vs 1800 g/d, for control and FBP groups respectively). Data on kids weight and ADG are shown in Table 2. The inclusion of fresh bergamot pulp in the diet of pregnant goat did not affect the birth weight of kids, as the initial body weight (BW) was comparable between groups (P = 0.784). Similarly, the final BW of kids was not statistically affected by FBP supplementation (P = 0.415). In turn, the average daily gain (ADG) was not influenced (P = 0.965) by dietary treatment.

The effects of the dietary treatment on the chemical composition of LTL are showed in Table 2. No significant differences were observed between the treatments for the muscle concentrations of 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*

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

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  $\alpha$ -linolenic acid (C18:3 n-3) and with a lower concentration of  $\gamma$ -

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 < 0.001

0.001 and P < 0.001, respectively) in the milk fat from goats fed FBP diet than in the milk fat from</li>animals fed the control diet.

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

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

On the whole, the milk from the FBP goats showed higher total polyunsaturated fatty acids (PUFA) content (P < 0.01) and lower level of saturated fatty acids (SFA; P < 0.05) then the milk from control goats. Finally, atherogenic and thrombogenic indexes were lower (P < 0.05) in the milk from goats fed FBP diet than in the milk from goats fed control diet.

210

# 211 *3.3. Meat fatty acid composition*

Intramuscular fatty acid composition is reported in Table 4. The SFA, stearic and palmitic acids 212 had a higher concentration (P < 0.05) in the intramuscular fat from kids fed control diet compared 213 to FBP group. The levels of myristoleic acid (C14:1 cis-9), palmitoleic (C16.1 cis-9) and oleic 214 (C18:1 *cis-* 9) acids were significantly higher (P < 0.05, P < 0.001 and P < 0.01, respectively) in the 215 intramuscular fat from FBP-group kids than in that from control-group kids. Among n-6 PUFA, 216 linoleic and arachidonic acids had higher levels (P < 0.001 and P < 0.05, respectively) in the 217 intramuscular fat from control group kids compared to their counterpart. Linolenic acid (C18:3 n-3) 218 content was higher (P < 0.01) in the intramuscular fat from FBP-group kids compared to the 219 220 control-group animals. Moreover, among the n-3 PUFA, eicosapentaenoic acid (C20:5 n-3) tended to be higher (P = 0.081) in the intramuscular fat of kids from FBP group compared to the 221 222 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.

The CLA *cis-9*, *trans-11* isomer was higher (P < 0.001) in the intramuscular fat from kids of the

- FBP group. The desaturation-CLA index showed a greater value (P < 0.05) also in intramuscular fat
- from kids from FBP-fed goats than in intramuscular fat from kids from only concentrate-fed goats.
- 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 252 of unsaturated FAs (Scerra et al., 2018), molasses and pectins (Postorino et al., 2002; Mandalari et 253 254 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 255 authors (Vasta et al., 2009; Vasta and Luciano 2011; Lanza et al., 2015) obseved that phenolic 256 257 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, 258 evaluated by the Folin-Ciocalteu assay (14.34 vs 1.35 g TAe/kg DM respectively). Consequently, it 259

would be reasonable to expect a change in the ruminal biohydrogenation pathway, and then in thebioavailability 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  $\Delta^9$ -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 FBPfed 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 292 293 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 294 of a-linolenic acid ingested by FBP might have affected rumen biohydrogenation process. 295 Furthermore, the inclusion of bergamot pulp in the diet increased the ingestion of phenolic 296 compounds in goats from FBP group, compounds that, as reported above, could impair the ruminal 297 298 biohydrogenation of PUFA, with a consequential increase of intermediate compounds (Vasta et al., 299 2009). In the milk fat from FBP-fed goats also the level of another intermediate from rumen 300 biohydrogenation such as vaccenic acid was higher than in milk fat from control goats. In this trial, 301 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 302 303 higher value of this saturated fatty acid was observed in the milk fat from control goats.

In addition to effects on ruminal biohydrogenation of PUFA, some authors observed that phenolic 304 compounds can increase the expression of the  $\Delta^9$ -desaturase enzyme (Vasta et al., 2009). So the 305 highest level of rumenic acid in milk from FBP fed goats could be linked both to its direct 306 307 formation in the rumen during biohydrogenation and also to its de-novo synthesis from vaccenic acid in goat udder through the action of the  $\Delta^9$ -desaturase enzyme. In our study, the desaturation-308 309 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 310 increased the rate of RA synthesis from VA in the mammary gland through the action of the 311

enzyme  $\Delta^9$ -desaturase. Also the levels C16:1 *cis-9*, fatty acid exclusively synthesized by the action of the  $\Delta^9$ -desaturase (Palmquist et al., 2004), in milk from goats fed the FBP diet was greater than in milk from goats from control group.

Despite the highest level of vaccenic acid in milk from goats from FBP group, no differences were 315 observed for this fatty acid in intramuscular fat from kids from both groups. Rumenic acid in kids 316 intamuscular fat was probably formed in different ways: (a) incorporated in the milk, after synthesis 317 in the rumen of goats, and subsequently in kid tissues; (b) formed in goat udder from vaccenic acid 318 by the action of  $\Delta^9$ -desaturase and subsequently incorporated in kid tissues; (c) formed directly in 319 kid muscle from milk trans-vaccenic acid by the action of  $\Delta^9$ -desaturase. This latter hypothesis of 320 formation of CLA in kid tissue could explain why the difference in vaccenic acid between 321 treatments was more important for milk than for meat. We suppose therefore that milk vaccenic 322 acid was partially desaturated to CLA in kids tissue by the action of  $\Delta^9$ -desaturase. This hypothesis 323 324 is supported by the value of the desaturation-CLA index that was strongly higher in intramuscular fat from kids from FBP-fed goats than in intramuscular fat from kids from control group. 325 Furthermore, a higher expression of the  $\Delta^9$ -desaturase enzyme could be supported by the greater 326 327 level of oleic acid in intramuscular fat of kids from FBP-fed goats than in intramuscular fat of kids from control goats, although its concentration was comparable between dietary treatments in milk 328 329 fat.

Regarding another important trans monounsaturated fatty acid that is formed in the rumen, the level of C18:1 *trans-10* was higher in milk fat from animal fed the control diet. The level of this fatty acid tended to be higher also in the intramuscular fat of kids of the Control group. In animals fed a diet characterized by a high concentrate inclusion the BH pathway may be altered causing an accumulation of C18:1 *trans-10* at the expense of C18:1 *trans-11* in the rumen, which is then reflected in the ruminant products.

In this study, the proportion of n-3 PUFA in meat fat was higher in the kids of the FBP group and this was mainly due to the level of  $\alpha$ -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  $\alpha$ -linolenic acid, the polyunsaturated 341 fatty acid EPA that derives from the elongation of  $\alpha$ -linolenic acid (Raes et al., 2004), tended to be 342 higher in the intramuscular fat of kids of the FBP group.

Instead, the levels of the most important n-6 PUFA such as linoleic and arachidonic acids were lower in intramuscular fat from kids of FBP group than in intramuscular fat from kids of control group, influencing the total n-6 PUFA that showed the lowest amount in intramuscular fat from kids of FBP group.

In accordance with higher levels of the n-3 polyunsaturated fatty acids in kids meat from FBP-fed goats, the n-6/n-3 ratio was significantly lower in this group. It is strongly recommended to decrease the PUFA n-6/n-3 ratio in food, which should not exceed the threshold of 4 (Department of Health, 1994). In this experiment the inclusion of approximately 350 g/day of bergamot pulp DM in the diet resulted in a PUFA n-6/n-3 ratio of 1.88, being this value lower compared to that observed in intramuscular fat of kids from only concentrate-fed goats where was equal to 4.77.

However, some of these results could be explained considering that the dietary treatment affectedthe intake of fatty acids of the goats.

Meat from kids reared by FBP-fed goats contained less unfavourable fatty acids for human health. This is well marked by the lower content of palmitic acid (P < 0.05), a fatty acid characterized by a high atherogenic potential. However, considering the highest level of this saturated fatty acid in FBP diet, this data was not expected.

In this trial, consequently to the positive effects of FBP on some desirable fatty acids, the atherogenic and thrombogenic indexes related to a lipid nutritional quality resulted lower in 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.

- 374
- 375
- 376
- 377
- 378
- 379
- 380
- 381
- 382
- 383
- 384
- 385
- 386
- 387
- 388
- ----
- 389

#### 390 **References**

- AOAC (Association of Official Analytical Chemists), 1995. Official Methods of Analysis (16th
  ed.). AOAC, Washington, DC, USA.
- Bañon, S., Vila, R., Price, A., Ferrandini, E., Garrido, M. D., 2006. Effects of goat milk or milk
  replacer diet on meat quality and fat composition of suckling goat kids. Meat Sci. 72, 216-221.
  https://doi.org/10.1016/j.meatsci.2005.07.004.
- Bessa, R.J.B., Alves, S.P., Jeronimo, E., Alfaia, C.M., Prates, J.A.M., Santos-Silva, J., 2007. Effect
- 397 of lipid supplements on ruminal biohydrogenation intermediates and muscle fatty acids in lamb.
- 398 Eur. J. Lipid Sci. Technol. 109, 868-883. https://doi.org/ 10.1002/ejlt.200600311.
- Brenes, A., Viveros, A., Chamorro, S., Arija, I., 2016. Use of polyphenol-rich grape byproducts in
  monogastric nutrition. A review. Anim. Feed Sci. Technol. 211, 1-17.
  https://doi.org/10.1016/j.anifeedsci.2015.09.016.
- 402 Department of Health, 1994. Nutritional aspects of cardiovascular disease. Report on Health and
  403 Social Subject no. 46. Her Majesty's Stationery Office, London.
- Ekiz, B., Ozcan, M., Yilmaz, A., Tölü, C., Savas, T., 2010. Carcass measurements and meat quality
  characteristics of dairy suckling kids compared to an indigenous genotype. Meat Sci. 85, 245-249.
  https://doi.org/10.1016/j.meatsci.2010.01.006.
- Folch, J., Lees, M., Stanley, G.H.S., 1957. A simple method for the isolation and purification of
  lipids from animal tissue. J. Biol. Chem. 226, 497-509. https://doi.org/ 10.1016/s00219258(18)64849-5.
- 410 Frutos, P., Hervás, G., Natalello, A., Luciano, G., Fondevila, M., Priolo, A., Toral, P.G., 2020.
  411 Ability of tannins to modulate ruminal lipid metabolism and milk and meat fatty acid profiles.
- 412 Anim. Feed Sci. Technol. 269, 114623. https://doi.org/10.1016/j.anifeedsci.2020.114623.
- 413 Gómez-Cortés, P., Guerra-Rivas, C., Gallardo, B., Lavín, P., Mantecón, A.R., De la Fuente, M.A.,
- 414 Manso, T., 2018. Grape pomace in ewes diet: Effects on meat quality and the fatty acid profile of
- 415 their suckling lambs. Food Res. Int. 113, 36-42. https://doi.org/ 10.1016/j.foodres.2018.06.052.

- 416 Griinari, J. M., Corl, B. A., Lacy, S. H., Chouinard, P. Y., Nurmela, K. V. V., Bauman, D. E., 2000.
- 417 Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by  $\Delta 9$ -desaturase. J.
- 418 *Nutr.* 130, 2285-2291. https://doi.org/10.1093/jn/130.9.2285.
- I.U.P.A.C., 1987. International Union of Pure and Applied Chemistry. Standard Methods for the
  Analysis of Oils, Fats and Derivatives. Pergamon Press, Oxford.
- 421 Kanes, K., Tisserat, B., Berhow, M., Vandercook, C., 1993. Phenolic composition of various tissues
- 422 of Rutaceae species. Phytochemistry 32, 967-974. https://doi.org/10.1016/0031-9422(93)85237-L.
- 423 Kawaii, S., Tomono, Y., Katase, E., Ogawa, K., Yano, M., 1999. Effect of citrus flavonoids on HL-
- 424 60 cell differentiation. Anticancer Res. 19, 1261-1269.
- 425 Lanza, M., Scerra, M., Bognanno, M., Buccioni, A., Cilione, C., Biondi, L., Priolo, A., Luciano, G.,
- 426 2015. Fatty acid metabolism in lambs fed citrus pulp. J. Anim. Sci. 93, 3179-3188.
  427 https://doi.org/10.2527/jas.2014-8708.
- Lobón, S., Joy, M., Sanz, A., Álvarez-Rodríguez, J., Blanco, M., 2019. The fatty acid composition
  of ewe milk or suckling lamb meat can be used to discriminate between ewes fed different diets.
  Anim. Prod. Sci. 59(6), 1108-1118. https://doi.org/10.1071/AN18082.
- Makkar, H.P.S., Blümmel, M., Borowy, N.K., Becker, K., 1993. Gravimetric determination of
  tannins and their correlations with chemical and protein precipitation methods. J. Environ. Sci.
  Health B 61, 161-165. https://doi.org/10.1002/jsfa.2740610205.
- 434 Mandalari, G., Bennett, R.N., Bisignano, G., Saija, A., Dugo, G., Lo Curto, R.B., Faulds, C.B.,
- 435 Waldron, K.W., 2006. Characterization of flavonoids and pectins from bergamot (Citrus bergamia
- 436 *risso*) peel, a major byproduct of essential oil extraction. J. Agricult. Food Chem. 54(1), 197-203.
- 437 https://doi.org/10.1021/jf051847n.
- 438 Natalello, A., Priolo, A., Valenti, B., Codini, M., Mattioli, S., Pauselli, M., Puccio, M., Lanza, M.,
- 439 Stergiadis, S., Luciano, G., 2020. Dietary pomegranate by-product improves oxidative stability of
- 440 lamb meat. Meat Sci. 162, 108037. https://doi.org/10.1016/j.meatsci.2019.108037.

- Palmquist, D.L., St-Pierre, N., McClure, K.E., 2004. Tissue fatty acids profiles can be used to
  quantify endogenous rumenic acid synthesis in lambs. J. Nutr. 134, 2407-2414.
  https://doi.org/10.1093/jn/134.9.2407.
- 444 Postorino, E., Finotti, E., Castaldo, D., Pirrello, A., 2002. La composizione chimica del
  445 "pastazzo"di Bergamotto. *Essenze derivati Agrumari*, 72, 15-19.
- Raes, K., De Smet, S., Demeyer D., 2004. Effect of dietary fatty acids on incorporation of long
  chain polyunsaturated fatty acids and conjugated linoleic acid in lamb, beef and pork meat: a
  review. Anim. Feed Sci. Technol. 113, 199-221. doi:10.1016/j.anifeedsci.2003.09.001.
- Raes, K., Fievez, V., Chow, T. T., Ansorena, D., Demeyer, D., De Smet, S., 2004. Effect of diet and 449 dietary fatty acids on the trasformation and incorporation of C18 fatty acids in doublemuscled 450 Belgian bulls. J. Agricult. 6035-6041. 451 Blue young Food Chem. 52, https://doi.org/10.1021/jf035089h. 452
- Salami, S.A., Luciano, G., O'Grady M.N., Biondi, L., Newbold, C.J., Kerry, J.P., Priolo, A., 2019.
  Sustainability of feeding plant by-products: A review of the implications for ruminant meat
  production. Anim. Feed Sci. Technol. 251, 37-55. https://doi.org/10.1016/j.anifeedsci.2019.02.006.
- Santos-Silva, J., Dentinho, M.T., Francisco, A., Portugal, A.P., Belo, A.T., Martins, A.P., Alves,
  S.P., Bessa, R.J., 2016. Replacing cereals with dehydrated citrus pulp in a soybean oil
  supplemented diet increases vaccenic and rumenic acids in ewe milk. J. Dairy Sci. 99, 1173-1182.
  https://doi.org/10.3168/jds.2015-9966.
- Scerra, M., Caparra, P., Foti, F., Galofaro, V., Sinatra, M.C., Scerra, V., 2007. Influence of ewe
  feeding systems on fatty acid composition of suckling lambs. Meat Sci. 76, 390-394.
  https://doi.org/10.1016/j.meatsci.2006.04.033.
- Scerra, M., Foti, F., Caparra, P., Cilione, C., Violi, L., Fiammingo, G., D'Agui', G., Chies, L.,
  2018. 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. Small Rumin. Res. 169, 160-166.
  https://doi.org/10.1016/j.smallrumres.2018.09.016.

- 467 Shahidi, F., Ambigaipalan, P., 2015. Phenolics and polyphenolics in foods, beverages and spices:
  468 Antioxidant activity and health effects–A review. J. Funct. Foods 18, 820-897.
  469 https://doi.org/10.1016/j.jff.2015.06.018.
- Siu, G.M., Draper, H.H., 1978. A survey of the malonaldehyde content of retail meats and fish. J.
  Food Sci. 43, 1147–1149. https://doi.org/10.1111/j.1365-2621.1978.tb15256.x.
- Sommella, E., Pepe, G., Pagano, F., Tenore, G.C., Marzocco, S., Manfra, M., Calabrese, G., 472 Aquino, R.P., Campiglia, P., 2014. UHPLC profiling and effects on LPS-stimulated J774A.1 473 macrophages of flavonoids from bergamot (Citrus bergamia) juice, an underestimated waste 474 anti-inflammatory 475 product with high potential. J. Funct. Foods 7, 641-649. https://doi.org/10.1016/j.jff.2013.12.021. 476
- 477 Sukhija, P. S., Palmquist, D. L., 1988. Rapid method for determination of total fatty acid content
  478 and composition of feedstuffs and feces. J. Agricult.Food Chem. 36, 1202-1206.
  479 https://doi.org/10.1021/jf00084a019.
- Tice, E. M., Eastridge, M. L., Firkins, J. L., 1994. Raw soybeans and roasted soybeans of different
  particle sizes. 2. Fatty acid utilization by lactating cows. J. Dairy Sci. 77, 166-180.
  https://doi.org/10.3168/jds.S0022-0302(94)76939-3.
- Tsiplakou, E., Zervas, G., 2008a. Comperative study between sheep and goats on rumenic acid and
  vaccenic acid in milk fat under the same dietary treatments. Livest. Sci. 119, 87-94.
  https://doi.org/10.1016/j.livsci.2008.03.009.
- Tsiplakou, E., Zervas, G., 2008b. The effects of dietary inclusion of olive ree leaves and grape mark
  on the content of conjugated linoleic acid and vaccenic acid in the milk of dairy sheep and goats. J.
  Dairy Res. 75, 270-278. https://doi.org/10.1017/S0022029908003270.
- Tsiplakou, E., Flementakis, E., Kalloniati, C., Papadomichelakis, G., Katinakis, P., Zervas, G.,
  2009. Sheep and goats differences in CLA and fatty acids milk fat content in relation with mRNA
  stearoyl-CoA desaturase and lipogenic genes expression in their mammary glad. J. Dairy Sci. 76,
- 492 392-401. https://doi.org/10.1017/S0022029909990100.

- Todaro, M., Alabiso, M., Scatassa, M.L., Di Grigoli, A., Mazza, F., Maniaci, G., Bonanno, A., 493 2017. Effect of the inclusion of fresh lemon pulp in the diet of lactating ewes on the properties of 494 Technol. 495 milk and cheese. Anim. Feed Sci. 225, 213-223. https://doi.org/10.1016/j.anifeedsci.2017.02.003. 496
- Ulbricht, T. L. V., Southgate, D. A. T., 1991. Coronary heart disease: Seven dietary factors. Lancet
  338, 985-992. https://doi.org/10.1016/0140-6736(91)91846-m.
- 499 Valenti, B., Luciano, G., Pauselli, M., Mattioli, S., Biondi, L., Priolo, A., Natalello, A., Morbidini,
- L., Lanza, M., 2018. Dried tomato pomace supplementation to reduce lamb concentrate in take: 500 Effects performance 501 on growth and meat quality. Meat Sci. 145, 63-70. https://doi.org/10.1016/j.meatsci.2018.06.009. 502
- 503 Valenti, B., Criscione, A., Moltisanti, V., Bordonaro, S., De Angelis, A., Marletta, D., Di Paola, F.,
- Avondo, M., 2019. Genetic polymorphisms at candidate genes affecting fat content and fatty acid
  composition in Modicana cows: Effects on milk production traits in different feeding systems.
  Animals 13 (6), 1332-1340. https://doi.org/10.1017/S1751731118002604.
- Valvo, M. A., Lanza, M., Bella, M., Fasone, V., Scerra, M., Biondi, L., 2005. Effect of ewe feeding
  system (grass vs concentrate) on intramuscular fatty acids of lambs raised exclusively on maternal
  milk. Anim. Sci. 81, 431-436. https://doi.org/10.1079/ASC50480431.
- Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent
  fiber, and non starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583-3597.
- 512 https://doi.org/10.3168/jds.S0022-0302(91)78551-2.
- Vasta, V., Mele, M., Makkar, H. P. S., Priolo, A., 2009. Ruminal biohydrogenation as affected by
  tannins in vitro. Brit. J. Nutr. 102, 82-92. https://doi.org/10.1017/ S0007114508137898.
- Vasta, V., Luciano, G., 2011. The effects of dietary consumption of plants secondary compounds on
  small ruminants' products quality. Small Rumin. Res. 101, 150-159.
  https://doi.org/10.1016/j.smallrumres.2011.09.035.

518	Velasco,	S., Caneque,	V., Perez,	, C., La	uzurica	, S., D1	az, M. T.,	Huidobro, F., Mar	nzanares, C.,
519	González	, J., 2001. F	atty acid o	composi	tion of	adipos	e depots o	of suckling lambs	raised under
520	different	production	systems.	Meat	Sci.	59,	325-333.	https://doi.org/10.	1016/S0309-
521	1740(01)0	00135-8.							

- Velasco, S., Cañeque, V., Lauzurica, S., Perez, C., Huidobro, F., 2004. Effect of different feeds on
  meat quality and fatty acid composition of lambs fattened at pasture. Meat Sci. 66, 457-465.
  https://doi.org/10.1016/S0309-1740(03)00134-7.
- 525 Zygoyiannis, D., Kufidis, D., Katsaounis, N., Phillips, P., 1992. Fatty acid composition of
- indigenous (*Capra prisca*) suckled Greek kids and milk of their does. Small Rumin. Res. 8, 83-95.
- 527 https://doi.org/10.1016/0921-4488(92)90010-2.

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

			Alfalfa	Bergamot
	Control diet	FBP diet	hay	pulp
Barley	300	150		
Maize	300	150		
Oat	150	80		
Soybean meal	70	90		
Faba bean	150	150		
Bergamot pulp	-	350		
Vitamin mineral premix <sup>1</sup>	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 <sup>2</sup> /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
<u>C20:0</u>	0.14	0.15	1.77	0.18

<sup>1</sup>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;

<sup>2</sup>Tannic acid equivalent

544

- 545
- 546
- 547
- 548

	treatment		- SEM <sup>1</sup>		
	Control	FBP	SEM	r value	
No. of kids	9	9			
initial BW <sup>2</sup> , kg	3.95	3.87	0.149	0.784	
Final BW <sup>2</sup> , kg	13.7	13.5	0.342	0.415	
ADG <sup>3</sup> (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	

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

<sup>1</sup>SEM= standard error of means; <sup>2</sup>BW=Body weight; <sup>3</sup>ADG=averange daily gain.

- -

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 <i>cis-9</i> , <i>cis-12</i> (LA) <sup>1</sup>	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) <sup>1</sup>	0.19	0.82	0.162	0.047
C20:5 n-3 (EPA) <sup>1</sup>	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) <sup>1</sup>	0.14	0.85	0.116	0.001
C22:6 n-3 (DHA) <sup>1</sup>	0.12	0.53	0.074	0.001
unknown	5.06	6.06	0.288	0.566

$\sum SFA^1$	70.16	61.70	1.63	0.012
$\sum MUFA^1$	22.83	26.42	1.19	0.065
$\sum PUFA^1$	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
$\sum PUFA^{1}/\sum SFA^{1}$	0.09	0.15	0.012	0.005
MUFA/SFA	0.33	0.43	0.027	0.032
Desaturation-CLA index <sup>2</sup>	43.75	50.40	3.860	0.032
Atherogenic Index <sup>3</sup>	2.55	1.83	0.176	0.033
Thrombogenic index <sup>4</sup>	2.83	1.62	0.230	0.002

<sup>1</sup>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.

<sup>2</sup>Desaturation-CLA index:  $100 \times [(cis-9, trans-11 C18:2 CLA)/(trans-11C18:1+cis-9, trans-11 C18:2 CLA)]$ 

<sup>3</sup>Atherogenic index: (C12:0 + 4\*C14:0 + C16:0)/(MUFA + PUFA n-6 + PUFA n-3)

<sup>4</sup>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)

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	1417	1470	<u> </u>	0.000
muscle	141/	14/8	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 <i>cis-9</i>	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 <i>cis-11</i>	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 <i>cis-9</i> , <i>cis-12</i> (LA) <sup>1</sup>	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
$C20:5 \text{ n-3} (EPA)^1$	1.00	1.41	0.120	0.081
C22:1	0.19	0.22	0.025	0.662
C22:5 n-3 (DPA) <sup>1</sup>	1.20	1.58	0.187	0.330
C22:6 n-3 (DHA) <sup>1</sup>	0.60	0.81	0.082	0.223
unknown	4.34	3.93	0.365	0.156
$\sum SFA^1$	45.61	38.34	1.46	0.005
$\sum MUFA^1$	31.62	42.35	1.92	0.001

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

<sup>1</sup>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.

<sup>2</sup>Desaturation-CLA index:  $100 \times [(cis-9, trans-11 C18:2 CLA)/(trans-11C18:1+cis-9, trans-11 C18:2 CLA)]$ 

<sup>3</sup>Atherogenic index: (C12:0 + 4\*C14:0 + C16:0)/(MUFA + PUFA n-6 + PUFA n-3)

<sup>4</sup>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)