Effect of the inclusion of quebracho tannins in a diet rich in linoleic acid on milk fatty acid composition in dairy ewes

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ABSTRACT

Despite controversy surrounding the ability of tannins to modulate the fatty acid (FA) profile of ruminant-derived products, reports on this issue are still very limited for dairy sheep. This study was conducted to examine the effect of the inclusion of quebracho tannins in a diet rich in linoleic acid on ewe performance and milk FA composition. Thirty-six lactating ewes were distributed into 6 lots and allocated to 2 treatments (3 lots/treatment): control or quebracho. All sheep received a total mixed ration based on alfalfa hay and a concentrate (forage:concentrate ratio of 40:60) supplemented with 20 g of sunflower oil/kg of dry matter plus 0 (control diet) or 20 g of an extract of quebracho tannins/kg of dry matter (QUE diet). Milk production and composition were analyzed on d 0, 3, 6, 9, 12, 15, 18, 21, 24, and 27 on treatments, and milk FA profile on d 0, 3, 6, 12, 18, and 27. On d 27, samples of rumen fluid were collected for pH, and lactate, ammonia, and volatile FA concentration analysis. Feeding the QUE diet had no apparent effect on animal performance and hardly modified ruminal fermentation characteristics, except for a reduction in the molar proportions of minor volatile FA. Dietary tannins increased the milk concentration of several 18:1 and 18:2 isomers and decreased that of branched-chain FA. Some of these changes were relatively constant throughout the experiment (e.g., *cis*-12 18:1 and *trans*-9,*cis*-12 18:2), whereas others varied over time (e.g., *trans*-10 18:1, which increased gradually with the QUE diet). Significant differences between treatments in *trans*-11 18:1 and *cis*-9,*trans*-11 conjugated linoleic acid were only observed on d 3. Overall, addition of quebracho tannins to a diet rich in linoleic acid did not prove useful to beneficially modify milk FA composition, especially over the long term.

Key words: biohydrogenation, condensed tannin, sheep, *trans* fatty acid

INTRODUCTION

Based on their potential benefits to consumer health, large interest exists in enhancing levels of some health-promoting FA in ruminant-derived products (Chilliard et al., 2007; Vasta and Luciano, 2011). Increased levels may be achieved through nutritional strategies inhibiting ruminal biohydrogenation (BH) of dietary long-chain PUFA or promoting the accumulation in the rumen of conjugated linoleic acid (CLA) precursors, such as *trans*-11 18:1 (vaccenic acid, VA; Lock and Bauman, 2004; Chilliard et al., 2007).

Some in vitro studies have suggested that tannins can exert these effects by impairing rumen microbial BH in general and the conversion of 18:1 to 18:0 in particular (Khiaosa-Ard et al., 2009; Vasta et al., 2009a; Buccioni et al., 2011). However, this has rarely been validated in vivo (Vasta et al., 2009b; Khiaosa-ard et al., 2011) and most experiments seem to point in a different direction (Kronberg et al., 2007; Benchaar and Chouinard, 2009; Cabiddu et al., 2009). In any case, reports on this issue are still limited and inconsistent.

In a recent study, Toral et al. (2011) examined the effect of the addition of hydrolysable and condensed tannins (namely, a proportional mixture of quebracho and chestnut tannin extracts; 10 g/kg of DM) to a diet containing sunflower oil (as a source of linoleic acid to increase ruminal formation of VA; Honkanen et al., 2012). Tannin supplementation had no effect on animal performance and was not able to enhance milk VA and CLA enrichment above that achieved with the presence of sunflower oil in the diet (Hervás et al., 2008; Toral et al., 2010). Given the great diversity in the structural features and, consequently, in the reactivity of different tannins (Mueller-Harvey, 2006), the lack of effects was attributed to the type and amount in the diet, which encourages further research to investigate the potential of these phenolic compounds to favorably modify ruminal BH and milk fat composition. Therefore, this study was conducted to examine the effect of the addition of an extract of condensed tannins (quebracho), at a higher inclusion rate (20 g/kg of DM), to a diet rich in linoleic acid, on lactating ewe performance and milk composition, with special focus on the FA profile.

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MATERIALS AND METHODS

Animals, Experimental Diets, and Management

Thirty-six Assaf ewes (BW = 81.2 ± 1.49 kg; DIM = 43 ± 0.7) were housed in individual tiestalls and distributed according to milk production, BW, DIM, number of lactation, and voluntary feed intake, in 6 balanced lots of 6 animals each. Lots were then randomly assigned to 2 experimental treatments (3 lots/treatment): control or supplemented with quebracho tannins (QUE).

Diets, prepared weekly, consisted of a TMR based on alfalfa hay (particle size >4 cm) and a concentrate (forage:concentrate ratio of 40:60), supplemented with 20 g of sunflower oil/kg of DM plus 0 (control diet) or 20 (QUE diet) g of a commercial extract of quebracho tannins (TanicolMOX; Agrovin SA, Alcázar de San Juan, Spain; declared tannin content >900 g/kg of DM). The ingredients and chemical composition of diets, which included molasses to reduce selection of dietary components, are given in Table 1. Before commencing the experiment, all animals received the control TMR during a 2-wk adaptation period. Clean water was always available and fresh diets were offered daily ad libitum at 0900 and 1900 h.

Ewes were milked at approximately 0830 and 1830 h in a 1×10 stall milking parlor (DeLaval, Madrid,

Spain). The trial lasted for 4 wk, and was conducted in accordance with Spanish Royal Decree 1201/2005 for the protection of animals used for experimental purposes.

Measurements and Sampling Procedures

Diets. Samples of offered and refused diets were collected every 4 d, stored at -30°C , and freeze dried.

Milk. Individual daily milk yield was recorded on d 0, 3, 6, 9, 12, 15, 18, 21, 24, and 27, both at morning and evening milkings. With the same frequency, milk samples were collected from each animal and stored at 4°C with a preservative (natamycin) until analyzed for fat, CP, lactose, and TS content. Milk FA composition was determined in unpreserved samples from each experimental lot that were collected on d 0, 3, 6, 12, 18, and 27, composited according to individual milk yield, and stored at -30°C until analysis.

Rumen Fluid. On d 29 of the experiment, ewes were milked and given free access to morning rations for 1 h. Thereafter, feeds were removed and 3 h later, samples of rumen fluid were collected from each ewe using a stomach tube. Immediately after collection, the fluid was strained through 2 layers of muslin cloth, the pH was measured, and 4 mL was acidified with 4 mL of 0.2 M HCl for ammonia concentration determination.

Table 1. Ingredients and chemical composition of the experimental diets¹

Item	Diet ²		SED ³	P-value ⁴
	Control	QUE		
Ingredient (g/kg of DM)				
Dehydrated alfalfa hay	396	389	—	—
Whole corn grain	181	177	—	—
Whole wheat grain	122	120	—	—
Soybean meal solvent 44% CP	147	144	—	—
Beet pulp, pellets	67	66	—	—
Molasses, liquid	40	39	—	—
Feed supplement ⁵	26	26	—	—
Sunflower oil ⁶	20	20	—	—
Quebracho tannin extract ⁷	0	20	—	—
Chemical composition (g/kg of DM)				
OM	901	905	3.1	NS
CP	173	170	3.2	NS
NDF	279	276	13.4	NS
ADF	181	178	10.9	NS
Ether extract	44	45	3.3	NS

¹For each experimental diet, n = 5.

²QUE = TMR containing 20 g of sunflower oil/kg of DM plus 20 g of a quebracho tannin extract/kg of DM.

³Standard error of the difference.

⁴NS indicates $P > 0.10$.

⁵Contained (g/kg): salts [NaHCO_3 (458.3), CaCO_3 (250.0), and NaCl (125.0)], minerals and vitamins (104.2), and wheat bran (62.5).

⁶Contained (g/100 g of FA): 16:0 (5.5), 18:0 (4.4), *cis*-9 18:1 (36.4), and *cis*-9,*cis*-12 18:2 (50.3).

⁷Condensed tannins extracted from quebracho (*Schinopsis lorentzii*) and reported to contain >900 g/kg (TanicolMOX; Agrovin SA, Alcázar de San Juan, Spain).

Further aliquots of 4 and 0.8 mL of ruminal fluid were taken for the analysis of lactic acid and VFA (deproteinized with 0.5 mL of 20 g of metaphosphoric acid/L and 4 g of crotonic acid/L in 0.5 M HCl). All samples were stored at -30°C until analysis.

Chemical Analysis

Diets. Diet samples were analyzed for DM (ISO, 1999a; ISO 6496:1999), ash (ISO, 2002; ISO 5984:2002), and CP (ISO, 2009; ISO 5983-2:2009) content. Neutral detergent fiber and ADF were determined by the methods described by Mertens (2002) and AOAC International (2006; method 973.18), respectively, using an Ankom²⁰⁰⁰ fiber analyzer (Ankom Technology Corp., Macedon, NY). Neutral detergent fiber was assayed with sodium sulfite and α -amylase and expressed with residual ash (the latter also for ADF). The content of ether extract in the diets was determined by the Ankom filter bag technology (AOCS, 2008; procedure Am 5-04).

Milk. Fat, CP, lactose, and TS concentrations were determined by infrared spectrophotometry (ISO, 1999b; ISO 9622:1999), using a MilkoScan FT6000 (Foss Electric A/S, Hillerød, Denmark). For milk FA composition analysis, lipids in 1 mL of milk were extracted using diethyl ether and hexane (5:4, vol/vol) and transesterified to FA methyl esters (**FAME**) using freshly prepared methanolic sodium methoxide, as outlined previously (Shingfield et al., 2003). Methyl esters were separated and quantified using a gas chromatograph (Agilent 7890A GC System; Agilent Technologies Inc., Santa Clara, CA) equipped with a flame-ionization detector and a 100-m fused silica capillary column (0.25-mm i.d., 0.2- μm film thickness; CP-SIL 88, CP7489; Varian Ibérica SA, Madrid, Spain) and hydrogen as the carrier gas. The total FAME profile in a 2- μL sample volume at a split ratio of 1:50 was determined using a temperature gradient program (Shingfield et al., 2003). Isomers of 18:1 were further resolved in a separate analysis under isothermal conditions at 170°C (Shingfield et al., 2003). Peaks were identified based on retention time comparisons with authentic standards (from Nu-Chek Prep., Elysian, MN; Sigma-Aldrich, Madrid, Spain; and Larodan Fine Chemicals AB, Malmö, Sweden), chromatograms reported in the literature (e.g., Shingfield et al., 2003; Kramer et al., 2008), and by comparison with milk samples for which the FA composition was determined based on gas chromatography analysis of FAME and GC-MS analysis of corresponding 4,4-dimethyloxazoline derivatives.

Rumen Fluid. Ammonia and lactic acid concentrations were determined by colorimetric methods and

VFA by gas chromatography, using crotonic acid as an internal standard, as reported in Toral et al. (2011).

Statistical Analysis

All analyses were performed using the SAS software package (version 9.2; SAS Institute Inc., Cary, NC). Data on DMI, milk yield and composition, as well as FA composition, were analyzed for repeated measures using PROC MIXED and assuming a covariance structure fitted on the basis of Schwarz's Bayesian information model fit criterion. The statistical model included the fixed effects of diet, time, and their interaction and the initial record measured at 0 wk (covariate). One-way ANOVA, using the PROC MIXED of SAS, was applied to data on rumen fermentation (pH, and ammonia, lactate, and VFA concentrations). In all cases, the lot was nested within the diet and used as the error term to contrast the effect of quebracho supplementation. Differences were declared significant at $P < 0.05$ and considered as tendencies toward significance at $P < 0.10$. Least squares means (adjusted for the covariance) are reported.

RESULTS

Inclusion of quebracho tannins in the diet supplemented with sunflower oil had no effect ($P > 0.10$) on any of the animal performance measurements monitored in this study. However, with the exception of DMI, all others (milk yield, and fat, protein, lactose, and TS proportions and yields) varied significantly over time (see Table 2).

Feeding the QUE diet caused only minor changes in ruminal fermentation characteristics. No differences ($P > 0.10$) were observed between ewes fed the control (first value of the following pairs) or the QUE (last value) diet for pH (6.77 vs. 6.76), or lactate (0.89 vs. 0.82 mmol/L) and total VFA (110.1 vs. 107.8 mmol/L) concentrations, or molar proportions of acetate, propionate, and butyrate (61.3 vs. 61.4, 19.9 vs. 20.3, and 15.7 vs. 15.8 mol/100 mol, respectively). Although the concentration of ammonia was numerically higher in control ewes (160.4 vs. 135.4 mg/L), it did not attain statistical significance. Only the molar proportion of minor VFA that mostly originated from deamination of some amino acids (namely, the sum of isobutyrate, isovalerate, valerate, and caproate) was reduced due to the addition of quebracho tannins (3.0 vs. 2.6 mol/100 mol; $P < 0.05$).

As reported in Tables 3 and 4, the inclusion of quebracho extract was not effective ($P > 0.10$) in modifying the concentration of major classes of milk FA according

Table 2. Dry matter intake, and milk yield and composition in ewes fed a TMR containing 20 g of sunflower oil/kg of DM plus 0 (control diet) or 20 g of a quebracho tannin extract/kg of DM (QUE diet)

Item	Diet		SED ¹	P-value ²		
	Control	QUE		D	T	D × T
DMI (g/d)	2,956	2,949	193.6	NS	NS	NS
Yield (g/d)						
Milk	2,242	2,278	47.4	NS	***	NS
Fat	122.8	124.3	3.48	NS	***	NS
CP	111.4	112.1	2.28	NS	***	NS
Lactose	115.7	117.8	2.64	NS	***	NS
TS	369.7	374.9	5.25	NS	***	NS
Composition (g/100 g)						
Fat	5.55	5.53	0.254	NS	***	NS
CP	4.97	4.94	0.047	NS	***	NS
Lactose	5.14	5.16	0.011	NS	***	NS
TS	16.55	16.54	0.289	NS	***	NS

¹Standard error of the difference for diet (D) effects.²Probability of significant effects due to experimental D, time on D (T), and interaction (D × T). NS indicates $P > 0.10$.*** $P < 0.001$.

to the degree of saturation [with average ($\pm$ SE) contents of 65.4 ± 0.22 , 27.3 ± 0.16 , and 7.3 ± 0.08 g/100 g of FA for SFA, MUFA, and PUFA, respectively]. However, the mean content of branched-chain FA was reduced with the QUE diet (1.80 vs. 1.67 g/100 g of FA; $P < 0.05$), whereas that of odd-chain FA remained unchanged ($P > 0.10$).

On the contrary, tannin supplementation altered the proportions of major classes of milk FA according to their origin ($P < 0.05$). Thus, a greater concentration of FA originated from mammary uptake of plasma lipids (i.e., with >16 carbons) in ewes fed the QUE diet in comparison with the control diet (average contents of 45.0 vs. 43.1 g/100 g of FA), which derived from increases in several 18:1 and 18:2 isomers and in most very long-chain n-6 PUFA (Table 4). Some of these changes were relatively constant throughout the experiment (e.g., *cis*-12 18:1, *trans*-9,*cis*-11 CLA, and *trans*-9,*cis*-12 18:2), whereas others were only evident after a long time on the QUE diet (e.g., *trans*-5, *trans*-10, and *trans*-13 and -14 18:1). In contrast, differences between treatments in *trans*-11 18:1 were only observed on d 3 of the experiment (Figure 1). The significant interaction for milk 18:0 concentration was limited to a transient decrease on d 6 in ewes fed the control diet.

In agreement with the increase in preformed FA, the addition of QUE slightly reduced ($P < 0.05$) the relative proportions of FA synthesized de novo in the mammary gland (i.e., with <16 carbons; -3.9%) or derived in equal amounts from both sources (i.e., with 16 carbons; -4.3%). Consistent reductions were also observed for most particular FA with 16 or fewer carbons affected by tannin supplementation (e.g., 10:0, *cis*-9 and *trans*-9 12:1, *cis*-12 14:1, 16:0, and *cis*-14 16:1).

DISCUSSION

The controversy in the literature surrounding the ability of tannins to modulate the FA profile of ruminant-derived products (Vasta and Luciano, 2011) is probably due to the great diversity in the structural features and, consequently, in the reactivity of these secondary compounds (Mueller-Harvey, 2006). Although attempts to attribute different nutritional effects to generic tannin classes have not proven useful (Mueller-Harvey, 2006), widespread perception exists that condensed tannins are less toxic and more efficient than hydrolysable ones, probably based on the ability of rumen microorganisms to degrade hydrolysable tannins (McSweeney et al., 2001; Makkar, 2003).

The dose-dependent effect of tannins is another major issue because of the difficulty in selecting concentrations to positively affect a particular parameter without conferring a negative response in others (e.g., in overall diet utilization; Makkar, 2003). The amount of quebracho tannin extract used in this experiment was chosen with 2 criteria: the ability to modulate ruminal BH of linoleic acid without impairing animal performance, and the practicality under farm conditions. Some in vitro studies (Khiaosa-Ard et al., 2009; Vasta et al., 2009a; Buccioni et al., 2011) have found a desirable effect of tannins on ruminal BH at very high doses (up to 18% of the DM incubated). However, such high levels would be impractical in terms of animal feeding.

In the present experiment, inclusion of 20 g of quebracho tannin extract/kg of diet DM had no apparent detrimental effects on animal performance, which is consistent with studies using low to moderate doses of condensed tannin extracts in ruminants (Benchaar et

Table 3. Saturated FA composition of milk from ewes fed a TMR containing 20 g of sunflower oil/kg of DM plus 0 (control diet) or 20 g of a quebracho tannin extract/kg of DM (QUE diet)

FA (g/100 g of FA)	Diet		SED ¹	P-value ²		
	Control	QUE		D	T	D × T
4:0	2.288	2.315	0.1476	NS	**	NS
5:0	0.019	0.016	0.0017	NS	*	†
6:0	2.596	2.608	0.0641	NS	***	NS
7:0	0.038	0.033	0.0025	NS	†	NS
8:0	2.860	2.893	0.1020	NS	**	NS
9:0	0.068	0.059	0.0049	NS	†	NS
10:0	8.878	8.365	0.1735	*	NS	NS
11:0	0.091	0.091	0.0112	NS	NS	NS
12:0	4.941	4.651	0.1108	†	NS	NS
iso 13:0	0.013	0.012	0.0013	NS	NS	NS
anteiso 13:0	0.008	0.008	0.0003	NS	**	NS
4,8,12-trimethyl 13:0	0.075	0.067	0.0104	NS	*	NS
14:0	9.893	9.634	0.1707	NS	*	NS
iso 14:0	0.084	0.090	0.0039	NS	**	NS
15:0	0.769	0.778	0.0210	NS	NS	NS
iso 15:0	0.161	0.141	0.0064	*	†	NS
anteiso 15:0	0.342	0.338	0.0085	NS	NS	NS
16:0	21.667	20.679	0.2435	**	*	NS
iso 16:0	0.253	0.306	0.0174	*	**	NS
3,7,11,15-tetramethyl 16:0	0.023	0.023	0.0011	NS	†	NS
17:0	0.486	0.505	0.0063	*	†	NS
iso 17:0	0.294	0.288	0.0090	NS	*	NS
anteiso 17:0	0.418	0.398	0.0080	*	**	NS
18:0	9.108	9.419	0.3400	NS	**	*
iso 18:0	0.054	0.056	0.0024	NS	†	NS
10-oxo-18:0	0.022	0.025	0.0052	NS	NS	NS
13-oxo-18:0	0.006	0.006	0.0009	NS	NS	NS
19:0	0.090	0.092	0.0031	NS	NS	NS
20:0	0.186	0.195	0.0027	*	**	NS
21:0	0.057	0.056	0.0015	NS	NS	NS
22:0	0.127	0.131	0.0029	NS	*	*
23:0	0.054	0.053	0.0027	NS	*	†
24:0	0.036	0.036	0.0040	NS	**	NS

¹Standard error of the difference for diet (D) effects.²Probability of significant effects due to experimental D, time on D (T), and their interaction (D × T). NS indicates $P > 0.10$.† $P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

al., 2008; Toral et al., 2011), and may be related to the lack of negative effects on ruminal fermentation. On the other hand, diet supplementation with quebracho was not able to beneficially modify milk FA composition, especially in the long term. Results on long-chain FA concentration in milk fat suggest that, in contrast with some studies in dairy cows (Benchaar and Chouinard, 2009) and ewes (Toral et al., 2011), the higher amount of quebracho extract used in this trial (2% DM) would have affected ruminal BH. Unfortunately, the goal of promoting the accumulation of *trans*-11 18:1 in the rumen to enhance milk CLA concentration would have been achieved only transiently, as pairwise comparisons between treatments for each sampling day indicate that the QUE diet only increased the relative milk content of *trans*-11 18:1 and its Δ^9 -desaturation product, *cis*-9,*trans*-11 CLA, on d 3 of the experiment (Figure 1). These time-dependent changes might be attributed to the effect of condensed tannins inhibiting the last step

of ruminal BH (i.e., the conversion of *trans*-11 18:1 to 18:0), as shown in in vitro assays (Khiaosa-Ard et al., 2009; Vasta et al., 2009a), as well as to a potential adaptation of rumen microbiota to the presence of tannins in the diet (McSweeney et al., 2001).

Results for milk FA composition were also compatible with changes in the putative pathways of ruminal BH of *cis*-9,*cis*-12 18:2, the most abundant FA in the sunflower oil included in the basal diet. On the one hand, feeding the QUE diet induced stable increases in milk fat concentration of *trans*-9,*cis*-12 18:2 and *cis*-12 18:1. These metabolites would derive from a minor BH pathway in which the *cis*-9 double bond of *cis*-9,*cis*-12 18:2 is initially isomerized and subsequently reduced in the rumen (Shingfield et al., 2010). The increase in *cis*-12 18:1 may also be accounted for by the direct reduction of the *cis*-9 double bond of *cis*-9,*cis*-12 18:2 or the hydrogenation of some 18:2 intermediates, such as *trans*-10,*cis*-12 or *cis*-10,*cis*-12 CLA (McKain et al.,

Table 4. Unsaturated FA composition of milk from ewes fed a TMR containing 20 g of sunflower oil/kg of DM plus 0 (control diet) or 20 g of a quebracho tannin extract/kg of DM (QUE diet)

FA (g/100 g of FA)	Diet		SED ¹	P-value ²		
	Control	QUE		D	T	D × T
MUFA						
<i>cis</i> -9 10:1	0.247	0.234	0.0067	NS	***	NS
<i>cis</i> -9 12:1	0.079	0.073	0.0026	*	*	NS
<i>trans</i> -9 12:1	0.036	0.034	0.0006	*	*	NS
<i>cis</i> -7 14:1	0.016	0.015	0.0011	NS	NS	NS
<i>cis</i> -9 14:1	0.139	0.134	0.0021	†	*	NS
<i>cis</i> -12 14:1	0.065	0.060	0.0019	*	NS	NS
<i>trans</i> -5 14:1	0.013	0.013	0.0012	NS	*	NS
<i>trans</i> -6 14:1	0.017	0.021	0.0010	*	NS	NS
<i>trans</i> -9 14:1	0.007	0.008	0.0005	NS	***	†
<i>trans</i> -5 15:1	0.125	0.126	0.0052	NS	***	NS
<i>trans</i> -6, -7 15:1	0.018	0.019	0.0007	NS	†	NS
<i>cis</i> -7 16:1	0.296	0.306	0.0032	*	NS	NS
<i>cis</i> -9 16:1	0.565	0.541	0.0097	†	*	NS
<i>cis</i> -11 16:1	0.010	0.011	0.0015	NS	***	NS
<i>cis</i> -14 16:1	0.111	0.093	0.0047	*	†	NS
<i>trans</i> -5 16:1	0.011	0.011	0.0014	NS	NS	NS
<i>trans</i> -6, -8 16:1	0.122	0.126	0.0122	NS	**	NS
<i>trans</i> -9 16:1	0.347	0.408	0.0189	*	*	NS
<i>cis</i> -9 17:1	0.154	0.145	0.0048	NS	NS	NS
<i>trans</i> -7 17:1	0.037	0.039	0.0010	*	NS	NS
<i>cis</i> -9 18:1	13.983	14.463	0.4336	NS	†	NS
<i>cis</i> -10 + <i>trans</i> -15 18:1	0.345	0.343	0.0266	NS	NS	NS
<i>cis</i> -11 18:1	0.472	0.483	0.0264	NS	NS	NS
<i>cis</i> -12 18:1	0.534	0.631	0.0339	*	NS	NS
<i>cis</i> -13 18:1	0.094	0.095	0.0023	NS	NS	NS
<i>cis</i> -15 18:1	0.097	0.096	0.0043	NS	NS	NS
<i>cis</i> -16 18:1	0.093	0.099	0.0045	NS	NS	NS
<i>trans</i> -4 18:1	0.034	0.035	0.0012	NS	NS	NS
<i>trans</i> -5 18:1	0.029	0.031	0.0019	NS	*	*
<i>trans</i> -6, -7, -8 18:1	0.518	0.541	0.0188	NS	NS	†
<i>trans</i> -9 18:1	0.463	0.455	0.0192	NS	NS	NS
<i>trans</i> -10 18:1	1.106	1.701	0.2532	†	NS	†
<i>trans</i> -11 18:1	4.670	5.233	0.2593	†	*	NS
<i>trans</i> -12 18:1	0.604	0.635	0.0221	NS	NS	NS
<i>trans</i> -13, -14 18:1	0.651	0.683	0.0150	NS	NS	*
<i>trans</i> -16 + <i>cis</i> -14 18:1	0.389	0.402	0.0166	NS	NS	NS
<i>cis</i> -9 19:1	0.015	0.016	0.0012	NS	†	NS
<i>cis</i> -9 20:1	0.020	0.024	0.0022	NS	**	NS
<i>cis</i> -11 20:1	0.012	0.011	0.0008	NS	†	NS
<i>trans</i> -11 20:1	0.032	0.036	0.0036	NS	†	NS
<i>cis</i> -15 24:1	0.008	0.009	0.0011	NS	**	NS
PUFA						
<i>cis</i> -9, <i>cis</i> -12 18:2	3.151	3.218	0.0831	NS	NS	NS
<i>cis</i> -9, <i>cis</i> -15 18:2	0.052	0.052	0.0021	NS	NS	NS
<i>cis</i> -9, <i>trans</i> -12 18:2	0.078	0.079	0.0016	NS	NS	NS
<i>cis</i> -9, <i>trans</i> -13 18:2	0.330	0.288	0.0253	NS	†	NS
<i>trans</i> -9, <i>cis</i> -12 18:2	0.054	0.063	0.0032	†	†	NS
<i>trans</i> -9, <i>trans</i> -12 18:2	0.016	0.016	0.0008	NS	NS	NS
9,14 18:2	0.130	0.123	0.0034	†	**	NS
10,14 18:2	0.077	0.079	0.0063	NS	†	NS
<i>trans</i> -11, <i>cis</i> -15 18:2	0.100	0.102	0.0093	NS	*	NS
<i>trans</i> -11, <i>trans</i> -15 18:2	0.017	0.019	0.0015	NS	NS	†
<i>cis</i> -9, <i>trans</i> -11 CLA ³	1.936	2.099	0.1048	NS	*	NS
<i>trans</i> -9, <i>cis</i> -11 CLA	0.034	0.041	0.0022	*	NS	NS
<i>trans</i> -10, <i>cis</i> -12 CLA	0.013	0.015	0.0017	NS	**	NS
<i>trans</i> -11, <i>trans</i> -13 CLA	0.012	0.012	0.0007	NS	**	NS
other <i>trans-trans</i> CLA ⁴	0.057	0.058	0.0016	NS	NS	NS
<i>cis</i> -6, <i>cis</i> -9, <i>cis</i> -12 18:3	0.064	0.061	0.0038	NS	NS	NS
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 18:3	0.624	0.637	0.0118	NS	**	NS
<i>cis</i> -9, <i>trans</i> -11, <i>cis</i> -15 18:3	0.019	0.019	0.0023	NS	†	*
<i>trans</i> -9 <i>trans</i> -12 <i>cis</i> -15 18:3	0.012	0.013	0.0017	NS	NS	NS
<i>cis</i> -11, <i>cis</i> -14 20:2	0.020	0.022	0.0008	*	NS	NS

Continued

Table 4 (Continued). Unsaturated FA composition of milk from ewes fed a TMR containing 20 g of sunflower oil/kg of DM plus 0 (control diet) or 20 g of a quebracho tannin extract/kg of DM (QUE diet)

FA (g/100 g of FA)	Diet		SED ¹	P-value ²		
	Control	QUE		D	T	D × T
<i>cis</i> -8, <i>cis</i> -11, <i>cis</i> -14 20:3	0.032	0.037	0.0023	†	**	NS
<i>cis</i> -11, <i>cis</i> -14, <i>cis</i> -17 20:3	0.015	0.015	0.0006	NS	**	NS
<i>cis</i> -5, <i>cis</i> -8, <i>cis</i> -11, <i>cis</i> -14 20:4	0.159	0.173	0.0048	*	NS	NS
<i>cis</i> -5, <i>cis</i> -8, <i>cis</i> -11, <i>cis</i> -14, <i>cis</i> -17 20:5	0.045	0.045	0.0021	NS	***	NS
<i>cis</i> -7, <i>cis</i> -10, <i>cis</i> -13, <i>cis</i> -16 22:4	0.021	0.024	0.0008	*	**	NS
<i>cis</i> -4, <i>cis</i> -7, <i>cis</i> -10, <i>cis</i> -13, <i>cis</i> -16 22:5	0.011	0.011	0.0014	NS	NS	NS
<i>cis</i> -7, <i>cis</i> -10, <i>cis</i> -13, <i>cis</i> -16, <i>cis</i> -19 22:5	0.100	0.101	0.0065	NS	***	NS
<i>cis</i> -4, <i>cis</i> -7, <i>cis</i> -10, <i>cis</i> -13, <i>cis</i> -16, <i>cis</i> -19 22:6	0.038	0.042	0.0044	NS	***	NS

¹Standard error of the difference for diet (D) effects.

²Probability of significant effects due to experimental D, time on D (T), and their interaction (D × T). NS indicates $P > 0.10$.

³Contains *trans*-7,*cis*-9 conjugated linoleic acid (CLA) and *trans*-8,*cis*-10 CLA as minor components.

⁴Sum of *trans*-7,*trans*-9 CLA + *trans*-8,*trans*-10 CLA + *trans*-9,*trans*-11 CLA + *trans*-10,*trans*-12 CLA.

† $P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

2010; Honkanen et al., 2012). In all cases, these results suggest changes in the rumen bacterial community of QUE-fed ewes favoring populations able to metabolize *cis*-9,*cis*-12 18:2 via mechanisms other than isomerization of the *cis*-12 double bond (McKain et al., 2010; Honkanen et al., 2012).

On the other hand, feeding the QUE diet induced time-dependent increases in other BH intermediates, such as *trans* 18:1 isomers that were particularly evident in the *trans*-10 18:1 (Figure 1) and indicate shifts in rumen bacterial populations and, consequently, in BH pathways due to the long-term consumption of tannins (McSweeney et al., 2001). Previous studies in lactating sheep fed condensed tannins have shown inconsistent results for *trans*-10 18:1 variations in milk fat, with no differences (Toral et al., 2011), increases (Abbeddou et al., 2011), or even slight decreases (Cabiddu et al., 2009), which could be attributed not only to the type and dose of tannins but also to the basal diet composition (Vasta et al., 2009b). Accumulation in milk of this *trans* FA has also been reported in ewes and cows fed marine lipids (Chilliard et al., 2007; Toral et al., 2010). However, the marked shift toward *trans*-10 18:1 formation at the expense of *trans*-11 18:1 in response to marine lipids is often accompanied by milk fat depression (Shingfield et al., 2010; Toral et al., 2010), whereas dietary quebracho tannins resulted in *trans*-11 18:1 concentrations always being much higher than those of *trans*-10 18:1 (Figure 1) and no effects on milk fat content. The health-related value of the increase in *trans*-10 18:1 in milk from ewes fed the QUE diet is not clear because of the uncertain involvement of this *trans* FA in cardiovascular disease (Gebauer et al., 2011).

The increase in *trans*-9,*cis*-11 CLA might also be of relevance due to its potential antilipogenic effect in the mammary gland (Perfield et al., 2007; Shingfield et al.,

2010), but, given its low concentration, no reduction in milk fat content was observed in this experiment. Although some studies have reported concomitant increases in *trans*-9,*cis*-11 CLA and *trans*-10 18:1 milk concentrations (Shingfield et al., 2010; Toral et al., 2010), no apparent association between both *trans* FA profiles was observed in the present study. All these changes in milk FA composition might be attributed to an effect of quebracho tannins on rumen microbiota, as supported by the variations in milk branched-chain FA, which are largely derived from bacteria leaving the rumen (Fievez et al., 2012).

Surprisingly, feeding the QUE diet also affected the concentration of most very long-chain n-6 PUFA. However, the fact that these FA are usually detected in very low concentrations in rumen digesta (Shingfield et al., 2003; Toral et al., 2012) would probably rule out a direct effect of tannins on their rumen outflow as the principal factor responsible for the increase. Under normal feeding conditions, PUFA with 20 and 22 carbons in milk fat would mostly derive from mammary uptake of FA synthesized and recirculated by other body tissues. In the particular case of very long-chain n-6 PUFA, whose hepatic synthesis involves alternating steps of elongation and desaturation of *cis*-9,*cis*-12 18:2 from dietary origin (Miyazaki and Ntambi, 2008), their increase might be mediated by a systemic action of QUE supplementation attributable to the low molecular weight phenolics included in the commercial extract. These compounds would be absorbed from the digestive tract (Makkar, 2003) and affect lipid metabolism in the liver or other body tissues. Finally, the slight reduction in the relative proportions of FA partially or totally synthesized de novo in the mammary gland (i.e., with 16 or less carbons) with the QUE diet may be explained by a dilution effect or by the changes

Figure 1. Temporal changes in milk *trans*-10 18:1, *trans*-11 18:1, and *cis*-9,*trans*-11 conjugated linoleic acid (CLA; g/100 g of FA) in ewes fed a TMR containing 20 g of sunflower oil/kg of DM plus 0 (control diet; ○) or 20 g of a quebracho tannin extract/kg of DM (QUE diet; ●). Values are the mean from 3 lots of 6 animals per lot; vertical bars represent the standard error of the difference.

in preformed FA, given the inhibitory effect of these metabolites (especially *trans* FA) on mammary de novo synthesis (Shingfield et al., 2010).

CONCLUSIONS

Overall, addition of QUE to a diet rich in linoleic acid did not prove useful to modulate milk FA composition, especially in the long term. Quebracho tannins

are proflavonoids with more compact, less accessible structures than other condensed tannins and, although they can exert beneficial effects in ruminants (Mueller-Harvey, 2006), they do not seem to strongly affect rumen microorganisms involved in BH. Therefore, further research is needed to investigate if other tannins, at practical doses, are more efficient at modulating ruminal biohydrogenation of dietary PUFA.

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