Fatty acid profile in the ruminal fluid and in the m. *longissimus dorsi* of lambs fed herbage or concentrate with or without tannins

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ABSTRACT - Twenty-eight male lambs were divided into two groups at age 45 d. Fourteen lambs were given fresh herbage (vetch); the remaining lambs were fed a concentrate-based diet. Within each treatment, seven lambs received a supplementation of quebracho tannins. At slaughter (age 105 d) the ruminal content and the muscle *longissimus dorsi* (LD) were collected. Ruminal fluid and LD fatty acid composition was determined by gas chromatography. Among the concentrates-fed lambs, tannins supplementation reduced ($P < 0.05$) the concentration of C18:0 (- 49 %) and increased vaccenic acid (VA; + 69 %) in the ruminal fluid. When tannins were included into the concentrate, the LD contained double levels of rumenic acid (RA) as compared to the LD of the lambs fed the tannins-free concentrate (0.96 *vs.* 0.46 % of total extracted fatty acids, respectively; $P < 0.05$). The concentration of PUFA was higher ($P < 0.05$) and SFA ($P < 0.01$) lower in the LD from lambs fed the tannin diets as compared to the animals receiving the tannin-free diets. In conclusion, tannins reduce the biohydrogenation of the PUFA in the rumen. This implies that tannins supplementation could be a strategy to increase the RA and PUFA content and to reduce the SFA into ruminant meats.

Key words: Conjugated linoleic acid, Fatty acids, Lamb, Tannins.

Introduction - Ruminant products are often blamed for their high content of SFA which are associated to increasing risk of cardiovascular diseases (Kromhout *et al.*, 1995). However, ruminant meat and milk contain the isomer cis-9, trans-11 of conjugated linoleic acid (rumenic acid, RA), which has been shown to exert favourable effects on human health (Ip *et al*., 1991). Rumenic acid arises from the ruminal biohydrogenation (BH) of cis-9, cis-12 18:2 (linoleic acid, LA) and of cis-9, cis-12, cis-15 18:3 (linolenic acid, LNA). Moreover, RA can also be formed endogenously in the muscle or in the mammary gland from trans-11 C18:1 (vaccenic acid, VA) through the action of Δ^9-desaturase enzyme (Corl *et al*., 2003). Several feeds used for ruminants contain tannins which affect the activity of ruminal microorganisms (Min *et al.,* 2003). It has been shown that dietary tannins reduce ruminal biohydrogenation in vitro (Vasta *et al*., 2009a) and affect muscular Δ^9-desaturase protein expression (Vasta *et al*., 2009b) in sheep. Here we have investigated the effect of dietary tannins, supplemented either in green herbage or in concentrates, on the fatty acid profile in sheep ruminal fluid and muscle.

Material and methods - Twenty-eight male Comisana lambs were weaned at 45 days of age (average initial BW 20.6 kg ± S.D. 3.35 kg) and divided according to a 2×2 factorial arrangement of treatments to

evaluate the effects of feeding system (herbage *vs.* concentrate) and supplementation (tannins *vs.* none). Over the 60-d experimental period, 14 lambs were fed vetch (*Vicia sativa*; a legume herbage not containing tannins) ad libitum. The remaining 14 lambs received a concentrate containing (as fed) barley (55.1 %), alfalfa hay (30.0 %), soybean meal (13.0 %) and mineral premix (1.9 %). Within each feeding system, half of the lambs (i.e., 7 herbage and 7 concentrate animals) received a supplementation of quebracho (*Schinopsis lorentzii*) tannins. The quebracho supplied to the lambs corresponded to 8.93% on the DM. Animals were slaughtered at age 105 days. Filtered ruminal fluid and the muscle *longissimus dorsi* (LD) were sampled at slaughter and stored at -80°C awaiting for fatty acids analyses. Ruminal fluid was methylated by direct transesterification procedure (Kramer *et al*., 1997). Intramuscular fatty acids were extracted as described in Vasta *et al*. (2009b). Fatty acids methyl esters were analyzed by gas chromatography using a GC 8000 TOP gas chromatograph (Thermo Fisher Scientific Inc., Milan, Italy) equipped with a 100-m WCOT CP-Select capillary column (i.d., 0.25 mm; film thickness, 0.25 µm; Chrompack, Middelburg, the Nederlands) and a flame ionization detector. Data were analyzed using the GLM procedure. The model included the feeding system (herbage or concentrate) and supplement (tannins or none) as fixed factors and the interaction between the two factors. When no significant interaction was found ($P > 0.05$), the model was reduced to main effects only. Means were compared using Tukey's test.

Results and conclusions - *Ruminal fluid fatty acid composition.* The concentration of C18:0 was lower in the ruminal fluid of the lambs fed concentrate with tannins compared to the lambs fed concentrate without tannins supplementation (19.84 *vs.* 39.00 % of total extracted fatty acids, respectively; $P < 0.05$; Table 1). Among the concentrate-fed lambs, VA was found at double concentration in the ruminal fluid of the lambs receiving tannins supplementation compared with the lambs not receiving tannins (5.50 vs. 2.80 % of total extracted fatty acids, respectively; $P < 0.05$). Among the lambs fed concentrates, the amount of total trans C18:1 fatty acids was double for the animals receiving the tannins supplementation as compared with those receiving the tannins-free concentrate (Table 1). Tannins supplementation resulted in a higher ($P < 0.05$) percentage of RA compared to the animals not receiving tannins. The concentration of LA tended to be higher in the ruminal fluid from lambs receiving the concentrate + tannins compared with those fed the tannin-free concentrate (2.72 vs. 1.41 % of total extracted fatty acids, respectively; $P = 0.061$). The inclusion of tannins into the concentrate strongly reduced (- 26%, on average; $P < 0.05$) the concentration of SFA and increased the concentration of MUFA ($P < 0.05$) as compared to the ruminal fluid of the lambs not receiving tannins (Table 1).

Muscle fatty acid composition. The inclusion of tannins into the concentrate resulted in a lower percentage of C18:0 compared to the intramuscular fat of the lambs fed the concentrate without tannins (- 18.8 %; $P < 0.0005$). When tannins were included into the concentrate the total trans C18:1 accumulated in the longissimus muscle at double percentage as compared to the muscle of the lambs fed the other treatments (5.23 *vs.* 2.57 % of total fatty acids, respectively). When tannins were included into the concentrate, the LD contained double levels of RA as compared to the LD of the lambs fed the tannins-free concentrate (0.96 vs. 0.46 % of total extracted fatty acids, respectively; $P < 0.05$). When tannins were added to the herbage the concentration of LA in the muscle was increased by 77 % compared to the lambs fed herbage without tannins ($P < 0.05$). The concentration of LNA was affected in tendency ($P = 0.076$) by tannins supplementation (Table 2). The tannins supplementation reduced the intramuscular SFA ($P < 0.01$) and increase the PUFA compared with the lambs fed a diet without tannins. In conclusion, the supplementation of quebracho tannins to lambs given a fresh herbage or a concentrate diet reduces ruminal biohydrogenation, leading to the accumulation of VA. This has major implications on meat fatty acid profile because VA acid is the precursor of the endogenous biosynthesis of RA. Also, the meat of the tannins-receiving lambs had higher percentages of PUFA and lower percentages of SFA as compared to the meat of the animals not supplemented with tannins, thus improving meat healthy properties.

Table 1. Effect of tannin supplementation on ruminal fluid fatty acids (% of total fatty acid methyl esters) of lambs fed herbage or concentrate diets.

	Feeding system (FS)							
	Herbage		Concentrate			*P*-value		
Supplement (S)	None	Tannins	None	Tannins	SEM	S	FS	S×FS
No of lambs	7	7	7	7				
C18:0	39.40 ^{y}	38.53 ^{y}	39.00 ^{y}	19.84 ^{z}	2.299	0.009	0.01	0.01
trans-11 C18:1	3.19 ^{z}	3.82 ^{zy}	2.80 ^{z}	5.50 ^{y}	0.300	0.002	0.18	0.04
Σ*trans* C18:1[a]	4.82 ^{z}	6.03 ^{z}	11.82 ^{z}	23.97 ^{y}	1.743	0.003	<0.0005	0.01
cis-9, *trans*-11 C18:2[b]	1.06	1.41	0.44	1.30	0.157	0.04	0.23	0.39
cis-9, *cis*-12 C18:2 n-6	2.17	2.00	1.41	2.72	0.189	0.11	0.95	0.04
cis-9, *cis*-12, *cis*-15 C18:3 n-3	1.48	0.42	1.68	0.68	0.165	0.40	0.001	0.90
ΣSFA	72.38 ^{y}	70.89 ^{y}	67.03 ^{y}	51.87 ^{z}	2.023	0.005	<0.0005	0.01
ΣMUFA	11.25 ^{z}	12.37 ^{z}	19.24 ^{z}	33.56 ^{y}	2.010	0.002	<0.0005	0.007
ΣPUFA	6.24	6.66	4.26	6.91	0.402	0.04	0.25	0.14

[a]Σtrans C18:1 = sum of trans 18:1 fatty acids, calculated as the sum of: trans-6 + trans-8 C18:1; trans-9 C18:1; trans-10 C18:1; trans-11 C18:1; trans-12 + cis-7 C18:1; trans-13 + trans-14 C18:1; trans-16 C18:1. [b]Conjugated linoleic acid. [x,y,z] Mean values within a row with unlike superscript letters were significantly different (P<0.05).

Table 2. Effect of tannin supplementation on longissimus muscle fatty acids (% of total fatty acid methyl esters) of lambs fed herbage or concentrate diets.

	Feeding system (FS)							
	Herbage		Concentrate			*P*-value		
Supplement (S)	None	Tannins	None	Tannins	SEM	S	FS	S×FS
No of lambs	7	7	7	7				
C18:0	14.76 ^{y}	14.75 ^{y}	14.16 ^{y}	11.49 ^{z}	0.334	0.006	<0.0005	0.006
trans-11 C18:1	1.31	1.04	0.69	1.32	0.109	0.38	0.41	0.039
Σ*trans* C18:1[a]	2.61 ^{z}	2.73 ^{z}	2.39 ^{z}	5.23 ^{y}	0.259	<0.0005	<0.0005	<0.0005
cis-9, *trans*-11 C18:2[b]	0.82	0.50	0.46	0.96	0.090	0.60	0.77	0.024
cis-9, *cis*-12 C18:2 n-6	8.16 ^{z}	14.46 ^{y}	9.23 ^{z}	10.67 ^{zy}	0.671	0.001	0.20	0.028
cis-9, *cis*-12, *cis*-15 C18:3 n-3	2.42	3.52	1.00	1.38	0.267	0.07	<0.0005	0.37
ΣSFA	43.00	39.36	41.72	40.30	0.482	0.007	0.84	0.20
ΣMUFA	37.34	30.93	40.88	40.17	1.104	0.048	0.001	0.10
ΣPUFA	19.22	29.30	17.12	19.17	1.438	0.017	0.016	0.10

[a]Σtrans C18:1 = sum of trans 18:1 fatty acids, calculated as the sum of: trans-4 C18:1; trans-6 + trans-8 C18:1; trans-9 C18:1; trans-10 C18:1; trans-11 C18:1; trans-12 + cis-7 C18:1; trans-16 C18:1. [b]Conjugated linoleic acid. [x,y,z] Mean values within a row with unlike superscript letters were significantly different (P<0.05).

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