



## PAPER

## Effect of Quebracho tannin extract on soybean and linseed oil biohydrogenation by solid associated bacteria: an *in vitro* study

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### Abstract

An *in vitro* trial was carried out to study the effects of Quebracho tannins extract (QE) on fatty acid profile of rumen solid adherent bacteria (SAB) during the fermentation of diets supplemented with soybean or linseed oil, as sources of linoleic (LA; 18:2 n-6) and  $\alpha$ -linolenic acid ( $\alpha$ -LNA; 18:3 n-3), respectively. Two control diets were prepared using a basal mixture of grass hay [760 g/kg on dry matter (DM)], soybean meal (55 g/kg DM), barley meal (130 g/kg DM), vitamin mineral premix (20 g/kg DM) and 35 g/kg DM of soybean (SOC diet) or linseed oil (LOC diet) as lipid supplement. Other two diets (SOCT and LOCT) were obtained by integrating SOC and LOC with QE (49 g/kg DM). The results confirmed that Quebracho tannins may be an effective method for reducing in SAB the biohydrogenation of LA (17.3 vs 34.5 g/100 g of fatty acid in SOC and SOCT, respectively) and LNA (10.7 vs 21.4 g/100 g of fatty acid in LOC and LOCT, respectively), but not for increasing the rumen accumulation of *cis*9, *trans*11 18:2 (0.77 vs 0.32 g/100 g of fatty acid in SOC and SOCT, respectively; 0.51 vs 0.43 g/100 g of fatty acid in LOC and LOCT, respectively) and *trans*11 18:1 (6.15 vs 3.64 g/100 g of fatty acid in SOC and SOCT, respectively; 5.53 vs 4.47 g/100 g of fatty acid in LOC and LOCT, respectively).

### Introduction

Rumen microbial community is a very complex system, which includes solid (SAB) and

liquid adherent bacteria (LAB). Solid adherent bacteria usually account for more than 70% of the total rumen bacteria (Yang *et al.*, 2001; Legay-Carmier and Bauchart, 1989) and many microbial species belonging to SAB are cellulolytic (Wallace *et al.*, 1999). Their relative proportion in rumen liquor (RL) is highly influenced by forage:concentrate ratio and by the addition of supplements in diet (Yang *et al.*, 2001). Solid adherent bacteria seems to play an interesting role in biohydrogenation (BH) of polyunsaturated fatty acids (PUFA) as linoleic acid (LA; *cis*9 *cis*12 *cis*15 18:2) because the fatty acid (FA) profile of these microorganisms is richer in *cis*9 *trans*11 18:2 [ruminic acid (RA)] than other rumen microbial species (Buccioni *et al.*, 2011, 2012; Vlaemink, 2006b; Kim *et al.*, 2002). Vlaemink *et al.* (2006b) found that total FA content in bacterial dry matter (DM) was 1.6 to 2.8 times higher in SAB than LAB, as a consequence of a lower proportion in SAB of gram-positive bacteria whose FA content in their cell wall was markedly lower than that of gram negative bacteria. This pattern is particularly evident when the diet is supplemented with high level of fat (Czerkawsky, 1976; Vlaemink *et al.*, 2006a). In literature the effect of oils rich in PUFA on rumen microflora and that of condensed tannins on lipid metabolism in ruminants is known, but little information is available on their contemporary effect on rumen lipid BH as a consequence of the disturbing activity on microbial community either of tannins or of the high PUFA amount contained in vegetable oils (Buccioni *et al.*, 2012; Patra and Saxena, 2009; Toral *et al.*, 2013). Therefore, it could be of interest to study the specific response of SAB to the contemporary tannin and vegetable oil challenge. The aim of this trial was to study *in vitro* the effects of Quebracho (*Schinopsis lorentii*) tannin extracts (QE) on FA profile of SAB when diets for ruminants are supplemented with soybean or linseed oil, as sources of LA and  $\alpha$ -linolenic acid ( $\alpha$ -LNA), respectively.

### Materials and methods

#### Feed composition and analysis

The feeds used in the present trial consisted of two basal diets composed of grass hay (760 g/kg DM), soybean meal (55 g/kg DM), barley meal (130 g/kg DM), vitamin mineral premix (20 g/kg DM) supplemented with soybean oil (SOC) or linseed oil (LOC) (35 g/kg DM) and of other two feeds (SOCT, LOCT) obtained by adding to SOC or LOC 49 g/kg DM of QE (containing 456 g of equivalent tannic acid/kg DM).

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Key words: Condensed tannins, Linoleic acid,  $\alpha$ -linolenic acid, *In vitro* rumen fermentation.

Received for publication: 13 January 2014.  
Accepted for publication: 1 July 2014.

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Italian Journal of Animal Science 2014; 13:207  
doi:10.4081/ijas.2014.3267

Feed samples (oven dried at 60°C for 24 h, grinded and 1 mm sieved) were analysed for crude protein (CP), starch, ash and ether extract (EE), according to the 954.01, 985.29, 954.05 and 920.39 procedures of AOAC (1990), respectively. Neutral detergent fibre (NDF) was determined using sodium sulfite and heat stable amylase; acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined in a sequential analysis. All data were expressed inclusive of residual ash (Van Soest *et al.*, 1991). Non protein nitrogen (NPN), soluble protein (SP), acid detergent insoluble protein (ADIP), neutral detergent insoluble protein (NDIP) and non-fibre carbohydrates (NFC) were determined according to Licitra (1996) and Sniffen *et al.* (1992). The chemical composition of the basal diet was (g/kg, all expressed on the DM basis): DM, 861; CP, 150; NPN, 11; SP, 42; EE, 50; NDF, 447; ADF, 276; ADL, 74; ADIP, 10; NDIP, 13; ash, 68; NFC, 419; starch, 113. The tannin content in the extract powder (456 g of equivalent tannic acid/kg DM) was determined according to Burns (1963). Fatty acid profile of feeds, determined according to Buccioni *et al.* (2006), was the following: SOC and SOCT contained respectively 53.75 and 53.69 g/100 g total FA of LA, 21.73 and 21.82 g/100 g total FA of *cis*9 18:1 [oleic acid (OA)], 7.51 and 7.55 g/100 g total FA of LNA; LOC and LOCT contained respectively 16.61 and 16.52 g/100 g total FA of LA, 16.72 and 16.75 g/100 g total FA of OA, 54.31 and 54.34 g/100 g total FA of LNA.

#### Rumen inoculum and fractionation procedure

Four ewes were used to provide rumen con-

tents, using a rumen fluid sampling pump. Animals were managed according to Institutional Animal Care and Use Committee of Florence University. Ewes were fed a basal diet formulated to shape rumen microflora and composed by grass hay (770 g/kg DM), soybean meal (55 g/kg DM) and barley meal (175 g/kg DM); animals had continuous access to water and mineral blocks. After 4 weeks of adaptation period, about 1 L of RL was collected from each ewe on the same day before the morning meal. The RL was immediately mixed and transferred to the laboratory in a thermostatic box (39°C). The RL was filtered through four layers of cheese cloth into a flask under a flux of CO<sub>2</sub> and an aliquot of the RL was buffered (1:3, v:v) by adding an artificial saliva solution (Buccioni *et al.*, 2011). Feeds (2 g DM) were incubated in triplicate with 200 mL of RL. The in batch incubator consisted of a thermostatic chamber (39°C) equipped with thirty-six 300 mL glass fermentation vessels provided with two inlets (one to release gas through a valve and one for the pH probe). Incubation times were 6, 12 and 18 h. The pH was continuously monitored during the fermentation. At the

start of the trial (t=0 min) and at each incubation time, samples of RL (200 mL) were collected in triplicates (the whole content of three fermenters per diet), in order to fractionate RL according to the procedure described by Buccioni *et al.* (2011).

Rumen liquor fractions containing SAB (about 150 mg) were extracted for lipid content according to Folch method (Folch *et al.*, 1957) and FA were methylated using a combination of methods according to Kramer *et al.* (2004): the first step consisted of an alkaline methylation with sodium methylate/methanol (1 mL of 0.5 mol/L sodium methoxide) to esterify glycerides. The second step involved an acid methylation with HCl/methanol (1.5 mL of 5% methanolic HCl, 10 min at 50°C) as catalyst to esterify free fatty acids. Fatty acid methyl esters (FAME) were extracted using n-hexane, with C19:0 methyl ester (Sigma Chemical Co., St. Louis, MO, USA) as the internal standard. Fatty acid methyl esters were separated and identified by gas chromatography (GC) according to Buccioni *et al.* (2010). The GC apparatus (3900 GC; Varian Inc., Palo Alto, CA, USA) was equipped with a flame ionisation detector

(FID) and a capillary column (CP-select for FAME: 100 m 0.25 mm i.d., film thickness 0.20 µm; Varian Inc.). The injector and FID temperatures were set at 270 and 300°C, respectively. The programmed temperature was 40°C for 4 min, increased to 120°C at a rate of 10°C/min, maintained at 120°C for 1 min, increased to 180°C at a rate of 5°C/min, maintained at 180°C for 18 min, increased to 200°C at a rate of 2°C/min, maintained at 200°C for 1 min, increased to 230°C at a rate of 2°C/min and maintained at this last temperature for 19 min. The split ratio was 1:100 and helium was the carrier gas with a flux of 1 mL/min. Standard mix (47792 Supelco; Sigma Chemical Co.) and published isomeric profiles (Destailas *et al.*, 2005) were used to identify the αLNA isomers. Two bacterial acid methyl ester mix [47080-U Supelco (Sigma Chemical Co.); GLC110 (Matreya LLC, Pleasant Gap, PA, USA)] and individual standard for methyl ester of iso C14:0, anteiso C14:0, iso C15:0 and anteiso C17:0 (21-1211-11, 21-1210-11, 21-1312-11 and 21-1415-11; Larodan, Malmo, Sweden) were used to identify branched FA profile. Inter and intra-assay coefficients of variation were cal-

**Table 1. Fatty acid composition of rumen solid adherent bacteria as affected by Quebracho extract tannin integration of feeds containing soybean or linseed oil.**

FA, g/100 g of total FA	Feed				SEM	P		
	SOC	SOCT	LOC	LOCT		Oil	Tannin	Oil×tannin
14:0	0.567 <sup>a</sup>	0.485 <sup>b</sup>	0.623 <sup>c</sup>	0.449 <sup>d</sup>	0.002	<0.0001	<0.0001	<0.0001
15:0	0.747 <sup>a</sup>	0.629 <sup>b</sup>	0.685 <sup>c</sup>	0.650 <sup>d</sup>	0.002	<0.0001	<0.0001	<0.0001
16:0	15.596 <sup>a</sup>	15.481 <sup>b</sup>	12.569 <sup>c</sup>	14.467 <sup>d</sup>	0.014	<0.0001	<0.0001	<0.0001
17:0	0.524 <sup>a</sup>	0.402 <sup>b</sup>	0.535 <sup>c</sup>	0.499 <sup>d</sup>	0.002	<0.0001	<0.0001	<0.0001
14:0 <i>iso</i>	0.010	0.010	0.010	0.010	0.002	0.9655	0.0560	0.0667
15:0 <i>iso</i>	0.143	0.049	0.081	0.068	0.024	0.401	0.038	0.1127
15:0 <i>ante</i>	0.435 <sup>a</sup>	0.312 <sup>b</sup>	0.525 <sup>c</sup>	0.365 <sup>d</sup>	0.002	<0.0001	<0.0001	<0.0001
16:0 <i>iso</i>	0.302 <sup>a</sup>	0.271 <sup>b</sup>	0.357 <sup>c</sup>	0.258 <sup>d</sup>	0.001	<0.0001	<0.0001	<0.0001
17:0 <i>iso</i>	0.028 <sup>a</sup>	0.008 <sup>b</sup>	0.028 <sup>a</sup>	0.016 <sup>c</sup>	0.001	<0.0001	<0.0001	<0.0001
17:0 <i>ante</i>	0.185 <sup>a</sup>	0.210 <sup>b</sup>	0.203 <sup>c</sup>	0.253 <sup>d</sup>	0.001	<0.0001	<0.0001	<0.0001
18:0	27.482 <sup>a</sup>	18.914 <sup>b</sup>	33.887 <sup>c</sup>	24.658 <sup>d</sup>	0.045	<0.0001	<0.0001	<0.0001
16:1	0.408 <sup>a</sup>	0.429 <sup>b</sup>	0.491 <sup>c</sup>	0.339 <sup>d</sup>	0.003	0.2894	<0.0001	<0.0001
18:1 <i>cis</i> 9	12.068 <sup>a</sup>	14.856 <sup>b</sup>	11.997 <sup>a</sup>	12.241 <sup>a</sup>	0.638	0.0459	0.0258	0.047
18:1 <i>cis</i> 11	0.732 <sup>a</sup>	0.982 <sup>b</sup>	0.639 <sup>c</sup>	0.628 <sup>d</sup>	0.002	<0.0001	<0.0001	<0.0001
18:1 <i>cis</i> 12	0.116 <sup>a</sup>	0.081 <sup>b</sup>	0.124 <sup>c</sup>	0.108 <sup>d</sup>	0.002	<0.0001	<0.0001	<0.0001
18:1 <i>cis</i> 15	0.278 <sup>a</sup>	0.233 <sup>b</sup>	0.546 <sup>c</sup>	0.369 <sup>d</sup>	0.002	<0.0001	<0.0001	<0.0001
18:1 <i>trans</i> 9	0.165 <sup>a</sup>	0.127 <sup>b</sup>	0.232 <sup>c</sup>	0.173 <sup>a</sup>	0.003	<0.0001	<0.0001	0.0004
18:1 <i>trans</i> 10	0.326 <sup>a</sup>	0.272 <sup>b</sup>	0.369 <sup>c</sup>	0.269 <sup>b</sup>	0.009	0.0286	<0.0001	0.014
18:1 <i>trans</i> 11	6.148 <sup>a</sup>	3.645 <sup>b</sup>	5.534 <sup>c</sup>	4.469 <sup>d</sup>	0.203	0.6094	<0.0001	0.0017
18:1 <i>trans</i> 12	0.311 <sup>a</sup>	0.203 <sup>b</sup>	0.493 <sup>c</sup>	0.344 <sup>d</sup>	0.003	<0.0001	<0.0001	<0.0001
18:2 <i>cis</i> 9 <i>cis</i> 12	17.309 <sup>a</sup>	34.539 <sup>b</sup>	7.452 <sup>c</sup>	10.708 <sup>d</sup>	0.038	<0.0001	<0.0001	<0.0001
CLA, total	0.767 <sup>a</sup>	0.320 <sup>b</sup>	0.514 <sup>c</sup>	0.425 <sup>d</sup>	0.011	<0.0001	<0.0001	<0.0001
18:3 <i>cis</i> 9 <i>cis</i> 12 <i>cis</i> 15	1.744 <sup>a</sup>	2.642 <sup>b</sup>	10.690 <sup>c</sup>	21.361 <sup>d</sup>	0.010	<0.0001	<0.0001	<0.0001
18:3 <i>cis</i> 9 <i>trans</i> 11 <i>cis</i> 15	0.107 <sup>a</sup>	0.145 <sup>b</sup>	0.190 <sup>c</sup>	0.299 <sup>d</sup>	0.040	0.0009	0.0100	0.0452
18:3 <i>trans</i> 11 <i>cis</i> 9 <i>cis</i> 15	0.061	0.073	0.081	0.084	0.008	0.010	0.010	0.010

FA, fatty acid; SOC, basal diet supplemented with soybean oil; SOCT, SOC supplemented with Quebracho tannin extract; LOC, basal diet supplemented with linseed oil; LOCT, LOC supplemented with Quebracho tannin extract; CLA, conjugated linoleic acid. Number of samples for each treatment at any time=3. <sup>ab</sup>Within a row, means with different superscript letters are significantly different at P<0.05.

culated by using a reference standard butter (CRM 164; Community Bureau of Reference, Bruxelles, Belgium) and detection threshold of FA was 0.01 g/100 g of FA. Geometrical and positional isomers of conjugated linoleic acid (CLA) were separated and identified by silver ion high-performance liquid chromatography (Sehat *et al.*, 1998). The stationary phase was a silver ion column (ChromSpher lipid column, 4.6 mm i.d. 250 mm stainless steel, 5  $\mu$  particle size; Varian Inc.). The mobile phase was a fresh mixture of acetonitrile in hexane (0.1% v/v). The injection loop was 50  $\mu$ L. The solvent flow rate was standardised at 1 mL min<sup>-1</sup> and UV was set at 233 nm. Conjugated linoleic acid mix standard (Sigma Chemical Co.) and high purity individual *cis*9, *trans*11 and *trans*10, *cis*12-18:2 (Matreya LLC) were used to identify the CLA isomers of interest. Since a reliable

internal standard for CLA is not yet available, the quantitative measurements were performed through a calibration and data were referred to the GC results. All FA composition results are expressed as g/100 g of FA.

### Statistical analysis

Data were processed by GLM of SAS (1999) using the following linear model with fixed factors: kind of oil, tannin presence, and incubation time as well as their interaction:

$$y_{ijz} = \mu + O_i + Q_j + T_z + O_i \times Q_j + O_i \times T_z + Q_j \times T_z + O_i \times Q_j \times T_z + e_{ijz}$$

where  $y_{ijz}$  is the observation;  $\mu$  is the overall mean;  $O_i$  the kind of oil ( $i$ =soybean or linseed oil);  $Q_j$  the presence of tannins ( $j$ =tannins or no tannins);  $T_z$  the incubation time ( $z$ =6h,12h,

18 h), and  $e_{ij}$  the residual error. For the sake of simplicity, only one level of probability ( $P < 0.05$ ) was adopted to test the significance of differences between means.

### Results and discussion

During the whole period of fermentation, pH values did not vary among fermenters containing different feeds ( $6.74 \pm 0.2$ , pH units). Oil supplementation, tannin integration and their interaction significantly affected the FA profile of SAB (Table 1).

In the present trial the inclusion of QE in feeds induced a decrease of 18:0 (stearic acid) production in SAB fraction, as a consequence of a decrease in BH of LA and of LNA whose

**Table 2. Effect of tannins integration of feeds containing soybean or linseed oil on main C18 fatty acids in rumen solid adherent bacteria, at different sampling times during the in vitro fermentation.**

FA, g/100 g total FA	Feed	Time, h				SEM
		0	6	12	18	
18:0	SOC	4.8 <sup>a</sup> $\alpha$	24.26 <sup>a</sup> $\beta$	25.61 <sup>a</sup> $\gamma$	32.73 <sup>a</sup> $\gamma$	0.01
	SOCT	4.79 <sup>a</sup> $\alpha$	16.44 <sup>b</sup> $\beta$	18.54 <sup>b</sup> $\gamma$	21.90 <sup>b</sup> $\gamma$	
	LOC	4.6 <sup>b</sup> $\alpha$	28.65 <sup>c</sup> $\beta$	35.33 <sup>c</sup> $\gamma$	37.61 <sup>c</sup> $\gamma$	
	LOCT	4.65 <sup>b</sup> $\alpha$	19.90 <sup>d</sup> $\beta$	22.93 <sup>d</sup> $\gamma$	31.12 <sup>d</sup> $\gamma$	
18:1 <i>trans</i> 10	SOC	nd	0.24 <sup>a</sup> $\alpha$	0.26 <sup>a</sup> $\alpha$	0.47 <sup>b</sup> $\beta$	0.03
	SOCT	nd	0.14 <sup>b</sup> $\alpha$	0.25 <sup>a</sup> $\beta$	0.42 <sup>a</sup> $\gamma$	
	LOC	nd	0.30 <sup>a</sup> $\alpha$	0.35 <sup>b</sup> $\alpha$	0.45 <sup>b</sup> $\beta$	
	LOCT	nd	0.15 <sup>b</sup> $\alpha$	0.23 <sup>a</sup> $\beta$	0.42 <sup>a</sup> $\gamma$	
18:1 <i>trans</i> 11	SOC	nd	3.56 <sup>a</sup> $\alpha$	5.26 <sup>a</sup> $\beta$	9.65 <sup>a</sup> $\gamma$	0.10
	SOCT	nd	2.14 <sup>b</sup> $\alpha$	3.25 <sup>b</sup> $\beta$	5.60 <sup>b</sup> $\gamma$	
	LOC	nd	3.99 <sup>c</sup> $\alpha$	4.97 <sup>c</sup> $\beta$	8.83 <sup>c</sup> $\gamma$	
	LOCT	nd	2.65 <sup>d</sup> $\alpha$	3.72 <sup>d</sup> $\beta$	7.04 <sup>d</sup> $\gamma$	
18:2 <i>cis</i> 9 <i>cis</i> 12	SOC	53.59 <sup>a</sup> $\alpha$	21.53 <sup>a</sup> $\beta$	16.24 <sup>a</sup> $\gamma$	14.16 <sup>a</sup> $\delta$	0.01
	SOCT	53.55 <sup>a</sup> $\alpha$	36.30 <sup>b</sup> $\beta$	36.33 <sup>b</sup> $\beta$	31.14 <sup>b</sup> $\gamma$	
	LOC	16.54 <sup>b</sup> $\alpha$	9.24 <sup>c</sup> $\beta$	7.54 <sup>c</sup> $\gamma$	5.54 <sup>c</sup> $\delta$	
	LOCT	16.47 <sup>b</sup> $\alpha$	13.01 <sup>d</sup> $\beta$	11.15 <sup>d</sup> $\gamma$	7.92 <sup>d</sup> $\delta$	
CLA tot	SOC	nd	0.21 <sup>a</sup> $\alpha$	0.32 <sup>a</sup> $\beta$	1.77 <sup>a</sup> $\gamma$	0.10
	SOCT	nd	0.26 <sup>b</sup> $\alpha$	0.37 <sup>b</sup> $\beta$	0.32 <sup>b</sup> $\gamma$	
	LOC	nd	0.43 <sup>c</sup> $\alpha$	0.54 <sup>c</sup> $\beta$	0.56 <sup>c</sup> $\beta$	
	LOCT	nd	0.35 <sup>d</sup> $\alpha$	0.39 <sup>b</sup> $\beta$	0.53 <sup>d</sup> $\gamma$	
18:3 <i>cis</i> 9 <i>cis</i> 12 <i>cis</i> 15	SOC	7.43 <sup>a</sup> $\alpha$	0.21 <sup>a</sup> $\beta$	3.57 <sup>a</sup> $\gamma$	2.05 <sup>a</sup> $\delta$	0.01
	SOCT	7.48 <sup>a</sup> $\alpha$	0.26 <sup>a</sup> $\beta$	3.36 <sup>a</sup> $\gamma$	4.30 <sup>b</sup> $\delta$	
	LOC	54.25 <sup>b</sup> $\alpha$	13.13 <sup>b</sup> $\beta$	10.51 <sup>b</sup> $\gamma$	8.34 <sup>c</sup> $\delta$	
	LOCT	54.28 <sup>b</sup> $\alpha$	25.92 <sup>c</sup> $\beta$	23.44 <sup>c</sup> $\gamma$	14.73 <sup>d</sup> $\delta$	
18:3 <i>cis</i> 9 <i>trans</i> 11 <i>cis</i> 15	SOC	nd	0.15 <sup>a</sup> $\alpha$	0.10 <sup>a</sup> $\beta$	0.07 <sup>a</sup> $\beta$	0.01
	SOCT	nd	0.12 <sup>a</sup> $\alpha$	0.20 <sup>b</sup> $\beta$	0.11 <sup>b</sup> $\alpha$	
	LOC	nd	0.11 <sup>a</sup>	0.10 <sup>a</sup>	0.09 <sup>a</sup> $\beta$	
	LOCT	nd	0.43 <sup>b</sup> $\alpha$	0.39 <sup>c</sup> $\beta$	0.37 <sup>c</sup> $\beta$	
18:2 <i>trans</i> 11 <i>cis</i> 15	SOC	nd	0.07	0.05	0.04	0.02
	SOCT	nd	0.08	0.07	0.07	
	LOC	nd	0.10	0.08	0.07	
	LOCT	nd	0.09	0.07	0.07	

FA, fatty acid; SOC, basal diet supplemented with soybean oil; SOCT, SOC supplemented with Quebracho tannin extract; LOC, basal diet supplemented with linseed oil; LOCT, LOC supplemented with Quebracho tannin extract; nd, not detected; CLA, conjugated linoleic acid. Number of samples for each treatment at any time=3. Means with different superscript Latin letters within the same column are significantly different at  $P < 0.05$ ; means with different superscript Greek letters within the same row are significantly different at  $P < 0.05$ .

content in SOCT and LOCT fermenters was constantly higher than that in fermenters containing control feeds (Table 1). The decrease of BH extent was confirmed also by the lowest content of total CLA, *trans*11 18:1 [vaccenic acid (VA)], *cis*9, *trans*11, *cis*15 18:3 (conjugated linolenic acid) and *trans*11, *cis*15 18:2 (vaccelenic acid) during the whole fermentation period in RL incubated with feeds integrated with tannin extract (Tables 1 and 2). However, data reported in literature are often referred to rumen mixed bacteria and seem to be rather controversial. Some studies demonstrated that the addition of QE tannin to a barley and lucerne hay based diet for sheep can induce an accumulation of VA in the rumen mixed bacteria and in the intramuscular fat (Vasta *et al.*, 2010), suggesting an inhibition of the last step of the BH of LA. In an *in vitro* study, in which

the rumen content was not fractionated, Kronenberg *et al.* (2007) observed that QE decreased LNA hydrogenation by nearly 43%. In a similar experiment, Khiaosa-Ard *et al.* (2009) reported that QE tannins are able to inhibit the last step of the BH of LNA. In the present study, the inhibition effect of QE tannins seems to act at the first steps of the BH process carried out by SAB and not in the final step, as reported in the previous trials. On the other hand, in this experiment unsaturated vegetable oils (soybean and linseed oil) were incubated alone or together with QE tannins, whereas in the previous experiments the effect of QE tannins was evaluated using a different lipid source since a basal diet was formulated including linseed grain as main lipid source. The different form of lipid supply (oil *vs* grains) may have affected the microbial

activity. The total CLA production was reduced by QE (Table 1) and the presence of tannins influenced the relative proportion of the single CLA isomers (Table 3) leading to an increase of the relative percentage of RA especially in SOCT, which was richer in LA. Conversely, other CLA isomers, such as *trans*, *trans* CLA and *trans*10, *cis*12 CLA decreased over time. As a consequence, *trans*10 18:1 as well, which are produced mainly by the BH of *trans*10, *cis*12 CLA, decreased. These results were in agreement with previous studies, which reported that the FA profile of SAB is characterised by a lower content of *trans*10 18:1 and 18:2 isomers (Buccioni *et al.*, 2011). This effect could be of interesting in the field application, because when soybean oil was used as energy supplement in high concentrate diets, *trans*10 18:1 and *trans*10, *cis*12 CLA content is usually

**Table 3. Effect of tannins integration of feeds containing soybean or linseed oil on main conjugated linoleic acid isomers in rumen solid adherent bacteria, at different sampling times during the *in vitro* fermentation.**

FA, g/100 g total CLA	Feed	Time, h			SEM
		6	12	18	
18:2 <i>trans</i> 10 <i>trans</i> 12	SOC	2.18 <sup>aα</sup>	1.84 <sup>aβ</sup>	3.21 <sup>aγ</sup>	0.06
	SOCT	1.87 <sup>bα</sup>	1.58 <sup>bβ</sup>	1.29 <sup>bβ</sup>	
	LOC	4.11 <sup>cα</sup>	20.3 <sup>cβ</sup>	38.7 <sup>cγ</sup>	
	LOCT	2.60 <sup>dα</sup>	1.44 <sup>dβ</sup>	2.86 <sup>dγ</sup>	
18:2 <i>trans</i> 9 <i>trans</i> 11	SOC	5.98 <sup>a</sup>	6.74 <sup>a</sup>	6.02 <sup>a</sup>	0.05
	SOCT	5.11 <sup>bα</sup>	5.84 <sup>bβ</sup>	6.22 <sup>bγ</sup>	
	LOC	2.42 <sup>cα</sup>	1.91 <sup>cβ</sup>	0.99 <sup>cγ</sup>	
	LOCT	1.47 <sup>dα</sup>	3.23 <sup>dβ</sup>	4.31 <sup>dγ</sup>	
18:2 <i>trans</i> 8 <i>trans</i> 10	SOC	5.53 <sup>aα</sup>	4.39 <sup>aβ</sup>	4.46 <sup>aβ</sup>	0.08
	SOCT	2.72 <sup>bα</sup>	1.98 <sup>bβ</sup>	1.50 <sup>bγ</sup>	
	LOC	0.81 <sup>cα</sup>	2.49 <sup>cβ</sup>	5.49 <sup>cγ</sup>	
	LOCT	0.59 <sup>dα</sup>	2.20 <sup>dβ</sup>	1.48 <sup>dγ</sup>	
18:2 <i>trans</i> 7 <i>cis</i> 9	SOC	1.84 <sup>aα</sup>	1.17 <sup>aβ</sup>	1.52 <sup>aγ</sup>	0.07
	SOCT	0.22 <sup>b</sup>	0.24 <sup>b</sup>	0.34 <sup>b</sup>	
	LOC	0.00 <sup>cα</sup>	0.09 <sup>cα</sup>	0.65 <sup>cβ</sup>	
	LOCT	0.00 <sup>cα</sup>	0.84 <sup>dβ</sup>	0.49 <sup>bγ</sup>	
18:2 <i>cis</i> 11 <i>trans</i> 13	SOC	7.53 <sup>aα</sup>	9.97 <sup>aβ</sup>	5.17 <sup>aγ</sup>	0.09
	SOCT	0.00 <sup>bα</sup>	8.93 <sup>bβ</sup>	5.90 <sup>bγ</sup>	
	LOC	10.51 <sup>cα</sup>	5.48 <sup>cβ</sup>	0.00 <sup>cγ</sup>	
	LOCT	8.72 <sup>dα</sup>	10.20 <sup>dβ</sup>	1.97 <sup>dγ</sup>	
18:2 <i>trans</i> 10 <i>cis</i> 12	SOC	8.73 <sup>aα</sup>	6.77 <sup>aβ</sup>	19.70 <sup>aγ</sup>	0.11
	SOCT	15.53 <sup>bα</sup>	9.05 <sup>bαβ</sup>	8.93 <sup>bβ</sup>	
	LOC	4.81 <sup>cα</sup>	2.76 <sup>cβ</sup>	0.55 <sup>cγ</sup>	
	LOCT	6.29 <sup>dα</sup>	12.90 <sup>dβ</sup>	1.34 <sup>dγ</sup>	
18:2 <i>cis</i> 9 <i>trans</i> 11	SOC	24.33 <sup>aα</sup>	46.41 <sup>aβ</sup>	45.31 <sup>aγ</sup>	0.12
	SOCT	34.40 <sup>bα</sup>	59.33 <sup>bβ</sup>	65.72 <sup>bγ</sup>	
	LOC	18.32 <sup>cα</sup>	30.91 <sup>cβ</sup>	42.21 <sup>cγ</sup>	
	LOCT	49.14 <sup>dα</sup>	43.60 <sup>dβ</sup>	41.83 <sup>dγ</sup>	
18:2 <i>trans</i> 7 <i>trans</i> 9	SOC	29.22 <sup>aα</sup>	2.99 <sup>aβ</sup>	2.75 <sup>aβ</sup>	0.09
	SOCT	6.80 <sup>bα</sup>	1.10 <sup>bβ</sup>	0.89 <sup>bβ</sup>	
	LOC	31.30 <sup>cα</sup>	5.49 <sup>cβ</sup>	1.68 <sup>cγ</sup>	
	LOCT	5.51 <sup>dα</sup>	2.36 <sup>dβ</sup>	1.96 <sup>cγ</sup>	

FA, fatty acid; CLA, conjugated linoleic acid; SOC, basal diet supplemented with soybean oil; SOCT, SOC supplemented with Quebracho tannin extract; LOC, basal diet supplemented with linseed oil; LOCT, LOC supplemented with Quebracho tannin extract. Number of samples for each treatment at any time=3. Means with different superscript Latin letters within the same column are significantly different at P<0.05; means with different superscript Greek letters within the same row are significantly different at P<0.05.

enhanced, as shown by *in vitro* and *in vivo* trials (Mele et al., 2006; Buccioni et al., 2012), increasing the risk of the milk fat depression syndrome. However, this effect should be tested in long term *in vivo* trials with the aim to evaluate the animal response. In literature, several studies reported that the presence of QE in feeds is able to induce a perturbation in rumen mixed bacterial strains with a decrease of the branched chain FA content in RL (Vasta et al., 2010). The ratio between *iso* and *anteiso* odd chain FA was strongly affected by QE, especially in the fermentation of SOCT feed (IAR=15*iso*+17*iso*/15*ante*+17*ante*; SOC=0.275 g/100 g FA vs SOCT=0.109 g/100 g FA P<0.05; LOC=0.149 g/100 g FA vs LOCT 0.139 g/100 g FA P<0.05), suggesting an inhibiting effect of QE tannins on cellulolytic bacterial strains, most of them belonging to SAB fraction (Vlaemink et al., 2006a, 2006b). Vasta et al. (2010) in a previous trial observed a similar trend in the RL of growing lambs when QE was added in the diet.

## Conclusions

Results from this *in vitro* trial showed that QE tannins are able to reduce the extent of LA and LNA BH by rumen bacteria. Hence, this information could be used to set up an effective feeding strategy for reducing hydrogenation of dietary PUFA when diet supplemented with soybean or linseed oil are used to improve FA profile of food from ruminants. However, further investigation is needed in order to better understand the mechanisms of action and the bacterial strains involved.

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