New insights on the metabolism of ricinoleic acid in ruminants

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ABSTRACT

Dairy goats were fed a total mixed ration with or without the inclusion of castor oil [40 g/kg of dry matter (DM)] to study the metabolism of ricinoleic acid (12-OH, *cis*-9–18:1). Ten goats, at 39.7 ± 4.0 d in milk, were individually penned and allocated at random to the 2 experimental diets. Goats were manually milked twice a day. Milk fatty acids (FA) were analyzed as methyl esters and hydroxyl groups were derivatized in trimethylsilyl ethers. Apart from ricinoleic acid, 6 FA were only detected in the milk of the castor oil group. Ricinoleic acid composed 0.3% of total FA in milk of the castor oil group, whereas the hydroxy-FA (8-OH-14:0, 10-OH-16:0, and 12-OH-18:0) and oxo-FA (8-oxo-14:0, 10-oxo-16:0, and 12-oxo-18:0) reached 7.5% of total FA in milk. We anticipate that these FA were derived from the metabolism of ricinoleic acid, although it was not clear if they were produced in the rumen or in the tissues. To confirm that, we conducted in vitro batch incubations repeated for 3 consecutive weeks with castor oil (40 g/kg of DM) and strained rumen fluid from 2 fistulated sheep. To examine the products formed over time, incubation tubes were stopped at 0, 6, 12, 24, 48, and 72 h. The results of the in vitro experiment showed that ricinoleic acid was metabolized in the rumen at a slow rate and the main products formed were 12-OH-18:0 and 12-oxo-18:0, by hydrogenation of the *cis*-9 double bond, followed by oxidation of the hydroxyl group, respectively. Our results suggest that the 12-OH-18:0 and 12-oxo-18:0 escape rumen and are further metabolized through partial β -oxidation in ruminant tissues. We propose that the 10-OH-16:0 and 8-OH-14:0 found in goat milk of the castor oil group are successive products of the β -oxidation of 12-OH-18:0, and the 10-oxo-16:0 and 8-oxo-14:0 are successive prod-

ucts of the 12-oxo-18:0 in tissues. Overall, our results indicate that ricinoleic acid is extensively metabolized in the rumen and tissues, producing mainly oxo- and hydroxy-FA that are further excreted in milk.

Key words: castor oil, ricinoleic acid, milk, rumen, fatty acid

INTRODUCTION

Castor oil plant (*Ricinus communis* L.) is one of the most important industrial crops in developing countries mostly due to its wide range of industrial uses. The castor oil, which is obtained by pressing the castor seeds, has unique characteristics including a high oxidative stability promoting longer shelf-life compared with other vegetable oils. Castor oil contains about 80 to 94% of ricinoleic acid (Binder et al., 1962), a hydroxy-unsaturated fatty acid (12-OH, *cis*-9–18:1), which gives the oil its distinct features. Ricinoleic acid is known to have antimicrobial properties (Novak et al., 1961); thus, it has been studied as a possible modulator of ruminal fermentation to be used as an alternative to ionophore additives to improve growth, feed intake, and efficiency. Indeed, inclusion of castor oil in diet of dairy cows (Gandra et al., 2014; de Jesus et al., 2016), bulls (Cruz et al., 2014; Silva et al., 2014), steers (Gandra et al., 2012), sheep (Maia et al., 2012a), or goat kids (Maia et al., 2012b) did not impair nutrient intake, digestibility, or growth performance.

Studies on the effects of dietary castor oil on the lipid composition of ruminant derived products are scarce. Prado et al. (2016) and Valero et al. (2014) reported that inclusion of a blend of castor oil and cashew nutshell liquid (*Anacardium occidentale* L.) in the diet of feedlot finishing bulls greatly affected the muscle fatty acid (FA) composition. However, in both studies the FA profile was limited and none of these studies identified the ricinoleic acid or other hydroxy-FA derivatives in muscle. Only Maia et al. (2012b) identified low quantities of ricinoleic acid in muscle of goat kids fed diets

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supplemented with 3% of castor oil. More scarce are the studies on the effects of castor oil on milk FA composition. It was reported that feeding 2 g/d of ricinoleic acid to dairy cows increased milk yield and milk fat content (Gandra et al., 2014); in contrast, Pereira et al. (2010) found that inclusion of 3% of castor oil in goats diet reduced milk fat content and density compared with the control. However, neither study reported the milk FA composition. Only an older study found trace amounts of hydroxy-FA in milk fat of dairy cows fed a diet containing 0.5% of castor meal and castor oil (Robb et al., 1974), and in another study, only the ricinoleic acid was reported in cheese of goats fed diets containing 4% castor oil (Medeiros et al., 2014).

Because castor oil contains about 80 to 90% of ricinoleic acid, we expect that it is metabolized in the rumen and tissues, and thus a large number of hydroxy-FA derivatives, together with minor amounts of ricinoleic acid, will be found in milk of ruminants fed castor oil. However, the metabolism of ricinoleic acid in the rumen or tissues has not been largely studied. In vitro experiments with rumen fluid and rumen bacteria were conducted to study the influence of ricinoleic acid on the metabolism of linoleic acid (Wallace et al., 2007; Ramos Morales et al., 2012). These studies were focused on the hypothesis that ricinoleic acid was an intermediate of CLA synthesis in the rumen, but no relevant attention was made to the hydroxy or other FA intermediates formed from ricinoleic acid. Hence, this work aims to study the effects of the inclusion of 40 g of castor oil/kg of DM in the diet of dairy goats on the milk FA composition with particular emphasis on the potential FA intermediates derived from ricinoleic acid. In addition, we also aim to explore the possibility that the FA metabolites found in milk could result from the metabolism of ricinoleic acid in the rumen. To investigate this hypothesis, we incubated castor oil with ruminal digesta in vitro for 6 to 72 h.

MATERIALS AND METHODS

Animal Experiment and Diets

Animal experiment was performed at the Field Station for Small Ruminant Research of the Federal University of Paraíba (João Pessoa, Brazil). Ten Saanen × Alpine goats, at 39.7 ± 4.0 DIM and weighing 46.8 ± 6.1 kg, were used. Goats were individually penned and allocated at random to 2 experimental diets: nonsupplemented (control) and supplemented with castor oil (40 g/kg of DM). Feed was supplied as a TMR twice a day in excess (20%) to ensure ad libitum intake and goats had continuous access to drinking water. Experimental diets (Table 1) were formulated according NRC (2007)

to meet the nutritional requirements of goats producing 2 kg of milk daily with 3% fat. The experiment lasted 7 d with an adaptation period of 15 d. Goats were manually milked twice a day. Before milking, the udder was cleaned and the strip-cup test was performed to detect mastitis. Post dipping was performed after milking using a commercial iodine solution. A composed milk sample per goat, representative of the last 2 d of the trial was prepared using proportional amounts of all milking samples. A composed sample per animal was collected using proportional amounts from morning and afternoon milking. Milk yield and DMI averaged 1.5 ± 0.32 kg/d and 1.5 ± 0.17 kg/d, respectively.

In Vitro Incubations

In vitro batch incubations were conducted at Faculty of Veterinary Medicine, University of Lisbon (Portugal). Ruminal fluid was collected from 2 fistulated sheep before the morning meal and immediately transferred to the laboratory in a thermostatic box at 39°C. Rumen fluid was immediately strained through 4 layers of cheesecloth and diluted (1:4, vol/vol) in the medium of Goering and Van Soest (1970) under CO₂ flux. The rumen buffered solution (6 mL) was distributed into Hungate tubes containing about 60 mg of a commercial TMR and 2.5 mg of castor oil (FJCampos, Pontinha, Portugal). The TMR contained dehydrated alfalfa (700 g/kg), wheat grain (105 g/kg), soybean meal (110 g/kg), and minerals and premix (25 g/kg). The chemical composition of the TMR was 902 g/kg of DM, 175 g of CP/kg of DM, 113 g of starch/kg of DM, 81 g of ether extract/kg of DM, and 213 g of crude fiber/kg of DM. For batch incubations, Hungate tubes were filled with CO₂ and closed with a butyl rubber stopper and screw cap, then tubes were incubated in a water bath (Unitronic, J.P. Selecta, Barcelona, Spain) at 39°C with gentle agitation for 0, 6, 12, 24, 48, and 72 h. After incubation, tubes were directly frozen and stored at −20°C. The allocation of tubes to the treatments, incubation time, order of filling with buffered ruminal fluid, and position in the water bath were randomized. The incubation procedure was replicated 3 times in 3 consecutive weeks with 2 tubes per treatment and time. Samples were freeze-dried (ScanVac CoolSafe, LaboGene ApS, Lyngby, Denmark) and stored at −20°C until analysis.

Fatty Acid and Dimethyl Acetal Analysis

Fatty acid methyl esters were prepared from freeze-dried milk fat samples by direct transesterification using potassium hydroxide (2 M) in methanol according to Rego et al. (2009). Fatty acid methyl esters and

dimethyl acetals (**DMA**) from the in vitro batch incubations were prepared by a direct transesterification procedure using sodium methoxide (0.5 M) in methanol followed by the addition of hydrogen chloride (1.25 M) in methanol according to Alves et al. (2013). The methyl nonadecanoate (1 mg/mL) was added as an internal standard. The hydroxyl groups of the FAME were derivatized in trimethylsilyl (**TMS**) ethers by reaction of the FAME extract with 10-fold excess by weight of N,O-Bis(trimethylsilyl)acetamide as silylating reagent in 1 mL of acetonitrile (Christie and Han, 2012). The mixture was left to react for about 30 min at room temperature and afterward it was dried in a stream of nitrogen. Samples were clean up by adding 4 mL of hexane and 1 mL of water. The hexane layer was dried over anhydrous sodium sulfate, decanted, and evaporated in a stream of nitrogen. Fatty acid methyl esters before and after silylation were analyzed by GLC with flame ionization detection using a Shimadzu GC 2010-Plus (Shimadzu, Kyoto, Japan) equipped with a SP-2560 (100 m × 0.25 mm, 0.20 µm film thickness, Supelco, Bellefonte, PA) capillary column. The chromatographic conditions were as follows: injector and detector temperatures were set at 250 and 280°C, respectively; helium was used as the carrier gas at 1 mL/min constant flow; the initial oven temperature of 50°C was held for 1 min, increased at 50°C/min to

150°C and held for 20 min, increased at 1°C/min to 190°C, and then increased at 2°C/min to 220°C and held for 40 min. Identification of FAME and DMA, as well as the position of the hydroxyl group, was achieved by analysis of the TMS derivatives by electron impact mass spectrometry using a Shimadzu GC-MS QP2010 Plus (Shimadzu) and according to published chromatograms (Alves et al., 2013). The chromatographic column and the GLC conditions were similar to the GC-flame ionization detector (**FID**) analysis. Additional mass spectrometer conditions were as follows: ion source temperature, 200°C; interface temperature, 240°C; and emission voltage, 70 eV. Fatty acids in milk fat samples were expressed as a percentage of total FA in the GC-FID chromatogram. Amounts of FA and DMA in incubation tubes were expressed as milligrams per flask and determined using the internal standard, assuming direct proportionality between GC-FID peak area and FA weight. Partial GC-MS chromatograms of the hydroxy-FAME and oxo-FAME regions are presented in Figures 1 and 2, respectively.

Calculations and Statistical Analysis

The milk fat melting point was estimated by computing the molar percentages of the milk FA and subsequently calculated the sum of the melting points

Table 1. Composition of the experimental diets and fatty acid composition of castor oil

Item	Diet		Castor oil (in vivo)	Castor oil (in vitro)
	Control	Castor oil		
Ingredient (g/kg of DM)				
Corn meal	304	259		
Soybean meal	165	170		
Castor oil	0	40		
Vitamin/mineral supplement ¹	15	15		
Elephant grass hay	516	516		
Chemical composition (g/kg of DM)				
DM	874	878		
CP	139	137		
Ether extract	26	63		
NDF	508	497		
Fatty acid (g/100 g of total fatty acids)				
16:0	24.0	23.6	1.7	1.7
18:0	4.9	4.8	1.3	1.7
<i>cis</i> -9–18:1	14.6	13.6	4.3	4.2
<i>cis</i> -11–18:1	0.9	0.9	0.6	0.7
18:2n-6	37.3	35.4	7.2	6.4
20:0	2.7	2.7	0.1	0.1
20:1	—	0.04	0.9	0.6
18:3n-3	10.8	10.8	0.4	0.3
22:0	1.9	1.9	—	—
23:0	0.7	0.7	—	—
24:0	2.3	2.3	—	—
12-OH, <i>cis</i> -9–18:1 (ricinoleic acid)	—	3.4	83.6	84.4

¹Composition per kilogram of DM: 180 g of Ca, 71 g of P, 28 g of K, 20 g of S, 20 g of Mg, 400 mg of Cu, 30 mg of Co, 10 mg of Cr, 250 mg of P, 150 mg of I, 1,350 mg of Mn, 15 mg of Se, 1,700 mg of Zn, 710 mg of Fe, 135,000 IU of vitamin A, 68,000 IU of vitamin D₃, and 450 mg of vitamin E.

Figure 1. Partial GC-MS chromatogram from the region between 55 and 77 min. (A) FAME + trimethylsilyl (TMS) derivatives from a milk fat sample of the control group; (B) FAME + TMS derivatives from a milk fat sample of the castor oil group; (C) FAME + TMS derivatives from 72-h incubations of castor oil with strained rumen fluid. *c* = *cis*; *t* = *trans*. I.S. = internal standard.

weighted by the respective FA molar percentages as described in Toral et al. (2013).

The proportionate disappearance of the biohydrogenation substrates (**BS**), 12-OH,*cis*-9-18:1, 18:2n-6, and 18:3n-3, during 6, 12, 24, 48, and 72 h of incubation was calculated as follows:

$$\text{disappearance (\%)} = [(BS_0 - BS_t)/BS_0] \times 100,$$

where BS is 12-OH,*cis*-9-18:1, 18:2n-6, or 18:3n-3 expressed in milligrams per milligram of total C18 FA in tubes at time 0 (BS_0) and time *t* (BS_t ; *t* = 6, 12, 24, 48, or 72 h).

Milk FA composition was analyzed using the MIXED procedure of SAS 9.0 (SAS Institute Inc., Cary, NC) with a model that included the treatment (control vs. castor oil) as the single fixed effect. When needed, the group option of the repeated statement was included in the model to accommodate the variance heterogeneity. In vitro data were analyzed using the MIXED procedure of SAS considering the weekly runs as random block and the incubation time as a categorical fixed effect. The linear, quadratic, and cubic effects of incubation time were evaluated using the respective polynomial orthogonal contrasts after computing the contrast coefficients with the Proc IML of SAS. Least squares means

Figure 2. Partial GC-MS chromatogram from the region between 80 and 120 min. (A) FAME + trimethylsilyl (TMS) derivatives of a milk fat sample from the control group; (B) FAME + TMS derivatives of a milk fat sample from the castor oil group; (C) FAME + TMS derivatives from 72-h incubations of castor oil with strained rumen fluid.

and standard error of the mean are reported, and treatment or contrast effects were considered significant at $P < 0.05$ and trends toward significance at $P = 0.05$ to 0.10.

RESULTS

Fatty Acid Composition of Milk

Milk FA composition of goats fed a control diet or with the inclusion of castor oil is presented in Table 2. As expected, we detected the ricinoleic acid (12-OH, *cis*-9-18:1) in milk of the castor oil group, reaching about 0.3% of total FA. We also detected and identified in milk fat of the castor oil group several

hydroxy-FA (i.e., 8-OH-14:0, 10-OH-16:0, and 12-OH-18:0) and oxo-FA (i.e., 8-oxo-14:0, 10-oxo-16:0, and 12-oxo-18:0), which were not detected in the control group. These hydroxy- and oxo-FA composed about 8% of total FA in the castor oil group. Additionally, the 10-OH-18:0 and 10-oxo-18:0 were identified in both groups; however, both proportions were higher ($P = 0.012$ and $P = 0.035$, respectively) in milk fat of the castor oil group compared with the control.

Nevertheless, the most abundant FA in milk were the *cis*-9-18:1, 16:0, and 18:0, which proportions averaged about 26, 21, and 18% of total FA, respectively. Both *cis*-9-18:1 and 18:0 proportions tended ($P = 0.058$ and $P = 0.064$, respectively) to decrease with the incorporation of castor oil in diet, whereas 16:0 was not af-

fects ($P = 0.607$). Moreover, castor oil incorporation decreased ($P < 0.05$) the proportions of 4:0, *cis*-9-14:1, *trans*-5-18:1, *trans*-16-18:1, and 18:2n-6 in milk. On the contrary, the proportions of 15:0, *cis*-7-16:1, *cis*-11-18:1, 20:1 were higher ($P < 0.05$) in milk of the castor oil group compared with control; however, these FA only represented 1.6% of total FA in milk. Regarding the partial sums, the proportions of total SFA was lower ($P = 0.009$) and *cis*-MUFA tended ($P = 0.075$) to be lower in the castor oil group compared with the control. In addition, we observed a difference ($P = 0.003$) for the estimated milk melting points, being 38.7°C for the control group and 41.4°C for the castor oil group.

In Vitro Batch Incubations

Incubation of castor oil with strained ruminal fluid for 6 to 72 h resulted in extensive metabolism of ricinoleic acid (Figure 3A), showing a linear and quadratic decrease ($P < 0.05$) from 1.7 mg at 0 h to 0.2 mg at 72 h (Table 3). Figure 3A also shows the main intermediates formed at the end of 72 h of incubation. The 12-oxo-18:0 linear and quadratic increased ($P < 0.05$) with time, reaching 1.4 mg per tube at 72 h, and the 12-OH-18:0 showed linear, quadratic, and cubic responses ($P < 0.05$) to incubation time (Table 3), reaching 0.4 mg per tube at 72 h. Figure 3B shows the kinetic curves of the other oxo- and hydroxy-FA that were also produced during 72 h of incubation with castor oil. We verified linear, quadratic, and cubic responses ($P < 0.05$) for the 10-OH-18:0, 13-OH-18:0, and 13-OH-18:1, quadratic and cubic responses for 12-OH-18:1, and both 13-OH-18:1 and 10-oxo-18:0 showed linear and quadratic responses ($P < 0.05$) with incubation time (Table 3).

The 18:2n-6 was also metabolized during in vitro incubation with castor oil (Figure 3C), decreasing from 800 µg at 0 h to 60 µg per tube at 72 h, under a kinetic curve with a linear, quadratic, and cubic response (Table 3). Figure 3C shows that only minor amounts of *cis*-9,*trans*-11-18:2 were detected in incubation tubes; however, it linearly and quadratically decreased from 11.2 µg at 0 h to 4.6 µg at 72 h of incubation. On the contrary, at the first 6 h a large amount of *trans*-18:1 isomers, particular *trans*-11, were formed (Figure 3C). Following this pattern, all the *trans*-18:1 isomers showed linear, quadratic, and cubic ($P < 0.05$) responses with time. Regarding the *cis*-9-18:1 and 18:3n-3, which are also known substrates for ruminal biohydrogenation, both decreased with incubation time (LQC contrasts, $P < 0.05$), and on the contrary the production of 18:0 increased (LQC contrasts, $P < 0.05$) reaching 1.9 mg at 72 h.

Figure 3D shows that incubation of castor oil with strained ruminal fluid for 72 h increased the amount of total FA and DMA in tubes (total FA+DMA). Compared with the branched-chain fatty acids (BCFA), the amount of DMA showed a rapid increase in the first 12 h of incubation followed by a slower decrease, whereas the BCFA showed a moderate increase (Q contrast, $P < 0.05$) during the 72 h of incubation. The majority of DMA followed the same pattern, presenting the highest amount at 12 h of incubation (Table 4), but the DMA iso-14:0, 14:0, 16:0, *trans*-11-18:1, and *cis*-9-18:1 showed linear, quadratic, and cubic responses; the DMA iso-15:0 showed linear and quadratic response; and the DMA 15:0 showed only linear response to incubation time. Regarding the effect of incubation time on the BCFA composition (Table 4), the iso-14:0, iso-17:0, and anteiso-17:0 linear and quadratic responses increased with incubation time, whereas iso-15:0, anteiso-15:0, and iso-16:0 linear, quadratic, and cubic responses increased with time. Table 4 also presents the effect of incubation time on other minor SFA during in vitro batch incubation with castor oil.

DISCUSSION

Several studies reported that the incorporation of castor oil in diet of ruminants (Gandra et al., 2012, 2014; Maia et al., 2012a,b; Cruz et al., 2014) did not affect nutrient intake, digestibility, or growth performance. However, the effect of the incorporation of castor oil in the FA composition of milk, particularly the specific FA resultant from the metabolism of ricinoleic acid, has not been studied. We hypothesized that ricinoleic acid, which in our experiment composed 84% of total FA in castor oil, is intensively metabolized in the rumen and tissues and that a large number of hydroxy-FA metabolites will be found in milk fat. Thus, we conducted a detailed FA analysis of milk fat from goats fed a TMR with or without incorporation of 40 g of castor oil/kg of DM, with particular emphasis on hydroxy-FA and its derivatives.

Our approach was to prepare FAME from milk fat samples and run on a cyanopropyl siloxane GLC capillary column currently recommended for the analysis of FAME from complex mixtures. This column allows the separation of most of the *trans*-18:1 isomers as well as the comparison with the FAME profile of the majority of the studies regarding ruminant milk fat. However, because the highly polar characteristics of this column did not allow detecting hydroxy-FAME, we derivatized the hydroxyl groups in TMS ether derivatives, which are for long being used for the analysis of hydroxy-containing compounds (Eglinton et al., 1968). Thus,

Table 2. Effect of castor oil on milk fatty acid composition (g/100 g of total fatty acids) and between milk fat melting point of goats

Item	Treatment ¹		SEM	P-value
	Control	Castor oil		
4:0	1.71	1.40	0.074	0.018
6:0	1.77	1.55	0.122	0.241
7:0	0.02	0.03	0.005	0.073
8:0	1.90	1.73	0.182	0.533
9:0	0.04	0.06	0.008	0.052
10:0	5.75	5.43	0.603	0.716
11:0	0.15	0.15	0.023	0.952
12:0	2.17	2.08	0.181	0.728
iso-13:0	0.02	0.02	0.003	0.667
13:0	0.09	0.08	0.011	0.717
iso-14:0	0.11	0.11	0.009	1.000
14:0	6.72	6.34	0.209	0.236
<i>cis</i> -9-14:1	0.06	0.04	0.005	0.033
iso-15:0	0.22	0.28	0.020	0.080
anteiso-15:0	0.38	0.41	0.016	0.165
15:0	0.68	0.80	0.033	0.029
iso-16:0	0.27	0.25	0.028	0.700
16:0	20.9	20.6	0.45	0.607
<i>trans</i> -9-16:1	0.06	0.06	0.012	0.825
<i>cis</i> -7-16:1	0.27	0.38	0.017	0.002
<i>cis</i> -9-16:1	0.36	0.38	0.021	0.449
iso-17:0	0.36	0.41	0.024	0.171
anteiso-17:0	0.35	0.34	0.009	0.295
17:0	0.42	0.46	0.018	0.153
<i>cis</i> -9-17:1	0.13	0.14	0.012	0.648
iso-18:0	0.05	0.04	0.008	0.735
18:0	19.3	16.0	1.09	0.064
<i>trans</i> -4-18:1	0.02	0.02	0.003	0.347
<i>trans</i> -5-18:1	0.02	0.01	0.002	0.035
<i>trans</i> -6/7/8 18:1	0.36	0.35	0.043	0.950
<i>trans</i> -9-18:1	0.31	0.35	0.036	0.410
<i>trans</i> -10-18:1	0.49	0.43	0.060	0.499
<i>trans</i> -11-18:1	1.18	1.64	0.205	0.151
<i>trans</i> -12-18:1	0.45	0.44	0.046	0.858
<i>trans</i> -15-18:1	0.20	0.16	0.018	0.169
<i>trans</i> -16-18:1 ²	0.31	0.24	0.019	0.027
<i>cis</i> -9-18:1	27.6	24.3	1.08	0.058
<i>cis</i> -11-18:1	0.27	0.39	0.022	0.004
<i>cis</i> -12-18:1	0.25	0.25	0.043	0.925
<i>cis</i> -13-18:1	0.01	0.02	0.004	0.182
<i>cis</i> -15-18:1	0.02	0.01	0.005	0.332
18:2n-6	2.02	1.59	0.092	0.011
Other-18:2	0.35	0.30	0.031	0.249
<i>cis</i> -9, <i>trans</i> -11-18:2 ³	0.60	0.79	0.093	0.195
18:3n-6	0.02	0.02	0.002	0.545
18:3n-3	0.08	0.08	0.004	0.740
20:0	0.37	0.37	0.014	0.769
20:1	0.05	0.07	0.002	<0.001
Δ 5,8,11-20:3	0.05	0.05	0.007	1.000
20:3n-6	0.02	0.01	0.004	0.105
20:4n-6	0.15	0.17	0.009	0.265
21:0	0.03	0.03	0.002	0.094
22:0	0.07	0.06	0.006	0.296
23:0	0.01	0.01	0.003	1.000
24:0	0.02	0.04	0.012	0.265
10-oxo-18:0	0.17	0.30	0.037	0.035
10-OH-18:0	0.08	0.15	0.015	0.012
8-oxo-14:0	ND ⁴	0.24	0.018	—
10-oxo-16:0	ND	0.84	0.046	—
12-oxo-18:0	ND	4.93	0.116	—
8-OH-14:0	ND	0.12	0.004	—
10-OH-16:0	ND	0.22	0.010	—
12-OH-18:0	ND	1.11	0.069	—
12-OH, <i>cis</i> -9-18:1	ND	0.26	0.020	—

Continued

Table 2 (Continued). Effect of castor oil on milk fatty acid composition (g/100 g of total fatty acids) and between milk fat melting point of goats

Item	Treatment ¹		SEM	P-value
	Control	Castor oil		
Other fatty acids	0.12	0.11	0.011	0.799
Partial sum				
SCFA ⁵	13.5	12.4	1.10	0.515
BCFA ⁶	1.75	1.86	0.070	0.326
SFA ⁷	48.6	44.8	0.80	0.009
<i>cis</i> -MUFA	29.1	26.0	1.08	0.075
<i>trans</i> -MUFA	3.40	3.71	0.418	0.621
PUFA	3.29	3.00	0.171	0.272
Total OH and oxo	0.25	8.18	0.084	<0.001
Melting point ⁸	38.7	41.4	0.45	0.003

¹Treatments: control = TMR; castor oil = TMR plus 40 g of castor oil/kg of DM.

²Contains *cis*-14–18:1 as minor component.

³Contains *trans*-7,*cis*-9–18:2 and *trans*-8,*cis*-10–18:2 as minor components.

⁴ND = not detected.

⁵Short-chain fatty acids, includes ≤12-carbon fatty acids.

⁶Branched-chain fatty acids, includes all iso- and anteiso-fatty acids.

⁷Includes linear SFA of ≥13-carbon chain length.

⁸Total fatty acid melting point (°C).

this approach permitted not only the detection of hydroxy-FAME in the cyanopropyl siloxane capillary column, but also the identification of the position of the hydroxyl group by analysis of their mass spectra (Supplemental Figure S1; <https://doi.org/10.3168/jds>

.2017-13117). Other approaches have been reported for the analysis of hydroxy-FA in milk and dairy products. Marquez-Ruiz et al. (2011) described a method where oxygenated FAME from milk fat are analyzed after fractionation by solid-phase extraction silica cartridges fol-

Figure 3. Influence of in vitro batch incubations of castor oil with strained rumen fluid during 0 to 72 h, on (A) ricinoleic acid (12-OH,*cis*-9–18:1) and the major oxo- and hydroxy-fatty acids (FA); (B) minor oxo- and hydroxy-FA; (C) 18:2n-6 and biohydrogenation intermediates, *trans*-18:1, *trans*-11–18:1, and *cis*-9,*trans*-11–18:2; (D) total FA + dimethyl acetals (DMA), total branched-chain fatty acids (BCFA), and total DMA.

Table 3. Effect of incubation time in C18 fatty acid composition ($\mu\text{g}/\text{tube}$) on 72-h in vitro batch incubations with castor oil

Item	Incubation time (h)						SEM	Contrast ¹
	0	6	12	24	48	72		
18:0	783	1,184	1,359	1,654	1,862	1,928	73.2	LQC
<i>trans</i> -6/7/8-18:1	4.12	18.7	18.8	19.7	18.2	17.2	0.861	LQC
<i>trans</i> -9-18:1	4.42	14.3	14.5	14.4	13.1	12.0	0.670	LQC
<i>trans</i> -10-18:1	7.21	21.6	22.9	25.9	26.8	24.8	4.450	LQC
<i>trans</i> -11-18:1	54.9	241	254	261	232	200	8.56	LQC
<i>trans</i> -12-18:1	4.33	14.6	15.4	16.5	14.9	13.8	0.459	LQC
<i>trans</i> -13/14-18:1	9.00	27.0	28.1	29.7	27.5	27.7	0.717	LQC
<i>trans</i> -15-18:1	3.73	11.2	11.4	12.7	11.3	10.7	0.442	LQC
<i>trans</i> -16-18:1 ²	5.44	11.8	12.6	14.4	13.4	14.0	0.464	LQC
<i>cis</i> -9-18:1	584	409	337	252	133	94.2	21.00	LQC
<i>cis</i> -11-18:1	54.3	48.8	43.3	32.0	23.3	18.0	3.38	LQ
<i>cis</i> -12-18:1	3.99	9.54	9.74	9.23	6.86	5.82	1.187	QC
<i>cis</i> -13-18:1	0.63	0.86	0.84	0.80	0.71	0.68	0.157	—
18:2n-6	808	386	267	157	73.3	55.7	37.56	LQC
<i>cis</i> -9, <i>trans</i> -11-18:2	11.2	6.43	8.56	3.12	3.48	4.61	2.209	LQ
Other 18:2	5.36	12.7	12.7	9.95	9.56	7.70	0.731	QC
18:3n-3	63.1	27.7	19.2	10.5	6.29	5.67	3.850	LQC
10-OH-18:0	3.57	20.9	25.3	28.8	32.9	30.0	1.856	LQC
12-OH-18:0	0.00	77.0	161	288	326	361	28.04	LQC
13-OH-18:0	0.00	1.61	2.34	2.62	2.85	2.59	0.203	LQC
12-OH- <i>trans</i> -18:1	3.84	22.5	23.1	22.2	18.9	12.7	2.143	QC
13-OH-18:1	0.00	2.12	2.84	3.94	4.43	4.61	0.560	LQ
12-OH, <i>cis</i> -9-18:1	1,661	1,545	1,196	674	456	233	120.1	LQ
10-oxo-18:0	2.63	13.6	22.3	41.1	55.7	62.8	3.094	LQ
12-oxo-18:0	0.37	154	361	799	1,161	1,365	82.31	LQ
Partial sum								
<i>trans</i> -18:1	92.2	360	377	394	357	320	10.46	LQC
Total OH	11.3	12.4	11.1	8.47	7.46	5.79	0.892	L
Total oxo	0.02	1.26	3.03	7.03	10.8	12.9	0.791	LQ

¹Indicates significant ($P < 0.05$) linear (L), quadratic (Q), or cubic (C) components of the response to incubation time.

²Contains *cis*-14-18:1 as minor component.

lowed by GLC using a medium polar capillary column. In other approach, lipids from milk fat samples were isolated by accelerated solvent extraction, then transesterified to obtain FAME, subsequently separated by solid-phase chromatography to obtain hydroxy-FAME, further converted into pentafluorobenzoyl derivatives, and analyzed by GLC with electron-capture negative ion mass spectrometry (Jenske and Vetter, 2008). These methods are more laborious and time consuming compared with our approach. Also, our method has the advantage that both hydroxy and non-hydroxy FA (e.g., the complex mixture of C18 FA) can be all separated and quantified in the same GLC chromatogram as illustrated in Figure 1.

Following this approach, we detected and identified 7 FA, together with ricinoleic acid, that were not detected in milk of the control group. These FA contained either a hydroxyl or an oxo (keto) group and composed about 7.7% of total FA in milk fat. Nevertheless, the ricinoleic acid was only found in minor amounts (0.26% of total FA), which suggests that it was extensively metabolized in the rumen or tissues. This is consistent with the

earlier studies of Robb et al. (1974) that identified trace amounts of hydroxy-FA in milk of dairy cows fed a diet containing 0.5% of castor meal and castor oil.

In addition, we identified the 10-OH-18:0 and 10-oxo-18:0 in milk fat of both control and castor oil groups. These FA were already detected in ruminant milk (Halmemies-Beauchet-Filleau et al., 2011; Marquez-Ruiz et al., 2011) and were reported to derive from microbial hydration of *cis*-9-18:1 in the rumen to produce 10-OH-18:0, followed by oxidation to produce the 10-oxo-18:0 (Jenkins et al., 2006; McKain et al., 2010). Thus, it was not surprising that we also detected both FA in milk of both groups.

To the best of our knowledge, this is the first time that the 8-oxo-14:0, 8-OH-14:0, and 10-OH-16:0 are identified in milk fat. The 10-oxo-16:0 was previously detected in minor quantities in milk of dairy cows fed red-clover-based diets (Halmemies-Beauchet-Filleau et al., 2011). These authors suggested that the 10-oxo-16:0 could be formed from hydration and oxidation of *cis*-9-16:1 in the rumen. Nevertheless, because we did not detect the 10-oxo-16:0 in milk of the control group, we

Table 4. Effect of incubation time on dimethyl acetal (DMA) and fatty acid (FA) composition ($\mu\text{g}/\text{tube}$), excluding C18 fatty acids, on 72-h in vitro batch incubations with castor oil

Item	Incubation time (h)						SEM	Contrast ¹
	0	6	12	24	48	72		
DMA								
iso-14:0	7.06	7.59	8.09	8.02	4.24	2.67	0.296	LQC
14:0	12.3	17.0	17.6	17.1	10.5	7.34	0.795	LQC
iso-15:0	7.37	7.70	8.07	7.53	5.17	5.10	1.241	LC
anteiso-15:0	18.9	28.7	45.5	43.1	55.9	50.3	21.99	—
15:0	3.76	4.14	3.79	3.76	2.56	1.88	0.740	L
16:0	19.4	29.1	30.0	29.5	20.7	15.2	2.12	LQC
18:0	9.21	2.96	3.63	4.85	3.54	2.09	2.62	—
<i>trans</i> -11–18:1	2.87	5.55	5.73	5.60	3.76	3.14	0.217	LQC
<i>cis</i> -9–18:1	2.51	4.43	4.99	4.76	3.21	2.18	0.304	LQC
FA								
12:0	95.0	68.5	65.0	58.3	56.2	57.3	3.41	LQC
13:0	0.49	1.12	1.10	1.14	1.94	2.11	0.166	L
iso-14:0	3.60	4.41	5.87	6.53	9.63	10.9	0.479	LQ
14:0	65.5	63.0	69.1	73.3	82.6	86.1	5.33	L
iso-15:0	11.3	16.5	17.9	20.6	24.1	25.2	0.75	LQC
anteiso-15:0	27.2	35.6	37.7	42.1	46.9	48.5	2.61	LQC
15:0	17.4	19.1	21.1	24.4	29.6	33.0	0.63	LQ
iso-16:0	9.77	10.6	11.3	13.7	16.8	17.4	0.386	LQC
16:0	810	836	852	893	918	908	22.2	LQ
<i>cis</i> -7–16:1	1.84	2.52	2.49	2.35	1.35	1.00	0.108	LQC
<i>cis</i> -9–16:1	5.74	4.48	4.45	3.61	2.49	1.59	0.306	LQ
iso-17:0	5.24	6.19	7.71	11.7	15.1	16.2	0.702	LQ
anteiso-17:0	17.3	18.8	20.7	26.6	30.9	31.3	1.11	LQ
17:0	10.2	12.3	12.8	15.3	17.3	17.7	1.38	LQ
20:0	19.9	21.2	22.9	25.7	27.3	27.8	0.81	LQ
20:1	12.5	11.1	9.91	7.89	6.33	5.80	0.832	LQ
22:0	12.2	12.1	12.2	12.6	12.6	12.4	0.272	—
23:0	3.41	3.45	3.33	3.22	3.29	3.03	0.193	—
24:0	10.4	11.2	11.5	12.0	12.0	11.9	0.617	LQC
26:0	19.4	20.6	21.2	24.4	26.0	26.5	0.741	LQ
28:0	7.80	6.41	7.56	6.42	6.55	7.16	0.676	—
Other	2.14	3.58	5.00	5.51	6.42	6.72	0.202	LQ
Partial sum (mg/tube)								
Total ²	5.33	5.58	5.59	5.80	6.00	5.96	0.001	LQ
DMA	0.08	0.11	0.13	0.13	0.11	0.09	0.025	Q
BCFA ³	0.07	0.09	0.10	0.12	0.14	0.15	0.003	LQ

¹Indicates significant ($P < 0.05$) linear (L), quadratic (Q), or cubic (C) components of the response to incubation time.

²Includes the sum of DMA and FA.

³Branched-chain fatty acids, including all iso-FA and anteiso-FA.

suspect that in our study it derived from the ricinoleic acid metabolism, but it was not clear if it is formed in the rumen or in tissues.

We conducted in vitro batch incubations of castor oil with strained rumen fluid to explore if the oxygenated FA found in milk of the castor oil group were formed in the rumen. The dose of the castor oil (40 g/kg of DM) used on the in vitro experiments was chosen to maximize the range of products formed and to reproduce the in vivo conditions. The 72 h of incubation was chosen taking into account preliminary results in our laboratory, which revealed that ricinoleic acid is metabolized at a slow rate. Hence, our experiments showed that the 12-oxo-18:0 and 12-OH-18:0 were the main products formed during the 72 h of in vitro batch incubations

with castor oil. Both 12-oxo-18:0 and 12-OH-18:0 increased as ricinoleic acid decreased, exceeding the quantity of ricinoleic acid after 24 h and 72 h of incubation, respectively (Figure 3A). Due the capacity of rumen bacteria to hydrogenate unsaturated double bonds and to oxidize hydroxy-FA (McKain et al., 2010), we suggest that the 12-OH-18:0 is formed by hydrogenation of *cis*-9 double bond of ricinoleic acid (12-OH,*cis*-9–18:1), followed by oxidation of the OH group to produce the 12-oxo-18:0. Indeed, Ramos Morales et al. (2012) reported that *Butyrivibrio proteoclasticus*, a known rumen bacteria, has the capacity to metabolize ricinoleic acid into 12-OH-18:0, but no other products were reported to be formed. However, we observed that 2 other hydroxy octadecenoic isomers (i.e., 12-OH-*trans*-18:1

and 13-OH-18:1) and 3 oxygenated octadecanoic acids (i.e., 13-OH-18:0, 10-OH-18:0, and 10-oxo-18:0) also increased with incubation time (Figure 3B). From these FA, we believe that only the 12-OH-*trans*-18:1 could result from the metabolism of ricinoleic acid in the rumen, probably by isomerization of the *cis*-9 into a *trans* monoenoic hydroxy-FA, an isomer of ricinoleic acid. The *trans* configuration of 12-OH-*trans*-18:1 was putatively identified based on the fact that during the GLC analysis it elutes before the ricinoleic acid, similarly to the elution pattern of *trans* and *cis* 18:1 isomers. Moreover, its kinetic curve also supports the hypothesis of an initial *cis/trans* isomerization of ricinoleic acid, as 12-OH-*trans*-18:1 rapidly increased at first 12 h and then slowly decreased with time, most likely due to further hydrogenation. Nevertheless, as 12-OH-*trans*-18:1 is detected in small amounts, particularly when compared with 12-OH-18:0, the metabolization pathway involving an initial *cis/trans* isomerization might be of minor importance compared with the direct reduction of *cis*-9 double bond.

The increased quantities of 10-oxo-18:0 and 10-OH-18:1 with incubation time was expected because both FA are known to be formed by the microbial action on *cis*-9-18:1 as already discussed (Jenkins et al., 2006; McKain et al., 2010). In addition, we predicted that the formation of both 13-OH-18:1 and 13-OH-18:0 results from the rumen biohydrogenation of linoleic acid (18:2n-6); indeed, it was already reported that rumen bacteria are capable of converting 18:2n-6 into 13-OH,*cis*-9-18:1 by hydration at position C12 (Hudson et al., 1998), and further hydrogenated, resulting in the formation of 13-OH-18:0. It would be expected to detect the final oxidation product (i.e., 13-oxo-18:0); however, it was

not possible to detect due to its low quantities and because it might co-elute with 12-oxo-18:0 in our chromatographic conditions.

A few studies explored the hypothesis that ricinoleic acid could be involved in the formation of rumenic acid (*cis*-9,*trans*-11-18:2) by rumen bacteria (Wallace et al., 2007; Ramos Morales et al., 2012) through mechanisms similar to that observed by lactic acid bacteria (Ogawa et al., 2005). However, neither Wallace et al. (2007) nor Ramos Morales et al. (2012) provided data to confirm that with rumen inocula. Consistent with these studies, we did not observe an increase in rumenic acid formation during the 72 h of in vitro batch incubations with castor oil. In contrast, the 18:2n-6 decreased with incubation time (Figure 3C); indeed, it is well-known that 18:2n-6 is extensively biohydrogenated in the rumen. Consequently, the *trans*-18:1, in particular the *trans*-11-18:1, greatly increased in the first hours, confirming that 18:2n-6 is rapidly metabolized in the rumen. Additionally, we compute the percentages of disappearance of ricinoleic acid, as well as of 18:2n-6 and 18:3n-3 (the major substrates of ruminal biohydrogenation), and we verified that at 6 h of incubation time more than 50% of 18:2n-6 and 18:3n-3 disappear, whereas ricinoleic acid did not reach 20% of disappearance (Figure 4). Only at 72 h of incubation did the disappearance reach similar values. These results indicate that ricinoleic acid is metabolized at a considerable lower rate than both 18:2n-6 and 18:3n-3. Similar findings were reported by Wallace et al. (2007) during incubations of mixed rumen bacteria with ricinoleic acid. These differences are probably due to different enzyme activity rates (i.e., isomerases vs. reductases). Indeed, it is known that isomerization of 18:2n-6 and 18:3n-3 is very fast and that hydrogenation rates are slower (Troegeler-Meynadier et al., 2006). Our results suggest that the first step of the metabolism of ricinoleic acid by rumen bacteria involves reduction of the *cis*-9 double bond rather than isomerization, so this might explain the slower disappearance rates of ricinoleic acid compared with 18:2n-6 and 18:3n-3. Despite the slow ricinoleic acid biohydrogenation rate, we found much lower concentrations of ricinoleic acid in milk than of its biohydrogenation derivatives. The reasons for that are not clear but might reflect differential absorption and transfer into the milk of ricinoleic acid and its derivatives. Moreover, biohydrogenation rate of ricinoleic acid might increase with adaptation of the ruminal microbial population to the presence of castor oil in diet.

Rumen microbial membranes contain plasmalogen lipids, which are a sub-class of phospholipids. Plasmalogens contains a long-chain FA attached at sn-2 position of glycerol and alk-1-enyl (vinyl) ether at the sn-1

Figure 4. Disappearance (%) of ricinoleic acid (12-OH,*cis*-9-18:1), linoleic acid (18:2n-6), and linolenic acid (18:3n-3) during 0 to 72 h of incubations of castor oil with strained rumen fluid.

position, whereas the usual phospholipids contain a FA attached at both sn-1 and sn-2 positions. During the HCl/methanol transesterification to prepare FAME, the DMA are also formed by cleavage of the vinyl ether chain of plasmalogenic lipids (Alves et al., 2013). Thus, the DMA composition, together with BCFA, might be used as rumen microbial internal markers. Castor oil, as the source of ricinoleic acid, does not seem to inhibit the growth and activity on rumen bacteria as the amount of total FA + DMA increased steadily with incubation time. Few studies reported that ricinoleic acid inhibited growth of specific rumen bacterial strains (Wallace et al., 2007; Ramos Morales et al., 2012). In our experiment the concentration of BCFA and DMA in the rumen showed different patterns with incubation time (Figure 3D). In the first hours, the amount of DMA was higher than the BCFA, but after 24 h of incubation an inversion occurred of the BCFA and DMA quantities, suggesting modifications on bacterial population or changes in the lipid composition of their cell membranes. Coincidentally, also at 24 h of incubation the 12-oxo-18:0 started to predominate over ricinoleic acid. It is tempting to hypothesize that alterations in the main oxygenated FA present in the incubation milieu are associated with shifts in bacterial population.

In summary, the detailed analysis of the products formed during *in vitro* batch incubations of castor oil with strained rumen fluid for 72 h showed that the 12-oxo-18:0, 12-OH-18:0, and 12-OH-*trans*-18:1 derived from the metabolism of ricinoleic acid in the rumen. Thus, the 8-OH-14:0, 10-OH-16:0, 8-oxo-14:0, and 10-oxo-16:0 that we found in milk fat of goats fed ricinoleic acid are most likely formed in tissues after absorption of the metabolites generated in the rumen. Other oxygenated FA with less than 18 carbons, as the 8-oxo-16:0 and 8-OH-16:0, were found in ruminant milk fat (Halme-mies-Beauchet-Filleau et al., 2011; Marquez-Ruiz et al., 2011). These authors suggested that both FA originated from partial β -oxidation of 10-oxo-18:0 and 10-OH-18:0 in tissues, respectively. Fatty acid β -oxidation is a multistep process by which FA are broken down close to the carboxyl group, producing FA with 2 carbons shorter than the original. Thus, we anticipate that the 10-OH-16:0 and 8-OH-14:0 found in goats milk are successive products of β -oxidation of 12-OH-18:0, and the 10-oxo-16:0 and 8-oxo-14:0 are successive products of β -oxidation of 12-oxo-18:0 in tissues. In Figure 5 we summarize the proposed metabolism of ricinoleic acid in dairy ruminants. Briefly, it is hydrogenated in the rumen forming the 12-OH-18:0, which is further oxidized to produce the 12-oxo-18:0 as the main product. Both oxygenated FA escape the rumen and are further absorbed and accessible in tissues to partial β -oxidation,

producing oxo- and hydroxy-FA that are 2 (10-oxo-16:0 and 10-OH-16:0) or 4 (8-oxo-14:0 and 8-OH-14:0) carbons shorter. Nevertheless, only a minor quantity of 12-oxo-18:0 and 12-OH-18:0 are oxidized in tissues and both FA are excreted in milk together with minor quantities of β -oxidation FA products.

It is well known that milk FA composition is greatly influenced by feeding factors, particularly by the addition of vegetable oils to the rations (Kliem and Shingfield, 2016). It has been proposed that the ability to maintain milk fat melting point within physiological values could play a role in the regulation of milk fat secretion when milk FA composition varies (Toral et al., 2013). However, our data indicate that this regulatory mechanism might be surpassed as the inclusion of castor oil in goat diets clearly increased the estimated milk melting point compared with control. Nevertheless, total FA milk melting points of both control and castor oil groups are within the range of $36.4^{\circ}\text{C} \pm 7.28$ (mean $\pm$ SD) found by Toral et al. (2013), which were calculated from 278 milk FA profiles from 3 dairy ruminant species and different dietary treatments. The increased melting point was probably due to the highest proportion of 12-oxo-18:0 in milk fat (4.9% of total FA) of the castor oil group, considering that the melting point of synthetic 12-oxo-18:0 averages 80°C (Grummitt and Siedschlag, 1949). The reduction in the proportion of SFA (44.8 to 48.6% of total FA) in milk of the castor oil group compared with the control did not seem to compensate for the high melting point of the oxygenated FA metabolites formed from ricinoleic acid. The high milk melting point estimated for the castor oil group might have technological implications in the derived dairy products, although these aspects were not evaluated in this study. However, previous studies showed that inclusion of castor oil in dairy goat diets had no effect on the physicochemical composition, rheological parameters, or sensory quality of cheese (Lacerda de Medeiros et al., 2013).

In our experiment, the incorporation of castor oil in goat diets did not negatively affect the nutritional quality of milk fat. Indeed, the proportion of total SFA decreased in milk of the castor oil group compared with the control; this decrease was mostly due to the reduction of 18:0, whereas the 14:0 and 16:0 that are the SFA associated with incidence of cardiovascular disease (FAO, 2003) did not differ from the control. Moreover, inclusion of castor oil in diet did not increase the proportion of *trans* FA in milk in contrast with the incorporation of other vegetal oils (Kliem and Shingfield, 2016). Nevertheless, the proportion of the 18:2n-6 decreased and the *cis*-9-18:1 tended to decrease in milk of the castor oil group compared with the con-

Figure 5. Proposed metabolism of ricinoleic acid (12-OH,*cis*-9-18:1) in dairy ruminants. Thick arrows represent the main metabolic pathways and thin arrows the putative pathways including all the oxo-fatty acids (FA) and hydroxy-FA detected in milk fat of goats fed castor oil, whereas the dashed arrows represent putative pathways including the hydroxy-FA detected in incubations of castor oil with strained rumen fluid.

trol, but this was compensated for by the increase of oxo- and hydroxy-FA, which may have some health benefits to humans. Indeed, ricinoleic acid is known for its anti-inflammatory and analgesic properties (Boddu et al., 2015), whereas hydroxystearic isomers have been reported to exert strong antiproliferative effects in human adenocarcinoma and osteosarcoma cell lines (Pagnotta et al., 2006; Calonghi et al., 2007; Parolin et al., 2012). However, more studies are needed to determine the health effects of these oxygenated FA to consumers.

CONCLUSIONS

Our results on the metabolism of ricinoleic acid in ruminants show that the microbial activity in the rumen metabolizes ricinoleic acid (12-OH,*cis*-9-18:1) to produce 12-OH-18:0, followed by oxidation to produce 12-oxo-18:0 as the main product. Both FA seem to flow to the abomasum, absorbed and further metabolized through partial β -oxidation forming oxo- and hydroxy-FA 2 or 4 carbons shorter than the initial 12-oxo-18:0 and 12-OH-18:0, respectively. Finally, large quantities of these 2 rumen-FA are excreted in milk together with

minor quantities of ricinoleic acid and the FA resulting from the β -oxidation. Overall, the incorporation of castor oil in goat diets led to the accumulation of the oxygenated FA in milk, which may have some beneficial health implications for consumers.

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