Fatty acid metabolism in lambs fed citrus pulp[1]

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ABSTRACT: In the present study, we have hypothesized that replacing barley with high proportions of dried citrus pulp in a concentrate-based diet for lambs could increase the intake of unsaturated fatty acids and could reduce the rate of the ruminal biohydrogenation of PUFA, with a consequent improvement of the intramuscular fatty acid composition. To test this hypothesis, 26 Comisana lambs were divided into 3 groups and for 56 d were fed a barley-based concentrate diet (CON; 8 lambs) or 2 diets in which barley was replaced with 24% (CIT24; 9 lambs) or 35% (CIT35; 9 lambs) dried citrus pulp. An overall improvement of the fatty acid composition of LM from lambs fed citrus pulp–containing diets was found. The PUFA/SFA ratio was lower ($P < 0.05$) in the LM from lambs in the CON group compared with both the CIT24 and CIT35 groups. The thrombogenic index was lower ($P < 0.05$) in meat from lambs fed the CIT35 diet compared with those fed the CON diet. The CIT35 diet increased the proportion of C20:5 n-3 in the LM ($P < 0.05$), whereas the CIT24 diet enhanced that of C22:6 *n*-3 ($P < 0.05$) compared with the CON diet. Some of these results might be explained considering that feeding the CIT24 and CIT35 diets increased the intake of total fatty acids ($P < 0.05$) and of C18:3 n-3 ($P < 0.01$) compared with feeding the CON treatment. On the other hand, phenolic compounds present in citrus pulp could have inhibited the ruminal biohydrogenation of PUFA. This is supported by the fact that regardless of the level of inclusion in the diet, citrus pulp increased the proportion of rumenic acid ($P < 0.001$) in LM compared with the CON diet. The plasma from lambs fed both CIT24 and CIT35 diets had a greater percentage of vaccenic acid (VA; $P < 0.001$) compared with that from lambs fed the CON diet, and the CIT35 diet increased the proportion of rumenic acid in plasma compared with the CON treatment ($P < 0.05$). In the ruminal fluid, stearic acid (SA) tended to decrease, and the sum of CLA tended to increase ($P = 0.09$) with increasing level of citrus pulp in the diets. Furthermore, the SA/(SA + VA) ratio tended to be lower ($P = 0.10$) in the ruminal fluid from lambs fed the CIT35 diet compared with that of the CON group. In conclusion, our results support the hypothesis that replacing barley with citrus pulp in the diet of growing lambs improves intramuscular fatty acid composition and underline the need for specific studies to clarify the mechanisms by which feeding citrus pulp affects the fatty acid metabolism in ruminants.

Key words: citrus pulp, fatty acids, lamb, meat quality, metabolism

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INTRODUCTION

Agroindustrial by-products represent potential low-cost feedstuffs that could replace conventional ingredients in ruminant diets (Vasta et al., 2008). Among these, citrus pulp is available in several areas, including the Americas and the Mediterranean. Citrus pulp is rich in readily degradable carbohydrates and can replace up to 30% of cereals in the diet of lambs with no adverse effects on animal growth, whereas

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productivity is reduced with greater levels of inclusion (45% or higher; Martínez-Pascual and Fernández-Carmona, 1980). Studies conducted in the last 20 yr have confirmed these findings; however, most of these studies focused on the effects of citrus pulp on animal growth and on nutritional aspects (Bampidis and Robinson, 2006), and little is known on the effects of citrus pulp on meat quality.

Citrus pulp is rich of bioactive compounds, such as unsaturated fatty acids (**UFA**), vitamins, and phenolic compounds (Balasundram et al., 2006; Ladaniya, 2008). Therefore, high levels of citrus pulp in concentrate-based diets for lambs would increase the intake of these compounds compared with conventional ingredients, with effects on meat quality. We found that 24% and 35% dried citrus pulp in a concentrate-based diet for lambs increased meat oxidative stability (Gravador et al., 2014; Inserra et al., 2014). Here we hypothesize that such levels of dried citrus pulp in the diet of lambs could increase the concentration of desirable PUFA in meat. This effect might be attributed to both a greater intake of UFA and an effect of phenolic compounds on the ruminal fatty acid metabolism (Raes et al., 2004; Vasta and Luciano, 2011). No experiments have been conducted so far to test this hypothesis; therefore, fatty acid content in ruminal fluid, plasma, and muscle was evaluated in the same lambs used by Inserra et al. (2014) and Gravador et al. (2014), which were fed a conventional concentrate-based diet or diets containing 24% or 35% dried citrus pulp.

MATERIALS AND METHODS

Experimental Design, Animals, and Diets

The trial was performed at a sheep farm located in an internal area of Sicily (Italy). The experimental protocol was approved by the University of Catania, and animals were handled by specialized personnel following European Union (2010) guidelines (EU Directive 2010/63). Twenty-six male Comisana lambs, born within 10 d, were naturally reared under their dams on pasture. From 50 d of age, lambs were fed concentrate feeds (25% broad bean, 25% whole wheat, 25% wheat bran, and 25% barley, on an as fed basis). At 90 d of age, animals were weighed (average initial BW 19.76 ± 3.84 [SD] kg) and individually penned indoor. Animals were randomly assigned to 3 dietary treatments and for 10 d were adapted to the experimental diets. Subsequently, for 56 d of the experimental period, lambs were fed a barley-based concentrate (**CON**; 8 animals), a concentrate including 24% as-fed dried citrus pulp in partial replacement of barley (**CIT24**; 9 animals), or a concentrate in which barley was further replaced by the inclusion of 35% dried citrus pulp (**CIT35**; 9 animals). The goal of this experiment was to replace as much barley as possible with the locally available citrus pulp. Therefore, on the basis of the available literature (Bampidis and Robinson, 2006) we have chosen 2 levels (24% and 35% on an as-fed basis) of inclusion of citrus pulp that would not impair animal productivity. We have previously analyzed each single ingredient and formulated the diets to be isonitrogenous and isoenergetic. The ingredients and chemical composition of the experimental diets are reported in Table 1.

All the ingredients were finely ground (5-mm screen) and mixed to avoid selection. Each day, the diets were supplied at 0900 h, and feed was continuously available in the feeders until 1800 h, after which feeders were removed from the individual boxes. For each animal, the amount of feed administered and refused was recorded daily to calculate DMI. Water was continuously available. Samples of the feedstuffs offered and refused were collected 4 times over the trial, vacuum packed, and subsequently stored at −30°C until analyses. Lamb weight was recorded at the beginning of the experiment and weekly until the end of the trial to calculate ADG. The weight was measured at 0800 h before supplying fresh feed.

After 54 d of experimental feeding, individual blood samples (approximately 8 mL) were collected from the jugular vein at 0800 h, before feeding. The tubes were kept refrigerated at 4°C during transport to the laboratory, where blood samples were centrifuged for 10 min at 2,500 × *g* at 4°C and the plasma was collected and stored at −80°C.

Slaughter Procedure and Sampling

At the end of the experiment (158 d of age) lambs were transported to a commercial abattoir, where they had access to the experimental feeds and water until 15 min before slaughtering. Animals were stunned by captive bolt, and following the procedure described by Vasta et al. (2009b), the ruminal fluid was collected from each animal immediately after slaughter, mixed, and accurately filtered through a cheesecloth layer. Ruminal fluid pH was measured by a pH meter (Orion 9106, Orion Research Inc., Boston, MA). Individual aliquots of ruminal fluid were stored at –80°C for fatty acid analyses.

Carcasses were halved, and the LM was excised from the left side within 20 min after slaughter, immediately vacuum packed, and stored at −30°C for fatty acid analyses.

Table 1. Ingredients and chemical composition of concentrate mixtures

	Experimental diet[1]		
Item	CON	CIT24	CIT35
Ingredient, % as fed			
Barley	60	35	23
Soybean meal	9	12	13
Dehydrated alfalfa	20	19	20
Wheat bran	11	10	9
Dried citrus pulp	—	24	35
Chemical composition			
DM, % as fed	88.9	89.3	90.6
CP, % DM	18.0	18.5	17.8
NDF, % DM	34.6	31.8	33.1
Ash, % DM	4.5	6.1	7.7
Total phenolic compounds[2]	4.0	6.7	7.9
Fat, % DM	1.5	1.8	2.3
ME, MJ/kg	10.4	10.5	10.5
Total fatty acids, mg/100 g DM	1,089	1,351	1,733
Individual fatty acids, % total fatty acids			
C12:0	0.6	0.2	0.2
C14:0	0.3	0.7	0.8
C16:0	22.2	22.5	23.2
C16:1	0.6	0.6	0.6
C18:0	1.3	3.1	2.0
cis-9 C18:1	14.6	18.2	17.9
C18:2 n-6	44.2	40.3	41.0
C18:3 n-3	13.8	12.6	12.5

[1]CON: control barley-based concentrate diet. CIT24 and CIT35: diets including 24% and 35% (as-fed) dried citrus pulp in partial replacement of barley, respectively.

[2]Expressed as grams tannic acid equivalents/kilogram DM.

Feedstuffs Analyses

Crude protein and ash of the experimental diets were determined according to AOAC procedures (AOAC, 1995) on pooled samples. The NDF was analyzed according to Van Soest et al. (1991). Fat was extracted by concentrates according to the procedure of Folch et al. (1957), and fatty acids were determined according to Gray et al. (1967). Total phenolic compounds (**PC**) were assessed according to Makkar et al. (1993) by an extraction from feedstuffs using aqueous acetone (70% vol/vol) and a susbsequent analysis by Folin-Ciocalteu reagent. The PC were expressed as grams of tannic acid equivalents per kilogram of DM. The ME was calculated using ASSIST.T software, version 1.3.1, developed by Centro Richerche Produzioni Animali (CRPA spa), Italy (http://www.crpa.it/assist)

Fatty Acid Analyses

Ruminal fluid (10 mL) was subjected to direct methylation using the 1-step procedure of Park et al. (2001), as modified by Buccioni et al. (2011). Fatty acid methyl esters (**FAME**) were extracted using *n*-hexane with C9:0 and C23:0 methyl ester (Sigma Chemical Co., St. Louis, MO) as internal standards and were maintained in vials with hermetic closure to avoid the loss of volatile components. The FAME were separated and identified by gas chromatography on a gas chromatograph (**GC**) equipped with a capillary column (CP-select CB for FAME, Varian, Middelburg, The Netherlands: length, 100 m; i.d., 0.25 mm; film thickness, 0.20 μm), according to Buccioni et al. (2015). A standard mix (47792, Supelco, Chemical Co., St. Louis, MO) and published isomeric profiles were used to identify the α-linolenic acid (**ALA**) isomers. Two bacterial acid methyl ester mixes (47080-U, Supelco, Chemical Co.; GLC110, Matreya, Pleasant Gap, PA) and individual standard for methyl esters of *iso* 14:0, *ante* 14:0, *iso* 15:0 and *ante* 17:0 (21-1211-11, 21-1210-11, 21-1312-11, and 21-1415-11, Larodan, Malmo, Sweden) were used to identify branched fatty acids.

Plasma fatty acids were methylated according to Kramer et al. (1997), as modified by Vasta et al. (2009b), using C9:0 and C23:0 methyl esters as internal standards. The FAME were extracted with hexane and were analyzed using a Varian (model Star 3400 CX) GC instrument equipped with a CP 88 capillary column (length, 100 m; i.d., 0.25 mm; film thickness, 0.25 μm) following the operative conditions detailed by Vasta et al. (2009b). The individual fatty acid peaks were identified by comparing retention times with those of known mixtures of standard fatty acids (37-component FAME mix, 18919-1 ampule, Supelco, Bellefonte, PA).

The intramuscular fat was extracted from LM samples according to the procedure of Folch et al. (1957). Duplicate samples of intramuscular lipids were methylated using methanolic KOH as described by Scerra et al. (2011), and C9:0 and C23:0 methyl esters were added as internal standards. Following the operating conditions detailed by Scerra et al. (2011), gas chromatographic analysis was performed on a Varian model Star 3400 CX instrument equipped with the same column described above for plasma fatty acid analysis. The individual fatty acid peaks were identified by comparing retention times with those of known mixtures of standard fatty acids (37-component FAME mix, 18919-1 AMP, Supelco). An index of the desaturation of *trans*-11 C18:1 (vaccenic acid, **VA**) to *cis*-9, *trans*-11 C18:2 CLA (rumenic acid, **RA**) was calculated as follows: 100 × [(RA)/(VA + RA)], according to Aldai et al. (2006). The

atherogenic index and the thrombogenic index were calculated according to Ulbricht and Southgate (1991) to evaluate the risk of atherosclerosis and the potential aggregation of blood platelets, respectively.

For all the analyses, inter- and intra-assay CV, detection limits, and correction factors were calculated by using a reference standard butter (CRM 164, Community Bureau of Reference, Brussels, Belgium; Contarini et al., 2013). Intra-assay CV ranged from 0.5% to 1.5%, whereas interassay CV ranged from 1.5% to 2.5%. The fatty acids were expressed as grams per 100 g of total fatty acids.

Statistical Analysis

All data were analyzed using a general linear model (**GLM**) procedure in which the dietary treatment was considered the fixed factor. The individual lamb was considered the experimental unit. When the overall effect of the dietary treatment in the GLM was found to be significant, multiple comparisons between means were performed using Tukey's adjustment. Data were reported as least squares means and SEM. Significance was declared at $P \leq 0.05$, whereas trends toward significance were considered when $0.05 < P \leq 0.10$. The statistical analysis was performed using Minitab, version 16 (Minitab Inc., State College, PA).

RESULTS

Growth Performance, Dry Matter, and Fatty Acids Intake

The dietary treatment did not affect the growth performance parameters of lambs, with comparable final BW and ADG being found between the 3 groups of animals (Table 2). No difference between treatments was found for DMI or for NDF intake, whereas the dietary treatment affected the fatty acid intake (Table 2). Specifically, the intake of total fatty acids was greater in the CIT35 ($P < 0.001$) and CIT24 ($P < 0.05$) groups compared with the CON group, whereas no difference was found between the CIT24 and CIT35 groups. Regarding the intake of individual fatty acids, the intake of linoleic acid (**LA**) for the CIT35 group tended to be greater ($P = 0.09$) than that for the CIT24 group and was greater ($P < 0.001$) than that for the CON group. The daily intake of ALA for lambs in the CIT35 group was greater ($P < 0.001$) and that for lambs in the CIT24 tended to be greater ($P = 0.10$) than that for the CON group, whereas no difference was found between the CIT24 and CIT35 groups. Finally, the daily intake of stearic acid (**SA**) was lower ($P < 0.001$) in the CON group than in both the CIT24 and CIT35 groups, with no difference being found between the latter.

Table 2. Effects of concentrates including dried citrus pulp on lamb performance and intakes

	Dietary treatment[1]				
Item	CON	CIT24	CIT35	SEM	*P*-value[2]
No. of animals	8	9	9		
Final weight, kg	29.6	29.6	28.4	1.040	0.876
ADG, g/d	175	178	179	0.009	0.985
DMI, g/d	666	790	756	0.033	0.317
NDF intake, g/d	230	251	250	0.011	0.693
Total FA intake, g/d	7.25[b]	10.67[a]	13.10[a]	0.695	<0.001
LA intake,[3] g/d	3.21[b]	4.30[a,b]	5.37[a]	0.274	0.001
ALA intake,[3] g/d	1.00[b]	1.34[a,b]	1.64[a]	0.083	0.002
SA intake,[3] g/d	0.09[b]	0.33[a]	0.27[a]	0.022	<0.001

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

[1]CON: control barley-based concentrate diet. CIT24 and CIT35: diets including 24% and 35% (as-fed) dried citrus pulp in partial replacement of barley, respectively.

[2]*P*-value of the effect of the dietary treatment tested using the general linear model.

[3]LA: linoleic acid (C18:2 n-6); ALA: α-linolenic acid (C18:3 n-3); SA: stearic acid (C18:0).

Fatty Acid Composition of Ruminal Fluid

Table 3 reports the pH and fatty acid profile of ruminal fluid. The dietary treatment did not affect ruminal fluid pH, with an average value of 6.56. The proportion of SA tended to be affected by the dietary treatment ($P = 0.09$), with the greatest numerical value being found in the ruminal fluid from lambs fed the CON diet compared with those fed the citrus pulp–containing diets. The percentage of RA was not affected by the dietary treatment. The proportion of *trans*-10, *cis*-12 C18:2 CLA increased ($P < 0.05$) in the ruminal fluid from lambs in the CIT24 group compared with the CON group, whereas values found for the CIT35 treatment were comparable to values for both CIT24 and CON. Overall, the sum of the identified CLA tended to be affected by the treatment ($P = 0.09$), with the lowest numerical value being found in the ruminal fluid of lambs fed the CON diet compared with those fed the CIT24 and CIT35 treatments. The percentage of VA was not affected by the dietary treatment; however, the SA/(SA + VA) ratio in the ruminal fluid from animals given the CIT35 diet was lower ($P < 0.05$) than in those fed the CIT24 group and tended to be lower ($P = 0.10$) than that of the CON group, with no difference being found between the latter (Table 3).

Table 3. Effects of concentrates including dried citrus pulp on ruminal fluid pH and fatty acids (g/100 g fatty acids)

	Dietary treatment[1]				
Item	CON	CIT24	CIT35	SEM	*P*-value[2]
No. of animals	8	9	9		
pH	6.57	6.55	6.56	0.045	0.987
Σ*iso* BCFA[3]	1.97	2.08	3.95	0.699	0.429
Σ*anteiso* BCFA[4]	1.62	1.61	1.36	0.094	0.445
C13:0	0.34	0.35	0.47	0.055	0.515
C14:0	0.93[a]	0.64[a,b]	0.53[b]	0.064	0.027
C14:0 *iso*	0.31[b]	0.57[a]	0.58[a]	0.043	0.013
C15:0	0.81	0.85	1.26	0.149	0.388
C15:0 *iso*	0.26	0.29	0.23	0.022	0.527
C15:0 *ante*	1.13[a,b]	1.29[a]	0.94[b]	0.059	0.032
C16:0	16.16	12.88	14.83	0.807	0.276
C16:0 *iso*	0.58	0.45	2.25	0.672	0.474
cis-9 C16:1	0.18	0.13	0.19	0.020	0.410
C17:0	0.51	0.55	0.43	0.028	0.178
C17:0 *iso*	0.81	0.77	0.88	0.073	0.828
C17:0 *ante*	0.48	0.31	0.42	0.056	0.486
C18:0 SA[5]	33.08	30.89	24.46	1.690	0.086
trans-5 C18:1	0.07	0.02	0.12	0.025	0.309
trans-6 to *trans*-8 C18:1	0.64	0.39	0.60	0.066	0.275
trans-9 C18:1	0.30	0.19	0.67	0.142	0.343
trans-10 C18:1	6.75	5.09	5.41	0.817	0.715
trans-11 C18:1 VA[5]	2.06	1.50	3.97	0.512	0.102
trans-12 + *cis*-7 C18:1	1.38	1.40	1.27	0.071	0.724
Σ*trans* C18:1[6]	10.32	7.66	11.27	0.987	0.303
cis-9 C18:1	3.03	2.24	3.36	0.299	0.290
cis-11 C18:1	0.49	0.55	0.53	0.022	0.549
cis-12 C18:1	0.62[a]	0.38[a,b]	0.28[b]	0.050	0.014
cis-15 C18:1	0.32	0.33	0.41	0.027	0.348
cis-9, *trans*-11 C18:2 CLA	0.558	1.07	1.29	0.168	0.205
trans-10, *cis*-12 C18:2 CLA	0.22[b]	0.49[a]	0.35[a,b]	0.034	0.003
ΣCLA	0.78	1.56	1.64	0.175	0.092
C18:2 n-6 LA[5]	2.57	1.86	1.43	0.289	0.290
trans-11, *cis*-15 C18:2	0.13	0.10	0.11	0.028	0.921
C18:3 n-3 ALA[5]	0.29	0.20	0.22	0.047	0.713
ΣSFA	56.13	50.51	47.92	2.090	0.283
ΣMUFA	16.01	12.32	16.89	1.170	0.240
ΣPUFA	4.14	4.16	3.90	0.371	0.950
SA/(SA + VA)[7]	0.94[a,b]	0.95[a]	0.86[b]	0.018	0.039

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

[1]CON: control barley-based concentrate diet. CIT24 and CIT35: diets including 24% and 35% (as-fed) dried citrus pulp in partial replacement of barley, respectively.

[2]*P*-value of the effect of the dietary treatment tested using the general linear model.

[3]Sum of *iso* branched chain fatty acids (BCFA): *iso* C14:0, *iso* C15:0, *iso* C16:0, *iso* C17:0.

[4]Sum of *anteiso* BCFA: *anteiso* C15:0, *anteiso* C17:0.

[5]SA: stearic acid; VA: vaccenic acid; LA: linoleic acid; ALA: α-linolenic acid.

[6]Σ*trans* C18:1 = sum of *trans* 18:1 fatty acids, calculated as the sum of *trans*-5 C18:1, *trans*-6 to *trans*-8 C18:1, *trans*-9 C18:1, *trans*-10 C18:1, *trans*-11 C18:1, *trans*-12 + *cis* 7 C18:1.

[7]SA: stearic acid (C18:0); VA: vaccenic acid (*trans*-11 C18:1).

Table 4. Effects of concentrates including citrus pulp on plasma fatty acid composition (g/100 g fatty acids)

	Dietary treatment[1]				
Item	CON	CIT24	CIT35	SEM	*P*-value[2]
No of animals	8	9	9		
C14:0	0.71[a]	0.57[a,b]	0.50[b]	0.030	0.012
C15:0	0.49	0.45	0.38	0.024	0.150
C16:0	16.11[a]	11.05[b]	11.81[b]	0.466	<0.001
cis-9 C16:1	0.58[b]	0.67[b]	1.05[a]	0.049	<0.001
C18:0 SA[3]	23.20[a]	18.65[b]	18.60[b]	0.462	<0.001
trans-11 C18:1 VA[3]	1.64[b]	3.07[a]	3.25[a]	0.176	<0.001
cis-9 C18:1	19.63[a]	17.52[a,b]	16.06[b]	0.432	0.002
cis-9, *trans*-11 C18:2 CLA	0.37[b]	0.42[a,b]	0.51[a]	0.021	0.019
C18:2 n-6 LA[3]	14.76[b]	24.12[a]	24.31[a]	0.885	<0.001
C18:3 n-3 ALA[3]	0.602[b]	1.99[a]	2.44[a]	0.173	<0.001
C20:4 n-6	2.99	3.71	3.06	0.141	0.067
C20:5 n-3 EPA[3]	0.80[b]	1.36[a]	0.43[c]	0.088	<0.001
C22:5 n-3 DPA[3]	1.04[b]	0.96[b]	1.81[a]	0.106	<0.001
C22:6 n-3 DHA[3]	1.60	1.64	1.68	0.041	0.762
ΣSFA	40.51[a]	30.73[b]	31.29[b]	0.899	<0.001
ΣMUFA	21.84	21.26	20.35	0.351	0.230
ΣPUFA	22.17[b]	34.20[a]	34.24[a]	1.120	<0.001
PUFA/SFA	0.55[b]	1.12[a]	1.10[a]	0.053	<0.001
Σ n-6	17.74[b]	27.83[a]	28.38[a]	0.931	<0.001
Σ n-3	4.05[b]	5.95[a]	6.36[a]	0.233	<0.001
n-6/n-3	4.40	4.75	4.36	0.121	0.365

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

[1]CON: control barley-based concentrate diet. CIT24 and CIT35: diets including 24% and 35% (as-fed) dried citrus pulp in partial replacement of barley, respectively.

[2]*P*-value of the effect of the dietary treatment tested using the general linear model.

[3]SA: stearic acid; VA: vaccenic acid; LA: linoleic acid; ALA: α-linolenic acid; EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid: DHA: docosahexaenoic acid

Fatty Acid Composition of Blood Plasma

As shown in Table 4, the proportions of palmitic acid (C16:0) and SA were lower ($P < 0.001$) in the plasma from animals given the diets containing citrus pulp compared with animals fed the CON diet, whereas no difference was found between the CIT24 and CIT35 treatments. Both the CIT24 and CIT35 diets increased the percentage of VA ($P < 0.001$) compared with the CON diet, and only the highest level of inclusion of citrus pulp (CIT35) increased the proportion of RA in plasma compared with the CON treatment ($P < 0.05$). No difference between the CIT24 and CIT35 treatments was found for the plasma content of both VA and RA. The percentage of oleic acid (*cis*-9 C18:1) was reduced ($P < 0.001$) by feeding lambs the CIT35 diet compared with the CON diet, whereas the proportion of oleic acid in plasma from lambs fed the CIT24 diet was not different ($P > 0.05$) from that of lambs

fed the CON and CIT35 treatments. Both ALA and LA percentages were found in lower proportions in plasma from animals fed the CON diet compared with animals fed the CIT24 and CIT35 diets, whereas no difference was found between the latter.

Regarding the long-chain PUFA, the CIT24 diet increased ($P < 0.001$) the plasma level of C20:5 *n*-3 (eicosapentaenoic acid, **EPA**) compared with CON and CIT35 diets. The concentration of EPA was lower in plasma from lambs fed the CIT35 diet compared with lambs fed the CON treatment ($P < 0.01$). The CIT35 diet increased the proportion of C22:5 *n*-3 (docosapentaenoic acid) in plasma compared with the CIT24 and CON treatments ($P < 0.001$), whereas no difference was found between the latter. No effect of the dietary treatment was found for the proportion of C22:6 *n*-3 (docosahexaenoic acid, **DHA**).

Lambs in both the CIT24 and CIT35 groups had similar percentages of total SFA in blood plasma, which were both lower than that found in plasma of lambs fed the CON diet ($P < 0.001$). The dietary treatment did not affect the proportion of total MUFA. Finally, the proportion of PUFA was lower ($P < 0.001$) in plasma from animals in the CON group compared with animals in the CIT24 and CIT35 groups, which, in turn, did not differ.

Fatty Acid Composition of Intramuscular Fat

The intramuscular fatty acid composition is reported in Table 5. The concentration of total intramuscular fat was not affected by dietary treatment. The CIT35 diet reduced the proportion of SFA ($P < 0.01$) compared with the CON diet, whereas feeding the CIT24 diet did not affect SFA compared with the CIT35 and CON diets. The proportion of total PUFA tended to be affected by the dietary treatment ($P = 0.06$), with the lowest numerical value found in the intramuscular fat from lambs fed the CON diet. The PUFA/SFA ratio was lower ($P < 0.05$) in the intramuscular fat from lambs in the CON group compared with lambs in the CIT24 and CIT35 groups, which, in turn, did not differ (Table 5). The proportion of MUFA was higher in the intramuscular fat from lambs fed the greatest proportion of citrus pulp (CIT35) compared with lambs fed the CIT24 treatment ($P < 0.05$), whereas the values found in the LM from the CON-fed lambs were comparable to those from both the CIT24 and the CIT35 treatments.

Regarding the individual fatty acids, for the class of MUFA, the proportion of *cis*-9 C14:1 was lower in muscle from lambs in the CIT24 group compared with those in the CIT35 group ($P < 0.05$), whereas its proportion in the LM from CON-fed lambs was comparable to that from lambs in both the CIT24 and CIT35 treatments. The LM from lambs in both the CIT24 and

Table 5. Effects of concentrates including dried citrus pulp on LM fatty acid composition (g/100 g fatty acids)

	Dietary treatment[1]				
Item	CON	CIT24	CIT35	SEM	*P*-value[2]
No. of animals	8	9	9		
Total intramuscular fat, g/100 g muscle	2.77	2.38	2.73	0.162	0.580
C10:0	0.39	0.44	0.37	0.024	0.463
C12:0	0.78	0.63	0.57	0.040	0.066
C14:0	4.60	4.12	3.92	0.194	0.366
cis-9 C14:1	0.12^{a,b}	0.09^{b}	0.15^{a}	0.010	0.048
C15:0	0.65	0.56	0.52	0.036	0.350
C15:1	0.20	0.16	0.18	0.017	0.624
C16:0	16.43	15.79	16.00	0.234	0.564
cis-9 C16:1	1.08	1.06	1.06	0.036	0.972
C17:0	1.04	1.01	0.94	0.029	0.377
cis-9 C17:1	0.39^{b}	0.65^{a}	0.69^{a}	0.045	0.011
C18:0 SA[3]	14.34	13.94	13.56	0.149	0.101
trans-11 C18:1 VA[3]	1.29	1.39	1.56	0.077	0.371
cis-9 C18:1	28.43	27.56	28.86	0.248	0.081
cis-9, *trans*-11 C18:2 CLA	0.71^{b}	1.08^{a}	1.02^{a}	0.039	<0.001
trans-9, *trans*-12 C18:2 n-6	0.58	0.56	0.48	0.025	0.216
C18:2 n-6 LA[3]	9.20	10.24	10.85	0.294	0.068
C18:3 n-6	0.27^{a,b}	0.34^{a}	0.14^{b}	0.030	0.012
C18:3 n-3 ALA[3]	1.00	1.21	0.99	0.059	0.236
C20:2 n-6	0.21^{a}	0.09^{b}	0.11^{b}	0.017	0.005
C20:3 n-6	0.47^{a}	0.19^{b}	0.20^{b}	0.031	<0.001
C20:3 n-3	0.13^{b}	0.15^{b}	0.23^{a}	0.016	0.005
C20:4 n-6	8.71	8.82	8.59	0.247	0.930
C20:5 n-3 EPA[3]	0.69^{b}	0.98^{ab}	1.06^{a}	0.056	0.012
C22:5 n-3 DPA[3]	1.95	1.83	1.89	0.106	0.909
C22:6 n-3 DHA[3]	0.73^{b}	1.39^{a}	1.29^{a,b}	0.105	0.021
ΣSFA	38.23^{a}	36.51^{a,b}	35.89^{b}	0.345	0.012
ΣMUFA	31.52^{a,b}	30.92^{b}	32.49^{a}	0.276	0.048
ΣPUFA	24.66	26.90	26.88	0.435	0.060
PUFA/SFA	0.65^{b}	0.74^{a}	0.75^{a}	0.017	0.027
Σ n-6	19.45	20.25	20.38	0.370	0.554
Σ n-3	4.50	5.57	5.48	0.207	0.077
n-6/n-3	4.38	3.85	3.81	0.160	0.320
Desaturation-CLA index[4]	35.53^{b}	45.43^{a}	40.20^{a,b}	1.620	0.046
Atherogenic index[5]	0.64	0.58	0.55	0.018	0.100
Thrombogenic index[6]	0.91^{a}	0.80^{a,b}	0.78^{b}	0.016	0.022

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

[1]CON: control barley-based concentrate diet. CIT24 and CIT35: diets including 24% and 35% (as-fed) dried citrus pulp in partial replacement of barley, respectively.

[2]*P*-value of the effect of the dietary treatment tested using the general linear model.

[3]SA: stearic acid; VA: vaccenic acid; LA: linoleic acid; ALA: α-linolenic acid; EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid: DHA: docosahexaenoic acid.

[4]Desaturation-CLA index: 100 × [(*cis*-9, *trans*-11 C18:2 CLA)/(*trans*-11 C18:1+ *cis*-9, *trans*-11 C18:2 CLA)] (Aldai et al., 2006).

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

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

CIT35 groups had a similar proportion of *cis*-9 C17:1, which was greater than that found in the LM from animals in the CON group ($P < 0.01$). No difference between treatments was found for the proportions of oleic acid (*cis*-9 C18:1) and VA. Regardless of the level of inclusion in the diet, citrus pulp increased ($P < 0.001$) the proportion of RA in LM compared with the CON diet, whereas no difference was found between the CIT24 and the CIT35 treatments. The dietary treatment affected the desaturation-CLA index, with a greater value ($P < 0.05$) found in muscle from lambs fed the CIT24 diet compared with that from lambs fed CON, whereas values found in the LM from CIT35-fed lambs were comparable to those of both the CON and CIT24 treatments (Table 5).

The percentage of LA tended to be affected by the dietary treatment ($P = 0.07$), with the lowest numerical values found in the LM from animals fed the CON diet compared with those fed the citrus pulp–containing diets. The proportions of C20:2 *n*-6 and C20:3 *n*-6 were greater in LM from lambs in the CON group compared with lambs in the CIT24 and CIT35 treatments ($P < 0.01$), with no difference being found between the latter. No effect of dietary treatment was found on the proportion of C20:4 n-6. For the n-3 fatty acids, no difference among groups was found for ALA percentage, whereas the long-chain n-3 PUFA were affected by the dietary treatment. Specifically, the intramuscular fat from lambs fed the CIT35 diet had a greater proportion of C20:3 n-3 compared with lambs fed the CON and CIT24 treatments ($P < 0.01$), whereas no difference between the latter was found. Moreover, the CIT35 diet led to a greater level of C20:5 n-3 (EPA) in the LM compared with the CON diet ($P < 0.05$), whereas the EPA proportion in the LM from lambs fed the CIT24 diet was comparable to that of both the CON and CIT35 treatments. The level of C22:6 n-3 (DHA) was greater in the intramuscular fat from lambs fed the CIT24 diet compared with lambs fed the CON diet ($P < 0.05$), whereas values found in the LM from lambs fed the CIT35 diet were comparable to those of both the CON and the CIT24 treatments. Overall, the proportion of total n-3 fatty acids in meat tended to be affected by the dietary treatment ($P = 0.08$), with the lowest numerical value found in the LM from lambs given the CON diet.

There was a tendency for atherogenic index to be affected by the dietary treatment ($P = 0.10$), with the greatest numerical value found in the LM from the animals fed the CON diet. Finally, the thrombogenic index was reduced ($P < 0.05$) by feeding lambs the CIT35 diet compared with the CON treatment, whereas the value found in the LM from the CIT24-fed lambs was comparable to that of both the CON and the CIT35 treatments.

DISCUSSION

Meat from ruminants is well recognized as rich in saturated fatty acids because of the biohydrogenation (**BH**) of PUFA occurring in the rumen (Harfoot and Hazlewood, 1988). Therefore, feeding strategies have been focused on enhancing the levels of desirable PUFA in meat from ruminants because of their favorable effects on human health (Bessa et al., 2007). The objective of this experiment was to verify if replacing barley with 24% and 35% citrus pulp in diets for growing lambs could affect fatty acid metabolism, thus improving intramuscular fatty acid composition. The reason why we have formulated this hypothesis is related to the greater content of unsaturated fatty acids and phenolic compounds of citrus pulp compared with barley. In agreement with this expectation, the most important finding of this experiment was the overall improvement of the meat fatty acid profile consequent to the replacement of barley with citrus pulp in the diet. In particular, the diet including the greatest level of citrus pulp (35%, as fed) made it possible to reduce the intramuscular concentration of SFA, whereas both CIT24 and CIT35 diets increased the PUFA/SFA ratio. Among the beneficial fatty acids, feeding lambs the citrus-containing diets increased the proportion of RA in muscle. Furthermore, compared with the CON diet, the CIT35 diet increased the proportion of EPA in the LM, and the CIT24 diet increased the deposition of DHA in the intramuscular fat. Moreover, including 35% citrus pulp in the diet decreased the thrombogenic index in the LM. These results suggest that including citrus pulp in the lamb diet modified the metabolism of fatty acids in the animals, which increased deposition of PUFA in the intramuscular fat compared with that in the CON diet.

On the one hand, in ruminants, differences in the proportions of the individual PUFA in muscle depend on differences in the intake of PUFA, whereby a high intake of PUFA with the diet results in a greater proportion of PUFA that escape saturation during the ruminal BH (Raes et al., 2004). For example, LA and ALA are essential fatty acids derived from the diet. In the present study, although the proportion of LA and ALA in the ruminal fluid was not statistically affected by the dietary treatment, their proportion was greater in the plasma from animals in the CIT24 and CIT35 treatments compared with those in the CON treatment, which is in agreement with the greater intake of these fatty acids for animals fed the citrus-supplemented diets. Furthermore differences in the intake of essential PUFA between treatments could partially explain the differences observed in the deposition of long-chain n-3 PUFA in the intramuscular fat. The greater propor-

tion of EPA and DHA in the muscle of the lambs fed the CIT35 and CIT24 diets, respectively, could be partially explained by the greater intake of ALA of these animals (Raes et al., 2004). Despite the greater intake of LA by feeding citrus pulp, the proportion of its long-chain n-6 derivative, arachidonic acid (**AA**), in plasma and muscle was not affected by the dietary treatment. Furthermore, the concentrations of other long-chain n-6 PUFA (C20:2 n-6 and C20:3 n-6) were lower in the intramuscular fat of lambs fed the citrus pulp–containing diets. These results could be explained considering that n-3 PUFA display a greater affinity than n-6 PUFA for the enzymes undertaking the synthesis of long-chain PUFA, which results in a greater rate of conversion of ALA to EPA/DHA compared with that of LA to AA (Kinsella et al., 1990; Arterburn et al., 2006).

On the other hand, possible effects of the diet on the ruminal BH of fatty acids could contribute to the deposition of PUFA in intramuscular fat. Some of our results could lead to the conclusion that feeding citrus-containing diets exerted an effect of the ruminal BH of PUFA. The first indication is given by the effect of the greatest level (35%) of dietary citrus pulp in reducing the SA/(SA + VA) ratio in the ruminal fluid, which suggests that the last step of LA and ALA BH (VA to SA) was impaired to a certain extent by feeding citrus pulp. In agreement with this observation, the proportion of SA and the sum of CLA in the ruminal fluid tended to be affected by the dietary treatment, with the greatest and lowest values being found, respectively, in the CON-fed lambs. Although the fatty acid profile of blood plasma might be rather different from that of the ruminal fluid, the analysis of plasma fatty acid profile could give insight into the effect of the dietary treatment on the fatty acid metabolism (Bauchart, 1993). We observed that the proportion of VA increased in plasma from lambs fed the citrus-supplemented diets compared with animals in the CON group and that the RA proportion was greater in plasma from lambs fed the CIT35 diet compared with those fed the CON treatment. Considering that the presence and concentration of these fatty acids in plasma partially depend on their formation in the rumen, these results further support the speculation that dietary citrus pulp, particularly the greatest level (35%), could have exerted an inhibitory effect on the ruminal BH. Other results found in plasma could lead to the hypothesis of an effect of the dietary treatment on the ruminal BH of PUFA. For instance, lambs fed the citrus-containing diets had a lower proportion of SA and a higher proportion of VA in plasma compared with those in the CON treatment.

A possible explanation for the observed effects could be related to the presence of plant secondary compounds in the dried citrus pulp. We found that diets containing citrus pulp had greater concentrations of total extractable phenolic compounds. The occurrence of phenolic substances in citrus fruit constituents (including pulp and peels) has been extensively documented, with most of these compounds being identified as flavonoids and phenolic acids (Abeysinghe et al., 2007; Tripoli et al., 2007). In the literature there is no evidence for the effect of dietary citrus pulp on the metabolism of fatty acids in ruminants; however, it has been demonstrated that plant secondary compounds, such as phenolic compounds, could impair the ruminal BH of PUFA, with a consequential increase of PUFA in muscle (Vasta and Luciano, 2011). Most of these studies have focused on tannins, suggesting that these phenolic substances could reduce the rate of PUFA BH via modification of the bacterial population in the rumen and the inhibition of BH steps, such as the conversion of VA to SA (Priolo et al., 2005; Durmic et al., 2008; Vasta et al., 2009a,b, 2010). Certainly, comparisons between studies on the biological effects of dietary plant secondary compounds should be made with caution, as the proportion of the different classes of these compounds can greatly vary between different sources. However, some of our results could lead to the speculation that secondary compounds in citrus pulp, such as phenolics, might have exerted an effect on fatty acid metabolism in lambs.

In ruminants, the deposition of most of the fatty acids in the intramuscular fat depends not only on the direct transfer from the feeds or on their formation in the rumen but also on the postabsorption metabolism and endogenous synthesis. The occurrence of RA in muscle, for example, is linked both to its direct formation in the rumen during BH and also to its synthesis from VA operated in muscle by the Δ9-desaturase enzyme (Bauman et al., 2000**;** Aldai et al., 2006). We found that the proportion of RA was greater in muscle from lambs fed the citrus-containing diets compared with those fed the CON treatment and that the desaturation-CLA index was greater in muscle from lambs fed the CIT24 diet compared with those fed CON. This finding might lead one to suppose that feeding the diet including 24% citrus pulp could have increased the rate of RA synthesis from VA in the muscle through the action of the enzyme Δ9-desaturase. This speculation could be supported by the higher content of phenolic compounds in citrus pulp compared with barley, as it has been demonstrated that some phenolic compounds, such as tannins, can increase the expression of the Δ9-desaturase enzyme (Vasta et al., 2009c). However, the results found for the concentration of *cis*-9 C14:1 are partially in contrast to the previous finding. Indeed, the *cis*-9 C14:1 is exclusively synthesized in muscle by the action of the Δ9-desaturase (Palmquist

et al., 2004), and we found that the concentration of this fatty acid in muscle from lambs fed the CIT35 diet was greater than that of the CIT24 group, whereas it did not differ from that of the CON group. We cannot propose a plausible explanation for this result, as the activity and expression of the Δ9-desaturase enzyme were not measured. Therefore, further research is needed to better clarify the possible effects of dietary citrus pulp on the endogenous synthesis of fatty acids in muscle. In this context, it would also be of interest to study a possible effect of citrus pulp on the synthesis and deposition in muscle of the very long chain n-3 fatty acids, such as EPA and DHA. Indeed, it has been demonstrated that the dietary administration of flavonoids to rats increased the synthesis of these fatty acids, although the exact mechanism was not elucidated (Toufektsian et al., 2011). Considering that flavonoids represent the main class of phenolic compounds in citrus fruits (Tripoli et al., 2007), it could be speculated that in the present study, a greater intake of flavonoids by the animals fed citrus pulp compared with those fed the CON diet could have contributed to the higher deposition of long-chain n-3 PUFA in the muscle.

In conclusion, our results support the hypothesis that replacing barley with high levels of citrus pulp in the diet of growing lambs increases the content of desirable fatty acids in muscle. These results could be linked to a higher intake of both unsaturated fatty acids and phenolic compounds by the lambs fed the citrus pulp–containing diets. Specific studies are needed to better clarify which specific bioactive components of citrus pulp could affect fatty acid metabolism in ruminants.

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