Effects of supplementation with vegetable oils, including castor oil, on milk production of ewes and on growth of their lambs[1]

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ABSTRACT: The objectives in this experiment were to compare the effects of castor oil, canola oil, or sunflower oil on lactation performance, milk composition, and milk fatty acid (**FA**) profile in Santa Inês ewes and on growth of lambs. Forty-four ewes (66.9 ± 4.7 kg of initial BW, mean ± SD) were penned individually with their lambs and used in a randomized complete block design with 11 blocks and four diets. The experimental diets were as follows: 1) basal diet without added oil (control), 2) 30 g FA/kg DM of canola oil (**CAN**), 3) 30 g FA/kg DM of sunflower oil (**SUN**), and 4) 30 g FA/kg DM of castor oil (**CAS**). The oils were added to a basal diet containing 50% of roughage. Once a week, from the 2nd to 8th wk of lactation, ewes were separated from their lambs, injected with oxytocin, and mechanically milked to empty the udder. After 3 h, using the same procedure, milk production was recorded, and milk was sampled for composition and FA profile determination. The growth of the lambs was monitored weekly. Ewes fed the control diet had greater ($P < 0.05$) dry matter intake (DMI) than those fed the oil-supplemented diets. No effect was observed on milk yield and on final BW of lambs. Milk fat and milk total solid concentrations were greater ($P < 0.05$) with the supply of CAS. Supplementation with CAN and SUN, but not with CAS, reduced ($P < 0.05$) the sum of FA with 14 or less carbon chains and increased ($P < 0.05$) the *c*9-18:1, 18:0 and most of the biohydrogenation intermediates, including the *t*10-18:1, *t*11-18:1, and *c*9,*t*11-18:2. All oil-supplemented diets reduced ($P < 0.05$) the content of 16:0 when compared with the control. Milk from ewes fed CAS presented only small proportion of 12-OH,*c*9-18:1 (0.31% of total FA) but much larger proportions of 12-OH-18:0 (1.58% of total FA) and particularly of 12-oxo-18:0 (2.95 % of total FA), which suggests that 12-OH,*c*9-18:1 was extensively metabolized in the rumen. Concluding, CAS increased milk fat and modified the milk FA composition by increasing the hydroxy- and oxo-FA. The potential health promoting proprieties and technological advantages of milk enriched with hydroxy- and oxo-FA are not know at present but deserve to be explored.

Key words: biohydrogenation, hydroxy fatty acids, oxo fatty acids, ricinoleic acid, sheep

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INTRODUCTION

The unsaturated oil supplementation of lactating ewes has been used to simultaneously increase energy density of diets, milk production, and to modify the fatty acid (**FA**) profile of milk

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by decreasing the medium chain saturated FA and increasing the content in vaccenic and rumenic acids (Sampelayo et al., 2007). In tropical regions, the lipid supplementation is expected to mitigate heat stress due to lower caloric increment (Renaudeau et al., 2012). However, supplementary fat to dairy ewes might depress intake, fiber digestion, milk fat (Sampelayo et al., 2007; Toral et. al., 2010), and promote a large accumulation of health deleterious *trans* FA (Toral et. al., 2010; Bichi et al., 2013). Public policies support the production of castor oil by small farmers in semiarid northeastern region of Brazil (César and Batalha, 2010). Castor oil is unique due to a very high proportion of ricinoleic acid (12-OH,*c*9-18:1) and is usually considered as nonedible (Severino et al., 2012). Nevertheless, Brazilian research have demonstrated that castor oil can be incorporated in the diet of small ruminants without any deleterious effects on digestion (Maia et al., 2012a), growth (Maia et al., 2012b), and milk production (Queiroga et al., 2010). Moreover, castor oil is less prone to oxidative degradation than other vegetable oil ensuring longer conservation in tropical farm conditions (Medeiros et al., 2013b). Despite that, castor oil availability in the market is still low, and its price is greater compared with other vegetable oils, it is not currently used in practical production conditions. The digestive and metabolic effects of ricinoleic acid in ruminants are still scarcely studied (Morales et al., 2012; Maia et al., 2012a). In vitro studies showed that it might inhibit the methanogenesis and modulate the biohydrogenation of other unsaturated FA (Morales et al., 2012), and eventually, it is converted into CLA, as demonstrated in pure cultures of *Lactobacillus plantarum* (Ando et al., 2003, 2004). Thus, we hypothesized that castor oil would allow similar productive performance to other common vegetable oils when included in diets of lactating ewes. In addition, we anticipated that CLA content in milk of ewes fed castor oil would be greater than in milk of ewes fed a control diet, without oil. To test these hypotheses, we assessed the effects of adding canola, sunflower, or castor oil in the diet of lactating ewes on dry matter intake (DMI), milk production, and milk FA profile and on the performance of ewes and their lambs.

MATERIALS AND METHODS

This study was conducted at the Sheep and Goat Intensive Production System of the Departamento de Zootecnia, Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo (ESALQ/USP), located in Piracicaba (22°42′24″ S and 47°37′53″ W), state of São Paulo, Brazil. The experimental protocol was revised and approved by the Institutional Animal Care and Use Committee at "Luiz de Queiroz" Agricultural College (Comitê de Ética no uso de Animais em Pesquisa; 2011/15).

Animals and Housing

Forty-four multiparous Santa Inês ewes (66.6 ± 4.9 kg of initial BW; 4.0 ± 1.8 lambings) were housed indoor and individually allotted with their lambs in pens (1.3 × 3.5 m) with a concrete floor, feed bunk, mineral box, and waterer. Twelve ewes gave birth to twins, and 32 ewes had single births. Twenty-one lambs were females, and 35 lambs were males. At lambing day, all ewes were dewormed with moxidectin 1% (Cydectin, Fort Dodge Saúde Animal Ltda, Campinas-SP, Brazil) according to label directions.

Experimental Design and Diets

After 7.0 ± 2.0 d in milk, ewes were divided into a randomized complete block design with 11 blocks and four diets. The blocks (four ewes each) were defined according to date of lambing (variation less than 7 d), type of birth (single or twin), sex of the offspring, and initial BW of the ewes and lambs. Five blocks were organized with ewes nursing single male lambs; three blocks included ewes nursing single female lambs, and three blocks for ewes nursing twins. Ewes within a block were randomly assigned to an experimental diet.

The treatments were defined by the addition of unprotected oil (DM basis), with canola oil, sunflower oil, or castor oil (Campestre Indústria e Comércio de Óleos Vegetais LTDA, São Bernardo do Campo, SP, Brazil) in the diet, maintaining the content of supplementation at 30 g FA/kg DM (Table 1). The oils were added to a basal diet that contained 50% DM of concentrate and 50% DM of forage (coastcross hay). The proportions of the ingredients and the chemical composition of the oil diets were the same, only the lipid source changed (Table 1). The experimental diets were as follows: 1) basal diet without added oil (control), 2) 30 g FA/kg DM of canola oil (**CAN**), 3) 30 g FA/kg DM of sunflower oil (**SUN**), and 4) 30 g FA/kg DM of castor oil (**CAS**).

The diets were formulated according to the recommendations of the National Research Council (2007). The metabolizable energy (ME) of the diets

Table 1. Ingredients, chemical composition of experimental diets and main FA profile of canola oil, sunflower oil, and castor oil

	Diets[a]			
Item	Control	CAN	SUN	CAS
Ingredients, g/kg DM				
Coastcross hay	500	500	500	500
Ground corn	388	351	351	351
Soybean meal	84	91	91	91
Oil	–	30	30	30
Urea	3	3	3	3
Limestone	6	6	6	6
Mineral supplement[b]	19	19	19	19
Analyzed chemical composition, g/kg DM				
Dry matter	885	887	887	887
Crude protein	153	147	146	145
Neutral detergent fiber	477	478	483	480
Ether extract	21	50	50	50
ME, Mcal/kg DM[c]	2.4	2.6	2.6	2.6
Added oil FAs(g/kg Fat)[d]				
16:0	–	4.5	6.6	1.3
18:0	–	4.2	4.4	5.1
*c*9-18:1	–	59.8	23.0	3.7
12-OH,*c*9-18:1	–	–	–	81.1
18:2n−6	–	18.7	60.2	5.6
18:3n−3	–	5.9	0.6	0.5
Others	–	6.9	5.2	2.7

[a]Diets = control: basal diet, no oil added; CAN: addition of 30 g FA/kg DM of canola oil; SUN: addition of 30 g FA/kg DM of sunflower oil and CAS: addition of 30 g FA/kg DM of castor oil.

[b]Composition: Ca 22%, P 5.5%, Mg 3.5%, S 2.2%, Cl 10.5%, Na 7.0%, Mn 1,500 mg/kg, Fe 500 mg/kg, Zn 1,550 mg/kg, Cu 440 mg/kg, Co 50 mg/kg, I 40 mg/kg, Se 20 mg/kg.

[c]Estimated by Small Ruminant Nutrition System (Cannas et al., 2004).

[d]Main FAs from canola oil, sunflower oil, and castor oil (Campestre Indústria e Comércio de Óleos Vegetais LTDA, São Bernardo do Campo, SP, Brazil) added to the diets.

was estimated using the Small Ruminant Nutrition System, v. 1.8.6 (Cannas et al., 2004).

Feeding Management and Data Collection

After forming the blocks, ewes were fed the control diet for 1 week, to adapt them to the experimental facilities and feeding management. After the adaptation period, the experimental period began, elapsing between the 14th ± 2 d of lactation and the 56th ± 2 d of lactation, completing 7 wk of data collection.

Corn was coarsely ground and mixed with soybean meal, urea, limestone, and mineral mixture using a horizontal mixer with a capacity of 500 kg (Lucato, Limeira, SP, Brazil). The oils were added to the concentrate just before feed delivery. The concentrate + oil and the coastcross hay were weighed separately (Marte, LC 100, São Paulo, SP, Brazil), mixed, and offered daily. Samples of each oil (20 mL) were collected weekly and then stored at −20 °C. After the end of the experiment, samples of each oil type were pooled for FA profile determination.

Animals had ad libitum access to feed and fresh water. Amounts of total mixed ration fed to animals were calculated according to previous DMI, and adjustments were made when needed so that refused feed did not exceed 10% of daily intake. Orts were recorded every week to determine the animal DMI. Feeds and orts were sampled weekly and frozen at −20 °C for later analysis. All ewes were weighed on a mechanical scale, accurate to 0.1 kg (Açôres 602 SM; Açôres Balanças Indústria e Comércio de Balanças Ltda, Cambé, PR, Brazil), for three consecutive days, always in the morning, without fasting, in the beginning and at the end of the experimental period. The initial BW and final BW were computed as the average of the three initial and the three final weight data, respectively.

To measure the milk production, once a week, the ewes were separated from their lambs and,

without delay, they were mechanically milked (Camp Agri, model GL300, São Paulo, SP, Brazil). Milk ejection was stimulated by the intravenous application of 10 international units (**IU**) of oxytocin (Univet S.A., Indústria Veterinária, São Paulo, SP, Brazil). The ewe's milking was performed at 10:00 a.m. and 1:00 p.m. The first milking was used to empty the udder, and the milk obtained was discarded. In the second milking, the milk amount produced was weighed to quantify the production during the 3 h interval, as described by Susin et al. (1995). Fat-corrected milk (6.5% fat; **FCM**) and fat- and protein-corrected milk (6.5% fat and 5.8% protein; **FPCM**) yields were calculated according to Pulina and Nudda (2004). The equations used were as follows: FCM (1,020 kcal/kg) = production, kg × (0.37 + 0.097 × fat, %), and FPCM (1,047 kcal/kg) = production, kg × (0.25 + 0.085 × fat, % + 0.035 × protein, %).

Two samples of milk per ewe (20 mL each), were collected weekly, from the second milking. One sample was preserved in Bronopol Broad Spectrum Microtabs II (2-bromo-2-nitropropano-1,3-diol, D & F Control Systems, Inc., Dublin, CA, USA) for milk composition determination, and the other one was stored at −20 °C for later FA profile analysis. Before FA determination, the weekly samples of milk were thawed and pooled by ewe.

After 2 wk of age, lambs had ad libitum access to a starter. The ingredients of the starter were as follows: 700 g/kg DM of corn, 238 g/kg DM of soybean meal, 15 g/kg DM of limestone, 10 g/kg DM of mineral mix, and 37 g/kg DM of molasses. The analyzed chemical composition of the starter was 885 g/kg of DM, 186 g/kg of CP, 126 g/kg of NDF, and 53 g/kg of ash. To control coccidiosis, sodium monensin (Rumensin 100; Elanco Brazil, São Paulo, SP, Brazil) was added to the starter at the rate of 25 mg/kg. The starter was available in a creep feeding system (0.80 × 1.0 m). To avoid access to the ewe's feed bunk, lambs were tied to the feeder, allowing them to nurse and to reach the creep feed and water. Lambs were weighted weekly, after a fasting period of 3 h (time elapsed between the two milkings), on an electronic scale, accurate to 0.01 kg (Marte LC-200; Marte Balanças e Aparelhos de Precisão Ltda, São Paulo, SP, Brazil) to calculate ADG. On the weighing day, the orts of the starter were quantified to determine the DMI by the lambs.

Chemical Analysis and Calculations

Samples of feeds and orts were grounded through a 1 mm Wiley Mill screen (Marconi, Piracicaba, SP, Brazil). The DM content of feed offered and orts was determined after oven-drying the samples at 105 °C for 24 h according to the Association of Official Analytical Chemists (AOAC, 1990; method 934.01). Ash was determined by incinerating the samples in a muffle furnace at 550 °C for 4 h (AOAC, 1990; method 942.05). Total nitrogen (N) concentration was determined using a LECO FP-528 Total Nitrogen Analyzer (LECO Corporation, St. Joseph, MI, USA; AOAC, 1990; method 968.06). The CP was obtained by multiplying the total N content by 6.25. The NDF was determined according to Van Soest et al. (1991), using heat-stable alpha-amylase and sodium sulfite using an Ankom 2000 Fiber Analyzer (Ankom Technology Corp., Fairport, NY, USA). The ether extract (**EE**) also was determined according to AOAC (1990; method 954.05).

Milk samples, previously preserved in Bronopol Broad Spectrum Microtabs II, were analyzed for the quantification of protein, fat, lactose, and total solids by infrared spectrometry Bentley 2000 instrument (Bentley Instruments, Chaska, MN; AOAC, 1990).

For FA analysis, milk samples per ewe were pooled, and total lipids were extracted following the methodology described by Feng et al. (2004). One aliquot of the lipid extract was methylated in two steps with 2 mL of 0.5 M sodium methoxide (10 min at 50 °C), and methanoic HCl was added (10 min at 80 °C), according to Kramer et al. (1997) and stored at −20 °C in 1.5 mL amber vials containing nitrogen to prevent possible oxidation.

The quantification and determination of FA were performed by GLC according to the general procedures described by Feng et al. (2004). The equipment used was an Agilent 7890A gas chromatograph equipped with a flame ionization detector (7683B) and a fused-silica capillary column (J&W 112-88A7, Agilent Technologies, Santa Clara, CA, USA), 100 m in length and 250 µm internal diameter, containing 0.20 µm cyanopropyl policiloxane. The data acquisition was performed using ChemStation software (Agilent Technologies, Santa Clara, CA, USA). The total chromatographic run time was 87.5 min divided into four heating ramps, as follows: 70 °C (1 min), 100 °C (5 °C/min, 2 min), 175 °C (10 °C/min, 40 min), 225 °C (5 °C/min), and 245 °C (20 °C/min, 20 min). The H_2 was used as carrier gas at a flow rate of 1.0 mL/min, the temperature of the injector and detector was 250 °C and 255 °C, respectively. The N_2 gas was used as Makeup with a flow of 30 mL/min. A split ratio of 50:1 was used. The identification of the FA methyl esters was performed based

on their retention time and by analogy with milk samples from goats fed castor oil analyzed with the same capillary column by gas chromatography coupled with mass spectrometry (Shimadzu GC-MS 2010-Plus, Shimadzu, Kyoto, Japan) as described by Alves et al. (2017). A mix standard Supelco® (Sigma–Aldrich, Bellefonte, PA, USA) of 37 FA and individual FA standards of *t*11-18:1, *c*9,*t*11-18:2, *t*10,*c*12-18:2, 12-OH,*c*9-18:1 (Nu-Chek Prep, Inc, Elysian, MN, USA) were used.

Statistical Analysis

The DMI, milk yield, and milk composition data were analyzed as repeated measurements over time using the MIXED procedure (SAS Inst. Inc, Cary, NC), according to the following statistical model: $y_{ijk} = \mu + D_i + b_j + e_{ij} + T_k + D_iT_k + e_{ijk}$, where μ = overall mean, D_i = the fixed effect of diet; b_j = random block effect, e_{ij} = subject level random error, T_k = fixed effect of time, D_iT_k = fixed effect of diet × time interaction, and e_{ijk} = within subject level random error. The covariance matrix used was the "autoregressive" (AR 1) and was selected using both Akaike corrected and Bayesian information fit criteria after adjusting models with the Autoregressive (1) (**AR [1]**), Heterogeneous AR (1) (**ARH [1]**), Ante-Dependence (**ANTE**), Compound Symmetry (**CS**), Heterogeneous CS (**CSH**), and Unstructured (**UN**) covariance. When significant treatments effects were found, a post hoc analysis using Tukey procedure was applied to identify significant differences ($P < 0.05$) among least square means. Least square means and standard error of the means of each treatment are presented in the tables.

The initial BW, final BW, BW change of the ewes throughout the experiment, the FA profile of the milk fat, and the variables related to the lambs were analyzed using the following statistical model: $y_{ij} = \mu + D_i + b_j + e_{ij}$, where μ = overall mean, D_i = fixed effect of diet, b_i = random effect of block and e_{ij} = the random error. The least square means shown were compared using a post hoc Tukey procedure, when significant ($P < 0.05$) treatment effects were detected.

RESULTS

The level of EE of the diets increased from 2.4% (control diet) to 5.1% (diets containing oil). Consequently, the three diets containing oil had a greater concentration of ME in relation to the control diet (2.6 vs. 2.4 Mcal/kg DM, respectively; Table 1), knowing that castor oil contains a ME content similar to the other common vegetable oils, such as canola and sunflower oils (Maia et al., 2012a).

Animals fed the oil-supplemented diets had lower DMI ($P < 0.05$) than those fed the control diet. Lipid supplementation increased EE intake ($P < 0.05$) and decreased intake of CP ($P < 0.05$) and NDF intake ($P < 0.05$) compared with animals fed the control diet (Table 2).

The ewe's body weight change over the experimental period averaged +1.2 kg and did not differ among diets (Table 2).

Milk production, FCM, and FPCM were similar between the control diet and those containing oil (Table 2). Adding oil to the diets did not affect milk production (g/3 h) of fat, protein, lactose, and total solids among diets. However, the content (%) of milk fat and total milk solids were greater ($P < 0.05$) when ewes were fed castor oil. The evaluation of the components in terms of their percentage showed that the milk protein and milk lactose contents were not affected by diets, but it was observed a week effect ($P < 0.05$), with decreased yield and concentrations of these components in the last experimental week. The interaction between week and diets was not significant for any of the variables.

The ADG and starter intake by lambs of ewes fed oil-supplemented diets were similar to those of ewes fed the control diet (Table 2).

FA profile and partial sums of FA of milk from ewes fed the different diets are presented in Table 3. Compared with control, supplementation with castor oil did not changed the proportions of major FA classes as the SFA, medium chain FA (MCFA, i.e., FA with carbon chain length ranging from 8 to 14 carbon atoms), *cis*-MUFA, PUFA, *trans*-FA, and biohydrogenation intermediates. This contrast with the effects of canola and sunflower oil supplementation that, compared with Control, reduced ($P < 0.05$) the MCFA and increased ($P < 0.05$) the *c*9-18:1, 18:0, and most of the biohydrogenation intermediates, including the *t*10-18:1, *t*11-18:1, and *c*9,*t*11-18:2, and also increased *cis*-MUFA compared with control and castor oil. However, all oil types reduced ($P < 0.05$) clearly the content of 16:0 when compared with the control. Nevertheless, milk samples from ewes fed castor oil presented several FA, which collectively comprise 6.2% of total FA, that were not present in the milk from ewe's fed the other diets. In these samples, the 12-OH, c9-18:1 comprised only 0.3% of total FA, whereas the 12-oxo-18:0 and 12-OH-18:0 comprised about

Table 2. Performance of ewes and suckling lambs fed a diet without oil or diets supplemented with canola, sunflower or castor oil

	Diets[a]					*P*-value		
Item	Control	CAN	SUN	CAS	SEM	Diet	Week	D × Wk[b]
Body weight of ewes, kg								
Initial	65.49	66.12	69.65	66.71	0.743	0.671	–	–
Final	66.80	67.81	70.35	67.71	0.842	0.748	–	–
BW change of ewes, kg	1.36	1.63	0.95	0.99	0.125	0.651	–	–
Intake of ewes								
DM, kg/d	2.49*	2.07†	2.18†	2.09†	0.024	0.004	0.028	0.154
CP, kg/d	0.38*	0.31†	0.32†	0.31†	0.004	0.005	0.049	0.144
NDF, kg/d	1.18*	0.97†	1.03†	0.98†	0.013	<0.001	0.002	0.155
Ether extract, kg/d	0.05†	0.11*	0.11*	0.11*	0.002	<0.001	0.002	0.118
ME, Mcal/d	5.90	5.23	5.62	5.32	0.058	0.174	0.028	0.145
Production, g/3 h								
Milk	192.6	184.3	167.9	155.2	3.631	0.136	<0.001	0.621
FCM[c]	220.5	218.4	187.5	197.7	4.975	0.260	<0.001	0.165
FPCM[d]	211.3	207.8	180.5	188.0	4.576	0.267	<0.001	0.183
Fat	15.4	15.5	13.0	14.5	0.394	0.301	<0.001	0.128
Protein	9.1	8.5	8.1	7.8	0.157	0.232	<0.001	0.789
Lactose	9.0	8.8	8.3	7.1	0.202	0.189	<0.001	0.262
Total solids	35.9	34.9	31.2	30.9	0.738	0.254	<0.001	0.253
Milk composition, %								
Fat	7.9†	8.2†	7.6†	9.3*	0.114	0.012	0.844	0.224
Protein	4.9	4.7	4.9	4.9	0.039	0.493	<0.001	0.176
Lactose	4.5	4.7	4.7	4.6	0.031	0.376	<0.001	0.119
Total solids	18.6†	18.7†	18.6†	20.0*	0.117	0.025	0.355	0.362
Lambs								
Initial age, d	10.2	9.1	10.0	9.2	–	–	–	–
Final age, d	59.2	58.1	59.0	58.2	–	–	–	–
Initial weight, kg	7.9	8.2	7.5	8.2	0.260	0.368	–	–
Final weight, kg	18.4	18.7	18.3	18.2	0.585	0.991	–	–
ADG, g	250.0	249.1	257.3	236.4	9.547	0.862	–	–
Starter intake, g DM/d	149.5	150.2	139.0	150.2	8.779	0.963	–	–

*–†Means in the same row followed by different letters differ by Tukey test ($P < 0.05$).

[a]Diets = control: basal diet, no oil added; CAN: addition of 30 g FA/kg DM of canola oil; SUN: addition of 30 g FA/kg DM of sunflower oil, and CAS: addition of 30 g FA/kg DM of castor oil.

[b]D × Wk = interaction between diet and week.

[c]FCM (6.5% fat-corrected milk) = production, kg × (0.37 + 0.097 × fat, %), according to Pulina and Nudda (2004).

[d]FPCM: 6.5% fat- and 5.8% protein-corrected milk = production, kg × (0.25 + 0.085 × fat, % + 0.035 × protein, %), according to Pulina and Nudda (2004).

3% and 1.6% of total milk FA. Thus, despite the decrease on 16:0 observed with castor oil supplementation when compared with control, the total SFA did not decrease due to presence of 12-oxo-18:0 and 12-OH-18:0. Conversely, the sum of SFA was reduced ($P < 0.05$) about 8.5 percent points by dietary canola and sunflower oils, compared with control. The decrease in SFA observed with canola and sunflower oils is mostly compensated by the increase ($P < 0.05$) in *trans*-FA (about 3.4 percent points) and cis-MUFA (3.4 percent points). The branched chain FA (BCFA) were not affected by diets.

DISCUSSION

The reduction of the DMI observed in animals fed lipid supplemented diets is probably due to a regulatory intake response to the greater energy density of lipid supplemented diets relative to control, as the estimated ME intake was similar to all diets. The similar ME intake explains the lack of effects of lipid supplementation on milk production, ewe's BW change, and lamb's growth, suggesting that intake was regulated by energy satiety mechanisms (Palmquist, 1994). Productive responses to castor oil were similar to the other more common oil sources, confirming previous reports that it can

Table 3. FA profile of milk and partial sums of milk FAs from ewes fed a diet without oil or diets supplemented with canola, sunflower or castor oil

	Diets[a]					
Item, % of total FA	Control	CAN	SUN	CAS	SEM	*P*-value
4:0	1.36	1.48	1.70	1.28	0.155	0.259
6:0	1.71	1.34	1.57	2.39	0.444	0.367
8:0	1.86*	1.40†	1.60*†	1.83*	0.081	0.001
10:0	6.85*	4.54†	5.20†	6.33*	0.262	<0.001
11:0	0.09*	0.06†	0.07*†	0.09*	0.006	0.006
12:0	4.46*	2.93†	3.26†	4.06*	0.195	<0.001
13:0	0.14†	0.14†	0.14†	0.19*	0.009	<0.001
14:0	11.03*	8.02†	8.37†	10.32*	0.408	<0.001
i-15:0	0.31*†	0.31*†	0.30†	0.35*	0.012	0.016
a-15:0	0.62*	0.51†	0.51†	0.58*†	0.027	0.017
*c*9-14:1	0.19*	0.11†	0.13*†	0.16*†	0.016	0.009
15:0	0.95*	0.81†	0.81†	0.90*†	0.026	0.001
i-16:0	0.31†	0.36*†	0.33†	0.40*	0.016	<0.001
16:0	26.2*	20.5†	20.4†	22.2†	0.534	<0.001
*t*9-16:1	0.09†	0.23*	0.23*	0.05†	0.037	0.002
i-17:0	0.44	0.49	0.45	0.43	0.019	0.164
*c*7-16:1	0.34	0.32	0.32	0.32	0.012	0.516
*c*9-16:1	1.03*	0.60†	0.72*†	0.75*†	0.109	0.049
a-17:0	0.57*	0.50*†	0.49†	0.50*†	0.019	0.018
17:0	0.70	0.63	0.60	0.64	0.026	0.077
i-18:0	0.09	0.10	0.08	0.10	0.006	0.222
*c*9-17:1	0.26	0.22	0.24	0.27	0.017	0.153
18:0	11.5†	15.7*	15.6*	11.3†	0.604	<0.001
*t*6/7/8–18:1	0.30‡	0.71*	0.51†	0.23‡	0.035	<0.001
t9-18:1	0.24†	0.55*	0.35†	0.22†	0.039	<0.001
*t*10-18:1	0.51†	1.41*	1.25*	0.40†	0.165	<0.001
*t*11-18:1	0.82†	2.41*	2.28*	0.77†	0.269	<0.001
*t*12-18:1	0.27†	0.59*	0.51*	0.25†	0.315	<0.001
*c*9-18:1	20.7†	24.5*	24.3*	21.0†	0.852	0.003
*c*11-18:1	0.50†	0.62*	0.48†	0.52†	0.024	0.002
*c*12-18:1	0.16†	0.27*	0.30*	0.19†	0.018	<0.001
*c*13-18:1	0.03‡	0.06*	0.05†	0.04†‡	0.003	<0.001
*t*16-/*c*14-18:1	0.25†	0.40*	0.42*	0.20†	0.016	<0.001
*c*15-18:1	0.10‡	0.22*	0.15†	0.10‡	0.009	<0.001
others 18:2[b]	0.28†	0.44*	0.35†	0.29†	0.019	<0.001
18:2n-6	1.82*†	1.73†	2.12*	1.63†	0.088	0.004
20:0	0.26‡	0.40*	0.26‡	0.33†	0.014	<0.001
18:3n-3	0.31*	0.26*†	0.24†	0.28*†	0.013	0.007
*t*7,*c*9-18:2	0.03†	0.09*	0.06*†	0.04†	0.013	0.017
*c*9,*t*11-18:2	0.52†	1.28*	1.20*	0.52†	0.099	<0.001
*t*10,*c*12-18:2	0.016	0.021	0.022	0.015	0.002	0.163
20:2n-6	0.02†	0.05*	0.05*	0.04*	0.004	<0.001
20:3n-6	0.03	0.04	0.04	0.03	0.002	0.323
22:0	0.07‡	0.09*†	0.10*	0.08†	0.003	<0.001
20:4n-6	0.27	0.25	0.25	0.26	0.012	0.334
20:5n-3	0.04	0.03	0.03	0.05	0.009	0.288
22:5n-3	0.08	0.07	0.07	0.08	0.006	0.052
22:6n-3	0.02	0.02	0.02	0.01	0.003	0.068
12-OH-18:0	–	–	–	1.58	–	–
12-oxo-18:0	–	–	–	2.95	–	–
12-OH,*c*9-18:1	–	–	–	0.31	–	–
SFA	69.6*	60.3†	61.9†	68.9*	0.941	<0.001

(Continued)

Table 3. (Continued)

Item, % of total FA	Diets[a]				SEM	*P*-value
	Control	CAN	SUN	CAS		
cis-MUFA	23.3[†]	26.8*	26.6*	23.3[†]	0.881	0.006
PUFA	2.59*[†]	2.45*[†]	2.81*	2.38[†]	0.125	0.029
n−3 PUFA	0.46*	0.38*[†]	0.37[†]	0.43*[†]	0.020	0.011
n−6 PUFA	2.13*[†]	2.07[†]	2.44*	1.96[†]	0.091	0.006
MCFA[c]	25.4*	17.9[†]	19.5[†]	25.4*	0.86	<0.001
BCFA[d]	2.33	2.26	2.18	2.37	0.069	0.201
trans-FA[e]	2.53[†]	6.41*	5.52*	2.26[†]	0.534	<0.001
Bioh. Intermed. FA[f]	3.52[†]	8.45*	7.44*	3.26[†]	0.640	<0.001
Castor oil derived FA[g]	–	–	–	6.17	–	–

*–‡Means in the same row followed by different letters differ by Tukey test ($P < 0.05$).

[a]Diets = control: basal diet, no oil added; CAN: addition of 30g FA/kg DM of canola oil; SUN: addition of 30g FA/kg DM of sunflower oil and CAS: addition of 30g FA/kg DM of castor oil /kg DM.

[b]sum of minor 18:2 isomers.

[c]Sum of short and medium chain FAs (i.e., with carbon chain length ranging from 8 to 14 carbon atoms).

[d]Sum of branched chain FAs.

[e]Sum of *trans* FA, excluding the CLA isomers.

[f]Sum of all 18:1 and 18:2 biohydrogenation intermediates.

[g]Sum of FA occurring exclusively in milk samples from ewes fed castor oil.

be used in ruminant production without any deleterious effects on digestion (Bris et al., 1969; Maia et al., 2012a), growth (Maia et al., 2012b), and milk production (Queiroga et al., 2010). Castor oil supplementation increased milk fat content, contrarily to what was reported for the milk of goats supplemented with castor oil (Pereira et al., 2010). Since milk fat yield remained constant among diets, the increase of milk fat content observed with the castor oil diet might be partially explained by the concentration of milk due to the nonsignificant milk volume reduction when compared with the other. However, other factors such as lower absorption of biohydrogenation intermediates and a putative specific effect of hydroxy- and oxo-FA derived from CAS cannot be excluded. The effects of week on protein and lactose production (g/3 h) followed the effect of milk production, decreasing in the 7th experimental week. The effects of week on protein and lactose contents are related to the reductions in their proportions in milk.

The FA composition of milk was greatly altered by oil supplementation. Canola and sunflower oil diets induced changes in milk FA profile very consistent with those well establish for unprotected lipid supplementation in lactating ruminants (Sampelayo et al., 2007; Gomez-Cortes et al., 2011a, 2011b) where FA de novo synthetized in the mammary gland like MCFA (i.e., those with 8 ≤ carbon chain ≤ 14) and also 16:0 are clearly reduced, whereas C18 FA including the 18:0, *c*9-18:1, and the biohydrogenation intermediates are increased when compared with the control. Although, the exact mechanism that triggers the reduction of MCFA in milk are not well established, it might involve the inhibition of de novo FA synthesis by C18 *trans* FA as suggested by Glasser et al. (2008), or simply to make use of the increased availability of circulating preformed C18 FA allowed by the lipid supplementation while maintaining the milk fat fluidity (Timmen and Patton, 1988; Toral et al., 2013). Differences in milk FA profiles between canola and sunflower oil diets are minor and mostly restricted to the concentration of 18:2n-6 that was greater in milk from ewes fed sunflower oil than in those fed canola oil. The larger concentration of 18:2n-6 in the milk of ewes supplemented with sunflower oil is easily explained by the FA composition of the sunflower oil and reported by others (Gomez-Cortes et al., 2011a).

The effects of dietary castor oil on milk FA profile were notable in which differ radically from the well-established pattern found for the largely unsaturated vegetable oils. In fact, castor oil supplementation did not depress the concentration of the MCFA and did not increase the *trans* biohydrogenation intermediates. Taken together, this seems to support the theory that *trans* biohydrogenation intermediates are responsible for the inhibition of the de novo synthesis of MCFA in mammary gland observed when unsaturated vegetable oils are fed to lactating ruminants (Glasser et al., 2008).

The failure of castor oil to increase the *trans*-18:1 and CLA isomers can be explained by the

lack of substrates to the normal ruminal biohydrogenation pathways, as the overwhelming FA of castor oil is the 12-OH,*c*9-18:1 (≈80%). The biohydrogenation of 12-OH,*c*9-18:1 is not well studied but apparently produces hydroxy-18:0 without the generation of *trans* and conjugated intermediates (Wallace et al., 2007). However, it has been reported that the gut bacterium *Lactobacillus plantarum* can generate CLA isomers from 12-OH,*c*9-18:1 (Ando et al., 2004), and in vitro data suggest that 12-OH,*c*9-18:1 might modulate the rumen biohydrogenation of regular C18 unsaturated FA leading to the increased concentration of *trans*-18:1 and CLA isomers (Morales et al., 2012). Thus, our hypothesis that castor oil supplementation should increase the CLA content in milk is not supported by the data reported here.

Milk from ewes fed castor oil contain several FA not present in the milk from the other treatments, including small amount of 12-OH,*c*9-18:1, other minor unidentified FA, and relevant amounts of 12-OH-18:0 and 12-oxo-18:0. The tentative identification of these FAs was done by analogy with the GC-MS identifications done in milk samples from goats fed castor oil (Alves et al., 2017). The presence of 12-OH,*c*9-18:1 in milk is easily explained by the absorption of this FA and its incorporation into milk fat. Increasing levels of unspecified hydroxy-FA in milk from cow fed castor oil have been reported (Robb et al., 1974). More recently, the presence of 12-OH,*c*9-18:1 have been reported in cheese made with milk from dairy goats fed castor oil (Medeiros et al., 2014) and in meat of goat kids (Maia et al., 2012b). This suggests that dietary 12-OH,*c*9-18:1 that reach the duodenum is absorbed and transferred to the tissues as already demonstrated in rodents and in man (Watson and Gordon, 1962; Rao et al., 1969). The 12-OH-18:0 and 12-oxo-18:0 are probably products of the ruminal biohydrogenation of the 12-OH,*c*9-18:1 transferred into milk.

For long that the occurrence of trace amounts of hydroxy- and oxo-FA have been reported in ruminant milk (Keeney et al., 1962; Marquez-Ruiz et al., 2011), however it might differ from those derived from 12-OH,*c*9-18:1 metabolism. The present data shows that the amount of hydroxy- and oxo-FA in milk comprises 6.2% of total FA when ewes are fed castor oil. The technologic and sensorial consequences of using milk with such a high concentration of hydroxy- and oxo-FA in cheese making is not known, although recent data with cheese made with goat milk suggests only minor effects (Pereira et al., 2010; Medeiros et al., 2013a). The potential health effects of consumption of milk and milk products enriched in those hydroxy- and oxo-FA is not known but deserves to be investigated. In fact, some hydroxystearic isomers have been reported to exert a strong antiproliferative effects in human adenocarcinoma and osteosarcoma cell lines (Calonghi et al., 2007), which suggests that milk enriched in hydroxy-FA might be health promoting.

Concluding, castor oil can be used in diets of nursing ewes allowing a productive response similar to canola and sunflower oils and with a positive response in milk fat content. However, contrarily to the other oil sources, castor oil supplementation did not depress the FA (8–14 carbon chain) and did not increase the *trans*-FA in milk, although showed greater hidroxy- and oxo-FA.

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