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Effect of forage/concentrate ratio and soybean oil supplementation on *in vitro* fatty acid profile of sheep rumen liquor

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ABSTRACT - In an *in vitro* trial, the effect of forage/concentrate ratio (F/C) and soybean oil (SO) supplementation on rumen liquor fatty acids profile was studied with the aim to evaluate changes in the profiles of biohydrogenation intermediates during the fermentation process. Feed samples of four diets were inoculated with sheep rumen liquor: i) high F/C and no SO; ii) high F/C and SO; iii) low F/C and no SO; iv) low F/C and SO. Fermentation times were 6, 12 h. Trans fatty acid were formed when oil supplement or a low forage content were present in the diets, but at different times of fermentation, as a consequence of a shift from the usual rumen biohydrogenation pathway. In fact, when a higher forage percentage was included in the diet, the alternative pathways of rumen biohydrogenation did not occur; trans10 C18:1 concentration was low and neither trans10, cis12 CLA nor trans, trans CLA appeared.

Key words: Forage/concentrate ratio, Trans fatty acids, Rumen biohydrogenation, Sheep.

Introduction - The biohydrogenation theory proposes that, in dairy cows, under certain dietary conditions, the typical pathways of rumen biohydrogenation are altered to produce trans fatty (trans 10 C18:1 and trans10 cis12 C18:2) acids that inhibit milk fat synthesis (Bauman *et al.*, 2003). Little information are available for dairy sheep. In a previous *in vivo* trial, Mele *et al.* (2006) studied the effect of F/C and SO supplementation on yield and fatty acid composition of milk from Sarda ewes; in this experiment, lactating ewes were fed four diets with different F/C and with or without SO. When oil was supplemented to a diet with a low F/C, the milk content of trans10 C18:1 and trans10, cis12 CLA increased, but the milk fat depression was not shown. Aim of present work is monitoring the fatty acid profile during in vitro rumen fermentation of samples of the same diets tested in the cited in vivo trial in order to evaluate changes in the profiles of biohydrogenation intermediates during the fermentation process in response to different lipid contents and F/C.

Material and methods - The composition of the four diets, studied *in vitro,* was: i) high F/C and no SO (HF/NO): lucerne hay 75% DM, barley meal 20% DM, soy bean meal 5% DM; ii) high F/C and SO (HF/O): lucerne hay 75% DM, barley meal 15% DM, soy bean meal 6% DM, SO 4% DM; iii) low F/C and no SO (LF/NO): lucerne hay 60% DM, barley meal 35% DM, soy bean meal 5% DM; iv) low F/C and SO (LF/O): lucerne hay 60% DM, barley meal 30% DM, soy bean meal 6% DM, soybean oil 4% DM. Samples were analysed for crude protein (CP), ash and ether extract (EE), according to AOAC (1990). Neutral detergent fibre (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined according to Van Soest *et al.* (1991) and non structural carbohydrates (NSC) were calculated according to Cornell Net Carbohydrate and Protein System (CNCPS; Licitra *et al.*, 1996); data are shown in table 1. The fatty acid profile of soybean oil, obtained according to Buccioni *et al.* (2006),

was characterized by the presence of cis9 C18:1 (OA), cis9 cis12 C18:2 (LA) and C18:3 cis9 cis12 cis15 (LNA) respectively 22.0, 50.5 and 6.7 (g/100 lipid extract). Feed samples were fermented according to Buccioni *et al.* (2006). Fermentation times were 6, 12 h. At the end of each fermentation period, the whole content of the fermentation flask was freeze-dried and methylated according to Kramer *et al.* (1997). The fatty acids methyl esters (FAME) were separated on a GC equipped with a capillary column (CP-select CB for FAME Varian, Middelburg, the Nederlands: 100 m x 0.25 mm i.d.; film thickness 0.20 µm) and quantified using C23:0 methyl ester as the internal standard. The injector and flame ionization detector temperatures were 270°C and 300°C. 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. Data were processed by GML of SAS (1999) using a linear model with two fixed factors with interaction: diet and fermentation time: $y_{ijn}=\mu+D_j+T_n+D_jT_n+e_{ijn,}$ where y_{ij} is the observation; μ is the overall mean; D_j the diet (j = 1, 2, 3, 4); T_n the fermentation time (n=1 to 2); D_jT_n the interaction between diet and fermentation time and e_{ijn} the residual error.

Table 1. Chemical composition of the four diets (g/100g DM).

	Diets					Diets			
	HF/NO	HF/O	LF/NO	LF/O		HF/NO	HF/O	LF/NO	LF/O
DM	85.6	86.1	85.7	86.1	ADF	28.0	27.6	28.4	23.2
CP	15.6	15.5	15.6	15.2	ADL	7.5	7.4	6.2	6.1
EE	1.8	5.5	1.6	5.2	NSC	30.5	27.5	36.8	34.0
NDF	45.2	44.7	39.5	39.3	Ash	7.0	6.8	6.4	6.2

Results and conclusions - The chemical composition of the diets affected the rumen liquor fatty acids profile. The fermentation was characterized by different amounts of trans isomers and by different times at which trans fatty acids reached the maximum yield (table 2). The presence of oil enhanced C18:1 trans fatty acids percentage in the oil supplemented diets, independently of F/C. In particular, LF/O showed times of biohydrogenation delayed with respect to the diets with a higher content of forage or without SO supplementation, inducing an accumulation of trans fatty acids. The main C18:1 isomer in rumen liquor was trans11 C18:1 (VA) for all diets, but with different concentrations.

Cis9 trans11 C18:2 (RA) was the main CLA isomers in rumen liquor in any case (table 2). In particular, RA reached the highest concentration at 6h in rumen liquor fermented with oil supplemented diets; on the contrary, with HF/NO and LF/NO the maximum was shifted at 12h. The coupling high forage and oil supplement in the diet produced also trans10 cis12 C18:2 isomer, whose appearance was only at 6h. The presence of oil in the diets or a low forage/concentrate ratio induced the production also of trans, trans CLA isomers.

As shown in literature, a diet with a high value of LA increases the RA and VA percentages in rumen liquor (Buccioni *et al.* 2006); the presence of trans, trans CLA, of trans10 cis12 CLA and trans10 C18:1 fatty acids were, probably, due to a shift in LA biohydrogenation as a consequence of an unusual lipids rumen biohydrogenation, induced by the presence of a low F/C or lipid supplementation. In agreement with Bauman and Griinari (2001), for HF/O, LF/O and LF/NO, the typical pathways of rumen biohydrogenation were altered. With high F/C, the alternative pathways of rumen biohydrogenation did not occur. In fact, in HF/NO rumen liquor neither trans10, cis12 CLA nor trans, trans CLA appeared. Moreover, the F/C or the inclusion of SO in the diets influenced the times of biohydrogenation

inducing different times of trans fatty acids synthesis; it could be due not only to a different chemical composition of the fermented organic matrix but also to the physical interaction between rumen bacteria and the oil soaked feed particles.

Table 2. Trans C18:1 and CLA isomers appearance in the rumen liquor at different fermentation times (mg/100 g DM). Data shown are the means of 3 replicates.

Fatty acid	Time	HF/NO	HF/O	LF/NO	LF/O	SEM
C18:1 t9	6 h	0.55 α a	2.61 β a	0.00 γ a	0.00 γ a	0.08
	12 h	0.00 α b	0.00 α b	0.67 β b	1.40 γ b	
C18:1 t10	6 h	0.55 α a	13.05 β a	1.55 γ a	0.00 δ a	0.04
	12 h	1.71 α b	0.00 β b	0.00 β b	5.04 γ b	
C18:1 t11	6 h	0.54 α a	59.16 β a	4.95 γ a	18.42 δ a	0.09
	12 h	12.87 α b	58.63 β b	0.57 γ b	27.30 δ b	
C18:1 t12	6 h	0.88 α a	3.34 β a	3.30 β a	0.00 γ a	0.05
	12 h	0.49 α b	4.29 β b	0.00 γ b	2.52 δ b	
CLA c9 t11	6 h	0.00 αa	97.59 βa	0.00 αa	99.09 γa	0.09
	12 h	80.15 αb	24.45 βb	49.78 γb	7.28 δb	
CLA t10 c12	6 h	0.00 α	6.38 βa	0.00 α	0.00 α	0.05
	12 h	0.00	0.00 b	0.00	0.00	
CLA t,t	6 h	0.00 α	24.07 βa	1.75 γa	10.53 δa	0.09
	12 h	0.00	0.00 b	0.00 b	0.00 b	

*Within a row, means with different Greek superscripts are significantly different (*P<0.05); within a column, means with different Latin superscripts are significantly different (*P<0.05).*

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