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Effect of dietary saponins from *Quillaja saponaria* L. on fatty acid composition and cholesterol content in muscle *Longissimus dorsi* of lambs

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The purpose of this study was to evaluate the effects of increasing levels of saponins from Quillaja saponaria *on fatty acid (FA) composition and cholesterol content in muscle* Longissimus dorsi *of lambs. A total of 24 Barbarine lambs were assigned to four dietary treatments: control diet (C) consisting of oat hay* ad libitum *and 400 g of concentrate (80% barley, 17.5% soybean meal and 2.5% vitamin and mineral supplement); C diet plus 30 ppm of* Q. saponaria *L. (QS30); C diet plus 60 ppm of* Quillaja *(QS60); C diet plus 90 ppm of* Quillaja *(QS90). Saponin supplementation reduced the concentration of C14:1* cis*-9 ($P = 0.001$) and of its desaturation index ($P = 0.002$). None of the FA intermediates of ruminal biohydrogenation (BH) was affected by* Quillaja *saponin supplementation ($P > 0.05$). The concentration of C20:4n-6 was higher in the meat of animals receiving 60 ppm of* Quillaja *than C and QS30 groups. Supplementing 60 ppm of* Quillaja *reduced the ratio between α-linolenic and linoleic acids compared with the C group ($P = 0.023$). We did not find any significant effect of* Quillaja *saponins on muscle cholesterol level. Further investigations are necessary to assess the metabolic fate of saponins in the rumen and to understand whether there is an effect of saponin on Δ^9-desaturase enzyme activity, ruminal BH and cholesterol metabolism in ruminants. Supplementing up to 90 ppm of* Quillaja *saponins did not produce detrimental effects on the overall meat FA profile.*

Keywords: saponins, fatty acid composition, cholesterol, meat, lamb

Implications

Supplementing up to 90 ppm of *Quillaja saponaria* L. did not affect the overall fatty acid profile and cholesterol content in lamb meat. The lower concentration of C14:1 *cis*-9 in the saponins-fed lambs suggests a possible effect of saponins on Δ^9-desaturase enzyme. Further research is needed.

Introduction

Saponins are secondary metabolites produced mainly by plants and also by lower marine animals and some bacteria (Yoshiki *et al.*, 1998). Their name derives from their ability to form stable foams in aqueous solutions. They are characterized by a sugar moiety that is glycosidically linked to a hydrophobic aglycone (named sapogenin), which may be triterpenoid or steroid in nature (Francis *et al.*, 2002). Saponins may be considered a part of plants' defense system as they have been shown to be antimicrobial, to inhibit mould and to protect plants from insect attacks (Francis *et al.*, 2002).

Many authors have studied the biological properties of saponins on animal and human nutrition (Cheeke, 2000; Francis *et al.*, 2002). It has been reported that saponins when supplemented in diets reduce cholesterol content in blood and tissues in monogastric mammals, such as rats, gerbils and humans (Sidhu and Oakenfull, 1986; Potter *et al.*, 1993; Harris *et al.*, 1997). This cholesterol-reducing effect is attributed to saponins ability to form insoluble complexes (micelles) with sterols (Sidhu and Oakenfull, 1986), such as cholesterol and bile acids. Several feedstuff used for livestock feeding, such as *Yucca schidigera* (Singer *et al.*, 2008; Holtshausen *et al.*, 2009), *Quillaja saponaria* (Holtshausen *et al.*, 2009), *Medicago sativa* (Klita *et al.*, 1996) and *Sapindus saponaria* (Abreu *et al.*, 2004) contain saponins and are used in

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animal nutrition with the purpose of inhibiting methanogenesis in the rumen (Patra and Saxena, 2010). Some studies have shown that saponins can be degraded by ruminal microflora (Gutierrez *et al.*, 1959; Makkar and Becker, 1997). Hitherto, there is no information with regard to the possible effects of dietary saponins on fatty acids (FAs) and cholesterol metabolism in ruminants and their effects on meat quality.

Some FAs present in ruminant products exert favorable effects on human health (McGuire and McGuire, 2000). Increasing levels of polyunsaturated FAs (PUFAs) in human diet reduce the risk of cardiovascular diseases and chronic pathologies (e.g. cancer, diabetes; Simopoulos, 1999). Besides PUFA, interest in conjugated linoleic acids (CLAs), especially in the C18:2 *cis*-9,*trans*-11 (rumenic acid, RA), has incremented considerably over the last years because of its beneficial effects on human health (reduction of carcinogenesis, atherosclerosis and body fat; McGuire and McGuire, 2000). The CLAs are synthesized during biohydrogenation (BH) of dietary C18:2 *cis*-9,*cis*-12 (linoleic acid, LA; Kepler and Tove, 1967), a process which is carried out by the ruminal bacteria.

It has been shown both in *in vivo* studies (Lu and Jorgensen, 1987; Wallace *et al.*, 1994; Klita *et al.*, 1996) and in *in vitro* ruminal fermentation systems (Makkar *et al.*, 1998; Wang *et al.*, 1998) that saponins have pronounced antiprotozoal activity. One of the potential consequences of antiprotozoal effect is the reduction of the predation by protozoa on rumen bacteria, thus modifying ruminal ecosystem. Therefore, it is plausible to suppose an effect of saponin supplementation on the appearance of FAs synthesized by bacteria during the BH pathway. However, so far, there are no studies on the possible effects of saponin on BH, with consequent effect on muscle FA composition. In addition, considering the interaction between saponins and cholesterol in other animal species, including humans, we have hypothesized an effect of saponins on cholesterol content in muscle *Longissimus dorsi* of lambs.

The aim of this study was to evaluate the effects of increasing levels of sapogenins present in a preparation of *Q. saponaria* on FA profile and cholesterol level in lambs.

Material and methods

Experimental design, animals and management

The experiment was carried out from April to June 2008 at the National Institute of Agricultural Research of Tunisia (INRAT, Tunisia). A total of 24 male Barbarine lambs, 5- to 6-month-old and with average initial live weight 18.6 ± 1.98 kg, were assigned to four dietary groups, each consisting of six animals. All groups received the same basal diet consisting of oat hay *ad libitum* and 400 g of concentrate (80% barley, 17.5% soybean meal and 2.5% mineral and vitamin supplement). Groups QS30, QS60 and QS90 were supplemented with 30, 60 or 90 ppm of a *Q. saponaria* preparation, respectively. The *Quillaja* saponins were administered as a water solution drench, prepared by dissolving the opportune amount of *Quillaja* extract in tap water. The *Q. saponaria* extract used in this experiment was purchased from Sigma Aldrich (batch: 024K2505, Santiago, Chile, USA) and it contained 20% sapogenin. Therefore, the sapogenin content administered to the groups QS30, QS60 and QS90 was 6, 12 and 18 mg per kg of feed (concentrate + hay) consumed, respectively. The control group (C) did not receive the *Quillaja* water solution. Lambs were allowed to the following experimental treatment: 15 days adaptation, 57 days growth trial, 4 days adaption to metabolic cages, 5 days digestibility measurement and 4 days resting (receiving the above diets). Data referring to the digestion and growth performance were presented in a companion paper by Nasri *et al.* (2010). At the end of the experimental period, animals were slaughtered in an experimental abattoir. After carcass refrigeration at 4°C for 24 h, the muscle *L. dorsi* was excised, wrapped in aluminum foil, vacuum packed and stored at −20°C until analysis. Meat FAs and cholesterol content were analyzed at the Sezione di Scienze Zootecniche, University of Pisa.

FAs analysis in the L. dorsi *muscle*

Total lipids of *L. dorsi* were extracted with Folch's method (1957) from 8 g of minced meat. FA methyl esters (FAMEs) were prepared by the base-catalyzed *trans*-methylation (Christie, 1982). Before methylation, C9:0 and C23:0 FAMEs were added together as internal GC 8000 Top standards. The FAMEs were determined by a ThermoQuest (Milan, Italy) gas-chromatograph (GC) apparatus equipped with a flame ionization detector (FID) and a high polar fused silica capillary column (WCOT fused silica CP-Select CB for FAMEs Varian, Middelburg, Netherland; 100 m × 0.25 mm i.d.; film thickness 0.25 μm). Helium was used as the carrier gas at a flow of 1 ml/min. The split ratio was 1 : 80. The GC conditions were: the oven temperature was programed at 150°C and held for 1 min, then increased up to 175°C at a rate of 0.8°C/min, held for 14 min, then increased up to 188°C at 2°C/min, held for 18 min, and then increased up to 230°C at a rate of 2°C/min, held for 13 min. The injector and detector temperatures were set at 270°C and 300°C, respectively. Meat FAs' results are expressed as percentage of total identified FAs.

Feedstuff analysis

Total lipids of hay and concentrate were extracted with chloroform–methanol, according to Folch's method (1957) The FAMEs were prepared by the base-catalyzed *trans*-methylation as described by Christie (1982). Feed FAs analysis was performed as described for meat samples. Feed FAs' profile is reported in Table 1 and the results are expressed as percentage of total identified FAs.

Cholesterol analysis

For cholesterol analysis, we have adopted the method described by Sander *et al.* (1989). The extracted total lipids (100 mg) were weighed in a 20 ml test tube, dried under nitrogen flow and then kept in vacuum and in the dark overnight. To this 500 μl of Betulin in chloroform (1 mg/ml) was added as an internal standard, and then 10 ml of 1N KOH in methanol was added and the mixture was vortexed for 30 s to make it free of dispersed fat particles. The tubes were shaken at room temperature for 18 to 20 h to allow the formation of a saponified

Table 1 *FA composition of control diet (% of total FAs)*

	Feed	
FA	Hay	Concentrate
C10:0	1.59	0.19
C12:0	1.49	0.20
C14:0	3.43	0.57
C15:0	0.70	0.11
C16:0	29.38	18.99
C16:1 *cis*-7	0.08	ND
C16:1 *cis*-9	0.48	0.11
C18:0	4.79	2.85
C18:1 *cis*-9	15.63	28.77
C18:1 *cis*-11	0.64	0.93
C18:2n-6	20.26	41.77
C18:3n-3	7.12	0.15
C20:0	1.22	0.28
C21:0	0.11	0.51
C20:4n-6	1.92	0.27
C22:0	1.28	0.28
C24:0	0.78	0.19
C24:1	0.12	0.10

FA = fatty acid; ND = not detectable.

fraction. Distilled water (10 ml) was then added to the saponified mixture, which was transferred to another test tube. The unsaponifiable fraction was extracted three times with 10 ml of diethyl ether, and the pooled diethyl ether extracts were washed twice with 5 ml of 0.5 N KOH and twice with 5 ml of saturated NaCl solution. After drying with anhydrous sodium sulfate for 1 h at 4°C, the extract was filtered, dried under vacuum using a rotary evaporator and then the dried material was kept for one night in the dark, in a glass bowl containing hygroscopic salts and under vacuum: this procedure allows the complete dryness of the samples before measuring the weight of the extracted lipids. The unsaponifiable fraction was then recovered in a solution of hexane : 2-propanol mixture (4 : 1 v/v) and stored at −20°C until analysis. Cholesterol was determined after silylation of unsaponifiable matter. Silylation solution was composed of a pyridine solution of hexamethyldisilazane and trimethylchlorosilane (5/2/1 v/v/v, respectively; Sweeley *et al.*, 1963) of the silylated sample. Derivatization was completed in 20 min at 40°C. About 0.4 μl was injected at a split ratio of 1 : 100. Trimethysilyl derivatives were identified and quantified by using a GC equipped with FID and an apolar 25 m × 0.25 mm i.d. capillary column according to Boselli *et al.* (2005). The injector and detector temperatures were set up at 325°C. The oven temperature was programed at 250°C and increased to 325°C at 3°C/min, with final temperature of 325°C held for 15 min. Helium was used as the carrier gas with a flow rate of 0.75 ml/min.

Statistical analysis

Data were analyzed by ANOVA as a completely randomized design and included in the model treatment effects and experimental error. Individual animals were considered as experimental units. When ANOVA was significant ($P < 0.05$), means were separated by the Pairwise comparison. The data were analyzed by the software MINITAB version 14.0.

Results

Effect of saponin supplementation on muscle FA composition and cholesterol content

Table 2a and b, show the effects of increasing levels of saponins on the FA profile and on the desaturation indexes (DIs) of lamb meat. The concentration of C14:0, C16:0 and C18:0 in meat was similar for the four groups of lambs (Table 2a). The concentration of total saturated FAs (SFAs) was unaffected by dietary treatments (Table 2b). The concentrations of *iso*-BCFA (branched chain FAs), *anteiso*-BCFA and total BCFA were not influenced by saponin supplementation ($P > 0.05$). None of the individual BCFA was affected ($P > 0.05$). The *iso*-BCFA/*anteiso*-BCFA ratio was similar in the meat of all experimental groups ($P = 0.225$; Table 2b).

When saponins were added to the diet, the concentration of C14:1 *cis*-9 in meat was lower ($P = 0.001$), whereas C16:1 *cis*-9 and C18:1 *cis*-9 were not affected by saponin supplementation ($P > 0.05$; Table 2a).

None of the FAs arising from ruminal BH was affected by saponin supplementation ($P > 0.05$). Neither the total *trans* C18:1 nor the individual *trans* C18:1 were influenced by saponin supplementation ($P > 0.05$). The concentration of RA was similar between the four groups of animals (Table 2a). The sum of the C18:1 *trans* and C18:2 with at least one double bound in *trans* configuration was not influenced ($P > 0.05$) by saponin supplementation (Table 2b).

The concentration of LNA (α-linolenic acid) and LA did not differ among the four treatments ($P > 0.05$). The ratio LNA/LA was lower ($P < 0.05$) in the group receiving 60 ppm of *Quillaja* than the C group (0.053 *v.* 0.083, respectively), and was found at intermediate value in the meat of lambs from the QS30 and QS90 groups (0.067 and 0.079, respectively; Table 2b). The concentration of C20:4n-6 in the meat of animals receiving 60 ppm of *Quillaja* was twice (2.22% of total FA) compared with that of meat from the C and QS30 groups (0.99% and 1.29% of total FA, respectively). The concentration of this FA in the meat of animals from the QS90 group was intermediate of the values (1.33% of total FA) of the other three groups. The concentration of C20:3n-6, C20:5n-3, C22:4n-6 and C22:5n-3 was unaffected by *Quillaja* supplement (Table 2a).

The concentrations of total UFAs (unsaturated FAs), PUFAs, medium-chain FAs and long-chain FAs were not influenced by saponin supplementation. Saponin supplementation did not affect ($P > 0.05$) the PUFA/SFA ratio (Table 2b).

The total DI, calculated as described by Kelsey *et al.* (2003) (product of Δ^9-desaturfase/[product of Δ^9-desaturase + substrate of Δ^9-desaturase]), was not affected by the dietary treatments ($P > 0.05$). The DI C14:1 *cis*-9/(C14:0 + C14:1 *cis*-9) was lower ($P < 0.005$) in QS30, QS60 and QS90 groups than in C group. The DI C16:1 *cis*-9/(C16:0 + C16:1 *cis*-9), the DI C18:1 *cis*-9/(C18:0 + C18:1 *cis*-9) and the DI RA/(vaccenic acid; VA + RA) were not influenced by saponin supplementation ($P > 0.05$; Table 2b).

Table 2a *Effect of saponin supplementation on* Longissimus dorsi *muscle individual FAs (% of total FAs)*

	Treatments*					
FA	C	QS30	QS60	QS90	s.e.m.	*P*-value
C10:0	0.21	0.18	0.18	0.18	0.008	0.367
C12:0	0.17	0.15	0.14	0.14	0.014	0.831
C14:0	2.20	1.99	1.78	1.83	0.151	0.233
C15:0	0.37	0.34	0.37	0.35	0.012	0.757
C16:0	23.70	22.52	21.63	22.86	0.337	0.181
C17:0	1.04	1.03	1.07	1.02	0.016	0.710
C18:0	21.46	22.84	22.16	24.36	0.567	0.325
C20:0	0.13	0.16	0.17	0.15	0.013	0.814
C14:0 *iso*	0.03	0.01	0.01	0.03	0.003	0.092
C15:0 *iso*	0.13	0.12	0.12	0.12	0.004	0.961
C16:0 *iso*	0.14	0.13	0.12	0.13	0.005	0.808
C17:0 *iso*	0.50	0.55	0.61	0.55	0.019	0.257
C18:0 *iso*	0.12	0.15	0.14	0.13	0.009	0.612
C15:0 *anteiso*	0.15	0.13	0.17	0.14	0.006	0.209
C17:0 *anteiso*	0.52	0.51	0.52	0.51	0.011	0.979
C14:1 *cis*-9	0.057^{A}	0.032^{B}	0.031^{B}	0.030^{B}	0.003	0.001
C16:1 *trans*-9	0.03	0.03	0.04	0.06	0.010	0.248
C16:1 *cis*-7	0.31	0.28	0.32	0.28	0.011	0.457
C16:1 *cis*-9	1.07	0.99	0.92	0.91	0.028	0.196
C16:1 *trans*-14	**0.027**	**0.008**	**0.012**	**0.007**	**0.004**	**0.160**
C17:1 *cis*-9	0.56	0.54	0.58	0.48	0.019	0.256
C18:1 *cis*-9	36.86	36.79	34.97	34.05	0.493	0.105
C18:1 *cis*-11	0.89	1.01	1.03	0.91	0.041	0.559
C18:1 *cis*-12	0.18	0.16	0.27	0.19	0.018	0.135
C18:1 *cis*-13	**0.036**	**0.004**	**0.031**	**0.047**	**0.007**	**0.221**
C18:1 *cis*-15	**0.027**	**0.010**	**0.008**	**0.032**	**0.004**	**0.097**
C18:1 *trans*-6,*trans*-8	0.22	0.24	0.27	0.24	0.009	0.349
C18:1 *trans*-9	0.23	0.24	0.24	0.26	0.006	0.241
C18:1 *trans*-10	0.30	0.30	0.39	0.34	0.020	0.362
C18:1 *trans*-11	0.90	0.78	0.81	1.03	0.041	0.116
C18:1 *trans*-12	0.31	0.33	0.36	0.35	0.014	0.675
C18:1 *trans*-15	0.11	0.15	0.13	0.13	0.007	0.401
C18:1 *trans*-16	0.18	0.15	0.18	0.22	0.011	0.162
C18:2 *trans*-8,*cis*-13	0.11	0.04	0.06	0.12	0.013	0.057
C18:2 *cis*-9,*trans*-12	**0.028**	**0.000**	**0.009**	**0.026**	**0.005**	**0.110**
C18:2 *trans*-11,*cis*-15	0.09	0.07	0.06	0.08	0.013	0.417
C18:2 *cis*-9,*trans*-11	0.28	0.24	0.26	0.30	0.016	0.686
C18:2 *cis*-9,*cis*-12	4.22	4.91	6.51	4.68	0.579	0.057
C18:3 *cis*-9,*cis*-12,*cis*-15	0.36	0.33	0.34	0.39	0.026	0.868
C20:1 *cis*-11	0.05	0.02	0.04	0.07	0.009	0.210
C20:3n-6	0.09	0.16	0.18	0.15	0.014	0.143
C20:4n-6	0.99^{b}	1.29^{b}	2.22^{a}	1.33^{ab}	0.262	0.027
C20:5n-3	0.09	0.11	0.27	0.17	0.030	0.154
C22:4n-6	0.06	0.03	0.06	0.07	0.011	0.494
C22:5n-3	0.22	0.26	0.43	0.36	0.033	0.088

FAs = fatty acids.
*Treatment: C = lambs receiving control diet; QS30 = lambs receiving C diet plus 30 ppm of *Quillaja*; QS60 = lambs receiving C diet plus 60 ppm of *Quillaja*; QS90 = lambs receiving C diet plus 90 ppm of *Quillaja*.
^{a,b}Different letters within the same row indicate significant differences ($P < 0.05$).
^{A,B}Different letters within the same row indicate significant differences ($P < 0.01$).

The effect of saponin supplementation on meat cholesterol content is shown in Table 3. The supplementation of saponins did not influence muscle cholesterol content ($P > 0.05$). The cholesterol content in meat ranged between 47.91 and 61.32 mg/100 g of muscle.

Discussion

Effect of saponins on muscle FA composition

In our study, we have focused on meat FA profile with particular emphasis on FAs arising from metabolism of rumen

Table 2b *Effect of saponin supplementation on* Longissimus dorsi *muscle FA indices (% of total FAs)*

	Treatments*					
FA	C	QS30	QS60	QS90	s.e.m.	*P*-value
SFA[1]	51.01	50.76	49.18	52.61	0.725	0.443
BCFA[2]	1.69	1.65	1.76	1.70	0.035	0.759
Iso-BCFA[3]	0.92	0.97	1.02	0.96	0.024	0.607
Anteiso-BCFA[4]	0.77	0.67	0.74	0.75	0.021	0.428
Iso-BCFA/*anteiso*-BCFA	1.20	1.49	1.40	1.28	0.053	0.225
Total *trans* C18:1[5]	2.25	2.18	2.37	2.57	0.082	0.370
Total *trans*[6]	2.53	2.23	2.48	2.83	0.096	0.180
LNA/LA	0.083[a]	0.067[ab]	0.053[b]	0.079[ab]	0.004	0.023
UFA[7]	48.71	49.00	50.56	47.09	0.718	0.422
PUFA[8]	6.55	7.23	10.21	7.68	0.538	0.074
MCFA[9]	31.09	29.18	28.36	29.46	0.402	0.098
LCFA[10]	68.68	70.60	71.46	70.34	0.407	0.095
PUFA/SFA	0.130	0.142	0.211	0.151	0.012	0.081
Total DI[11]	0.44	0.44	0.44	0.41	0.006	0.320
DI C14:1 *cis*-9/(C14:0 + C14:1 *cis*-9)	0.025[A]	0.016[B]	0.017[B]	0.016[B]	0.002	0.002
DI 16:1 *cis*-9/(C16:0 + C16:1 *cis*-9)	0.04	0.04	0.04	0.04	0.001	0.638
DI C18:1 *cis*-9/(C18:0 + C18:1 *cis*-9)	0.63	0.63	0.61	0.58	0.008	0.202
DI RA/(VA + RA)	0.24	0.24	0.24	0.22	0.011	0.848

FAs = fatty acids; SFA = saturated FAs; BCFA = branched chain FAs; LNA = α-linolenic acid; LA = linoleic acid; UFA = unsaturated FAs; PUFA = polyunsaturated FAs; MCFA = medium-chain FAs; LCFA = long-chain FAs; DI = desaturation index; RA = rumenic acid; VA = vaccenic acid.
*Treatment: C = lambs receiving control diet; QS30 = lambs receiving C diet plus 30 ppm of *Quillaja*; QS60 = lambs receiving C diet plus 60 ppm of *Quillaja*; QS90 = lambs receiving C diet plus 90 ppm of *Quillaja*.
[a,b]Different letters within the same row indicate significant differences ($P < 0.05$).
[A,B]Different letters within the same row indicate significant differences ($P < 0.01$).
[1]Sum of SFA: C8:0; C10:0; C12:0; C14:0; C15:0; C16:0; C17:0; C18:0; C20:0.
[2]Sum of *iso*- and *anteiso*- BCFA: *iso*-BCFA; *anteiso*-BCFA.
[3]*Iso*-BCFA = C14:0 *iso*; C15:0 *iso*; C16:0 *iso*; C17:0 *iso*; C18:0 *iso*.
[4]*Anteiso*-BCFA = C15:0 *anteiso*; C17:0 *anteiso*.
[5]Total *trans* C18:1 = sum of *trans* C18:1: C18:1 *trans*-6,*trans*-8; C18:1 *trans*-9; C18:1 *trans*-10; C18:1 *trans*-11; C18:1 *trans*-12; C18:1 *trans*-15; C18:1 *trans*-16.
[6]Total *trans* = sum of total *trans* FAs: C16:1 *trans*-14; C18:1 *trans*-6,*trans*-8; C18:1 *trans*-9; C18:1 *trans*-10; C18:1 *trans*-11; C18:1 *trans*-12; C18:1 *trans*-15; C18:1 *trans*-16; C18:2 *trans*-8,*cis*-13; C18:2 *cis*-9,*trans*-12.
[7]Sum of UFA: C14:1 *cis*-9; C16:1 *trans*-9; C16:1 *cis*-7; C16:1 *cis*-9; C16:1 *trans*-14; C17:1 *cis*-9; C18:1; C18:1 *trans*-6,*trans*-8; C18:1 *trans*-9; C18:1 *trans*-10; C18:1 *trans*-11; C18:1 *trans*-12; C18:1 cis-9; C18:1 *trans*-15; C18:1 *cis*-11; C18:1 *cis*-12; C18:1 *cis*-13; C18:1 *trans*-16; C18:1 *cis*-15; C18:2 *trans*-8,*cis*-13; C18:2 *cis*-9,*trans*-12; C18:2 *trans*-11,*cis*-15; C18:2 *cis*-9,*cis*-12; C18:3 *cis*-9,*cis*-12,*cis*-15; C20:1 *cis*-11; C20:3 *cis*-8,*cis*-11,*cis*-14; C20:5 *cis*5,*cis*-8,*cis*-11,*cis*-14,*cis*-17; C22:4 *cis*-7,*cis*-10,*cis*-13,*cis*-16; C22:5 *cis*-7,*cis*-10,*cis*-13,*cis*-16,*cis*-19.
[8]Sum of PUFA: C18:2 *trans*-8,*cis*-13; C18:2 *cis*-9,*trans*-12; C18:2 *trans*-11,*cis*-15; C18:2 *cis*-9,*cis*-12; C18:3 *cis*-9,*cis*-12,*cis*-15.
[9]Sum of MCFA (10 < C > 17): C12:0; C14-*iso*; C14:0; C15-*iso*; C14:1 *cis*-9; C15-*anteiso*; C15:0; C16-*iso*; C16:0; C16:1 *trans*-9; C16:1 *cis*-7; C16:1 *cis*-9; C16:1 *trans*-14; C17-*iso*; C17-*anteiso*; C17:0; C17:1 *cis*-9.
[10]Sum of LCFA (C > 17): C18-iso; C18:0; C18:1 *trans*-6,*trans*-8; C18:1 *trans*-9; C18:1 *trans*-10; C18:1 *trans*-11; C18:1 *trans*-12; C18:1 *cis*-9; C18:1 *trans*-15; C18:1 *cis*-11; C18:1 *cis*-12; C18:1 *cis*-13; C18:1 *trans*-16; C18:1 *cis*-15; C18:2 *trans*-8,*cis*-13; C18:2 *cis*-9,*trans*-12; C18:2 *trans*-11,*cis*-15; C18:2 *cis*-9,*cis*-12; C18:3 *cis*-9,*cis*-12,*cis*-15; C20:0; C18:2 *cis*-9,*trans*-11; C20:1 *cis*-11; C20:3 *cis*-8,*cis*-11,*cis*-14; C20:4 *cis*-5,*cis*-8,*cis*-11,*cis*-14; C20:5 *cis*-5,*cis*-8,*cis*-11,*cis*-14,*cis*-17; C22:4 *cis*-7,*cis*-10,*cis*-13,*cis*-16; C22:5 *cis*-7,*cis*-10,*cis*-13,*cis*-16,*cis*-19.
[11]Total DI calculated as (C14:1 *cis*-9 + C16:1 *cis*-9 + C18:1 *cis*-9 + C18:2 *cis*-9,*trans*-11)/(C10:0++ C14:0 + C14:1 *cis*-9 + C16:0 + C16:1 *cis*-9 + C18:0 + C18:1 *cis*-9 + C18:1 *trans*-11 + C18:2 *cis*-9,*trans*-11).

bacteria (e.g. BCFA) or those FAs, which are intermediates in the process of ruminal BH.

The concentration of BCFA was not affected by the dietary treatments. BCFAs are formed by ruminal bacteria from the propionate that originates in the rumen by the fermentation of dietary carbohydrates (Scaife *et al.*, 1978) and their synthesis is enhanced when animals are fed concentrate-based diets (Vlaeminck *et al.*, 2004). The concentration of BCFA in the rumen also depends on ruminal protozoal population (Or-Rashid *et al.*, 2007), which is sensitive to saponins (Wallace *et al.*, 1994; Makkar *et al.*, 1998). Therefore, in our study, we would have expected an interaction between saponin supplementation and the BCFA in the muscle. Probably the levels of saponin supplementation chosen for this study were not sufficient to have an impact on those microorganisms responsible for BCFA synthesis.

The concentrations of C16:1 *cis*-9 and C18:1 *cis*-9 were not affected by *Quillaja* supplementation; these FAs derive both from feed and from the desaturation of C16:0 and C18:0, operated by Δ^9-desaturase enzyme in the muscle (Choi *et al.*, 2001). Conversely, C14:1 *cis*-9 is exclusively synthesized endogenously in muscle by Δ^9-desaturase enzyme (Palmquist *et al.*, 2004) and therefore the DI C14:1 *cis*-9/(C14:0 + C14:1 *cis*-9) has been proposed as an evaluation index of Δ^9-desaturase enzyme activity in lamb muscle (Palmquist *et al.*, 2004). The lowering effect of saponins on the concentration of C14:1 *cis*-9 and of its DI was an unexpected result. Nevertheless, saponin supplementation did not affect the

Table 3 *Effect of saponins on* Longissimus dorsi *muscle cholesterol*

	Treatments*					
	C	QS30	QS60	QS90	s.e.m.	*P*-value
Cholesterol mg/100 g muscle	61.32	53.12	47.91	52.46	2.48	0.295
Cholesterol g/100 total lipids	**4.25**	**4.30**	**4.21**	**3.78**	0.208	0.825

*Treatments: C = lambs receiving control diet; QS30 = lambs receiving C diet plus 30 ppm of *Quillaja*; QS60 = lambs receiving C diet plus 60 ppm of *Quillaja*; QS90 = lambs receiving C diet plus 90 ppm of *Quillaja*.

other DIs. The fact that the DI C14:1 *cis*-9/(C14:0 + C14:1 *cis*-9) was the only DI affected by saponins might be explained by considering that Δ^9-desaturase enzyme has higher affinity for 16 and 18 carbon atoms' FAs than FAs with a shorter carbon chain (Enoch *et al.*, 1976). Even though we did not observe a dose-response effect of saponin supplementation, maybe in the presence of saponins, Δ^9-desaturase enzyme activity was weaker for C14:0, which is not among the Δ^9-desaturase enzyme preferred substrates of action. We did not observe changes between the four treatments in C16:1 and C18:1 accumulation probably because the high concentration of these FAs could have masked a variation of Δ^9-desaturase activity. In addition, it is plausible to suppose that saponins (or their aglycone) may influence directly or indirectly Δ^9-desaturase enzyme activity. In fact, an enzyme-inhibitory effect of saponins has been previously described, for example, *in vitro* for elastase and hyaluronidase enzymes (Facino *et al.*, 2006) and *in vivo* for the cytochrome P450 enzymatic system in rats (Kim *et al.*, 1997). In a previous study, we have found that supplementing lambs with tannins (another group of plants' secondary compounds, which are not absorbed through the digestive tract) reduced the Δ^9-desaturase enzyme protein expression (Vasta *et al.*, 2009). Further research is needed to elucidate it in context to saponins. In addition, RA is endogenously synthesized from the desaturation of VA by Δ^9-desaturase enzyme. According to our hypothesis of a possible inhibition of Δ^9-desaturase enzyme by saponins, we would have expected a lower concentration of RA in the meat of the saponins-supplemented lambs, but this was not the case. Palmquist *et al.* (2004) have shown that up to 90% of the RA in lamb intramuscular fat originates through endogenous synthesis.

Ruminant products are significant sources of dietary *trans* FAs, which originate from BH of dietary UFAs by the activity of ruminal microbes (Mosley *et al.*, 2002). In this study, none of the FAs that originated during the BH was affected by saponin supplementation. For most of the *trans* FAs the exact synthetic pathways are still unclear. The conversion of LA and LNA to VA is carried out by '*group A*' bacteria (mostly Gram-positive bacteria), whereas the following step of the BH is carried out by '*group B*' bacteria (mostly Gram-negative bacteria; Kemp and Lander, 1984). As Wang *et al.* (2000) noted, the Gram-negative bacteria are more resistant to saponins than Gram-positive bacteria. Therefore, we would have expected an effect of dietary saponins on ruminal BH, but at the inclusion levels of saponins chosen for this study we did not observe any interference of saponins on ruminal BH. Gutierrez *et al.* (1959) reported that *Butyrivibrio fibrisolvens* – one of the bacteria involved in the BH of PUFA in the rumen – strains isolated from cattle rumen was able to degrade alfalfa saponins. Therefore, it is possible that in this study saponins were ineffective because they were degraded by ruminal microorganisms.

C20:4n-6, whose concentration in this study was higher in the meat of QS60 lambs compared with that of C and QS30 lambs, is mainly derived from LA through elongation and desaturation reactions (Brenner, 1989). Arachidonic acid is preferentially stored in cell membrane phospholipids, rather than in triglycerides (Aurousseau *et al.*, 2004). There was a tendency to have a leaner meat in QS60 group compared with the other groups (1.16 for QS60 *v.* 1.5 for C, 1.25 for QS30, 1.43 for QS90 g of total intramuscular fat/100 g of muscle; $P < 0.1$). Leaner meat contains greater proportions of phospholipids than triglycerides (Aurousseau *et al.*, 2004) and this would explain the higher concentration of C20:4n-6 in the meat of the QS60 lambs compared with the other groups.

Effect of saponins on muscle cholesterol composition

A large number of studies have shown an hypocholesterolemic effect of saponins in mammals (Sidhu and Oakenfull, 1986). However, so far, there is no information on the effects of saponins on cholesterol metabolism in ruminants. There are two school of thoughts emerging for the hypocholesterolemic effect of saponins: (i) a direct binding between saponins and dietary cholesterol in the gut, which prevents its absorption from the small intestine and (ii) a binding between saponins and bile acids in the gut, which decreases the enterohepatic circulation of bile acids and increases cholesterol excretion with feces (Sidhu and Oakenfull, 1986). These hypotheses were formulated through studies with monogastric animals. Considering that ruminants' diets do not contain cholesterol, the first of the hypotheses above mentioned is not valid. Moreover, saponins are structurally modified by ruminal microorganisms (Makkar and Becker, 1997; Wang *et al.*, 1998) and it is likely that the aglycone remaining after saponin degradation is not as effective as the entire saponin in binding bile acids in the gut. This would explain why we did not observe any hypocolesterolemic effects of saponins in lamb. It is also likely that the inclusion level of saponins was not enough for interfering with cholesterol metabolism.

Conclusions

Saponin supplementation did not affect the overall FA profile of lamb meat. However, we have observed that saponin supplementation reduced the concentration of C14:1 *cis*-9 and the respective DI (C14:1 *cis*-9/(C14:0 + C14:1 *cis*-9)). We hypothesize an effect of saponins on Δ^9-desaturase enzyme activity, although the other DIs were not affected by saponin supplementation. None of the FAs arising from ruminal BH was affected by *Q. saponaria*. Supplementing lambs with 60 ppm of *Quillaja* increased C20:4n-6 in meat. Thus, an influence of saponin on LA metabolism is possible. The concentration of

cholesterol in meat was not influenced by saponin supplementation. This result could be attributed to insufficient dose of saponin supplemented in the diet or to structural modification of saponins carried out by ruminal microorganisms. Further investigations should be undertaken to evaluate the effect of saponins on ruminal BH, with emphasis on Δ^9-desaturase enzyme activity, and on cholesterol levels of lamb meat.

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